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UV-A sunscreen from red algae for protection against premature skin aging

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Abstract

In summer time at the beach, people know that they have to protect themselves with sun creams against sunburn. But sunlight affects our skin every day of the year, which finally leads to premature skin aging, also called photo-aging. This is shown by the fact that permanent exposed skin sites, such as the face and hands show in general more aging signs than covered skin. The principal etiologic factor of premature skin aging is UV-A. Unlike UV-B, UV-A is not dependent on time of day or season, and it penetrates rather well through clouds and window glass. Skin therefore need protection against daily exposure to UV-A.

Cosmetic ingredients intended for everydady use should be absolutely safe. Mycosporine-like amino acids are UV-A sunscreens produced by certain algae, corals and zooplankton. We have found the red alga *Porphyra umbilicalis*, a traditional sea vegetable better known as 'Nori', to be a rich source of mycosporine-like amino acids. A preparation of mycosporine-like amino acids in liposomes was tested in a study on UV-A-induced skin aging. In this study a UV-A dose was applied that corresponds to everyday situations in central Europe. Comparing skin lipid oxidation and skin aging parameters such as elasticity, wrinkle depth and roughness, a cream with liposomal mycosporine-like amino acids performed as well as a cream with a synthetic UV-A sunscreen.

The skin's own UV defence system is not perfect

The solar radiation that reaches the earth's surface ranges from 290 to 4000 nm. It can be separated into UV-B (290 – 320 nm), UV-A (320 – 400 nm), visible light (400 – 700 nm) and infrared radiation (700 – 4000 nm). UV radiation is damaging to a wide variety of biological systems. The highly energetic photons in these wavelengths cause damage to macromolecules such as DNA, proteins and membrane lipids. Nature has evolved a number of defense mechanisms to cope with UV radiation. Synthesis of UV-screening compounds is almost ubiquitous. In humans, specified epidermal cells, melanocytes, produce melanins in response to UV light. (Melanins are polymeric structures that are synthesized by the enzymatic oxidation of tyrosine.)

Protection by melanins is not always sufficient. Especially in summer time, higher energy UV-B can cause acute sunburn after exposure to direct sunlight. Whereas UV-B is retained in the upper epidermis UVA can reach the dermis where it is responsible for the premature skin aging effects of sun light. The fact that the skin on exposed areas such as the face and hands shows aging signs much earlier than protected skin sites clearly indicates UV as the principal etiologic factor in premature skin aging and indicates that melanin cannot completely protect against UV-A irradiation.

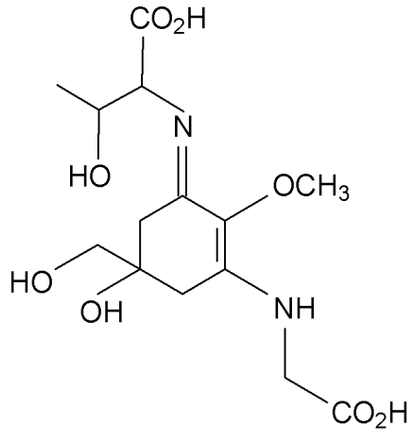
UV-A is the principal etiologic factor for premature skin aging

Because UV-B is completely absorbed in the epidermis, it is UV-A that causes aging changes such as wrinkling, dryness and pigment abnormalities. UV-A alters the expression of certain genes by the generation of reactive oxygen species (ROS) and/or stimulation of pro-inflammatory cytokines such as IL-1β and TNF-α (Tebbe *et al.*). It has been shown that UV-A activates the

transcription factors NF-κB and AP-1 (Djavaheri-Mergny & Dubertret). This results in the induction of a series of collagen and elastin degrading enzymes, the so called matrix metalloproteinases (MMPs). A decrease in collagen content and fiber fragmentation ultimately leads to the typical signs of photoaging. ROS are formed after absorption of UV through skin chromophors, like urocanic acid or DNA. When UV light is absorbed by trans-urocanic acid (absorption maximum at 345 nm), singlet oxygen is generated. This highly reactive molecule can react with proteins or with lipids. The reaction products, such as lipid peroxides, are themselves ROS and have lost cellular functionality (for a review see Pinnell & Durham).

While UV-B is highly dependent on the season, daytime, cloudiness and latitude, UV-A is relatively constant during the year. In central Europe, erythemal UV-B is of minor importance in daily skin care products, but UV-A is present all year round at potentially harmful levels inside buildings as well as in the open air since UV-A easily penetrates window glass. To estimate individual, daily UV exposure, test persons were equipped with small UV dosimeters (Rudolph & Träger). Exposure was measured on typical working days, taking into account indoor UV radiation. Their data show that inside buildings, UV-B levels are insignificant. A daily UV-B dose of 2.5 MEDs at an open window on a sunny summer day is reduced to 0.2 MED if the window is closed. In contrast, about 40% of the outdoor UV-A radiation dose could still be received indoors. Of real relevance for daily skin care are therefore not SPF factors but UVA protection.

Figure 1 : Chemical structure of the mycosporine-like amino acid Porphyra-334



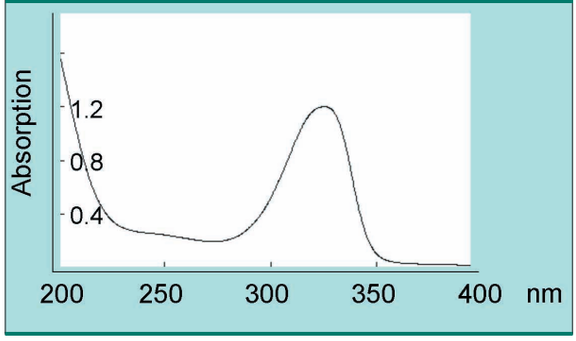
This argues for the application of a daily UV-A protection ingredient. Although the percutaneous absorption of synthetic UV-A sunscreens is low (Benech-Kieffer *et al*), there will be a substantial systemic accumulation when they are used daily, because day creams with UV-protection contain up to 1 to 5% pure synthetic UV-A sunscreens. Natural UV-absorbing substances are therefore a suitable alternative for daily skin care. Trials to commercially utilize melanins in cosmetic formulations failed because the substance is insoluble and the particles are dark brown. The UV-screening substances of plants, phenolic acids and polyphenols, are already in use as the active molecules in a series of cosmetic products against photo-aging.

Red algae produce a technically and commercially applicable UV-A sunscreen

The strongest UV-A-absorbing compounds in nature are the mycosporine-like amino acids (MAAs). These are water-soluble substances found in a number of lower organisms such as cyanobacteria, red algae, dinoflagellates, corals and many marine invertebrates. The basic cyclohexanone or cyclohexenimine chromophore is responsible for UV absorption. Incorporation of various amino acids or iminoalcohol groups results in a diversity of about 20 MAAs (for a review see Cockell & Knowland). Corals that live in clear shallow water, a UV-intense environment, are found to produce 13 different MAAs (Shick and Dunlap 2002). Mountain lakes are another UV-intense environment, because exposure to UV increases with altitude and because the water is normally very clear so that UV can penetrate to depths of 20 m. Seven different MAAs were detected in the zooplankton of mountain lakes in the Central Alps (Sommaruga).

The red alga *Porphyra umbilicalis* is reported to produce the MAAs Porphyra-334 (**Figure 1**) and Shinorine (Gröniger *et al.*). Their absorption coefficients (εmolar) at 334 nm are 42'300 and 44'700 respectively (for the spectrum see **Figure 2**). Their filter capacity is therefore similar to that of synthetic UV-A sunscreens such as butyl methoxydibenzoylmethane (ε_{molar} 40'000) and terephthalylidene dicamphor sulfonic acid (ε_{molar} 45'000). *P. umbilicalis* lives in all oceans at the shore area on rough-surfaced rocks. It is a small alga, up to 20 cm, greenish

Figure 2 : Absorption spectrum of 1% MAA's encapsulated in liposomes



when young and later purplish-red, with an irregularly shaped frond that is membranous but tough. *P. umbilicalis*, commonly known as Purple Laver in America and Europe or as Nori in Asia, is the most widely consumed seaweed in the world.

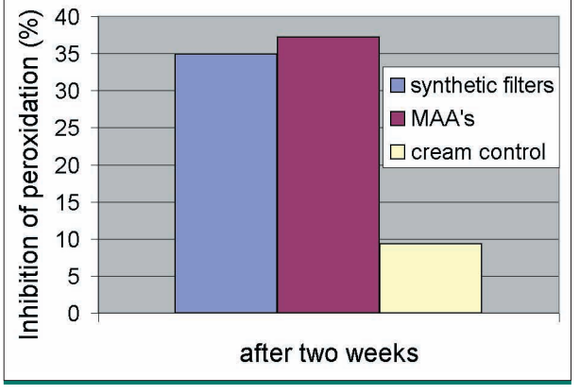
The MAAs in *P. umbilicalis* from the Bretagne in France were analysed by HPLC. This algae material contained the MAAs Porphyra-334 and Shinorine, in a ratio of 2 : 1. The total MAA concentration was 1.4% of dry mass. This is considerably more than the reported typical concentration range of 0.16 to 0.84% (Cockell & Knowland).

Study on the activity of MAAs to protect against photo-aging

Normal UV-A exposure at non-protected skin areas is between 3 and 20 J/cm^2. Rudolph and Träger measured an UV-A exposure of 8 J/cm^2 at the outside of a forearm facing an open window during weather that was a mixture of cloudy and sunny periods. The irradiation dose that was applied in this study (two times 10 J/cm^2 per week) therefore corresponds to an average UV-A exposure at a non-protected skin site. Thus, the study mimics daily photo-aging of the skin.

UV-A radiation is a powerful generator of harmful radicals in our skin cells. The first targets are the lipids in the cell membranes that are oxidized to lipid peroxides. These lipid radicals stand at the beginning of a deleterious reaction sequence that finally ends in loss of skin resilience and formation of wrinkles (for a review see Pinnell & Durham). The formation of lipid peroxides in the test areas was measured after 28 days. Compared to the untreated test area, treatment with a cream containing 0.005% MAAs encapsulated in liposomes reduced lipid peroxidation by 37% (**Figure 3**). The

Figure 3 : Inhibition of lipid peroxidation compared to the beginning of the study and the untreated area



capacity of the test product with MAAs to neutralize free radicals was as good as that of a product with synthetic filters (35% reduction), which contained 1% UV-A filter and 4% UV-B filter.

Free radicals that are produced upon exposure to UV radiation finally lead to photo-aging of the skin. These alterations in the skin are expressed in reduced firmness and smoothness. Application of the test product with MAAs encapsulated in liposomes could not only counteract these processes but even improve skin parameters during the study period. Both firmness and smoothness were significantly improved after four weeks, by 10% and 12% respectively (**Figures 4 and 5**). Using these parameters, the MAA test product performed clearly better than the product with the synthetic filters.

Figure 4 : Improvement of skin firmness compared to the beginning of the study and the untreated area

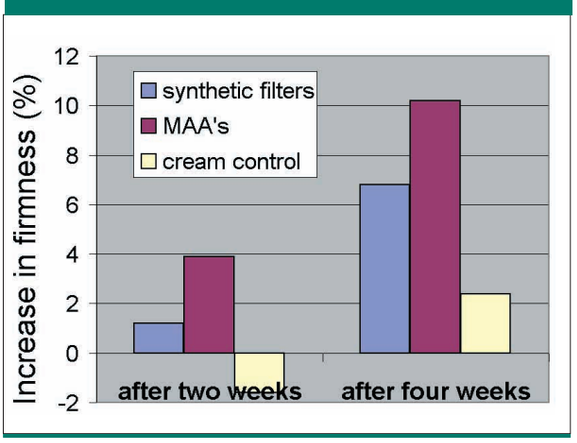
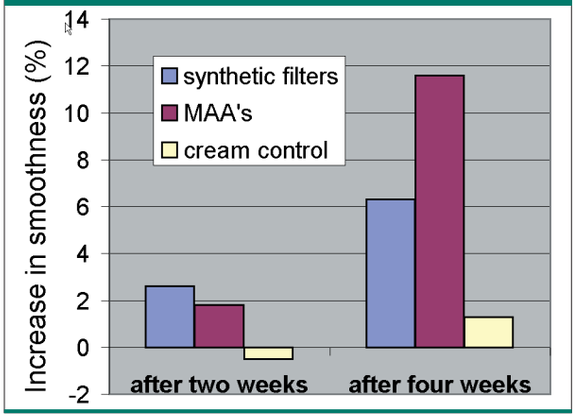


Figure 5 : Improvement of skin smoothness compared to the beginning of the study and the untreated area



The study clearly demonstrates that a cream with 0.005% MAAs can neutralize UV-A effects as efficiently as a cream with 1% synthetic UV-A filters and 4% UV-B filters. The UV-B sunscreens are not relevant to this study because the test areas were only exposed to UV-A irradiation. But as outlined in the introduction, this corresponds to normal working day situations in central Europe where only UV-A effects are signficant. Considering the UV-A-filtering capacity, the cream with 1% synthetic UV-A filter is 200 times more active than the MAA test product. The fact that this was not reflected in the study results (or even in the opposite way) indicates that the absorption capacity is not the only factor that is important for skin protection against photo-aging. Highly important is what happens to the screening compounds when they switch energy levels during absorption. The ideal sunscreen rapidly and effectively transforms absorbed UV into harmless thermal energy with no subsequent loss of protective power. It has been reported that butyl methoxydibenzoylmethane can partially decompose into inactive reaction products upon UV exposure (Chatelain & Gabard) and generates free radicals when illuminated (Damiani *et al.*). Analysis of the photophysical and photochemical properties of MAAs showed their qualification as photo-protectors because they do not produce reactive intermediates upon irradiation (Conde *et al*). Antioxidants present in the MAA extract might have also exerted a protective role in the photo-aging study. One of these antioxidants could be the MAA precursor molecule in the biosynthesis, 4-deoxygadusol. (Dunlap *et al.*, 4th International Marine Biotechnology Conference, Sorrento, September 23, 1997).

Methods

HPLC-Analysis of MAAs

Separation of MAAs was done on a Superspher 100 RP 18 column (4 _m, 254 x 4 mm) with a LiChrospher 100 RP 18 endcapt precolumn (5 _m). The mobile phase was 0.02% acetic acid and the flow rate 1.0 ml/min. The detection wavelength was 334 nm. Identification was done by comparison with MAA standards that were provided by Prof. Dr. D.-P. Häder (Friedrich-Alexander University of Erlangen, Germany).

Human study

Liposome encapsulated MAAs were prepared by extraction of dried *P. umbilicalis* in water and subsequent ultrafiltration and ion exchange chromatography of the extract. For encapsulation this material was mixed with lecithin by high pressure homogenization. The test product was a cream with 5% MAA extract. The final MAA concentration in the test product was 0.005%. The cream with MAAs was compared to a cream with 4% ethylhexyl methoxycinnamate and 1% butyl methoxydibenzoylmethane. The cream without actives served as control.

The study was conducted with 20 women in the age range 36 – 54. The test products were applied twice daily on defined sites on the inner side of the forearm. The test areas were irradiated twice weekly with UV-A (10 J/cm^2). Skin elasticity (firmness) was measured with a Cutometer SEM 575. Skin roughness (smoothness) was determined with the digital micromirror device 'PRIMOS' (GFMesstechnik GmbH, Teltow, Germany). Skin hydration was measured with the Corneometer CM 825 PC. Lipid peroxidation was measured by HPLC.

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