Mycosporine-like amino acids (MAAs): Chemical structure, biosynthesis and significance as UV-absorbing/screening compounds

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Continuous depletion of the stratospheric ozone layer has resulted in an increase in ultraviolet-B (UV-B; 280-315 nm) radiation on the earth’s surface which inhibits photochemical and photobiological processes. However, certain photosynthetic organisms have evolved mechanisms to counteract the toxicity of ultraviolet or high photosynthetically active radiation by synthesizing the UV-absorbing/screening compounds, such as mycosporine-like amino acids (MAAs) and scytonemin besides the repair of UV-induced damage of DNA and accumulation of carotenoids and detoxifying enzymes or radical quenchers and antioxidants. Chemical structure of various MAAs, their possible biochemical routes of synthesis and role as photoprotective compounds in various organisms are discussed.

Keywords: Cyanobacteria, Macroalgae, Mycosporine-like amino acids (MAAs), Phytoplankton, UV radiation

Absorption of solar energy to drive photosynthesis exposes photosynthetic organisms to potentially damaging ultraviolet (UV) radiation in their natural habitats. Continued depletion of stratospheric ozone layer due to atmospheric pollutants such as chlorofluorocarbons (CFCs), chlorocarbons (CCs) and organobromides (OBs) has resulted in an increase in ultraviolet-B (UV-B; 280-315 nm) radiation on earth’s surface1-4. Ozone depletion has been reported both in Antarctic and Arctic regions, declining by more than 70% during late winter and early spring due to a phenomenon known as polar vortex5,6. Harmful doses of UV-B radiation penetrate deep into a water column influencing the photosynthetic denizens7. The optical properties of water determine the extent of penetration. Depth of 20 m in oceanic water and only a few centimeters in brown humic lakes and river affords 90% protection against UV-B irradiation8. UV-B is the most effective radiation causing cell damage by directly meddling with the DNA and proteins and indirectly via producing reactive oxygen species9-11. The survival, growth, pigmentation, photosynthetic oxygen production, motility, N2 metabolism, phycobiliprotein composition and 14CO2 uptake are the main physiological and biochemical processes affected by UV-B radiation12-17.

A number of photosynthetic organisms which are simultaneously exposed to visible and UV radiation have evolved mechanisms to circumvent the damaging effects of UV radiation. These mechanisms include light driven repair of UV-induced damage of DNA18,19, spore germination and reproduction20, accumulation of carotenoids, detoxifying enzymes, radical quenchers and antioxidants21,22, and synthesis of UV-absorbing or screening compounds23,24. Among UV-absorbing/screening compounds mycosporine-like amino acids (MAAs) have received much attention for their putative role in UV photoprotection, which were originally identified in fungi as having role in UV-induced sporulation25. The following text reviews the available information on the structure, biosynthesis and evolution of MAAs and their photoprotective role in various organisms.

Structure and biosynthesis of MAAs

The MAAs, found in various organisms from tropical to polar regions23,26,27, are small (<400 Da), colorless, water-soluble compounds composed of cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acids or its imino alcohol28. There are more than 20 different MAAs found in various organism some of which are shown in Table 1. Generally, the ring system contains a glycine subunit at third carbon atom. Some MAAs also contain sulfate esters or glycosidic linkages through the imine substituents28. Difference in the absorption
Table 1—MAAs with their corresponding absorption maxima and molecular structure

<table>
<thead>
<tr>
<th>Mycosporine-like amino acids</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
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<tbody>
<tr>
<td>Mycosporine-taurine</td>
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<td>Asterina-330</td>
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<tr>
<td>Mycosporine-glycine</td>
<td>310</td>
<td><img src="example.png" alt="Mycosporine-glycine" /></td>
<td>Mycosporine-glutamic acid-glycine</td>
<td>330</td>
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<tr>
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<td><img src="example.png" alt="Palythine" /></td>
<td>Palythinol</td>
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<td>Palythine-serine-sulfate</td>
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<td><img src="example.png" alt="Palythine-serine-sulfate" /></td>
<td>Mycosporine-2-glycine</td>
<td>334</td>
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<tr>
<td>Palythine-serine</td>
<td>320</td>
<td><img src="example.png" alt="Palythine-serine" /></td>
<td>Shinorine</td>
<td>334</td>
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<tr>
<td>Mycosporine-methylamine-serine</td>
<td>327</td>
<td><img src="example.png" alt="Mycosporine-methylamine-serine" /></td>
<td>Porphyra-334</td>
<td>334</td>
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<tr>
<td>Mycosporine-methylamine-threonine</td>
<td>327</td>
<td><img src="example.png" alt="Mycosporine-methylamine-threonine" /></td>
<td>Mycosporine-glycine-valine</td>
<td>335</td>
<td><img src="example.png" alt="Mycosporine-glycine-valine" /></td>
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spectra of MAAs is due to the variations in the attached side groups and nitrogen substituents. The most common methods for MAA detection and quantification are high-performance liquid chromatography (HPLC) based on their retention times and absorption maxima and obtaining entire UV scans via diode array detection (DAD)\textsuperscript{24,29,30}. The electrospray ionization mass spectrometry coupled with liquid chromatography (LC/MS) has also been used to analyze the MAAs in aquatic environment\textsuperscript{31,32}. Recently Torres et al.\textsuperscript{33} have reported the structure and molecular formula of Porphyra-334 by the application of MS in conjunction with \textsuperscript{1}H and \textsuperscript{13}C NMR data. A number of MAAs are still to be chemically characterized and an exhaustive survey of organisms may reveal enormous diversity of these compounds.

The biosynthesis of MAAs is presumed to be occurring via the first part of shikimate pathway (Fig. 1), but concluding evidences are lacking to a great extent. It has been found that 3-dehydroquinate, which is formed during shikimate pathway, acts as a precursor for the synthesis of fungal mycosporines and MAAs via gadusols\textsuperscript{26,34}. This view is supported by the inhibition of MAAs synthesis in Stylophora pistillata by the application of glyphosate which is a specific shikimate pathway inhibitor\textsuperscript{35}. The primary MAA mycosporine-glycine thus synthesized by shikimate pathway is then transformed through chemical and/or biochemical conversions into secondary MAAs\textsuperscript{36-38} (Fig. 1). The synthesis of MAAs occurs in bacteria, cyanobacteria, phytoplankton and macroalgae but not in animals, because they lack the shikimate pathway.

### Role of MAAs in photoprotection

The strong UV absorption maxima between 310-362 nm, high molar extinction coefficient (ε = 28,100-50,000) and photostability in distilled and sea water in presence of photosensitizers\textsuperscript{39}, support the contention that MAAs have a photoprotective role\textsuperscript{40,41}. These compounds are capable of effectively dissipating absorbed radiation without producing reactive oxygen species\textsuperscript{42}. As these compounds provide photoprotection in organisms having shikimate pathway, certain marine animals such as arthropods, rotifers, molluscs, fish, cnidarians, tunicates, eubacteriobionts, poriferans, nemertineans, echinoderms, platyhelminthes, polychaetes, bryozoans and protozoans have also been reported to protect themselves from UV radiation by MAAs. However, studies have shown that in these shikimate pathway lacking animals these compounds are derived from their algal diet\textsuperscript{43,44}. Thus, MAAs provide protection from UV radiation not only in their producers but also to primary and secondary consumers\textsuperscript{45}. MAAs have also been shown to be highly resistant against abiotic stressors such as temperature, UV radiation, various solvents and pH\textsuperscript{46,47}.

### MAAs in cyanobacteria

Cyanobacteria have a cosmopolitan distribution in both aquatic and terrestrial ecosystems ranging from hot springs to the Antarctic and Arctic regions. The prominent role of N\textsubscript{2}-fixing cyanobacteria in improving the soil fertility is well documented\textsuperscript{48}. They are also significant constituents of marine ecosystems and account for a high percentage of oceanic primary productivity. Palaeobotanical studies reveal their appearance to the Precambrian era and the presence of

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<tr>
<td>Usujirene</td>
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<tr>
<td>Palythene</td>
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<tr>
<td>Euhalothece-362</td>
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Fig 1—Biosynthetic routes for the formation of MAAs via the shikimate pathway and their feasible chemical and/or biochemical conversion. Broken line represents the putative biosynthetic connection between Dehydroquinate, gadusols and MAAs. PEP: Phosphoenolpyruvate; DAHP: 3-deoxy-D-arabinoheptulosinate-7-phosphate; DHQ: dehydroquinate; EPSP: 5-enolpyruvyshikimate-3-phosphate.
UV-absorbing compounds like MAAs presumably may have supported their existence at that time when there was no stratospheric ozone layer. The accumulation of large amounts of MAAs in cyanobacteria was first reported in 1969 by Shibata49. MAA-producing cyanobacteria are abundant in hyper saline environments. Garcia-Pichel et al50 examined the morphology, physiology and 16s rRNA gene sequence of 13 MAAs-containing strains of unicellular halophilic cyanobacteria. These strains shared a common complement of MAAs. From a community of unicellular cyanobacterium (Euhalothece sp.) inhabiting the upper layer of a gypsum crust developing on the bottom of a hyper saline saltern pond, two MAAs having absorption maximum at 331 and 362 nm, were isolated when grown at high light intensities. The compound absorbing at 331 nm was purified and identified as mycosporine-2-glycine. This was the first report of mycosporine-2-glycine in the cyanobacteria51. Recently Volkmann et al52 have identified and given the structure of another 362 nm absorbing compound (euhalothece-362). It seems that Euhalothece sp. produces two UV absorbing MAAs, mycosporine-2-glycine and euhalothece-362. Four different mycosporine-like amino acids have also been detected in members of the halophilic filamentous genus Microcoleus53. Zhang et al54 isolated and characterized three MAAs, M-tau (UV-B sunscreen), dehydroxylusujirene and M-343 in Synechocystis sp. PCC 6803 and observed that dehydroxylusujirene and M-343 induction was mainly UV-A dependent but M-tau could be induced by both UV-A and UV-B. The MAAs such as asterina-330, shinorine, palythinol and mycosporine-glycine have been found to play a photoprotective role in epilithic cyanobacteria from a freshwater lake55. Microcystis aeruginosa, a common bloom forming cyanobacterium was able to synthesize MAAs (shinorine and porphyra-334) which directly absorbed UVR. This enabled cyanobacterium to develop and maintain surface bloom even in the presence of high solar irradiance including ultraviolet radiation56.

The Leptolyngbya mat in Antarctica showed significant photochemical inhibition under supplemental UV-B but was less prominent in a Phormidium mat because it was found to contain 25 times the concentration of UV protecting MAAs than to the Leptolyngbya mat57. A polychromatic action spectrum for the induction of MAAs in a rice-field cyanobacterium Anabaena sp. showed a single prominent peak at 290 nm58. A prominent peak at 290 nm and a smaller peak at 312 nm in an action spectrum for MAAs induction have been shown for Nostoc commune59. Similarly Portwich and Garcia-Pichel60 have shown a peak at 310 nm in an action spectrum of MAA synthesis in Chlorogloeopsis PCC 6912 and proposed a reduced pterin as a putative candidate for the induction of MAA shinorine. UV-B dependent induction of MAAs synthesis experiments in Anabaena sp., Nostoc commune and Scytonema sp. have revealed the presence of shinorine, which is a bi-substituted MAA, containing both a glycine and serine group with an absorption maximum at 334 nm59.

Sinha et al61 reported UV-B induced synthesis of two MAAs, shinorine and porphyra-334 (both having absorption maximum at 334 nm) in three species of Nodularia (N. baltica, N. harveyana and N. spumigena) by using the 395, 320 and 295 nm cut-off filters. In most of the cyanobacteria the exact location of MAAs is not known but in Nostoc commune it has been shown to be extracellular and linked to oligosaccharides in the sheath28. These glycosylated MAAs represent perhaps the only known example of MAAs that is actively excreted and accumulated extracellularly acting as true screening compounds62. The MAAs in single celled cyanobacterium Gloeocapsa sp. absorb only 10-30% of incident photons but its screening efficiency may be substantially increased in colony and mat forming cyanobacteria63,64. The MAAs are osmotically active compounds and their accumulation in the cell is regulated by osmotic mechanism. This is reflected by the fact that field populations of halotolerant cyanobacteria accumulate high concentration of MAAs65. Thus MAAs not only play a significant role as photoprotective compounds but also act as osmoregulators in certain cyanobacteria.

MAAs in phytoplankton

Phytoplanktons are by far the major biomass producers in the oceans forming the base of the food web. Their productivity matches all terrestrial ecosystems taken together66. In addition, several genera of phytoplankton produce volatile substances, mainly dimethyl-sulfide (DMS). These volatile compounds act as precursors of cloud condensation nuclei and thus counteract the greenhouse effect. The cumulative effect of marine biota in the reduction of
CO₂ concentration and emission of DMS has been estimated to cool the atmosphere by up to 6°C. Like cyanobacteria, phytoplanktons are also exposed to the harmful effects of UV radiation because these radiations can penetrate up to euphotic zone. Therefore, phytoplanktons have developed certain resistance mechanisms against UV radiation by inducing the MAAs synthesis.

The probability that MAAs act as UV-B absorbing compounds has been derived from the fact that the distribution of MAAs in marine organisms shows a significant correlation with the depth, which in turn regulates the dosage of UV or PAR radiation. So far MAAs have been reported to occur predominantly in members of the Dinophyceae, Bacillariophyceae and Haptophyceae. In larger cells, the MAAs are more effective because of large surface area, the smaller cells compensate it by forming dense populations. In the dinoflagellate *Alexandrium excavatum*, isolated from the continental shelf near Buenos Aires, transfer from low to high PAR caused a change in MAAs composition and overall increase in UV absorption. The *Prorocentrum micans* (dinoflagellate) showed high concentrations of MAAs when grown in presence of UV radiation. Morrison and Nelson presented time-series data collected at the Bermuda Atlantic Time-series study (BATS) site to document seasonal induction of enhanced UV absorption by MAAs in phytoplankton in the summer stratified surface water of the Sargasso Sea.

In the dinoflagellate *Gyrodinium dorsum*, the accumulation of MAAs was stimulated by PAR and UV radiation while in *Phaeocystis antarctica* the induction of MAAs synthesis occur only by UV-A and UV-B radiation. The protective function of MAAs is not necessarily restricted to the phytoplankton producing them because MAAs have been shown to be released from phytoplankton grown in cultures. MAAs have been identified in colored or chromophoric dissolved organic matter (CDOM) of coastal water, where they might contribute up to 10% of the CDOM absorption at 330 nm. Thus MAAs not only benefit their producers but also provide protection to other organisms against lethal UV-B in their habitats.

**MAAs in macroalgae**

Macroalgae are the major biomass producers along the coastlines and on the continental shelves. They are exploited commercially on a large scale and provide habitat for larval stages of economically important animals. Both short and long-term exposure to solar radiation inhibits growth in adult stages of several species of macroalgae due to the destruction in chloroplast, mitochondria, and occurrence of metabolites produced under UV stress. The photoprotective role of UV-absorbing compounds in macroalgae was first reported in 1961. Since then many qualitative and quantitative studies have been carried out to survey the distribution of MAAs among commercially important macroalgae. The MAAs content in macroalgae varies between classes and with increasing depth and latitudes. Deep water algal species do not contain MAAs. MAA content is an important factor controlling the biogeographic distribution of macroalgae, since species from lower, high-solar latitudes always exhibit more MAAs than individuals from higher, low-solar latitudes.

Many macroalgae produce one or several UV absorbing substances. Most MAA-producing macroalgae belong to Rhodophyceae, followed by Phaeophyceae, and only a few green algae produce MAAs. Three different types of protection by MAAs have been reported: (i) the sublittoral algae which are not likely to be exposed to higher doses of solar UV do not synthesize MAAs at all, (ii) majority of the supralittoral algae with maximum exposure to UV-B radiation produce high amount of MAAs. They cannot be further induced by exposure to any radiation, and (iii) some supralittoral algae in which MAAs production can be induced by solar radiation. Hoyer et al. studied the formation of MAAs as a photoprotective strategy against harmful UV radiation in 18 species of Antarctic red macroalgae by giving radiation treatment at 400-700, 320-700 and 295-700 nm and reported induction and accumulation of MAAs only in 8 species, while remaining ten, mainly deep water species, did not remain unaffected. Table 2 shows different algal groups divided into three physiological response type, based on MAA synthesis in response to radiation treatment.

Recently Arróniz-Crespo et al. have reported the occurrence of MAAs (porphyra-334 and mycosporine-glycine) in red alga *Lemanea fluviatilis*. They found that mycosporine-glycine was specifically induced by UV-B radiation. Korbee et al. studied the effect of light quality on accumulation of UV-absorbing MAAs in red alga *Porphyra leucosticta* and found that blue light favored the accumulation of porphyra-334, palythine and asterina-330 whereas the
white, yellow, green and red lights favored the accumulation of shinorine. Induction of mycosporine-glycine has also been found in *Laminaria saccharina*\(^9\). Volkmann et al\(^9\) reported the synthesis of mycosporine-glutaminol-glucoside in presence of photosynthetically active radiation and UV radiation, suggesting a photoprotective function\(^9\). In the chlorophyte *Dasycladus vermicularis*, UV-absorbing compounds have been detected\(^9\). A polychromatic action spectrum was determined for the induction of MAAs in the chlorophyte *Prasiola stipitata* showing a clear maxima at 300 nm\(^9\). The sub-aerial green macroalga *Prasiola crispa* sub sp. *antarctica* contained high concentration of a unique UV-absorbing compound with an absorption maxima at 324 nm. It was characterized as a putative MAA due to chromatographic properties\(^9\). This MAA has been recently identified in other green algae closely related to *Prasiola* sp. except in *Myrmecia incisa* which had 322 nm-MAA\(^9\). Han and Han\(^9\) reported UV-B absorbing compounds with a prominent absorption maximum at 294 nm in green alga *Ulva pertusa* by using different cut-off filters. These compounds were synthesized only in response to UV-B treatment but their chemical structure is yet to be determined.

**Evolution of MAAs as UV-absorbing compounds**

The level of UV-C and UV-B radiation flux was higher on earth’s surface prior to the formation of ozone layer that occurred when oxygen concentration increased during the early Proterozoic by cyanobacterial photosynthesis. It was therefore necessary for these aquatic photosynthetic organisms to protect themselves against UV-induced destruction of complex organic molecules\(^9\), through the synthesis of organic molecules like MAAs\(^9\). The chemistry of the first specific UV-screening molecules on Archean Earth is not well known but it is hypothesized that aromatic-containing reaction centers were some of the earliest UV-screens that later on started to perform a light harvesting role in photosynthesis\(^9\).

The evolutionary origin of UV-screening compounds is still unknown. It is presumed that many of them evolved for other physiological roles but later adapted to UV-screening function under selection pressure. MAAs function as osmotic regulators in some cyanobacteria\(^9\) and such alternative roles may have given rise to the first UV-screening MAAs\(^9\). Many of the simpler MAAs such as mycosporine-glycine might be predominantly absorbing in UV-B region but later as the oxygen level increased the absorption of UV-A became necessary since it resulted in the formation of oxygen free radicals\(^9\). This was done by replacing the ketone function by nitrogen atom in UV-B absorbing compounds. This had a greater mesomeric effect on the benzene ring shifting the absorbance to UV-A zone. In addition, mutation in UV-B screening compound might have also caused a shift in UV-A absorption. In eukaryotic algae, the MAAs have been thought to be passed by cyanobacteria in the plastidic line\(^9\) and if green algae were the true origin of land plants, it may be
speculated that early land plants might have initially been depended on MAAs instead of flavanoids which protect them from UV radiation in present time.

Conclusion
UV-absorbing MAAs are prevalent throughout the microbial world especially in cyanobacteria, phytoplankton and macroalgae. These compounds not only protect the producers but the consumers also, against the harmful effects of UV radiation. A number of photoprotective compounds from diverse organisms are yet to be chemically characterized. Although, shikimate pathway has been suggested for the synthesis of MAAs, it is still to be well documented. The loci for synthesis and storage of MAAs in a cell have to be ascertained. Presently, only in *Porphyra yezoensis*, the MAAs are known to be located in chloroplasts. Distribution of MAAs in phylogenetically related algae can perhaps prove to be of chemotaxonomic value.

Although, presence of higher concentration of MAAs in organisms exposed to intense solar radiation has been described as a contraption against UV damage, there is no specific proof suggesting the MAAs in organisms exposed to intense solar radiation. Although, presence of higher concentration of MAAs in a cell have to be ascertained. Presently, only in *Porphyra yezoensis*, the MAAs are known to be located in chloroplasts. Distribution of MAAs in phylogenetically related algae can perhaps prove to be of chemotaxonomic value.

Although, presence of higher concentration of MAAs in organisms exposed to intense solar radiation has been described as a contraption against UV damage, there is no specific proof suggesting the exclusive role of MAAs as sunscreen. It is presumed that they play more than one role in the cellular metabolism. The UV-A absorption properties of the crude methanolic extract of Porphyra-334 have been determined against two commercial sun care products in terms of mean critical wavelength, mean UV-A/UV-B ratios and UV-A protection category. The data suggest that it can serve as a UV-A protecting sunscreen by providing wide protection against UV radiation. It has also been reported that MAAs may also act as antioxidants to prevent cellular damage resulting from UV-induced production of active oxygen species. Further work is needed to test the potentialities of MAAs as antioxidants and thereby opening the way for industrial or pharmacological development of the biological sunscreens and antioxidants.

Acknowledgement
K.L.S. is thankful to UGC, New Delhi for the award of Junior Research Fellowship.

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