Public Health and Toxins from Marine Blue-Green Algae

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Marine blue-green algae belonging to the Oscillatoriaceae are frequently toxic. Lyngbya majuscula, for example, is the causative agent of a severe contact dermatitis that sometimes affects swimmers and bathers at beaches on the windward side of Oahu, Hawaii during the summer months. In August, 1980 an outbreak of this dermatitis occurred and 86 persons with symptoms were reported to the Hawaii Department of Health. The active principles in the blue-green alga are aplysiatoxin and debromoaplysiatoxin, two highly inflammatory acetogenic substances that were first found in the digestive tract of the sea hare Stylocheilus longicauda. A smaller amount of a third inflammatory substance, lyngbyatoxin A, an indole alkaloid that is structurally related to teleocidin B from Streptomyces mediocidicus, is also present in L. majuscula and is responsible in part for the dermatitis. Aplysiatoxin, debromoaplysiatoxin, and lyngbyatoxin A have been shown to be potent tumor promoters in vivo, comparable in potency and biological effects with 12-O-tetradecanoylphorbol-13-acetate (TPA) from Croton oil. The discovery of the cocarcinogenic properties of these toxins suggests that L. majuscula may be an important public health concern.

Only one marine blue-green alga, Lyngbya majuscula, poses any potential public health concern. This filamentous cyanophyte is the causative agent of a severe contact dermatitis that affects several swimmers and bathers using the windward beaches of the Hawaiian Islands during the summer months (1-3). The active principles have been isolated and identified as two phenolic bislactones, aplysiatoxin and debromoaplysiatoxin (4,5), and an indole alkaloid, lyngbyatoxin A (6). All three of these substances have been shown to be potent irritants, producing erythema, blisters and necrosis when applied to the skin (6,7).

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The most recent major outbreak of this dermatitis on the windward side of Oahu occurred in August, 1980 at Kailua, Kalama, and Pilapu beaches. A total of 86 cases were reported to the Hawaii State Department of Health. The dermatitis was described as similar to a burn and generally involved the genital and perianal areas. The initial symptoms, which appeared after a few hours, were erythema and a burning sensation, followed by blister formation and deep desquamation which lasted for several days. Aplysiatoxin and debromoaplysiatoxin were identified as the dermatitis-producing agents of this outbreak, since relatively large quantities of both toxins were isolated from specimens of L. majuscula floating in the ocean at the time (8). Debromoaplysiatoxin had already been shown to be present in the L. majuscula that was responsible for an outbreak of seaweed dermatitis at Laie Bay on windward Oahu in 1977 (9).

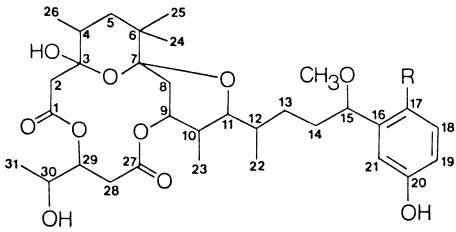
Lyngbyatoxin A is generally a minor component in dermatitis-producing L. majuscula (10). Lyngbyatoxin A, however, is the major irritant in a variety of L. majuscula growing at Kahala beach near Diamond Head, Oahu. Curiously outbreaks of seaweed dermatitis have not been reported in the Kahala area.

L. majuscula is a common blue-green alga which grows abundantly in many areas of the sub-tropical and tropical Pacific Basin and also in the Caribbean. Outside of the Hawaiian Islands, however, seaweed dermatitis has only been reported in Japan. A large outbreak of skin dermatitis occurred at Gushikawa beach in Okinawa in July, 1968 and affected 242 persons (11,12). Although a blue-green alga had been considered to be one of the possible causes of this mass attack, samples of L. majuscula were not collected and examined at the time of the outbreak and so it is not absolutely certain that L. majuscula was the causative agent. Debromoaplysiatoxin and aplysiatoxin, however, have been shown to be present in L. majuscula growing at Gushikawa beach by Fujiki at the National Cancer Center Research Institute in Tokyo, Japan (private communication).

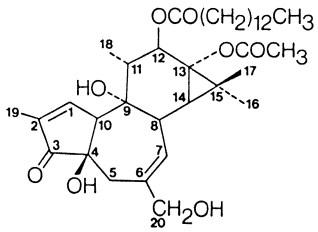
Structure Determination

Debromoaplysiatoxin and aplysiatoxin were first isolated from the midgut gland of the sea hare Stylocheilus longicauda by Kato and Scheuer in 1974 (4). The two toxins were accompanied by the relatively non-toxic compounds, anhydrodebromoaplysiatoxin and anhydroaplysiatoxin, which Kato and Scheuer found to be the products of mild acid treatment of the corresponding toxins. From elegant chemical and spectral studies, Kato and Scheuer deduced the gross structures for the two toxins and two anhydrotoxins in this four-component mixture without ever isolating any of the pure compounds. Although they strongly suspected that the aplysiatoxins had an algal origin, the dietary source was not determined.

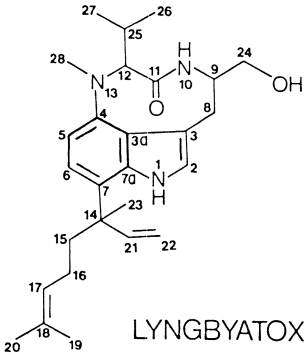
In a search for new antineoplastic agents from blue-green algae, Mynderse isolated a cytotoxic substance that was active against P-388 lymphocytic mouse leukemia from a deep-water variety of L. majuscula growing on the pinnacles in Enewetak Atoll and showed that it was identical with debromoaplysiatoxin (9). Since Kato and Scheuer had not isolated pure debromoaplysiatoxin, comparison of the 20,30-diacetates was made to establish their



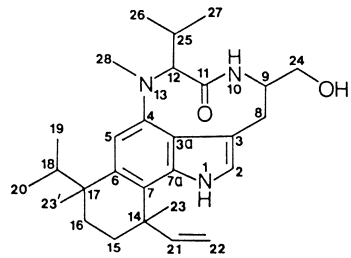
APLYSIATOXIN, R = Br
DEBROMOAPLYSIATOXIN, R = H



TPA



LYNGBYATOXIN A



TELEOCIDIN B

identities. Mynderse managed to crystallize debromoaplysiatoxin, but to date it has not been possible to solve its structure by X-ray crystallography. Circular dichroism and difference nOe ^1H NMR studies and chemical degradation, however, indicated that the absolute stereochemistry was probably 3S,4R,7S,9S,10R,11R,12S,15S,-29R,30R (10). Recently 19,21-dibromoaplysiatoxin, formed by bromination of debromoaplysiatoxin in aqueous methanol at pH 6, was crystallized by C. Cheuk in my laboratory and X-ray studies by G. Matsumoto and J. Clardy at Cornell University have now verified the proposed absolute stereochemistry.

Lyngbyatoxin A was first isolated from the variety of L. majuscula growing at Kahala beach, Oahu (6). Its structure, which was deduced mainly from spectral data, proved to be similar to that of teleocidin B (13), a highly irritating substance that is produced by certain Streptomyces, e.g. S. mediocidicus. These soil fungi had been shown to be responsible for a contact dermatitis affecting workers in the antibiotic industry. Recently Fijiki and Sugimura at the National Cancer Center Research Institute in Japan have found that one of the two components of teleocidin A, another dermatitis-producing agent from these Streptomyces, is identical with lyngbyatoxin A. The absolute stereochemistries of the teleocidins and lyngbyatoxin A are unknown at this writing; however, optical studies indicate that the absolute configuration of the nine-membered lactam ring is the same in both the fungal and algal toxins (6).

Tumor-Promoting Properties

In two-stage chemical carcinogenesis, certain chemical agents (tumor promoters), which alone do not cause cancer, amplify the development of tumorous cells from cells that have been initially exposed to a single, subcarcinogenic dose of carcinogen. Skin tumors on mice can be made to appear in about 10-12 weeks following a single sub-carcinogenic application of carcinogen with twice weekly applications of a tumor promoter. Tumors are not formed if the order of treatment is reversed, i.e. months of twice weekly applications of tumor promoter are followed by a onetime application of a subcarcinogenic dose of carcinogen. Unlike carcinogens which act directly on the cellular DNA, tumor promoters exert their effects by binding to receptors associated with the cell membrane (14). These receptors somehow control cell growth and differentiation, for some cells can be induced to proliferate while others are induced to differentiate on treatment with very small quantities of tumor promoter. The most well known tumor promoter is 12-O-tetradecanoylphorbol-13-acetate (TPA), a deterpenoid ester from Croton oil.

About four years ago phorbol esters and related diterpenes were the only class of tumor promoters known to act at hormonal levels. Fujiki and Sugimura at the National Cancer Center Research Institute in Tokyo, Japan then discovered that teleocidin B and its dihydro derivative were powerful tumor promoters (15), acting also at hormonal levels and sharing with TPA many of the same biological effects. This was a very interesting finding as the teleocidins were indole alkaloids and had structures that were quite different from the phorbol esters. Subsequent collaborative studies between our laboratory and the Japanese established that lyngbyatoxin A was also a potent tumor promoter (16) and furthermore that debromo-aplysiatoxin and aplysiatoxin belonged to a third class of powerful tumor promoters which behaved like the phorbol esters and teleocidin alkaloids (17).

Lyngbyatoxin A, debromoaplysiatoxin, and aplysiatoxin induce irritancy in mouse skin to the same degree as TPA (16,17). Significant reddening of mouse ear skin is observed after 24 hours when 0.1 nmol of toxin is applied. Each compound exhibits a potency similar to that of TPA in inducing ornithine decarboxylase (ODC) activity in dorsal mouse skin. Increased ODC activity is characteristic of fast-growing neoplasms and uptake of putrescine by tumorous cells is much more rapid compared with normal cells (18). Each toxin shows the same effects as TPA in several cell culture systems, including stimulation of arachidonic acid release, prostaglandin production, and choline turnover (19,20); formation of superoxide anions and hydrogen peroxide (21); induction of Epstein-Barr virus expression and enhancement of Epstein-Barr virus-induced transformation (22); induction of terminal differentiation and adhesion of HL-60 cells (17,23); aggregation of human lymphoblastoid cells (22); inhibition of terminal differentiation of Friend erythroleukemia cells (16); aggregation of NL-3 cells (17); stimulation of 2-deoxyglucose transport; enhancement of transformation by adenovirus; enhanced cloning efficiency of adenovirus-transformed cells; inhibition of melanogenesis in B16 cells and inhibition of myogenesis in human myoblast cultures (24);

stimulation of immune interferon, T cell growth factor (interleukin-2), and lymphotoxin production in human peripheral blood lymphocytes (25); and inhibition of binding of [^{125}I]EGF (epidermal growth factor) to its membrane receptors in mouse and rat embryo cell lines (20). Lyngbyatoxin A, debromoaplysiatoxin, and aplysiatoxin have also been found to inhibit the specific binding of [^{3}H]-phorbol-12,13-dipropionate (26) and [^{3}H]-phorbol-12,13-dibutyrate (20) to membrane-associated cellular receptors at potencies similar to those of TPA. These findings provide evidence that phorbol esters, teleocidin alkaloids, and aplysiatoxin bis-lactones have certain structural features in common which enable them to bind to the same receptors, thereby producing similar biological responses in the cell.

Recent studies suggest that the phorbol ester, teleocidin, and aplysiatoxin tumor promoters operate by activating a phospholipid and calcium ion dependent phosphorylating enzyme, protein kinase C (27-30). The activity of protein kinase C is also stimulated by unsaturated diacylglycerol. Since these tumor promoters induce increased turnover of membrane phospholipids, diacylglycerol may be generated to further increase protein kinase C activity. It has been suggested that the putative endogenous analog of these tumor promoters might actually be a diacylglycerol (31) and that protein kinase C may be a receptor for the tumor promoters or at least a component of the receptor complex (31). When intact cells are treated with the tumor promoter, protein kinase C appears to move from the cytosol to the cell membranes. Whether the tumor promoter is bound to protein or phospholipid in the apparent quaternary complex of phospholipid, protein kinase C, calcium ion, and tumor promoter has not been established unambiguously.

To date the evidence seems to favor the binding of tumor promoter to phospholipid in the cell membrane. Specific binding of [^{3}H]TPA to mouse epidermal particulate matter is susceptible to phospholipases C and A_2, less susceptible to protease, and completely resistant to glycosidase (32). Photoaffinity labelling studies with [20-^{3}H]-phorbol 12-p-azidobenzoate 13-benzoate indicates that the irreversible binding of this photolabile phorbol ester to mouse brain membrane is predominantly to the phospholipid (specifically phosphatidylethanolamine and phosphatidylserine) portion rather than to the protein portion (33).

It is interesting that the digestive tracts of sea hares and fish that feed upon L. majuscula are not affected by the aplysiatoxins and lyngbyatoxin A. Preliminary studies by Fujiki suggest that the cells of sea hares may lack the tumor promoter-binding receptors that are so common in mammalian cells (34).

X-ray structural data are available for TPA (35) and dihydroteleocidin B (36-37), but only the absolute stereochemistry of TPA is known. As mentioned above the structure of aplysiatoxin has been solved by X-ray crystallography and its absolute stereochemistry has been determined from other data (10). These three tumor promoters, which represent three distinct classes of natural products, appear to have common structural features that enable it to bind to the same membrane receptors.

Evidence in the literature (38-40) indicates that the α,ß-unsaturated keto group at C-3, the primary allylic hydroxyl at C-20, the tertiary hydroxy group at C-4, and the long-chain

ester group at C-12 are needed for the high activity of TPA. The Weinstein group at Columbia University has used computer graphic analysis to compare the three-dimensional structures of TPA and dihydroteleocidin B (41). Their best fit is obtained when the absolute configurations of the two amino acid residues in dihydroteleocidin B are both D. In their model the C-11 carbonyl, N-13, N-1, and the OH on C-24 in dihydroteleocidin occupy very similar positions in space with the C-3 carbonyl, the OH at C-4, the OH at C-9, and the OH on C-24 in TPA, respectively; the monoterpenoidal portion of dihydroteleocidin B and the C_{14} ester group of TPA, which are essential for effective binding of these promoters to the hydrophobic regions of the receptor, are also in similar positions.

Possible Human Intoxication

It is not clear if the toxins associated with L. majuscula enter the human food chain. Hashimoto had observed rabbitfish (Siganus fuscescens) feeding on sea grasses entangled with L. majuscula during his investigation of seaweed dermatitis in Okinawa and wondered if there could be any connection between the toxicity of L. majuscula and human intoxication in the Ryukyus Islands resulting from ingestion of the viscera of rabbitfish (11). No follow-up study, however, was made.

There is a recent case report of a local resident of Hawaii who inadvertently attempted to orally ingest L. majuscula (42). Upon placing the alga in his mouth, he noted an instant burning sensation and several hours later the mucous membranes of the anterior portion of his mouth appeared as if they had been scalded. The discomfort persisted for three days, but after two weeks all manifestations had completely disappeared. Interestingly, L. majuscula grossly resembles the edible, filamentous green alga Enteromorpha prolifera, known as limu'ele'ele to the Hawaiians (43).

During our collections we have noticed that L. majuscula is frequently entangled with other seaweeds, some of which are edible. One wonders if some of the seaweed that is eaten by the Hawaiians could be contaminated by small amounts of L. majuscula. Unlike in Japan where increased urbanization has resulted in a decrease in stomach cancer, the Hawaiian race continues to show the highest incidence of gastrointestinal cancer in the world. The Hawaiians eat at least two seaweeds that contain carcinogenic and mutagenic halogencontaining compounds, viz. Asparagopsis taxiformis (Hawaiian name: limu kohu) (44,45) and Laurencia nidifica (Hawaiian name: limu mane'ono'o) (46,47). In their diet alone the Hawaiians may be getting all of the necessary agents for the initiation and promotion of gastrointestinal cancer. Epidemiological studies, however, are needed to evaluate the actual role of seaweed diet in the incidence of stomach cancer in Hawaii (48).

To date there is no evidence that the toxins of L. majuscula are involved in the development of human cancer. Certainly frequent ingestion of edible seaweeds that are contaminated with L. majuscula could increase the probability of gastrointestinal cancer. There is precedence for this. The black and Creole population of the Caribbean island of Curacao suffers from an exceedingly high rate of esophageal cancer which appears to be related to the daily intake of a tea prepared from the leaves of a

bush Croton flavens. Analysis of the leaf extract shows the presence of diterpene di- and triesters that are structurally related to TPA (49). The diesters exhibit strong tumor promoting activity in dorsal mouse skin. Each cup of tea contains more tumor promoter than is required to maintain chronic irritation of the human esophagus, a necessary requirement for the promotion of esophageal cancer.

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