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ADENOSINE TRIPHOSPHATE EXTRACTION PATENTS

https://en.wikipedia.org/wiki/Adenosine_triphosphate

Adenosine_Triphosphate

Adenosine triphosphate (ATP) is a nucleotide, also called a nucleoside triphosphate, is a small molecule used in cells as a coenzyme. It is often referred to as the "molecular unit of currency" of intracellular energy transfer.[1]

ATP transports chemical energy within cells for metabolism. Most cellular functions need energy in order to be carried out: synthesis of proteins, synthesis of membranes, movement of the cell, cellular division, transport of various solutes etc. The ATP is the molecule that carries energy to the place where the energy is needed. When ATP breaks into ADP (Adenosine diphosphate) and Pi (phosphate), the breakdown of the last covalent link of phosphate (a simple -PO4) liberates energy that is used in reactions where it is needed.

It is one of the end products of photophosphorylation, aerobic respiration, and fermentation, and is used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division.[2] One molecule of ATP contains adenine, ribose, and three phosphate groups, and it is produced by a wide variety of enzymes, including ATP synthase, from adenosine diphosphate (ADP) or adenosine monophosphate (AMP) and various phosphate group donors. Substrate-level phosphorylation, oxidative phosphorylation in cellular respiration, and photophosphorylation in photosynthesis are three major mechanisms of ATP biosynthesis.

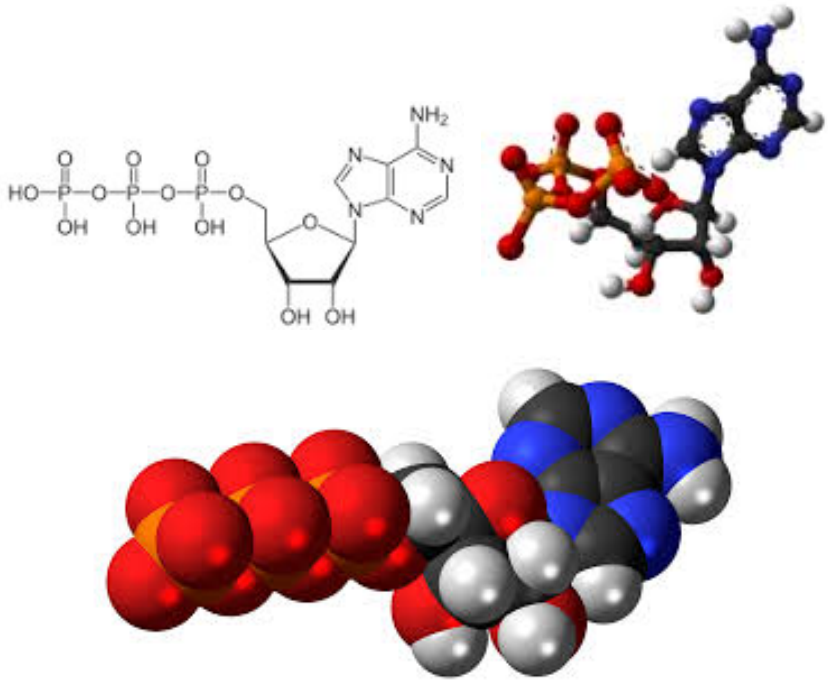
Metabolic processes that use ATP as an energy source convert it back into its precursors. ATP is therefore continuously recycled in organisms: the human body, which on average contains only 250 grams (8.8 oz) of ATP,[3] turns over its own body weight equivalent in ATP each day.[4]

ATP is used as a substrate in signal transduction pathways by kinases that phosphorylate proteins and lipids. It is also used by adenylate cyclase, which uses ATP to produce the second messenger molecule cyclic AMP. The ratio between ATP and AMP is used as a way for a cell to sense how much energy is available and control the metabolic pathways that produce and consume ATP.[5] Apart from its roles in signaling and energy metabolism, ATP is also incorporated into nucleic acids by polymerases in the process of transcription. ATP is the neurotransmitter believed to signal the sense of taste.[6]

The structure of this molecule consists of a purine base (adenine) attached by the 9′ nitrogen atom to the 1′ carbon atom of a pentose sugar (ribose). Three phosphate groups are attached

at the 5′ carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter-convert ATP, ADP and AMP. When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.

ATP was discovered in 1929 by Karl Lohmann,[7] and independently by Cyrus Fiske and Yellapragada Subbarow of Harvard Medical School,[8] but its correct structure was not determined until some years later. It was proposed to be the intermediary molecule between energy-yielding and energy-requiring reactions in cells by Fritz Albert Lipmann in 1941.[9] It was first artificially synthesized by Alexander Todd in 1948.[10]...



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http://www.webmd.com/vitamins-supplements/ingredientmono-1067-adenosine.aspx?activeingredientid=1067&activeingredientname=adenosine

ADENOSINE

Overview

Adenosine is a chemical that is present in all human cells. It readily combines with phosphate to form various chemical compounds including adenosine monophosphate (AMP) and adenosine triphosphate (ATP). People use it for medicine.

AMP is taken by mouth for treating shingles (herpes zoster infection) and a blood disorder called porphyria cutanea tarda.

ATP is used under the tongue to increase physical energy. It is also given intravenously (by IV) for treating acute kidney failure, multiple organ failure, high blood pressure in lungarteries (pulmonary hypertension), cystic fibrosis, lung cancer, weight loss associated

with cancer, and controlling blood pressure during anesthesia and surgery. It is also used for cardiac stress tests.

Healthcare providers give adenosine intravenously for treating surgical pain and nerve pain, pulmonary hypertension, and certain types of irregular heartbeat. It is also given for controlling blood pressure during anesthesia and surgery and for heart tests called cardiac stress tests.

Adenosine is injected into the space around the spinal cord to treat nerve pain.

Adenosine phosphate is given by injection into the muscle (intramuscularly) for treating varicose veins, bursitis, pain and swollen tendons (tendonitis), itchiness, multiple sclerosis (MS), neuropathy, shingles (herpes zoster infection), cold sores and genital herpes (herpes simplex infections), and poor blood circulation.

How does it work?

Adenosine blocks faulty circuitry in the heart, which causes irregular heart rhythm. Adenosine triphosphate (ATP) might prevent changes in energy metabolism that cause weight loss in people with advanced cancer.

Uses & Effectiveness

Effective for:

Treating certain kinds of irregular heartbeat (as a prescription-only intravenous medicine).

Possibly Effective for:

Treating weight loss in people with advanced cancer. Intravenous ATP seems to improve appetite, food intake, and quality of life in people with advanced non-small-cell lung cancer and other tumors.

Wounds, usually in the legs, due to poor circulation (venous stasis ulcers). Intramuscular AMP might relieve fluid retention, itchiness, swelling and redness due to venous stasis ulcers.

Insufficient Evidence for:

Shingles (herpes zoster infection). Early research suggests that AMP given by injection into the muscle might be effective for treating herpes zoster (shingles) infection and for preventing nerve pain that follows these infections. Intramuscular AMP might also be effective for treating other kinds of herpes infections, according to limited research.
Lung cancer. Developing studies suggest that ATP is not effective for treating non-small-cell lung cancer.
Pain.
Other conditions.
More evidence is needed to rate the effectiveness of adenosine for these uses.

Side Effects & Safety

Adenosine appears to be safe for most people when given by injection by qualified healthcare givers. It can cause breathing problems and chest pain, particularly when given at high doses. Headache, heart pounding, low blood pressure, nausea, sweating, flushing, lightheadedness, sleep problems, coughing, and anxiety can also occur.
Special Precautions & Warnings:
Pregnancy and breast-feeding: Not enough is known about the use of adenosine during pregnancy and breast-feeding. Stay on the safe side and avoid use.

Gout: ATP can raise the level of uric acid in the blood stream and in the urine, and this might trigger a case of gout. Gout causes red, hot, tender, swollen joints. The joint that is most often affected is at the base of the big toe.

Heart disease: ATP can cause reduced blood flow to the heart and chest pain. It might worsen symptoms in patients with heart diseases such as chest pain and heart attack...

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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3483284/
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doi: 10.1186/1550-2783-9-48

Adenosine-5'-triphosphate (ATP) supplementation improves low peak muscle torque and torque fatigue during repeated high intensity exercise sets

John A Rathmacher

Abstract
Background

Intracellular concentrations of adenosine-5’-triphosphate (ATP) are many times greater than extracellular concentrations (1–10 mM versus 10–100 nM, respectively) and cellular release of ATP is tightly controlled. Transient rises in extracellular ATP and its metabolite adenosine have important signaling roles; and acting through purinergic receptors, can increase blood flow and oxygenation of tissues; and act as neurotransmitters. Increased blood flow not only increases substrate availability but may also aid in recovery through removal of metabolic waste products allowing muscles to accomplish more work with less fatigue. The objective of the present study was to determine if supplemental ATP would improve muscle torque, power, work, or fatigue during repeated bouts of high intensity resistance exercise...

Results

No differences were detected in high peak torque, power, or total work with ATP supplementation; however, low peak torque in set 2 was significantly improved ($p < 0.01$). Additionally, in set 3, a trend was detected for less torque fatigue with ATP supplementation ($p < 0.10$).
Conclusions

Supplementation with 400 mg ATP/d for 15 days tended to reduce muscle fatigue and improved a participant’s ability to maintain a higher force output at the end of an exhaustive exercise bout...

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Adenosine 5′-triphosphate (ATP) supplements are not orally bioavailable: a randomized, placebo-controlled cross-over trial in healthy humans

Ilja CW Arts

Abstract

Background

Nutritional supplements designed to increase adenosine 5′-triphosphate (ATP) concentrations are commonly used by athletes as ergogenic aids. ATP is the primary source of energy for the cells, and supplementation may enhance the ability to maintain high ATP turnover during high-intensity exercise. Oral ATP supplements have beneficial effects in some but not all studies examining physical performance. One of the remaining questions is whether orally administered ATP is bioavailable. We investigated whether acute supplementation with oral ATP administered as enteric-coated pellets led to increased concentrations of ATP or its metabolites in the circulation.

Results

ATP concentrations in blood did not increase after ATP supplementation via enteric-coated pellets or naso-duodenal tube. In contrast, concentrations of the final catabolic product of ATP, uric acid, were significantly increased compared to placebo by ~50% after administration via proximal-release pellets ($P = 0.003$) and naso-duodenal tube ($P = 0.001$), but not after administration via distal-release pellets.

Conclusions

A single dose of orally administered ATP is not bioavailable, and this may explain why several studies did not find ergogenic effects of oral ATP supplementation. On the other hand, increases in uric acid after release of ATP in the proximal part of the small intestine suggest that ATP or one of its metabolites is absorbed and metabolized. Uric acid itself may have ergogenic effects, but this needs further study. Also, more studies are needed to determine whether chronic administration of ATP will enhance its oral bioavailability...

Background

Nutritional supplements designed to increase adenosine 5′-triphosphate (ATP) concentrations are commonly used by athletes as ergogenic aids. ATP is the primary source of energy for the cells, and supplementation may enhance the ability to maintain high ATP turnover during high-intensity exercise. ATP is also released from cells to act as a local regulator of neurotransmission, inflammation, and nociception via interaction with purinergic receptors [1,2]. ATP is present in substantial concentrations in a number of foods (e.g. meat, soy, mushrooms) [3] and in breast milk [4,5]. Furthermore, capsules containing ATP are currently registered in France for the treatment of low back pain of muscular origin, and supplements containing ATP are marketed on the internet for various purposes including the restoration of

energy.

Oral ATP supplements have beneficial effects in some but not all studies examining physical performance. In an experimental study by Jordan et al.[6], three groups of nine healthy men received ATP (150 or 225 mg) or placebo for 14 days. Physical performance and muscular strength were positively affected. Another study investigated the effects of supplementation with an ATP-containing registered drug for 30 days (Atépadène®, 90 mg daily) [7,8]. The questionnaire-based outcome indicated that it provided benefit to patients with subacute low back pain. In contrast to these beneficial findings, Herda et al. [9] found no improvements in muscle strength, power output, or endurance after supplementation of 24 healthy men with a commercially available treatment intended to increase ATP. The authors suggested that the lack of an effect in this double-blind, placebo-controlled crossover trial, might be caused by breakdown of ATP in the gastrointestinal tract. Because they did not collect blood samples from the participants, the authors could not verify whether ATP concentrations in the blood circulation had been altered as a result of supplementation [9].

Evidence on the oral availability of ATP supplements is limited. In the study by Jordan et al. [6], no changes in whole blood and plasma ATP concentrations were detected, but the dosages administered were modest (225 mg or less). Animal studies reporting alterations in cardiac, vascular and pulmonary function after 30 days of oral ATP supplementation, also found no increases in systemic concentrations of plasma or erythrocyte ATP [10,11]. However, the concentration of ATP in plasma taken from the portal vein of rats increased rapidly up to a 1000-fold after instillation of ATP in de small intestine [11]. The identification of a number of nucleoside transporters in the small intestine further suggested that orally administered ATP may be absorbed and utilized by the human body [12].

We have previously shown that ATP is bioavailable after intravenous administration in humans [13]. ATP concentrations in erythrocytes increased in a dose-dependent manner by ~60% after 24 h of continuous infusion. We now report the results of a randomized, placebo-controlled, cross-over trial in 8 healthy humans, designed to assess the oral bioavailability of an ATP nutritional supplement. The ATP was administered as a single dose that was high enough to enable its detection in whole blood (5000 mg). Furthermore, an acid-resistant enteric coating of the multi-particulate supplement was used to prevent the degradation of ATP in the acidic environment of the stomach. As a comparison, ATP was also directly instilled in the small intestine via a naso-duodenal tube....

CN103163122
Portable extracting device of adenosine triphosphate

Inventor(s): WAN DONGYUN +

The invention relates to a novel extracting device of cell adenosine triphosphate (ATP). The portable extracting device of the adenosine triphosphate aims to overcome the defects of a chemical extracting method and a physical extracting method in the existing bioluminescent analysis method and provides the novel extracting device of the adenosine triphosphate. Based on a boiled extraction principle, by means of exquisite designs of an extractor structure of the adenosine triphosphate, a heating system and the like, the portable extracting device of the adenosine triphosphate achieves convenient operation of extraction of the adenosine

triphosphate, and has the advantages that the required samples are less, the operation is simple, people do not need to worry about that the concentration of the adenosine triphosphate is influenced by outflow of steam due to boiling, the steam influences the laboratory environment and the like, and the defects that inhibition of decomposing enzyme of the adenosine triphosphate and protection of luciferase can not be achieved together in the chemical extracting method are overcome.

DESCRIPTION

The present invention relates to a novel cell Adenosine triphosphate (ATP) extraction device. This device provides a new adenosine triphosphate extraction device for the shortcomings of the existing bioluminescence analysis methods, the adenosine triphosphate chemical extraction method and the physical extraction method. The device is based on the principle of boiling extraction, through the careful design of adenosine triphosphate extractor structure, heating system, etc., to achieve the cell adenosine triphosphate extraction of the deliberate operation, with less sample, simple operation, do not worry about boiling lead to steam spill effects of samples of adenosine triphosphate And the impact of steam on the laboratory environment and many other advantages, but also to overcome the chemical extraction methods exist on the inhibition of adenosine triphosphate enzyme inhibition and luciferase protection can not have both drawbacks.

Technical field

The present invention belongs to the field of biochemical analysis, and more particularly to a novel cell adenosine triphosphate extraction device and method.

Background technique

Microbiological testing is of great importance both in clinical testing and in industrial applications. At present, the detection of microbes mainly uses the traditional plate culture counting method, which takes a long time to cultivate the microorganisms under certain conditions, and then the plate count to obtain the concentration of microorganisms. The method steps cumbersome, time-consuming, often difficult to meet the requirements of the rapid detection of the scene. Adenosine triphosphate biofluorescence detection method has been developed in recent years, a new microbial detection method, it has a fast, accurate, sensitive and many other advantages.

Adenosine triphosphate (ATP) as an important energy molecule exists in all organisms, by determining the amount of adenosine triphosphate under certain conditions, can indirectly infer the number or concentration of microorganisms. The adenosine triphosphate biofluorescence assay utilizes the complex biochemical reactions between adenosine triphosphate (ATP) and fluorescein-luciferase in the microbial cells to produce bioluminescence, and then the fluorescence intensity is measured by a fluorometer or a liquid scintillation tester. In the case where the external conditions such as fluorescein, luciferase, temperature, and pH are the same, the fluorescence intensity is proportional to the amount of adenosine triphosphate.

Before performing the above-mentioned photo-light reaction to test the amount of adenosine triphosphate, it is first necessary to break the cell wall and the cell membrane to release adenosine triphosphate by certain physical and chemical means. At present, taking into

account the factors conducive to the operation, usually the use of chemical means to release adenosine triphosphate. Commonly used chemical release agents, including, for example, surfactants, acids, bases, and even organic solvents. In addition to the release of the release of adenosine triphosphate in the release of the purpose, but also should have the following two effects: First, should be quickly passivated release process of the cell's own adenosine triphosphate protease (ATPase), reduce or eliminate its adenosine triphosphate Of the decomposition; the second is not the follow-up of light in the reaction of luciferase adverse effects. The above two requirements for the release of the liquid to meet the two opposite effects of the biological enzyme lead to the choice of the release fluid is difficult to complete, usually taking into account the two components after the compromise, thereby reducing the sensitivity of bioluminescence detection, No technology to completely avoid the above shortcomings.

Physical means is another option for the extraction of adenosine triphosphate, such as mechanical extrusion, ultrasound, boiling, etc., but some of these shortcomings have led to its lack of access to widespread use, such as mechanical extrusion, ultrasound, etc., and can not make ATPase , It will hydrolyze the microbial adenosine triphosphate, affecting the detection sensitivity; although the heating method can make ATPase denaturation, extraction rate is high, but there are cumbersome, repeatability is not good, will produce steam effects of sample size and laboratory environment and other shortcomings. Therefore, it is very important to find a more convenient and effective method for the extraction of adenosine triphosphate with both ATPase inhibition and luciferase protection, which is important for the improvement and promotion of bioluminescence analysis.

The contents of the invention

The object of the present invention is to provide an effective and simple extraction device for the problems existing in the physical and chemical release means of adenosine triphosphate in the presence of adenosine triphosphate biofluorescence detection. The device should have the advantages of easy to use, less sample consumption, good repeatability, and almost no steam spillover.

In order to achieve the above object, the technical solution of the present invention is to provide a portable extraction device for adenosine triphosphate, which comprises a three-step adenosine triphosphate extractor, a heating system, and a temperature control system.

Boiling is a good way to extract the microbial adenosine triphosphate, the extraction rate is high, and can be extracted with adenosine triphosphate at the same time, making ATPase denaturation, inhibition of adenosine triphosphate hydrolysis, but commonly used tube, beaker and other boiled containers (extractor) is usually used external contact heat transfer (Such as resistance wire furnace, etc.), it is difficult to precisely control the boiling time, resulting in a large change in extraction rate, sample test repeatability is poor; the same time, test tubes, beakers and other boiling containers for the open state, resulting in steam spill, the impact of extraction of adenosine triphosphate sample concentration The
In view of the above-mentioned drawbacks, the present invention adopts and combines the following three ways to precisely control the boiling time: 1) the use of infrared radiation heating, far infrared penetration is strong, in sufficient high radiant heat state, can achieve almost instantaneous boiling; (3) small sample pool (<0.3mL capacity spherical container); 4) in the heating system outside the set of infrared reflection system to improve the effective utilization of infrared radiation heat. The above method allows the heat to reach and focus

quickly on the sample position, so that the sample is almost instantaneous boiling, will be heated to boiling time is almost reduced to zero, reducing the calculation of boiling time error in order to improve the precise control of adenosine triphosphate extraction rate.

In view of the shortcomings of the sample, the slender sample is the self-condensing system, and the additional condensing tube is not attached to ensure the sufficient condensation of the sample steam. And back to the sample cell, maintaining the sample uniformity of the volume and concentration of the contents. At the same time, in order to ensure the self-condensation effect, the heating system of the extraction device will only focus the heating position of the sample pool position, almost no heat from the self-condensation section.

The above heating system and adenosine triphosphate extractor, in the sample microbial adenosine triphosphate accurate and rapid extraction at the same time, to ensure that the concentration of adenosine triphosphate concentration is consistent and repeat the sample.

The detection step of the high sensitivity bioluminescence detection method provided by the invention is:
1) With a pipette to accurately measure a certain amount of samples, into the adenosine triphosphate extractor;
2) Start heating system heating for a certain time, such as 2min;
3) With a pipette to accurately measure a certain amount of samples, mixed with fluorescein - luciferase bioluminescence detection.
The adenosine triphosphate extractor is an infrared transparent material, which is derived from crystalline silicon, crystalline germanium, MgF2, CsI, silicate glass, aluminate glass, gallate glass, chalcogenide glass, borate glass, quartz Glass, Al2O3 transparent ceramics, yttrium oxide transparent ceramics, polyethylene, polypropylene, polyvinyl chloride, polystyrene, ABS resin, EVA resin, PET resin, PBT resin, polyurethane, polyphenylene sulfide, polyphenylene ether, poly Formaldehyde resin, polystyrene, polycarbonate, polyamide, plexiglass, nylon, polyvinylidene fluoride, polytetrafluoroethylene, polysulfone and the like.

Wherein the bottom of the sample is a spherical sample cell and the upper part is a helical self-condensing tube. The bottom of the spiral self-condensing tube is connected with the top of the spherical sample cell, and the top of the sample cell is connected with the inside of the spiral self-

Wherein the inner spherical diameter of the bottom spherical sample cell is 5 to 10 mm and the wall thickness is 0.01 to 1 mm; the expansion length of the upper spiral condenser is 1 to 1000 cm, the inner diameter of the cross section of the condenser tube is 0.1 ~ 5mm, wall thickness of 0.01 ~ 1mm.

The adenosine triphosphate extractor is charac
terized in that the amount of each treated sample is 0.03 to 0.3 mL.

The heating system is characterized in that the heating mode is mainly heated by infrared heat radiation with a power of 50 to 5000W.

The infrared ray emitting material is made of graphite, carbon fiber, carbon nanotube fiber, graphene, silicon carbide, alumina, silica, zirconium boride, zirconium boride, mixed oxide infrared radiation material, tungsten oxide ceramic , Infrared radiation enamel selected.

The heating system is characterized in that the heating system is spherical and concentric with the sample cell and allows the cell of the adenosine triphosphate extractor to be partially inserted, with a gap between 0 and 100 mm.

The heating system is characterized in that the periphery of the heating system comprises an infrared reflecting system which is spherical and concentric with the sample cell and the heating system. The infrared reflecting material is selected from the group consisting of metallic aluminum, copper, nickel and titanium.

Description of the drawings

Figure 1 shows a schematic diagram of a self-made adenosine triphosphate extraction device.

Figure 2 shows the fluorescence intensity at different extraction times using a self-made adenosine triphosphate extraction device.

Figure 3 shows the results of comparison using a self-made adenosine triphosphate extraction device with a conventional chemical extraction method.

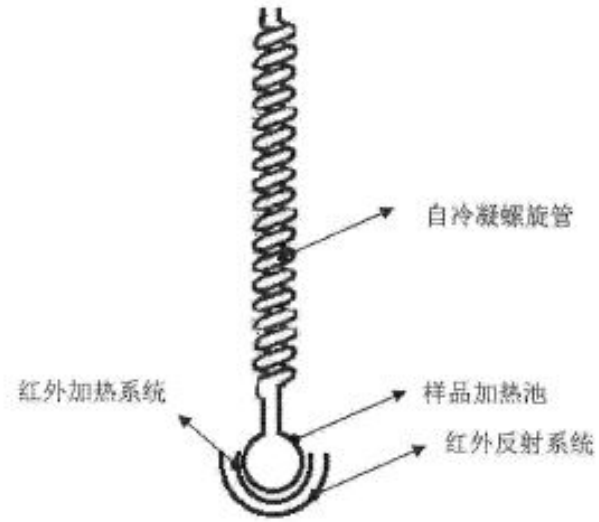


图 1

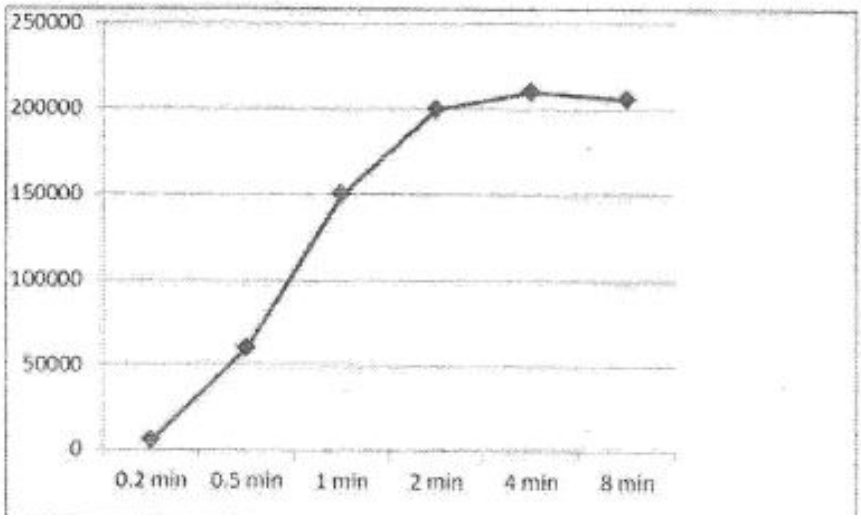


图 2

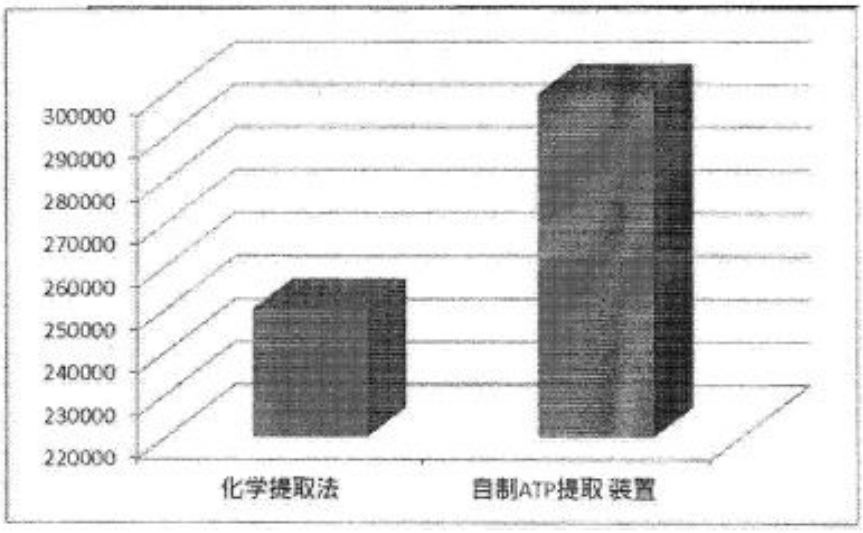


图 3

Detailed description

The features and advantages of the present invention will be further described below with reference to specific embodiments. It should be understood, however, that these examples are merely illustrative of the invention and are not to be construed as limiting the scope of the invention. The test methods not specified for the specific conditions in the following examples are generally in accordance with conventional conditions or in accordance with the conditions recommended by the manufacturer.

The invention has the advantages that:

In the existing bioluminescence analysis method, the method of chemical extraction of adenosine triphosphate in the presence of passivation of adenosine triphosphate decomposing enzyme and protection of luciferase is difficult to take into account. The physical extraction method is cumbersome and reproducible, and the amount of steam and the laboratory environment Shortcomings, provided a new adenosine triphosphate extraction device. The device is required to use less sample, at least 0.03mL; easy to operate, only need to inject the sample adenosine triphosphate extractor, start the heating system, a short extraction time can be (2min); each time the sample test repeatability; Compromise inhibition of ATPase and luciferase protection; there is no need to worry about the effects of steam spillage on the concentration of adenosine triphosphate and the effect of steam on the laboratory environment...

CN102584923
Method for extracting compounds from Chondrus ocelltus

Inventor(s): GEFEI ZHOu, et al.

The invention relates to a method for extracting compounds from Chondrus ocelltus, and belongs to the technical field of medical plant chemical extraction processes. A method for separating and preparing a monomer component from Chondrus ocelltus comprises the following steps: extracting Chondrus ocelltus with ethanol, carrying out extraction of the extract with petroleum ether, ethyl acetate and n-butanol, subjecting the n-butanol extract to silica gel column chromatography, eluting with a mixture of chloroform, methanol and water at a ratio of 7:3:0.1, collecting section by fractioning according to color and Rf value, combining same parts, concentrating under reduced pressure to obtain seven fractions C1-C7, wherein C2 is subjected to silica gel column chromatography again and eluting with a mixture of chloroform, methanol and water at a ratio of 9:2:;0.1 to obtain five fractions, and C2-C4 are loaded on an Sephadex LH-20 column and eluting with a mixture of chloroform and methanol at a ratio of 1:1 to obtain white powered material adenosine triphosphate with a purity higher than 98%.

DESCRIPTION

The present invention relates to a method for extracting compounds from the red algae, which belongs to the technical field of chemical extraction of pharmaceutical plants. The extraction of carrageenan by ethanol was carried out. The extract was extracted with petroleum ether, ethyl acetate and n-butanol. The n-butanol phase extract was treated with silica gel column The elution was carried out with chloroform: methanol: water = 7: 3: 0.1, and the same fraction was collected according to the color and Rf values. The fractions were concentrated under reduced pressure: C1-C7. Where C2 was again chromatographed on silica gel, chloroform: methanol: water = 9: 2: 0.1 to give five fractions. Wherein C2-4 is eluted with Sephadex LH-20 column via chloroform: methanol = 1: 1 to give adenosine triphosphate as a white powder, with a purity of more than 98%.

A method for extracting compounds from the red algae

Technical field

The present invention relates to a method for extracting compounds from the red algae, which belongs to the technical field of chemical extraction of pharmaceutical plants.

Background technique

Carrageenan, red algae, cedarae, carrageenan, intertidal seaweed, commonly known as sea fungus. "Chinese marine drug dictionary" records <[2]>: Carrageenan all edible, medicine, with laxative, and blood swelling, analgesic myogenic effect, attending chronic constipation, fractures, bruises, etc. disease. Carrageenan contains a variety of antibacterial and antiviral ingredients, not only can prevent colds, and can eradicate a wide range of infectious diseases, influenza B and mumps have a strong ability to suppress the virus. With the deepening of the pharmacological activity of natural products, carrageenan many physiological activities were found, has attracted domestic and foreign scholars and product research and development personnel attention.

Carrageenan is an important economic algae, mainly used for the production of carrageenan (also known as carrageenan). But so far, the study of the active ingredients of carrageenan is limited to the routine analysis of the basic components and some of the early isolated

compounds. Therefore, it is necessary to carry out more in-depth study on the chemical composition of carrageenan.

The contents of the invention

It is an object of the present invention to provide a method for extracting adenosine triphosphate from a red algae and a method for extracting the present invention. The present invention further studies the chemical composition of the carrageenan by comparing the nutritional components of the carrageenan at different locations in the same season The use of modern spectroscopy techniques for the identification of the structure of the extract, and anti-bacterial and immune activity of the study, aimed at the development and utilization of carrageenan A certain basis.

The present invention is achieved by the following technical solutions

A compound extracted from the red algae, specifically in that the compound is named adenosine triphosphate having the chemical formula of the following structure:
[Image]
A method for extracting adenosine triphosphate from the genus Cabbage, which is characterized by the following steps:

（1）The red algae carrageenan powder placed in 3-5 times the quality of its solvent in the cold leaching extract to ensure that the solvent concentration after soaking 80% -90%, filtered to remove algae residue, combined extract, evaporated under reduced pressure Solvent, dark green oily extract;
（2） Followed by petroleum ether, ethyl acetate and n-butanol extraction extract concentrate;
（3) The n-butanol phase extract was purified by silica gel column chromatography eluting with chloroform: methanol: water = 7: 3: 0.1. TLC thin layer chromatography was used to track the samples according to Rf and color. The Rf and color The same part, concentrated under reduced pressure seven mixture fraction C1-C7, take C2 spare;
（4）C2-2, C2-3, C2-4, C2-5; after a large number of screening, take the two, then take the silica gel column chromatography, with chloroform: methanol: water = 9: 2: 0.1, C2-4 was eluted with a Sephadex LH-20 column via chloroform: methanol = 1: 1, where C2-4c was repeatedly washed with chloroform to give a white powdery substance.
According to the detector ultraviolet spectrum, the same samples were collected and the same samples were obtained for each chromatographic peak obtained, and the monomer substance adenosine triphosphate with purity greater than 98% was obtained.

The specific steps of the above extraction process are as follows:

（1）The red algae carrots powder placed in 3-5 times the quality of its solvent at room temperature cold soak 7-10 days to ensure that the solvent concentration after soaking 80% -90%, continuous solvent for 3-5 times, that is, 3-5 times for extraction;
Said solvent is ethanol; normal temperature is generally 20-30 ° C;
（2） The extract in step 1 is filtered to remove the algae residue, and the above-mentioned 3-5 times of the extract is added. The solvent is evaporated under reduced pressure, that is, the alcohol in the extract is evaporated under reduced pressure to obtain a dark green oily extract;
（3）The dark green oily extract obtained in step 2 was first suspended by water and then

extracted with the same amount of petroleum ether as the suspension for 4-8 times. The petroleum ether phase was combined and concentrated under reduced pressure to obtain a dark green extract.

（4）The aqueous phase obtained in step 3 is added to the same amount of ethyl acetate as the aqueous phase at room temperature for 4 to 8 times, the ethyl acetate phase is combined and concentrated under reduced pressure to give a brownish extract;

（5）And the aqueous phase obtained in step 4 is then added with the same amount of n-butanol as the water for 4 to 8 times, the n-butanol phase is combined and concentrated under reduced pressure to obtain a yellow extract;

(6), take 100-200 mesh silica gel, activated at 110 ° C ± 5 ° C for 25-35 minutes, mixed with petroleum ether, ultrasonic to bubble, and then wet into the column, the column size: 50 × 600mm , Packed column height: 400mm, standing 60-80 hours;

（7）The n-butanol phase extract in step 5 was collected, dissolved in methanol, triturated with three times its mass of 60-100 mesh silica gel and dried on a dry basis with chloroform: methanol: water = 7: 3: 0.1 Gradient elution, collected in 50 mL tubes, 45 mL per tube, collected by TLC thin layer chromatography, segmented according to Rf and color, combined with Rf and the same color, and concentrated under reduced pressure to obtain seven mixtures fraction C1 -C7; C1: No. 1-6 pipe merged; C2: Section 7-19 tube merged; C3: Section 20-34 tube merger; C4: 35-47 tube merger; C5: 48-61 tube merger; C6: 62- 72 pipe merger; C7: 73-89 tube merger;

(8), take silica 200-300 mesh, according to the process of step 6 after the activation of the wet column, column size: 20 × 400mm, loading column height: 280mm;

(9), take step 7 to separate the resulting fraction C2, dissolved with methanol, with three times its mass of 60 to 100 mesh silica gel sample, dry loading, chloroform: methanol: water = 9: 2: 0.1 Gradient elution, with 20mL test tube segment collection, each tube collection of 18mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and the same color parts, concentrated under pressure five mixture fraction C2 -1, C2-2, C2-3, C2-4, C2-5;

C2-1: the first 1-3 tube merged; C2-2: the first 4-12 tube merged; C2-3: 13-21 tube merged; C2-4: 22-29 tube merged; C2-5: 30 -39 tube merge;

（10） Take Sephadex LH-20 gel column, column size: 15 × 800mm; loading column height: 700mm, take step 9 to separate the fractions C2-4, with chloroform: methanol = 1: 1 dissolved, Eluted with chloroform: methanol = 1: 1, collected in 10 mL tubes, 5 mL per tube, analyzed by TLC thin layer chromatography, segmented according to Rf and color, merged with Rf and the same color, -17 tube for the C2-4c, a white solid precipitation, repeated washing with chloroform to get white powder material, the detection of adenosine triphosphate.

The method comprises the following steps: firstly, extracting the carrageenan with ethanol, the extract is extracted with petroleum ether, ethyl acetate and n-butanol, and the n-butanol phase extract is obtained by silica gel column Chromatography with chloroform: methanol: water = 7: 3: 0. 1, eluted according to the color and Rf values, and the same fraction was combined and concentrated under reduced pressure to give seven fractions: C1-C7. Where C2 was again chromatographed on silica gel, chloroform: methanol: water = 9: 2: 0.1 to give five fractions. Wherein C2-4 was eluted with Sephadex LH-20 column via chloroform: methanol = 1: 1 to give adenosine triphosphate as a white powder with a purity of more than 98%.

The invention further studies the chemical composition of the carrageenan, extracts and separates the physiologically active substances in the carrot with modern separation means, uses the modern spectroscopic technique to identify the structure and conducts the research

on the antibacterial and immunological activity , For the development and utilization of carrageenops to provide a basis.

Description of the drawings

Figure 1: Schematic diagram of the solvent distribution of the ethanol extract of the red algae

Figure 2: Separation flow chart of n-butanol phase extract of red algae.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Hereinafter, specific embodiments of the present invention will be described with reference to the accompanying drawings for a further explanation of the constitution of the present invention.

Example 1

（1）The red algae carrageenan powder placed in 3 times the quality of its solvent at room temperature for 10 days to ensure that the solvent concentration after soaking 85%, continuous solvent 4 times, that is, 4 times to extract;
Said solvent is ethanol; normal temperature is generally 20-30 ° C;
（2） The extract in step 1 is filtered to remove the algae residue, and the above four times of the extract is added. The solvent is evaporated under reduced pressure, that is, the alcohol in the extract is evaporated under reduced pressure to obtain a dark green oily extract;
（3）The dark green oily extract obtained in step 2 was first suspended by water and then extracted with the same amount of petroleum ether as the suspension at room temperature for 6 times. The petroleum ether phase was combined and concentrated under reduced pressure to obtain a dark green extract.
（4）And the aqueous phase obtained in Step 3 was further added with the same amount of ethyl acetate as the aqueous phase at room temperature for 6 times, and the ethyl acetate phase was combined and concentrated under reduced pressure to give a brownish extract;
（5）And the aqueous phase obtained in step 4 is added with the same amount of n-butanol as the aqueous phase at room temperature for 6 times, the n-butanol phase is combined and concentrated under reduced pressure to obtain a yellow extract;
(6), take 100-200 mesh silica gel, 110 ° C ± 5 ° C activation for 30 minutes, mixed with petroleum ether, ultrasonic to bubble, and then wet into the column, the column size: 50 × 600mm, installed Column height: 400mm, standing 60-80 hours;
（7） The n-butanol phase extract in step 5 was collected, dissolved in methanol, triturated with three times its mass of 60-100 mesh silica gel, and dried in the chromatographic column of step (6) in chloroform, Methanol: water = 7: 3: 0.1 gradient elution, with 50mL test tube segment collection, each tube collection of 45mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and color the same part, And the mixture was concentrated under reduced pressure to obtain seven mixture fractions C1-C7; C1: No. 1-6 pipe merged; C2: Section 7-19 tube merged; C3: Section 20-34 tube merger; C4: 35-47 tube merger; C5: 48-61 tube merger; C6: 62- 72 pipe merger; C7: 73-89 tube merger;
(8), take silica 200-300 mesh, according to the process of step 6 after the activation of the wet column, column size: 20 × 400mm, loading column height: 280mm;
(9), the fraction F2 from the step 7 was separated, dissolved with methanol, mixed with 60 to

100 mesh silica gel with its mass of 60 to 100 mesh, and dried in the chromatographic column of step (8) Methanol: water = 9: 2: 0.1 gradient elution, with 20mL test tube segment collection, each tube collection of 18mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and color the same part, The mixture was concentrated under reduced pressure to obtain five mixture fractions C2-1, C2-2, C2-3, C2-4, C2-5;
C2-1: the first 1-3 tube merged; C2-2: the first 4-12 tube merged; C2-3: 13-21 tube merged; C2-4: 22-29 tube merged; C2-5: 30 -39 tube merge;
（10) Take Sephadex LH-20 gel column, column size: 15 × 800mm; loading column height: 700mm, take step 9 to separate the fractions C2-4, with chloroform: methanol = 1: 1 dissolved,
Eluted with chloroform: methanol = 1: 1, collected in 10 mL tubes, collected 5 mL per tube, analyzed by TLC thin layer chromatography, segmented according to Rf and color, merged with Rf and the same color, -17 tube for the C2-4c, a white solid precipitation, repeated washing with chloroform to get white powder material, adenosine triphosphate.

Example 2

（1） The red algae carrots powder placed in 5 times the quality of its solvent at room temperature for 10 days to ensure that the solvent concentration after soaking 85%, continuous solvent for 5 times, that is, 5 times to extract;
Said solvent is ethanol; normal temperature is generally 20-30 ° C;
（2） The extract in step 1 is filtered to remove the algae residue, and the above five times of the extract is added. The solvent is evaporated under reduced pressure, that is, the alcohol in the extract is evaporated under reduced pressure to obtain a dark green oily extract;
（3）The dark green oily extract obtained in step 2 was first suspended by water and then extracted with the same amount of petroleum ether as the suspension at room temperature for 8 times. The petroleum ether phase was combined and concentrated under reduced pressure to obtain a dark green extract.
（4 The aqueous phase obtained in Step 3 was added to the same amount of ethyl acetate as the aqueous phase at room temperature for 8 times, and the ethyl acetate phase was combined and concentrated under reduced pressure to give a brownish extract;
（5）And the aqueous phase obtained in step 4 was added to the same amount of n-butanol at room temperature for 8 times. The n-butanol phase was combined and concentrated under reduced pressure to obtain a yellow extract.
(6), take 100-200 mesh silica gel, activated at 110 ° C ± 5 ° C for 35 minutes, mixed with petroleum ether, ultrasonic to bubble, and then wet into the column, the column size: 50 × 600mm, Column height: 400mm, standing 60-80 hours;
（7） The n-butanol phase extract in step 5 was collected, dissolved in methanol, triturated with three times its mass of 60-100 mesh silica gel, and dried in the chromatographic column of step (6) in chloroform, Methanol: water = 7: 3: 0.1 gradient elution, with 50mL test tube segment collection, each tube collection of 45mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and color the same part, And the mixture was concentrated under reduced pressure to obtain seven mixture fractions C1-C7; C1: No. 1-6 pipe merged; C2: Section 7-19 tube merged; C3: Section 20-34 tube merger; C4: 35-47 tube merger; C5: 48-61 tube merger; C6: 62- 72 pipe merger; C7: 73-89 tube merger;
(8), take silica 200-300 mesh, according to the process of step 6 after the activation of the wet column, column size: 20 × 400mm, loading column height: 280mm;
(9), the fraction F2 from the step 7 was separated, dissolved with methanol, mixed with 60 to

100 mesh silica gel with its mass of 60 to 100 mesh, and dried in the chromatographic column of step (8) Methanol: water = 9: 2: 0.1 gradient elution, with 20mL test tube segment collection, each tube collection of 18mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and color the same part, The mixture was concentrated under reduced pressure to obtain five mixture fractions C2-1, C2-2, C2-3, C2-4, C2-5;
C2-1: the first 1-3 tube merged; C2-2: the first 4-12 tube merged; C2-3: 13-21 tube merged; C2-4: 22-29 tube merged; C2-5: 30 -39 tube merge;
（10） Take Sephadex LH-20 gel column, column size: 15 × 800mm; loading column height: 700mm, take step 9 to separate the fractions C2-4, with chloroform: methanol = 1: 1 dissolved,
Eluted with chloroform: methanol = 1: 1, collected in 10 mL tubes, 5 mL per tube, analyzed by TLC thin layer chromatography, segmented according to Rf and color, merged with Rf and the same color, Tube for the C2-4c, a white solid precipitation, repeated washing with chloroform to get white powder material, adenosine triphosphate.

Example 3

（1 30 kg of red algae carrageenan powder placed in 4 times the quality of its solvent at room temperature for 7 days, to ensure that the solvent concentration after soaking 90%, continuous solvent 4 times, that is, 4 times to extract;
Said solvent is ethanol; normal temperature is generally 20-30 ° C;
（2） The extract in step 1 is filtered to remove the algae residue, and the above four times of the extract is added. The solvent is evaporated under reduced pressure, that is, the alcohol in the extract is evaporated under reduced pressure to obtain a dark green oily extract;
（3) The dark green oily extract obtained in step 2 was first suspended by water and then extracted with the same amount of petroleum ether as the suspension at room temperature for 6 times. The petroleum ether phase was combined and concentrated under reduced pressure to obtain a dark green extract.
（4）And the aqueous phase obtained in Step 3 was further added with the same amount of ethyl acetate as the aqueous phase at room temperature for 6 times, and the ethyl acetate phase was combined and concentrated under reduced pressure to give a brownish extract;
（5）And the aqueous phase obtained in step 4 is added with the same amount of n-butanol as the aqueous phase at room temperature for 6 times, the n-butanol phase is combined and concentrated under reduced pressure to obtain a yellow extract;
(6), take 100-200 mesh silica gel, activated at 110 ° C ± 5 ° C for 25-35 minutes, mixed with petroleum ether, ultrasonic to bubble, and then wet into the column, the column size: 50 × 600mm , Packed column height: 400mm, standing 60-80 hours;
（7) The n-butanol phase extract in step 5 was collected, dissolved in methanol, triturated with three times its mass of 60-100 mesh silica gel, and dried in the chromatographic column of step (6) in chloroform, Methanol: water = 7: 3: 0.1 gradient elution, with 50mL test tube segment collection, each tube collection of 45mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and color the same part, And the mixture was concentrated under reduced pressure to obtain seven mixture fractions C1-C7 : C1: No. 1-6 pipe merged; C2: Section 7-19 tube merged; C3: Section 20-34 tube merger; C4: 35-47 tube merger; C5: 48-61 tube merger; C6: 62- 72 pipe merger; C7: 73-89 tube merger;
(8), take silica 200-300 mesh, according to the process of step 6 after the activation of the wet column, column size: 20 × 400mm, loading column height: 280mm;
(9), the fraction F2 from the step 7 was separated, dissolved with methanol, mixed with 60 to

100 mesh silica gel with its mass of 60 to 100 mesh, and dried in the chromatographic column of step (8) Methanol: water = 9: 2: 0.1 gradient elution, with 20mL test tube for segment collection, each tube collection of 18mL, the use of TLC thin layer chromatography tracking detection, according to Rf and color segmentation, combined Rf and color the same part , Concentrated under reduced pressure to obtain five mixture fractions C2-1, C2-2, C2-3, C2-4, C2-5;
C2-1: the first 1-3 tube merged; C2-2: the first 4-12 tube merged; C2-3: 13-21 tube merged; C2-4: 22-29 tube merged; C2-5: 30 -39 tube merge;
（10) Take Sephadex LH-20 gel column, column size: 15 × 800mm; loading column height: 700mm, take step 9 to separate the fractions C2-4, with chloroform: methanol = 1: 1 dissolved,
Eluted with chloroform: methanol = 1: 1, collected in 10 mL tubes, 5 mL per tube, analyzed by TLC thin layer chromatography, segmented according to Rf and color, merged with Rf and the same color, Tube for the C2-4c, a white solid precipitation, repeated washing with chloroform to get white powder material, adenosine triphosphate.
The compound of the present invention, which is extracted from the genus Cabbage, is a coenzyme. Have improved the role of body metabolism, involved in body fat, protein, sugar, nucleic acid and nucleotide metabolism, but also the main source of energy in the body. Applicable to cell damage caused by cell damage after the disease. Animal test found that the goods on the electrophysiology of cardiomyocytes have a significant effect, can inhibit the slow reaction of calcium influx cells flow, block and extend the atrioventricular node back to the loop of the forward conduction, high dose can block the compartment The way of reentry, with the role of enhanced vagus nerve, available ventricular tachycardia. ATP may be used as a nanotechnology and irrigated energy. Artificial pacemakers may benefit from this technology without the need for battery power.
In poultry farming: 1. For broiler, meat duck, pig, beef cattle, sheep, fish, shrimp and other fleshy animal fertility, promote growth; For the disease caused by animal drinking water, feed intake decreased, and quickly replenish the body energy level; The use of this product can promote the rapid recovery of animal disease after the onset; Applicable to animals due to disease, drugs, toxins and other pathogenic factors caused by liver damage, kidney damage, intestinal mucosal injury, fallopian tube injury after repair.

UA13519

METHOD FOR INCREASING EFFICACY OF EXTRACTING ADENOSINE TRIPHOSPHATE FROM MUSCULAR TISSUES OF MEAT OF SLAUGHTERED ANIMALS

Inventor(s): KRAVTSIV ROMAN YOSYPOVYCH, et al.

The method for increasing efficacy of extracting adenosine triphosphate from the muscular tissues of the meat of the slaughtered animals consisted in the loading the minced meat into the maceration tank followed by extraction of adenosine triphosphate. The area of the actual contact between the extractant and the raw material increases due to the fluidized state of extraction process. With this aim, the tank is rotated with the angular speed that is optimal for specific raw material and the extant of its mincing.

JPH02290299
METHOD FOR EXTRACTING ADENOSINE TRIPHOSPHATE IN MICROORGANISM

Inventor(s): SUGIZAKI MASAHITO, et al.

PURPOSE:To extract effectively adenosine triphosphate contained in microorganism in active sludge at room temperature with a simple operation in a short time by adding trichloroacetic acid solution to the active sludge slurry as an extraction agent. CONSTITUTION:Trichloroacetic acid solution is added to an active sludge slurry as an extraction agent so as to extract adenosine triphosphate contained in microorganism in the active sludge. This method can be carried out at room temperature, furthermore, its operation is simple, its operation time is short and an efficiency in extracting adenosine triphosphate is high.

JPH03236797
ATP EXTRACTING AGENT

Inventor(s): SUGI TAKUMI, et al.

PURPOSE: To make it possible to measure number of microorganism even in a system having a few number of microorganism because of much extraction amount of the microorganism or ATP by using a specific alkylene oxide adduct of an alkylamine as an extracting agent.

CONSTITUTION:The aimed ATP-extracting agent for extracting adenosine triphosphate(ATP) from bacterium, filamentous fungus, Basidiomycetes, yeast, algae, etc., containing an alkylene oxide adduct of an alkylamine expressed by the formula (R is 8-22C alkyl or alkenyl; m, n, p and q are each 0 or integer and m+p=0-20, n+q=1-20 and m+n+p+q>=2; addition of alkylene oxide may be block addition or random addition and when it is block addition, either one of ethylene oxide block and propylene oxide block may be first added to the alkylamine).

DESCRIPTION

The present invention relates to an ATP extractant for extracting ATP (adenosine triphosphate) without destroying cells from microorganisms. [Related Art] In recent years, analytical instruments have been developed in which ATP is extracted from microorganisms and the amount of ATP is quantified from the amount of luminescence generated by the reaction with luciferin-luciferase to measure the number of microorganisms in a short period of time . Here, in order to measure low concentration of microorganisms, an ATP extracting agent that extracts microorganisms efficient efficiency <ATP is required. Conventionally, surfactants such as ethylene oxide adducts of alkylphenols, ethylene oxide adducts of alkylamines, ethylene oxide adducts of quaternary ammonium salts and the like are known as ATP extractants for extracting ATP from microorganisms (Japanese Patent Publication No. 62 -4120). However, with the conventional ATP extractant, the amount of ATP to be extracted was small, so the detection limit of the number of microorganisms was about 10 '/ -. Therefore, when measuring the number of microorganisms of a system having a small

number of microorganisms such as cooling water or white water of the pulp and paper industry, for example, there is a problem that the measurement method utilizing the ATP amount can not be applied in many cases. DISCLOSURE OF THE INVENTION Problems to be Solved by the Invention An object of the present invention is to solve the above problems, and it is an object of the present invention to provide a microorganism capable of measuring the number of microorganisms even in a system with a large extraction amount of ATP extracted from microorganisms and a small number of microorganisms In order to obtain an ATP extractant. [Means for Solving the Problem] The present invention is an ATP extracting agent comprising an alkylene oxide adduct of an alkylamine represented by the following general formula (1). (In the formula, R represents an alkyl group or alkenyl group having 8 to 22 carbon atoms, m, n, p and q are each 0 or a positive number, m + p = o to 20.n + q = 1-20, m + n + p + q ≧ 2 The alkylene oxide adduct may be a block adduct or a random adduct, and in the case of a block adduct, either an ethylene oxide block or a propylene oxide block may be added to the alkylamine in advance. In the present invention, the alkylamine to which the alkylene oxide is added is represented by the following general formula (Il) RNH, ... (n) wherein R is as defined above. Specific examples thereof include octylamine, decylamine, dodecylamine, tetradecylamine, hexadecylamine, octadecylamine, eicosylamine, oleylamine and the like. The alkylene oxide added to such an alkylamine is propylene oxide, propylene oxide or ethylene oxide, but other alkylene oxides may be added together with these alkylene oxides, as represented by the above-mentioned general formula (1) , In the present invention, it is possible to use those obtained by adding both ethylene oxide and propylene oxide to alkylamine, or those to which propylene oxide is added singly, but it is preferable that both are added.

The addition form is not particularly limited and may be a random adduct randomly added with an alkylene oxide or a block adduct in which an alkylene oxide is continuously added blockwise. In the case of a block adduct, , Ethylene oxide may be added first or propylene oxide may be added first. In general formula (1), 1 m and p, or n and q may be the same or different from each other It is good. The alkylene oxide adduct of the alkylamine represented by the general formula (1) can be obtained by adding propylene oxide alone to the alkylamine represented by the general formula [1], or by adding propylene oxide and ethylene oxide randomly or arbitrarily In the following order. The ATP extractant of the present invention contains an alkylene oxide adduct of an alkylamine represented by the above general formula (I), and an alkylene oxide adduct of alkylamine may be used alone, or another component may be blended In any case, it can be dissolved in a solvent such as water to prepare a solution, which can be contacted with a microorganism to extract ATP. In the present invention, examples of microorganisms subject to ATP extraction include bacteria, filamentous fungi, basidiomycetes, yeasts, deformed bacteria. Algae, protozoans and the like. The ATP extractant of the present invention can extract ATP with a high extraction rate without destroying cells of microorganisms and does not inhibit the enzymatic activity of luciferase. Therefore, the ATP extractant of the present invention extracts ATP from a microorganism, reacts this ATP with luciferin-luciferase, measures the amount of luminescence generated, quantifies the amount of ATP, and measures the number of microorganisms from the amount It can be used for measuring the number of microorganisms. In order to measure the number of microorganisms using the ATP extracting agent of the present invention, the ATP extracting agent of the present invention is added to a solution containing a microorganism in an amount of 0.01 to 2% by weight of the compound represented by the general company (1) Add 0.05 to 0.3% by weight, bring it into contact for about 10 seconds to 2 minutes to extract ATP, add luciferin and luciferase, measure the amount of luminescence using a luminescence amount measuring instrument As shown in FIG. The system for measuring the number of microorganisms using the ATP extracting

agent of the present invention is not particularly limited as long as it is a system containing microorganisms, and industrial products such as white water system, cooling water system, aqueous adhesives and the like in one pulp and paper mill, various fermentation Equipment, activated sludge apparatus and the like. When the ATP extracting agent of the present invention extracts ATP from the microorganisms in large amounts and the ATP extracting agent is used for measuring the number of microorganisms utilizing the amount of ATP, the amount of luminescence by the luciferin-luciferase reaction increases, and 104 A / - to the number of microorganisms.

Therefore, even in a system with a small number of microorganisms to which this method could not be applied conventionally, the number of microorganisms can be measured using the amount of ATP.

EFFECT OF THE INVENTION

According to the present invention, since an alkylene oxide adduct of a specific alkylamine is used, an ATP extractant capable of extracting ATP from a microorganism with high extraction rate is obtained. Therefore, by using the ATP extractant of the present invention, it is possible to measure the number of microorganisms using ATPm even in a system with a small number of microorganisms which could not be applied to date.

COMPARATIVE EXAMPLES 1 TO 4

White water (PH 6, 5, microorganism number 2.4 X 10 '/ / * * fl) 0.1 - collected from the paper valve plant and 0.2% by weight aqueous solution of the ATP extractant shown in Table 1 0.10 was placed in a cuvette and allowed to stand for 1 minute to carry out ATP extraction operation from microorganisms. An enzyme solution containing luciferin-luciferase was added to this solution, and after 10 seconds, the amount of luminescence was obtained using a biocounter M2O10 (manufactured by Rumac). The results are shown in Table 1. Table 1 14B: propylene oxide addition (block working meat) after addition of ethylene oxide. -50: Simultaneous addition of ethylene oxide and propylene oxide (random adduct). * 6 C 5 H 1 s o - 0 - (CH * 201 ° e e 1 m, p indicates 1, 1 Akebono 4 K (summer) m, p. -2 n, q represents nm Q of the general formula (1). -3 A: Addition of propylene oxide, addition of ethylene oxide (block L um 1 m). 70: 30 (! Mixture of IJ Mu υ. From the results in Table 1, it can be seen that the ATP extractant used in Examples has a higher luminescence amount than the ATP extractant used in Comparative Example, and exhibits a good ATP extraction effect. Example 8, Comparative Example 5 Bacteria (Pseudos + onas sp,) separated from a cooling water system were cultured to obtain a culture solution with a bacterial count of 10 "4 g / -. This culture solution was centrifuged, and the separated bacteria were suspended in sterilized water, and further diluted with sterilized water to obtain a bacterial suspension having a predetermined concentration. 0.1 mM of this bacterial suspension and 0.1 - 0.4% by weight aqueous solution of the following ATP extractant were placed in a cuvette, and 30 seconds later, an enzyme solution containing luciferin-luciferase was added. After 10 seconds, the amount of luminescence was determined using the biocounter M2O10. The relationship between the number of bacteria (1) in 1 - and the relative luminescence (RLLI) is shown in FIG.

As the ATP extractant, the following ones were used. Example 8 Randomly added 3 moles of propylene oxide and 2 moles of ethylene oxide to 1 mole of cxmozJ <.

COMPARATIVE EXAMPLE 5:

1 Mole of CUS! 'I <2> with 2 mol of ethylene oxide added. As can be seen from the results in FIG. 1, with the AT'P extractant used in the comparative example, there is no difference in the relative luminescence amount with the number of bacteria of 105 / - or less, it is not possible to measure the bacterial count from the relative luminescence amount In the ATP extraction used in the example, there is a correlation between the bacterial number and the relative luminescence amount up to 10 '/ -, and it is possible to measure the number of bacteria in a system with a small number of bacteria as compared with the conventional product Is possible.

Brief Description of the Drawings

FIG. 1 is a graph showing the results of Example 8 and Comparative Example 5.

US2472130
Process for the preparation of a mixture of nucleotides containing predominantly adenosintriphosphate

[PDF]

Inventor: EMERICH SZENT-GYORGYI ALBERT

GB1182280 / US3432487
Process for Extracting Hydrophilic Substances

A process for a hydrophilic substance, particularly adenosine triphosphate, from a material containing the hydrophilic substance bound or sequestered to a water-insoluble substance comprises preparing a mixture of the material with water and an organic liquid extractant, the water being partially soluble in the extractant and the extractant being present in an amount sufficient to dissolve part but not all of the water, whereby there are formed as separate phases an aqueous phase containing the dissolved hydrophilic substance and an organic liquid extractant phase, separating the phases and recovering the aqueous phase. The process is particularly applicable to hydrophilic substances found in living organisms, e.g. nucleotides, nucleosides, flavins or watersoluble cofactors. Specified extractants are 1-butanol, 2-butanol, t-butyl alcohol, n-amyl alcohol, t-amyl alcohol and 3-pentanol, anhydrous 1-butanol being preferred. Example describe the extraction of adenosine triphosphate from a culture of Escheriahia coli.

Description

We, E.I. Du PONT DE NEMOURS AND COMPANY, a corporation organised and existing under the laws of the State of Delaware, United States of America, of Wilmington, State of Delaware, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be

particularly described in and by the following statement:-

This invention relates to a process for obtaining aqueous extracts of various hydrophilic substances from biological or nonbiological materials in a manner which also provides any desired concentrations of these extracted substances in the aqueous solutions obtained.

A more specific aspect of this invention relates to a process for obtaining aqueous extracts and for concentrating aqueous solutions of biological hydrophilic substances, i.e. hydrophilic substances which can be found in living organisms, such as nucleotides (e.g., adenosine triphosphate, hereinafter referred to as ATP), nucleosides, flavins, water-soluble vitamins, and watersoluble co-factors.

Hydrophilic substances are frequently present in a material in combination with various water-insoluble substances. The water-insoluble substances may be linked to or sequester the hydrophilic substances so as to render them relatively inaccessible for aqueous extraction. For example, ATP is present in biological cells in which membrane and other material prevent its quantitative extraction by water alone.

ATP is a nucleotide which is present in all living organisms. By determining whether there is ATP present in a certain environment. it is possible to detect the existence of living organisms in that environment. Thus by monitoring for the presence of ATP, the existence of microorganisms may be determined. This determination is useful in the detection of biological warfare agents; in the determination of background levels of microorganisms in the environment such as air, water, food, clean assembly areas, hospital rooms and germ free areas, and the detection of any increased contamination in any of these enviroments; and in monitoring the effectiveness of sterilization procedures and the sterilization of compounds and apparatus. Further, the determination of ATP is useful for the study of cell aggregates, for example tissues of higher animals.

One method for determining the presence of ATP is by means of the phenomenon of firefly bioluminescence-i.e. by the reaction of ATP with firefly lantern extract. In practicing this method, a sample of the environment to be tested or assayed for living organisms, such as an aqueous extract of the material which is suspected to contain living organisms, is mixed in the presence of oxygen with firefly lantern extract. If a biological material is present in the sample, this fact will be indicated by the emission of light. The amount of bioluminescent light which is emitted is directly proportional to the amount of ATP present in the material being tested. However, since the ATP is, to some extent, sequestered by or linked to other substances, the total ATP is not available quantitatively in an extracellular free state for reaction in the firefly bioluminescent assay.

In testing for the presence of biological material, since the amount of bioluminescent light which is emitted is directly proportional to the amount of intracellular ATP in the material tested, it is desirable to bring about as high a degree of release of the ATP contained within the material suspected of containing biological material as possible.

ATP is the primary energy donor for all metabolic processes and is also useful, in and of itself, in biochemical research, in inhibitS ing enzymatic browning of potatoes and as a pharmacological agent. However, the extraction of ATP from biological material is difficult.

One method by which ATP has been extracted from biological material is disclosed in U.S.

Patent 2,472,130. This method involves treating finely comminuted cells with a water-soluble agent such as ethyl alcohol, discarding the alcohol and subsequently extracting the ATP from the residual cell material into water. However, this procedure is inconvenient since it must be performed in a plurality of separate steps and, moreover, since it does not provide a convenient means for concentrating ATP in the aqueous phase.

According to the invention a process is provided for extracting a hydrophilic substance from a material containing the hydrophilic substance bound or sequested to a water-insoluble substance. The process comprises preparing a mixture of a material containing the hydrophilic substance, water and an organic liquid extractant which will liberate the hydrophilic substance from the material in which it is contained. The water in the admixture may be initially present (e.g., when the material containing the hydrophilic substance is in the form of an aqueous preparation), or the water may be added at the time the admixture is prepared. The organic liquid extractant is chosen so that a portion of the water in the admixture will be dissolved in the extractant. The extractant is used in an amount sufficient to dissolve part but not all of the water. There is thereby formed a separate aqueous phase in a quantity less than that originally present in the admixture. This aqueous phase contains dissolved therein the hydrophilic material which has been released from the substance in which it was originally contained. The aqueous phase may then be separated from the organic liquid phase and recovered. The aqueous phase may be used as such (e.g., aqueous extracts of ATP may be assayed as hereinafter described) or the hydrophilic material may be recovered therefrom, such as by evaporation of the water.

The practice of this invention may be used to extract water-soluble, hydrophilic substances from a material containing the same using an appropriate organic liquid extractant which is not completely miscible with water and which will act to liberate the hydrophilic substance from the material.

Such organic liquid extractants include compounds such as ketones, alcohols, aldehydes, esters, nitroparaffins, phenols or derivatives of any of such compounds which are substituted with groups or atoms such as fluorine, 1 chlorine or phenyl. In a preferred embodiment of this invention, the process is employed to extract biological hydrophilic substances (hydrophilic substances found in living organisms), such as neucleotides, nucleosides, flavins, water-soluble vitamins or watersoluble cofactors, from materials containing such substances. We use the term "favin" herein to mean isoalloxazine, quercetin or one of a group of yellow plant pigments such as lacto flavins, lumi flavins, protein flavins, purine flavins and carbohydrate flavins.

Thus, by the practice of this invention, aqueous extracts of the following may be obtained: low molecular weight organic compounds such as acetic acid from petroleum using an ester such as ethyl acetate as the liquid extractant; water-soluble pesticides such as 2,4-dichlorophenoxyacetic acid from soil using a nitroparaffin such as nitroethane; inorganic compounds such as sodium iodide from dimethylsulfoxide using an alcohol such as 1-butanol; nucleotides such as ATP from tissue cells using 1-butanol; nucleosides such as adenosine from reaction products resulting from the synthesis of the same, using an aldehyde such as butyraldehyde as the extractant; flavins such as riboflavin from spinach using an alcohol such as 5-fluoro-l-pentanol; water-soluble vitamins such as ascorbic acid from citrus using phenol; and water-soluble cofactors such as coenzyme R from egg yolk using a ketone such as 3-pentanone.

This invention will be further described with specific reference to the extraction of ATP from a biological material although it is to be understood that the invention is not restricted thereto.

The biological material from which ATP may be extracted and concentrated may be obtained from tissues taken from a living or recently living animal, from a bacterial or viral culture, suspensions of microorganisms, blood, urine, water or beverages suspected of contamination, tissue cells, patient exudates, air suspected of containing microorganisms, food or other contaminated environments.

Organic liquids which may be used as extractants include, for example, monohydric alcohols containing from four to seven carbon atoms. These are well known compounds and include 1-butanol, 2-butanol, tbutyl alcohol, n-amyl alcohol, t-amyl alcohol, and 3-pentanol. Anhydrous 1-butanol is the preferred alcohol and is advantageously used in an amount sufficient to give a water: alcohol ratio of from 1 1 to 1:5.

The method of preparing the admixture of sample material containing (or suspected to contain) biological material, the alcohol and water is not critical. Thus, the sample material may be added to a mixture of the alcohol and water or it may be added first to one of these components and then the other component added. It is generally preferred that an aqueous suspension of the sample material first be prepared and that the alcohol be added to this suspension.

Where the number of cells per test volume of the sample and, consequently the amount of ATP, is below the limit of detectability, a preferred method of operation is to filter an adequate volume of an aqueous suspension of the cells through a suitable filter, such as a membrane filter of the submicron size.

The filter, together with the material retained on it, is then placed into an appropriate container to which an appropriate quantity of the organic liquid extractant is added. The mixture is shaken and then allowed to stand for from one to several minutes. Sufficient water is then added and mixed with the organic liquid to saturate the latter and create the desired volume of aqueous phase.

The mixture may then be centrifuged if further phase separation is desired. The ATP will now be in the aqueous phase. Aliquots of the aqueous phase can be removed by syringe or other means and used for assay in the firefly bioluminescent reaction. Thus, the cells contained in relatively large volumes of suspension can be collected, extracted, and the ATP concentrated for assay.

The relative amounts of organic liquid extractant and water used in the practice of this invention are important. Sufficient organic liquid is used to bring about the release of the ATP contained in the cells and sufficient water is used to provide the separate aqueous phase in which is carried the ATP released from the cells. The amount of organic liquid must be sufficient to dissolve part, but not all, of the water. If it is desired to obtain more concentrated solutions of ATP, lesser amounts of water and greater amounts of organic liquid may be used, provided that sufficient water remains undissolved in the organic liquid so as to provide the separate aqueous phase.

The organic liquid extractant in the admixture apparently dissolves lipids or other materials in the cell membranes, thereby making them permeable to the ATP contained in the cells. The

ATP is then extracted almost completely into the aqueous phase. Moreover, the organic liquid reduces the volume of the aqueous phase by dissolving a portion of the water but not the ATP. Thus, by appropriate adjustment of the amount of organic liouid used, the desired concentration of ATP in the aqueous phase can be achieved.

The separation of the aqueous phase from the organic liquid phase occurs when the admixture is allowed to stand for a short period of time. However, if it is desired to speed up the separation, this may be accomplished by centrifuging the admixture.

By the practice of this invention, the ATP content of biological material may be recovered substantially quantitatively so that it is available for participation in the lightproducing response of the firefly bioluminescent reaction. This invention also provides a mechanism for concentrating the ATP to increase the sensitivity of the overall assay method.

The aqueous extract may be assayed by means of the firefly bioluminescent technique by contacting an aliquot of the extract in the presence of oxygen with firefly lantern extract, which extract contains luciferin, luciferase and magnesium, and monitoring for the emission of light. The aqueous reaction medium will generally contain enough oxygen to allow the bioluminescent reaction to take place. The amount of light emitted may be measured to determine the amount of ATP present. This provides a measure of the number of cells present.

The firefly bioluminescent reaction may be carried out utilizing crude firefly lantern extracts or the purified constituents therefrom which participate in the bioluminescent reaction. A sufficiently high degree of sensitivity may be attained using the primary extract of the firefly lantern. Lyophilized firefly lantern extract may be obtained commercially. This material may be prepared for use by dissolving it in distilled, deionized water to the desired concentrations. The extracts used in the examples which follow, are obtained by dissolving 70 mg. of lyophilized firefly lantern extract in 5 ml. of water. The lyophilized preparation also contains MgSO4 and potassium arsenate in amounts sufficient to result in concentrations of 0.OlM and O.05M, respectively. The pH of such a solution is 7.4. The solutions may be further diluted to give any desired concentration of firefly lantern extract. The firefly lantern extract which may b_ used may also be prepared in the laboratory from dessicated firefly tails. The firefly tails are first ground to a fine powder with a mortar and pestle with a small amount of washed silica. The powder is then extracted with 0.05M potassium arsenate-0.O1M MgSO4 at pH 7.4.

In order to observe and record small amounts of light produced by a positive response between the material to be assayed and the firefly lantern extract and to make quantitative measurements of the amount of light emitted, instruments which will sense and record the intensity of the emitted light may be used. In order to detect and record the intensity of emitted light, one procedure consists of injecting the aqueous extract prepared in accordance with the practice of this invention into a cuvette containing the firefly lantern extract. The extract is held at pH 7.4 with potassium arsenate buffer. The light emitted as the result of the reaction between any ATP in the aqueous extract and the firefly lantern extract strikes the photosensitive surface of a photomultiplier tube giving rise to an electric potential which can be measured and recorded by either an oscilloscope photograph or a chart recorder.

A convenient unit for measuring the response produced by the intrument is the millivolt.

Because the response (i.e., light emission) is almost instantaneous when the aqueous extract contaiinng ATP is contacted with the firefly lantern extract, the firefly lantern extract should be positioned in front of the light detection system prior to the introduction of the material to be assayed. The bioluminescent response with ATP is determined by measuring the maximum intensity of the emitted light, which after reaching this maximum value, decays logarithmically. With all -other factors constant, the maximum intensity is directly proportional to the concentration of ATP.

The instrumentation necessary for the quantitative measurement of bioluminescence consists of a photomultiplier tube for the conversion of light energy into an electrical signal, a device for determining the magnitude of the signal, and a light-tight chamber for presentation of the bioluminescent reaction to the photomultiplier tube.

In one system, part of the assembly consists of a composite sensing and reaction chamber which contains a photomultiplier tube, with appropriate circuitry, and a rotary cylinder mounted in a block of aluminum in a manner which permits removal of the reaction chamber without exposing the phototube to light. A section of the cylinder wall is cut out to accommodate a standard rectangular cuvette. Immediately above the cuvette holder is a small injection port sealed with a replaceable light-tight rubber plug. The entire unit is painted black to reduce light reflection. The photomultiplier converts the light energy into an electrical signal. An oscilloscope, which records the magnitude of the signal from the photomultiplier, is provided with an adjustable vertical deflection scale which will allow an adjustment in system sensitivity. There is a multiple switching arrangement at the oscilloscope input which makes it convenient to adjust the system zeros and balances. The differential input to the oscilloscope provides a means to balance the dark current output of the phototube. The response to the firefly luminescent system displayed on the oscilloscope screen is recorded with a camera which mounts directly onto the front of the oscilloscope. To observe and record the reaction, the cuvette containing the necessary reagents is positioned in the cuvette - carrier without exposing the phototube. Rotation of the carrier positions the cuvette in front of the phototube. The extract presumed to contain ATP is then added through the injection port and the magnitude of the response, if any, is recorded by the camera. The procedure for assaying aqueous extracts prepared according to the practice of this invention utilizing electronic apparatus to detect and record the intensity of the bioluminescent reaction, which procedure is employed in the examples, is described below:

One tenth ml. of a 0.5% buffered aqueous solution of commercially available lyophilized firefly lantern extract is placed into a cuvette which is then positioned in a light detection chamber. The extract contains luciferase, luciferin and magnesium. Sufficient dissolved oxygen for the bioluminescent reaction is present in the solution. Ten microliters of the aqueous extract to be assayed are then drawn into a hypodermic syringe and immediately injected through the light-proof seal into the cuvette. The reaction reaches maximum light intensity in less than one second and then decreases logarithmically for several minutes. The entire procedure can be executed and the response through its maximum amplitude recorded in less than 2 minutes.

In order to make quantitative determinations of the amount of ATP present, the instrument used to measure the light response may be calibrated using known concentrations of ATP. A calibration may be plotted by injecting 1/100 ml. portions of known concentrations of ATP through the lightproof seal into the cuvette by means of a hypodermic syringe. The light response in millivolts is plotted against the ATP concentration. A straight linear function is

obtained. For example, if the response from 10-1 gamma of ATP is 20,000 millivolts, and that from 10-2 is 2,000 millivolts.

In order that the invention will be fully understood, the following Examples are 110 given by way of illustration only.

EXAMPLES 1 TO 3

A culture of Escherichia coft is incubated for 24 hours. To 1 ml. portions of the resultant aqueous suspension of bacterial cells 115 are added the amounts of anhydrous 1butanol set forth in Table 1. The mixtures are shaken for 1 minute and centrifuged in a clinical centrifuge for one minute to separate the aqueous phase from the butanol phase. Ten microliters from both the butanol and the aqueous phases of each example are assayed for ATP content as previously described and the results are set forth in Table 1.

TABLE 1

Amount Response (MV) Example Butanol Aqueous Phase Butanol Phase 1... 2 ml. 1220 No response 2... 3 ml. 1900 No response 3... 4 ml. 2700 No response Upon the addition of approximately 5 or more ml of butanol, no aqueous phase remains.

EXAMPLE 4

A 24-hour culture of Escherichia coli is prepared. To a 1 ml. portion of the culture, there are added 4 ml. of anhydrous 1-butanol. The mixture is shaken for 1 minute and allowed to stand for 15 minutes to separate the aqueous phase from the butanol phase. A 10 microliter portion is assayed for ATP content as previously described. A response of 2600 MV is obtained.

By way of contrast, another 1 ml. portion of the culture is admixed with 4 ml. of distilled water; the mixture is subjected to ultrasonic oscillation for 1 minute (another means used for releasing ATP content from cells) and a 10 microliter portion of the mixture is assayed for ATP. A response of only 46 MV is obtained.

EXAMPLE 5

This example illustrates the advantage of using anhydrous 1-butanol.

To a 1 ml. portion of a 24-hour culture of Escherichia cali, there is added a 4 ml. portion of 1-butanol which is saturated with respect to water. To another 1 ml. portion of the same culture, there is added a 4 ml. portion of anhydrous 1-butanol. The two mixtures are shaken for 1 minute and then centrifuged to separate the aqueous phase from the alcohol phase. Three 10 microliter aliquots from each aqueous phase are assayed for ATP content as previously described. The results are set forth in Table 2.

TABLE 2

Response (MV) of Aqueous Extracts Taken from Mixture with:

Water-Saturated Butanol Anhydrous Butanol 170 180 960 1080 1000 EXAMPLES 6 TO 8 A 24-hour culture of Escherichia coli is suspended in distilled water to give approximately

1.3Ux10 cells/ml. In each example, a 10 ml. portion of the cell suspension is filtered through a membrane filter having a diameter of 1 inch and a pore size of 0.45 micron. Each filter is then immersed in 1 ml. of anhydrous 1-butanol, shaken for 1 minute and then the amounts of water indicated in Table 3 are added. The mixture is again shaken and centrifuged for 1 minute and the aqueous phase is recovered. The volume of the aqueous phase obtained in each example is set forth in Table 3. A 10 microliter aliquot of the aqueous phase obtained in each example is assayed for ATP as previously described. The results are set forth in Table 3.

TABLE 3

Vol. Water Vol. of Added to Resulting Butanol Extract Aqueous Phase Response Example (ML.) (ML.) (MV.) 6... 0.25 0.10 4,800 7.. 0.20 0.04 10,200 8... 0.20 0.04 9,600

EXAMPLES 9 TO 17

These examples illustrate the variety of alcohols which may be used as the organic liquid extractant. A 24-hour culture of S Escherichia cali is suspended in distilled water to give approximately 2.4x 10 cells/ ml. In each example, a 10-ml. portion of the cell suspension is filtered through a membrane filter having a diameter of one inch and a pore size of 0.3 micron. Each filter is then immersed in 5 ml. of one of the anhydrous alcohols listed in Table 4. shaken for one minute and sufficient water added to give an aqueous phase of 0.1 ml. Each mixture is again shaken and centrifuged for one minute and the aqueous phase is recovered.

A 10-microliter aliquot of the aqueous phase obtained in eich example is assayed for ATP as previodsry described. The results are set forth and-compared in Table 4.

TABLE 4

Example Response (MV) No. Alcohol Aqueous Phase 9... 1-butanol 7500 10... 2-methyl-2-propanol 3000 11... 1-pentanol 2900 12... 3-pentanol 3200 13... 2-methyl-1-butanol 6600 14... 3-methyl-2-butanol 1900 15... 1-hexanol 5800 16... 1,1-dimethyl-l-propanol 3900 17... 1-heptanol 3300

DE10250754

Production of composition with high ATP N-glycosidase and immunomodulatory activity, useful as antiinfective or anticancer drug, comprising material from marine sponges, e.g. Axinella polypoides

Inventor(s): SCHATTON WOLFGANG, et aL

Production of a composition (I) with high adenosine triphosphate (ATP) N-glycosidase activity and a broad immunomodulatory spectrum involves collecting specific classes of marine sponges; and processing the collected product (without isolation of individual compounds) to give pharmaceutical, cosmetic or nutritional supplement compositions or biochemical reagents. Production of a composition (I) with high APT-N-glycosidase activity and a broad immunomodulatory spectrum involves collecting marine sponges of the Hyalospongiae, Demospongiae, Hadromeridae, Peocilosclerida e, Haplosclerida e, Spongiidae, Clionidae, Axinellidae, Raspailiidae, Esperiopsidae, Halichondriidae, Mycaliidae or

Myxillidae classes; and processing the collected product (without isolation of individual compounds) to give pharmaceutical, cosmetic or nutritional supplement compositions or biochemical reagents. Independent claim is included for an immunomodulatory or antiinfective pharmaceutical or cosmetic composition, biochemical reagent or nutritional supplement composition, comprising an aqueous homogenate of sponges of at least one of the above classes, from which components have not been removed by organic solvent extraction followed by chromatography.

DESCRIPTION

The present invention relates to the simple process described in the claims for the preparation of a composition with high ATP-N-glycosidase activity from marine sponges, agents containing these and their use. In particular, according to the invention, individual substances are not isolated but the collected sponge material is used. An agent which exhibits particular anti-infective, immunomodulatory effects can thus be obtained. Specifically, sponges of the classes Hyalospongiae, Demospongiae, Hadromeridae, Peocilosclеridae, Haplosclеridae, Spongiidae, Clionidae, Axinellidae, Raspailiidae, Esperiopsidae, Halichondriidae, Mycaliidae and Myxillidae are collected and the collected product is isolated into pharmaceutically / cosmetically acceptable compositions without isolating individual compounds Extracts, processed. Thus, an ATP-N-glycosidase, determined for the first time in the animal kingdom, Enzyme activity which is also suitable for food supplements, for organ targeting or also as a catalyst in the production of phosphorylated products.

Background of the invention.

The basic principle of anti-infective therapy, which is acknowledged as the state of knowledge, is the principle of selective toxicity of anti-infectives established by Paul Ehrlich: when micro-organisms, such as Gram-positive or Gram-negative bacteria, are specifically stained, as Robert Koch had shown , There should also be substances which inhibit or kill microorganisms in growth without the host organism, (Eg, humans, plants or animals) (cf. Mutschler, E. et al. "Drug Effects" 2001, B. Auflage, WVG, Stuttgart).

[1] Based on this, the selective toxicity of the anti-infectives currently used therapeutically is based on the attack on structures which are not present in the host organism or at least in a substantially different form than in the pathogenic pathogens. There are currently four basic mechanisms for the action of anti-infectives: 1. Inhibition of cell wall synthesis, Eg by beta-lactam antibiotics, glycopeptides, fosfomycin, 2. Disturbance of the permeability of the cytoplasmic membrane, eg. For example by polypeptide antibiotics, polyene antibiotics, 3. Blockade of protein biosynthesis, For example by aminoglycosides, tetracyclines, chloramphenicol, macrolides, lincosamides, 4. Inhibition of nucleic acid synthesis, Eg by rifampicin, sulfonamides, gyrase inhibitors, flucytosine, antiviral polymerase inhibitors. 1. Inhibition of cell wall synthesis, Eg by beta-lactam antibiotics, glycopeptides, fosfomycin, 2. Disturbance of the permeability of the cytoplasmic membrane, For example by polypeptide antibiotics, polyene antibiotics, 3.

Blockade of protein biosynthesis, For example by aminoglycosides, tetracyclines, chloramphenicol, macrolides, lincosamides, 4. Inhibition of nucleic acid synthesis, Eg by rifampicin, sulfonamides, gyrase inhibitors, flucytosine, antiviral polymerase inhibitors. 1. Inhibition of cell wall synthesis, Eg by beta-lactam antibiotics, glycopeptides, fosfomycin, 2. Disturbance of the permeability of the cytoplasmic membrane, For example by polypeptide

antibiotics, polyene antibiotics, 3. Blockade of protein biosynthesis, For example by aminoglycosides, tetracyclines, chloramphenicol, macrolides, lincosamides, 4. Inhibition of nucleic acid synthesis, Eg by rifampicin, sulfonamides, gyrase inhibitors, flucytosine, antiviral polymerase inhibitors. 1. Inhibition of cell wall synthesis, Eg by beta-lactam antibiotics, glycopeptides, fosfomycin, 2. Disturbance of the permeability of the cytoplasmic membrane, eg. For example by polypeptide antibiotics, polyene antibiotics, 3. Blockade of protein biosynthesis, For example by aminoglycosides, tetracyclines, chloramphenicol, macrolides, lincosamides, 4.

Inhibition of nucleic acid synthesis, Eg by rifampicin, sulfonamides, gyrase inhibitors, flucytosine, antiviral polymerase inhibitors.

The field of action of the modern anti-infectives extends to bacterial infections, mycoses, protozoa and diseases.

A serious drawback of the present selective toxic therapy with anti-infectives is the great problem that in the course of the therapy of the disease exciter adaptations to the selective principle of action, which lead to resistances against the anti-infektivum. Many antibiotics have already become ineffective in the course of the years of their application, since resistant germs have formed during the therapy cycles.

Therefore, new anti-infectives with new as possible mechanisms of action are required, especially those with a low risk of resistance formation. Such new principles are also being studied, especially in marine ecosystems, especially in organisms that live like sea squids in symbiotic communities with fungi, algae and microorganisms. This is therefore promising as effective mechanisms of exchange-based growth control and controlled survival have evolved over the course of evolution. This is usually done by low molecular weight secondary metabolites.

Summaries can be found in various reviews, B. Krebs, H.Chr. Recent Developments in the Field of Marine Natural Products with Emphasis on Biologically Active Compounds, 1986, Progress in the Chemistry of Organic Natura) Products 49, 151-363, or Sarma, A. S. et al. Edit. Secondary Metabolites from Marine Sponges, 1993, Ulstein Mosby, Berlin.
As can be seen from this, marine sea swamps form numerous secondary low molecular weight metabolites with some new complex lead structures which are difficult to access by classical chemical methods.

The () describes isolable chemical compounds of the group of substituted azepines, which are known as protein kinase C, from various sponges such as Phakellia flabellate (Great Barrier Reef, Australia), Hymeniaeiidon aldis (Okinawa), Axinella verrucosa (Mediterranean) and Acan thella aurantiaca (Red Sea) Inhibitors have in particular anti-viral and anti-tumor properties. Such an effect is based on the kinase-specific activity of the energy supply of the cells by the elimination of phosphate from the adenosine triphosphate (ATP). If this cleavage - The known ATPase reaction - Is inhibited by appropriate inhibitors as mentioned above, an interruption of the signal transfer in the cell occurs.
After the (), discodermolide compounds (terpene lactones) from Discodermia disoluta are described which are used to modify the immune system and inhibit tumor growth. The substances are recovered analogously as above by extraction with methanol / toluene, distillation between water / ethyl acetate and chromatography of the acetone phase.
In the (), spongistatins are lactones) (2,3,5,7,8,9) by isolation from Spongiidae,

Demospongidae (2,3) and Spirastrella spirulifera by extraction with an organic solvent such as alcohol, methylene chloride, toluene, distributing the organic phase between water / organic solvent and chromatographic purification of the organic phase. The substances are said to show antitumor activity.

The above-described methods are distinguished by the fact that certain chemical substances of the group of azepines, lactones from the mentioned marine organisms are isolated with organic solvents and chromatographic methods and these are then used as pharmaceutical active ingredients. Several cleaning methods have to be used. The isolated compounds act via the known inhibition of the phosphate cleavage (ATPase reaction) and the interruption of the energy supply of the cell caused thereby. However, this mechanism is not specific, as can cells which are not to be switched off from the power supply, such as, for example, B. Host cells in infections or benign cells in tumor diseases.
It is therefore an object of the present invention to provide a pharmaceutical, cosmetic, or even a food or supplementary agent or biochemical reagent with the aid of a simple method with which the supply of the cell is changed efficiently and specifically, in particular the energy supply of, Eg by infection or tumor growth, can be interrupted without the metabolic processes of the host / the healthy cell being substantially impaired or, on the other hand, with an effective dietary supplementation in patients, Eg with pathogenically colonized intestinal flora.

This object is achieved according to the invention by the fact that in particular sponges of the classes of Hyalospongiae, Demospongiae, Hadromeridae, Peocilosclerida, Haplosclerida, Spongiidae, Clionidae, Axinellidae, Raspailiidae, Esperiopsidae, Halichondriidae, Mycaliidae, Myxillidae (such as, For example, described in Rupert Riedel, Fauna and Flora of the Mediterranean Sea, Verlag Paul Parey 1983), and without isolation of individual compounds, the collected product as such can be processed into pharmaceutically / cosmetically acceptable agents, food supplements, biochemical reagents.

Surprisingly, it has been found that products which, in contrast to the substances described so far, have an extremely active novel ATP-cleaving enzymatic activity, are obtained here, see Examples 1 and 2.

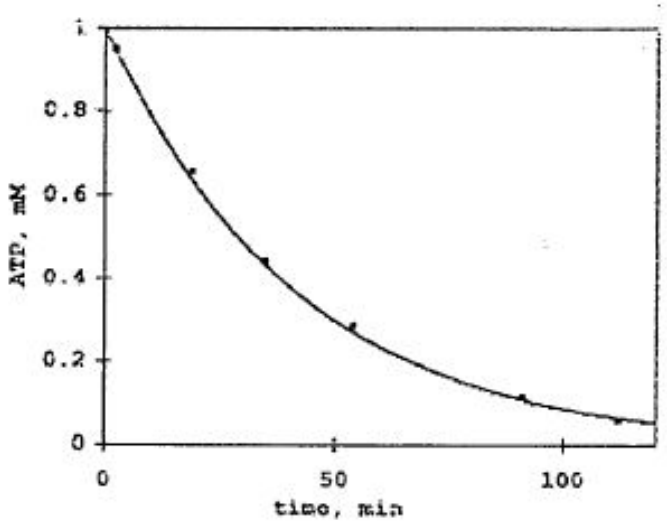
The incubation of aqueous or, For example aqueous alcoholic preparations of sponges or Sponge homogenates catalyze the cleavage of the ATP substrate directly into adenine without any dephosphorylation step. The incubation also provides directly D-ribose 5-triphosphate, which has never been described as a metabolic ATP product, see also Table 1.
Accordingly, the method according to the invention provides a composition whose novel effect is based on the previously unknown catabolic metabolic pathway for ATP which is present in sponges. This activity can be attributed to an enzyme (protein) that catalyzes the hydrolysis of the N-glycosidic binding of ATP. ATP N-glycosidase can be mentioned in this respect, and the products according to the invention thus have an ATP N-glycosidase-Activity.

Although various enzymes are known to cleave the N-glycosidic bond between adenine and ribose, it has not yet been shown to decompose ATP in its most energetic form directly into adenine and D-ribose tri-phosphate.

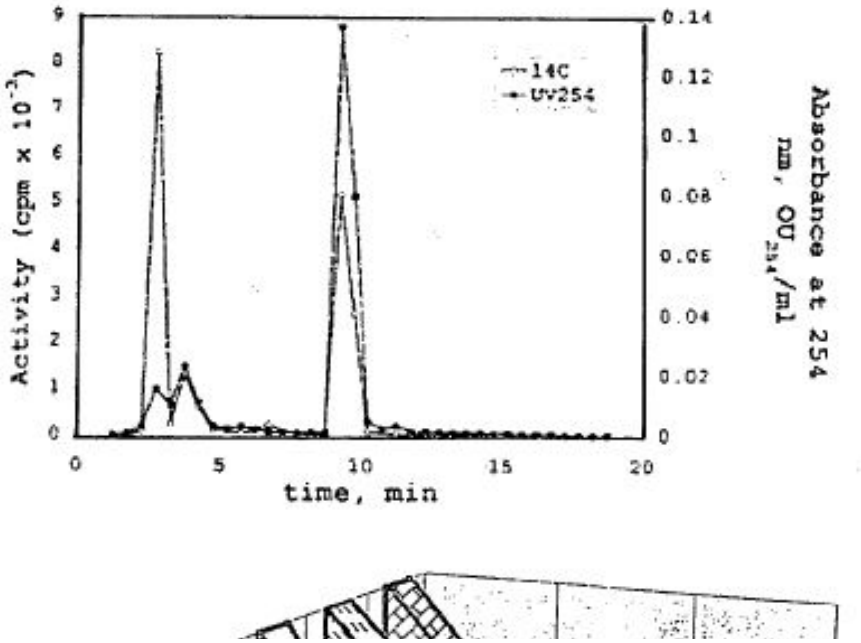
According to the present invention, an enzyme which has hitherto been unknown in the animal kingdom has been found for the first time in a readily available product, Activity

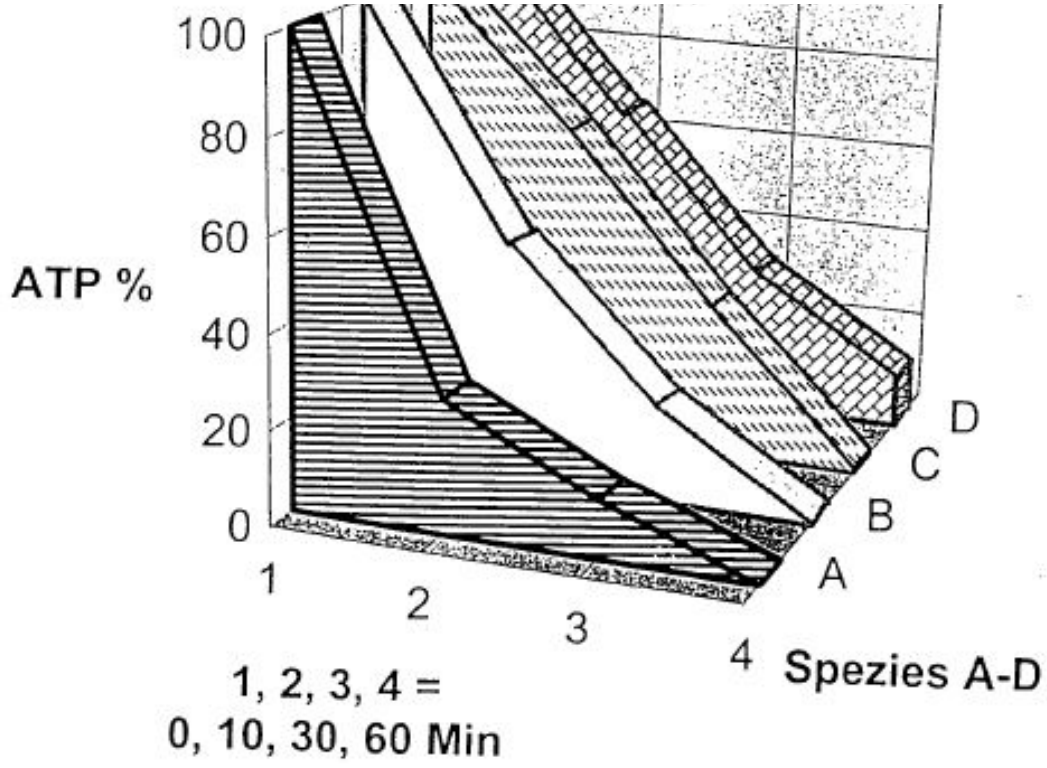
attributable to the presence of an ATP N-glycosidase found in the lowest animal cell organisms, sea floes. Surprisingly, it has been found that the enzymatic activity is such that N-glycosidic bonds of adenosine nucleotide in its most energetic form (ATP), which is the most widespread and ubiquitous adenine-containing cell component, are cleaved.
ATP plays a role as a transmitter in cell-cell communication, in humans, among other things. In the central nervous system and in the area of the smooth or transverse muscle (see Fig. Mutschler).

On the other hand, ATP provides the body with the energy required for life. The surprisingly found new enzymatic activity- Completely in contrast to the known ATPase activity, which gradually provides the metabolism energy to the cell by elimination of phosphate residues, All ATP-energy-dependent metabolic processes of the cells can be selectively solved with very high enzymatic activity within a very short time.



Figur 2

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