

Why Do Runners Use eATP?

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Product Name: eATP

Recombinant Protein Name: cDysglobin kinase

Abstract

Long distance-runners undergo strenuous year-round training to prepare for one important race in a year. In an era where physiological and technical aspects of running have been mastered, athletes look to improve small factors to achieve their goals; whether it be a PR or winning an important race. eATP (pronounced *eat-t-p*) is a running gel that contains cDysglobin kinase, a recombinant protein composed of dystrophin, myoglobin, and creatine kinase, muscle (CK-MM) that redefines the traditional gel by improving skeletomuscular performance alongside refueling the body with quick carbohydrates. cDysglobin kinase is created by combining DNA sequences with restriction enzymes, mass-replicated using PCR, and translated in vitro. Important active sites are preserved structurally, allowing the subunits to collectively improve power output, aerobic threshold, and energy stores. It is recommended that the user consults a professional regarding appropriate use and the side effects before using the product in any competition setting.

Background

October 12, 2019. On this day, Eliud Kipchoge shattered the marathon 2-hour barrier at the INEOS 1:59 challenge, finishing with a historic time of 1:59:40h*. This run shocked the running world; the 34-year-old Kenyan had just broken what many universally considered an unbreakable barrier in athletics, among the likes of Wilt Chamberlain's 100 point game or Wayne Gretzky's 163 assists in a season.

Kipchoge's incredible run was in optimal conditions. He ran on a flat course equipped with Nike Vaporflys Next%, the best marathon race shoes, and was paced by previous WR holders and top runners from around the world. He consumed gels and drinks throughout the race to replenish carbohydrate and electrolyte stores. In an era where athletes have maximized physical prowess and fine-tuned technical aspects, athletes turn to performance enhancers to break personal records.

Running gels have been used by endurance runners since the late 80s as quick carbohydrate fuel during races. eATP (pronounced *eat-t-p*) is a gel containing recombinant protein cDysglobin kinase which elevates the traditional use of a running gel by increasing muscle power output, aerobic threshold, and energy stores in muscles. It doubles as a fuel and allows runners to run faster than ever during long races while reducing injury rates.

Materials and Methods

DNA for dystrophin, myoglobin, and CK-MM are isolated from muscle tissue using a test tube containing water, detergent, ethanol, and protease. The fragments are placed in separate wells with necessary enzymes and ddNTPs to sequence the strands via dideoxy termination sequencing. Dystrophin and myoglobin sequences are placed in a test tube with samples of restriction enzyme HindIII and Mg^{2+} . The digestion reaction is incubated at $\sim 72^{\circ}C$ for 1 hour, then stopped by heating at $\sim 65^{\circ}C$ for 15 minutes. Digested sequences are separated by restriction fragment length polymorphism analysis. The digestion process is repeated with digested myoglobin and creatine kinase using restriction enzyme EcoRV. All digested DNA fragments are placed in a test tube with T4 DNA ligase along with necessary cofactors / buffers, and incubated to complete ligation. Then, the reaction is incubated at $\sim 70^{\circ}C$ to denature the ligase. Recombinant DNA template is placed in a test tube with Taq polymerase, DNA primers, and a deoxynucleotide mix. The reaction is heated to $\sim 95^{\circ}C$ to denature DNA strands, then reduced to $\sim 55^{\circ}C$, and finally raised to $\sim 72^{\circ}C$. The cycle is repeated 30 times to produce a billion copies of the recombinant DNA. The DNA is dyed with ethidium bromide, and is placed under gel electrophoresis to isolate the successful recombinant DNA. Recombinant DNA is placed with sufficient ribosomes, tRNA, and amino acids to synthesize the recombinant protein in vitro.

Discussion

eATP is a gel composed of carbohydrates, creatine, water, and cDysglobin kinase designed to improve runner performances in endurance races. cDysglobin kinase is a recombinant protein of dystrophin, myoglobin, and creatine kinase, muscle (CK-MM) with a purpose to increase concentrations of these proteins in muscle cells. Dystrophin is a protein that anchors actin filaments to the cell membrane in order to maintain cell structure during muscle contraction, transmit force generated by muscle movement, and

signals muscle repair and growth. An increase of dystrophin will directly improve power output, and therefore stride length. Myoglobin stores and transports oxygen needed for oxidative phosphorylation and generates ATP needed for muscle contraction. An increased concentration of myoglobin allows muscle cells to undergo aerobic cellular respiration for longer periods of time and reduce lactate buildup while running faster paces. CK-MM facilitates the reversible reaction of high-energy phosphocreatine and ADP to generate creatine and ATP. Phosphocreatine is an energy buffer that can recharge ADP to form ATP. This provides a steady stream of energy for runners throughout a race. Overall, cDysglobin kinase functions to improve power output, aerobic threshold, and energy stores.

The structure of cDysglobin kinase is V-shaped where subunits structurally similar to myoglobin and CK-MM are connected to the N-terminus of dystrophin. The C-terminus is proline-rich allowing for interactions between dystroglycan and membrane-bound proteins to form signaling pathways. These signaling pathways are responsible for improving myelination along axons related to running, thus improving the neuron transmission speed. Actin binding sites are found at the bend of the protein. The N-terminus arm contains various polar and acidic binding sites forming an active site that facilitates substrate-level phosphorylation, as well as an aromatic pyrrole nitrogen ring that covalently binds to O₂, preventing early release. The proteins are combined from N to C termini in a way that preserves the original protein's important binding sites in order to improve skeletomuscular and neuromuscular performance.

The stability of the CK-MM subunit is susceptible to denature in high lactic acid concentrations, slowing down energy produced. Myoglobin lowers the body's usage of fermentation and therefore the likeliness of CK-MM denaturation. Ca²⁺ is supplemented with the eATP gel to stabilize and strengthen the tertiary structure of the dystrophin subunit.

It should be noted that use of eATP should not exceed the recommended amount of 2 gels per day. Use of eATP may cause thirstiness, upset stomach, diarrhea, and bloating. Creatine found in the gel induces osmosis similar to Na⁺ which will draw water into muscle cells, and leaves you feeling dehydrated and bloated. It's highly recommended that water is consumed with the gel to reduce the effects of digestion related side effects and dehydration. Consult a doctor before using this product to discuss if it is right for you.

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