Ultraviolet-B radiation effects on cyanobacteria and the role of sunscreen pigments in its protection

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Abstract

Depletion of the stratospheric ozone layer due to anthropogenically released chemicals such as chlorofluorocarbons (CFCs) has resulted in an increase in ultraviolet-B (UV-B; 280-315 nm) radiation on the Earth’s surface. A number of physiological and biochemical processes such as growth, survival, cell differentiation, motility, pigmentation, photosynthesis, nitrogen metabolism, proteins and DNA have been reported to be affected by UV-B radiation. However, response of cyanobacteria towards UV-B radiation differs in various species. Most probably differential effects are mainly due to protective mechanisms which various species of cyanobacteria adopt under natural condition upon sensing the stress of UV-B radiation. Various protective mechanism(s) such as morphological, avoidance, synthesis of sunscreen pigments, DNA repair by photoreactivation etc. have been reported in diverse species of cyanobacteria to counteract the damaging effects of UV-B radiation. This chapter deals with the general effects of UV-B radiation on cyanobacteria and the role of sunscreen pigments as protective mechanism against UV-B mediated damages.

Introduction

Cyanobacteria are the first photosynthetic oxygen-evolving gram-negative prokaryotes which appeared during the Precambrian era (between 2.8 and 3.5×10⁹ years ago) when the ozone shield was absent. They presumably faced high fluxes of ultraviolet radiations (UVR), which must have acted as an evolutionary pressure leading to the selection for effective protecting mechanisms. Cyanobacteria are the most important biomass producers and play globally significant role in biogeochemical cycle of nitrogen, carbon and oxygen (Häder et al., 2007). These prokaryotes are excellent source of a wide range of biologically active compounds of significant values that has fascinated researchers for their pharmaceutical and biotechnological exploitation (Gademann and Portmann, 2008; Rastogi and Sinha, 2009).

During past few decades rapid industrialization has resulted in an increase in anthropogenically released atmospheric pollutants such as chlorofluorocarbons, chlorocarbons and organobromides that cause depletion of stratospheric ozone layer (Weatherhead and Anderson, 2006). The rapid depletion of ozone layer causes increase in the highly energetic UV radiation on the Earth’s surface which is absorbed by biomolecules such as nucleic acids and proteins and ultimately shows lethal effects on the biological systems (Häder et al., 2007).

On the basis of the mechanisms of interaction with living matter and its overall severity, the solar UV spectrum can be divided into ultraviolet-A (315–400 nm), ultraviolet-B (280–315 nm), and UV-C (100-280 nm). UV-A effects occur through sensitizing molecules, which on absorption can interact with oxygen to create reactive oxygen species (ROS) these in return can oxidize a number of cellular biomolecules. UV-B damage, by contrast, results from direct absorption by the target molecules (mainly DNA and proteins) and does not depend on the presence of oxygen (Rastogi et al., 2010). Highest energetic ultraviolet-C radiation does not reach the Earth’s surface at present, owing to absorption by ozone and losses through atmospheric scattering.

General effects of UV-B radiation on cyanobacteria

Several studies conducted under laboratory and natural conditions have revealed the harmful effects of UV-B radiation on growth, survival, motility, development, pigmentation, nutrient uptake, and various other metabolic processes of cyanobacteria (Häder et al., 2007). These effects are in part due to the direct effect on membrane proteins, photosystem II, DNA, enzymes, growth regulators or due to indirect effect through the formation of reactive oxygen species. Of several targets of UV-B damage that have been reported, DNA and photosynthesis are recognized as the most predominant action sites (Singh et al., 2010a). It has been elegantly demonstrated that when Anabaena aequalis cultures are exposed to UV-B radiation there is delayed differentiation of vegetative cells to heterocyst and further into akinetes (Blakefield and Harris, 1994). Growth and survival of several cyanobacteria are severely affected by UV-B exposure although degree of growth inhibition and survival vary significantly in different genera (Sinha et al., 1995). Virtually, most of the experiments on UV-B radiation have been conducted under controlled laboratory conditions, a few workers reported that growth is inhibited up to 40 % under solar UVR in Anabaena sp. PCC 7120 and certain other nitrogen-fixing cyanobacteria (Gao et al., 2007).

One of the crucial detrimental effects of UV-B radiation in cyanobacteria include photobleaching of photosynthetic pigments, decrease in phycobiliprotein content and disassembly of phycobilisome complex (Sinha et al., 2007).
et al., 2005). SDS-PAGE analysis of phycocyanin and its linker polypeptides in Anabaena sp. revealed loss of αβ monomers of phycocyanin after 1 h of UV-B exposure (Sinha et al., 2003). Additionally, several studies have demonstrated that UV-B radiation affects spectral properties of pigments specifically chlorophyll a and phycobiliproteins of cyanobacteria (Gao et al., 2007). Together with this, various other photosynthetic parameters such as $^{14}$CO$_2$ uptake, O$_2$ evolution and ribulose-1, 5 bisphosphate carboxylase/oxygenase (RUBISCO) activity are also down regulated (Sinha et al., 2008). The D1 and D2 proteins that are major constituent of PSII reaction centers are degraded by exposure of UV-B. Equally important effects of UV-B radiation include inhibition of nitrogen fixation in almost all the species so far tested. Kumar et al.,(2003) reported complete loss of nitrogenase activity within 25-40 min of UV-B exposure in several rice field cyanobacteria. It was demonstrated that the activity of UV-B inhibited nitrogenase did not appear upon transfer of cultures to fluorescent light, suggesting that the inhibition may be due to specific inhibition of the enzyme (Kumar et al., 2003). Using inhibitors of protein synthesis and PS-II activity, they demonstrated that restoration of nitrogenase activity in UV-B exposed cultures occurred by fresh synthesis of nitrogenase polypeptide. Interestingly, other enzymes of nitrogen metabolism such as nitrate reductase, glutamine synthetase and glutamate synthase are less sensitive to elevated level of UV-B intensity (Kumar et al., 1996).

Total protein profile of cyanobacteria showed significant alterations following exposure of cultures to UV-B radiation (Sinha et al., 2005). Several protein bands disappeared and a few new protein bands appeared in the gel. Total proteome analysis of Synechocystis sp. PCC 6803 by 2-dimensional (2-D) gel electrophoresis showed different expression level of proteins in the cytoplasm under short and long-term UV-B stress (Gao et al., 2009). One hundred and twelve differentially expressed protein spots were identified by mass spectrometry to match 75 diverse protein species. The above study focused on amino acid biosynthesis, photosynthesis and respiration, energy metabolism, protein biosynthesis, cell defense, and other functional groups (Gao et al., 2009).

Several types of DNA damages have been identified which probably develop mainly due to direct absorption of UV-B radiation by the native DNA molecule and indirectly by oxidative stress. The main DNA damages include formation of dimeric photoproducts such as cis-syn cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) and their Dewar isomers (Sinha and Hädé, 2002). Damage to DNA was also evident in the study conducted by Kumar et al., (2004) who found loss of template activity of genomic DNA in Anabaena BT2 after UV-B exposure. They employed simple and rapid technique of PCR assay such as RAPD (random amplified polymorphic DNA), rDNA amplification and ARDRA (amplified ribosomal DNA restriction analysis) to reveal damage caused to DNA by UV-B treatment. Recently a modified version of the fluorometric analysis of DNA unwinding (FADU) assay has been described by Rastogi et al., (2010) that enabled the measurement of DNA strand breaks and DNA repair in a reliable and convenient manner. Huang et al., (2002) have made critical analysis of global gene expression profile of the cyanobacterium Synechocystis sp. strain PCC 6803 in response to irradiation with UV-B and white light. The study showed that several families of transcripts were altered by UV-B treatment, including mRNAs specifying proteins involved in light harvesting, photoprotection and heat shock response. There are several other processes in cyanobacteria which are affected by UV-B radiation, herein we have highlighted only general effects.

**Defense mechanisms developed by cyanobacteria against UV-B radiation**

As described above cyanobacteria have many cellular targets that are severely affected by harmful UV radiations however these microorganisms developed various defense mechanisms to overcome harmful effects of these UV radiations. In this section various defense mechanisms adopted by cyanobacteria against UV radiations are described.

Cyanobacteria in their natural habitat adopted avoidance as a first line of defense which includes migration from high level to low level of UV intensity in water column, formation of mats, changes in morphology to provide self shading and synthesis of extracellular polysaccharides. The avoidance of damaging UVR provides protection up to some extent as this mechanism includes motility and mat forming capability of cyanobacteria as well as on the turbidity and depth of the water column.

However, once UVR reaches inside the cell it interacts with oxygen and other organic compounds to generate toxic ROS such as superoxide (O$_2^-$), hydroxyl radical (OH) or hydrogen peroxide (H$_2$O$_2$) which causes oxidative stress. To overcome these oxidative stresses a second line of defense comes into play which comprise both non-enzymatic and enzymatic antioxidants. Non-enzymatic antioxidants in the cells are ascorbate (vitamin C), tocopherol (vitamin E), carotenoids and reduced glutathione. The enzymatic antioxidants are superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and the enzymes involved in the ascorbate-glutathione cycle to detoxify the ROS such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (He and Hädé, 2002).
A third line of defense operates through the synthesis of UV-absorbing compounds by several cyanobacteria (Sinha and Häder, 2008). Mycosporine-like amino acids (MAAs) and scytonemin are well known UV-absorbing screening compounds that provide photoprotection against UV-B and/or UV-A radiation (Sinha and Häder, 2008). This line of defense mechanism is described in forthcoming section.

When repair of harmful UVR-induced damages (dampages of biomolecules such as DNA and proteins) escape from first, second and third line of defense mechanisms, a fourth line of defense mechanism i.e., DNA repair by photoreactivation plays vital role. Although the presence of multiple copies of genomic DNA in the cyanobacteria may nullify the effect of single mutations by UVR, the existence of several DNA repair mechanisms strengthen them even more to cope with this radiation (Sinha and Häder, 2002). These mechanisms include photoreactivation by photolyase which converts UV induced dimers into monomers, the dark or excision repair and the recombinational repair. The presence of a UV-inducible photoreactivation system has been reported in strains of Anabaena sp. such as Anabaena sp. PCC 7120, A. variabilis PCC 7937, Anabaena sp. M-131 and A. variabilis sp. PCC 7118 (Levine and Thiel, 1987). The genes for photolyase homologs have been identified in Synechocystis sp. PCC 6803 and were functionally characterized for their roles in photoreactivation (Ng and Pakrasi, 2001). Han et al., (2001) have also reported photoreactivation of UV-B induced inhibition of photosynthesis in Anabaena sp. An increase in the transcript level of recA and the concomitant increase in the abundance of the corresponding 37–38-kDa polypeptide were reported in A. variabilis after UV exposure (Oottrrim and Coleman, 1989). The gene for the DNA repair enzyme Fpg (formamidopyrimidine-DNA glycosylase) has been reported from Synochoccus elongatus and was suggested to be involved in photoprotection against oxidative damage (Mühlenhoff, 2000). Repair of cyclopurimidine dimers (CPDs) is light and temperature dependent since these lesions were removed more efficiently in visible light than in darkness and the optimum temperature for the repair was found at 20 °C (Rastogi et al., 2011).

In addition, recent studies showed that cyanobacteria may also undergo apoptosis or programmed cell death (PCD) when a cell is damaged beyond repair. An autocatalytic PCD has been shown to operate in the nitrogen-fixing cyanobacterium Trichodesmium sp. and was found to be induced by high irradiance, iron starvation and oxidative stress (Berman-Frank et al., 2004). Similarly, recent data on Microcystis aeruginosa showed that H₂O₂ treatment induces PCD in this organism, and catalase was capable of inhibiting PCD, implicating the role of PCD under oxidative stress (Ross et al., 2006). Several genes encoding for caspases, enzymes involved in PCD in eukaryotes, have also been recently reported in the sequenced genome of M. aeruginosa (Franguel et al., 2008). The caspase activity as well as proteins reacting to human caspase-3 antibodies was reported in Trichodesmium sp. (Berman-Frank et al., 2004). The freshwater cyanobacterium Anabaena has also been found to activate PCD and increases general protease activity after exposure to univalent-cation salts (Ning et al., 2002). Thus, it is clear that the process of PCD plays an important role under oxidative stress in cyanobacteria, however further study is needed to find out its advantageous role under UV stress or UV-mediated oxidative stress.

**Sun-screening compounds in cyanobacteria**

One of the most important defense mechanisms towards UV-B radiation effects in cyanobacteria is the production of secondary metabolites which act as sunscreen. UV sunscreen compounds have two main properties: (a) it must absorb in the UV range with a high absorption coefficient, and (b) its concentration in the organism must be sufficient to cause a substantial reduction in the UV dose received by the organism. In addition to above properties, the compound should not sensitize photodamage, and should be present in a conformation that is optimal for screening and is produced specifically and/or in response to UV light exposure. Brief description of sunscreen compounds is given below under separate heading.

A. **Scytonemin: a sunscreen pigment**

Although several workers reported sheath pigments from a number of cyanobacterial species but the nature, physiological role and universality of scytonemin remained unexplored till 1993. Garcia-Pichel and Castenholz (1991) for the first time characterized in detail the structure and function of scytonemin from a few cyanobacteria. Scytonemin is a yellow to brown, lipid-soluble, non-fluorescent, very stable pigment that is excreted and deposited in the extracellular polysaccharide sheaths of some cyanobacteria (Garcia-Pichel and Castenholz, 1991). Microspectrophotometric measurements of the transmittance of pigmented sheaths and the screening of excitation of phycocyanin fluorescence under UV light demonstrated that the pigment is effective in shielding the cells from incoming near-UV and blue radiation, but not from green or red light, which is needed for photosynthesis. The screening effect of scytonemin among many cyanobacteria is substantial, particularly among colony-forming species (Garcia-Pichel and Castenholz, 1993). In the model organism Chloroglooeopsis sp., its presence is associated with amelioration of photo-inhibition of photosynthesis, reduced photobleaching of chlorophyll a and faster growth rates under UV-A radiation. The beneficial effects also
remain under conditions of physiological inactivity. Structurally scytonemin is a symmetrical indole-alkaloid consisting of fused heterocyclic units, connected through a carbon–carbon bond, with a molecular mass of 544 g mol\(^{-1}\). Scytonemin is redox sensitive, changing between a greenish brown oxidized form and a red reduced form, although the physiologically relevant form is in the oxidized state. The complex ring structures with conjugated double-bond distribution allow strong absorption in the UV-A range, with a maximum around 370 nm in vivo (Proteau et al., 1993).

**Distribution and physiological ecology**

Scytonemin can be readily detected by microscopic observation, and it has been reported from more than 300 cyanobacterial species, including all major taxonomic groups (Garcia-Pichel and Castenholz, 1991). It is common in natural populations that are exposed to high irradiance, population from extreme environment, such as recurrent desiccation or low temperatures. Scytonemin has yet to be found in planktonic populations, either marine or freshwater. Its content can reach up to 5% of the cellular dry weight in cultivated isolates; much larger accumulations are encountered in field populations (Sinha et al., 2001). Typical habitats for scytonemin-bearing cyanobacteria are intertidal mats, epilithic biofilms and biological soil crusts, but many others exist. Scytonemin is produced in response to UV-A exposure in all cases examined (Ehling-Schulz et al., 1997). Certain environmental stresses can modulate scytonemin synthesis in apparently complex regulatory interactions. Temperature or photo-oxidative stresses alone do not induce scytonemin synthesis, but can increase the levels attained during UV-A induction (Dillon et al., 2002). Osmotic stress results in a moderate induction of scytonemin production in one strain, but not in many others (Garcia-Pichel and Castenholz, 1991). Periodic desiccation or lack of fixed nitrogen can also enhance the level of scytonemin attained during UV-A induction in some strains (Fleming and Castenholz, 2007; 2008).

**Biosynthesis**

Preliminary studies based on structural, genetic, radiotracer techniques and evidences from the pre-genomics era revealed that the biosynthesis of scytonemin is related to that of the aromatic amino acids (Mandalka, 1999). Proposition of biosynthetic pathway became possible after isolation of a scytonemin-less mutant strain of *Nostoc punctiforme* ATCC 29133 (PCC 73102) by transposon mutagenesis. The mutation was formally traced to open reading frame (ORF) NpR1273 (now known as *scyD*). Subsequently, it was demonstrated that *scyD* was the part of a gene cluster of unknown function, involving 18 contiguous ORFs (NpR1276 to NpR1259, including *scyA-scyF*) (Fig. 1). Possible involvement of aromatic amino biosynthetic pathway is also evident from gene cluster denoted in Fig. 1 where the homologues of genes in the shikimate pathway and aromatic amino acid pathway are located downstream of the scytonemin biosynthetic genes (*scyA-scyF*). It is presumed that the synthesis of scytonemin starts in the cytoplasm from supply of tryptophan and p-hydroxyphenylpyruvate. During the first step of biosynthesis ScyB, a homologue of amino acid dehydrogenase, (an NADH-dependent oxidoreductase) catalyses the oxidative deamination of Trp (tryptophan) to provide indole-3-pyruvic acid (I3P). The second required precursor p-hydroxyphenylpyruvic acid (HPP), is most probably made available through NpR1269, a putative prephenate dehydrogenase which catalyses oxidation of prephenate to form HPP. It is also felt that as ScyA (NpR1276) exhibits strong amino acid sequence similarity to a thiamine pyrophosphate (TPP)-requiring enzyme known as acetalactate synthase, most probably it is involved in the formation of α-acetalactate from two molecules of pyruvate (Chipman et al., 1998). In the next step of reaction, ScyC (NpR1274) mediates the transformation of α-acetalactate (β-keto acid adduct) to the monomer of scytonemin skeleton involving a series of reaction such as decarboxylation, cyclization and oxidation (Balskus and Walsh, 2009). Finally, the enzymes encoded by *scyD, scyE* and *scyF* transform the resulting skeleton to the final product in the form of scytonemin.

After the elucidation of hypothetical pathway, certain important findings have emerged pertaining to scytonemin biosynthesis. Studies based on comparative genomics and direct sequencing of several strains have revealed that the scytonemin cluster of *Nostoc* ATCC 29133 is well conserved among cyanobacteria (Sorrels et al., 2009; Soule et al., 2009). Additionally, five additional genes were found located in the cluster of several strains (Soule et al., 2009), homologues of which are also conserved in *Nostoc* ATCC 29133. Much work is still needed to decipher the exact biosynthetic pathway and role of individual gene in scytonemin synthesis. Equally important is the localization of this compound, how does it accumulate in sheath of many cyanobacteria.
B. Mycosporine-like amino acids: a diverse family of sunscreens

Mycosporine-like amino acids (MAAs) constitute a diverse family of water soluble, colorless natural products that share a 5-hydroxy-5-hydroxymethyl-cyclohex-1, 2-ene ring and have a methoxy-substituent in C2 (Table 1). They are always substituted in C3 with an amino compound and in C1 with either an oxo or an imino moiety. Sometimes the term ‘mycosporine’ refers to those with a ketone at C1 (also known as oxo-mycosporines or mono-substituted mycosporines), whereas MAA is reserved for those with an imino-group (such as imino-mycosporines and bi-substituted mycosporines) (Gao and Garcia-Pichel, 2011). MAAs can sometimes be glycosylated or covalently bound to oligosaccharides. They present unique absorption spectra with a single, narrow and strong absorption band that has a maximum absorbance between 309 and 362 nm.

Fig. 1. Biosynthetic pathway for scytonemin.
Table 1. Absorption maxima and molecular structure of MAAs

<table>
<thead>
<tr>
<th>Name of MAAs</th>
<th>Absorption maxima (nm)</th>
<th>Molecular Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA-glycine</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>Mycosporine-taurine</td>
<td>309</td>
<td></td>
</tr>
<tr>
<td>Mycosporine-2 glycine</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>Mycosporine-alanine</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>Mycosporine-glutamine</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>Mycosporine–serinol</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Value</td>
<td></td>
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<tr>
<td>------------------------</td>
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<tr>
<td>Palythene</td>
<td>360</td>
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</tr>
<tr>
<td>Palythine</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Palythine-threonine</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Palythenic acid</td>
<td>337</td>
<td></td>
</tr>
<tr>
<td>Palythinol</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>Porphyra-334</td>
<td>334</td>
<td></td>
</tr>
</tbody>
</table>
MAAs dissipates energy as heat without generating ROS (Conde et al., 2004). Most MAAs are stable, but mycosporine-Gly and mycosporine-Gln can undergo photosensitized hydrolysis (Bernillon et al., 1990). MAAs are most typically accumulated as solutes in the cytoplasm, but derivatives covalently bound to oligosaccharides can be excreted into the ‘sheath’ (glycocalyx), as in Nostoc commune (Böhm et al., 1995). Mycosporine content often reaches several per cent of the cellular dry mass of the producing organism (Favre-Bonvin et al., 1987). Various aspects of MAA biology have recently been comprehensively reviewed (Shick and Dunlap, 2002; Carreto and Carignan, 2011; Gao and Garcia-Pichel, 2011). Recently a database has been developed on the distribution of mycosporine and MAAs in marine and freshwater organisms (Sinha et al., 2007).

**Phylogenetic and ecological distribution**

For the first time mycosporines were reported in the mycelia of fungi basidiomycetes that had been induced to sporulate by exposure to UV-B radiation (Favre-Bonvin et al., 1987). Since then, mycosporine-like natural products from red algae, sea-stars, corals, phytoplankton, dinoflagellates, lichens, sponges and cyanobacteria have been reported and now there is a vast array of microorganisms that have mycosporine-like secondary metabolites (Shick and Dunlap, 2002). However, no reports are available of the presence of MAA in bacteria, plants and archaea. Animals do not synthesize MAAs directly as they lack shikimate pathway but they receive them from their diet or from symbiotic microorganisms. Bi-substituted mycosporines are prevalent in algae and cyanobacteria, but they are absent from fungi, in which a considerable diversity of mono-substituted mycosporines exists (Garcia-Pichel, 1998).

**Ecology and physiology of MAAs**

Screening can be enhanced substantially in N. commune (Ehling-Schulz and Scherer, 1999), in which the MAAs are laid down extracellularly, and in most other species owing to growth as a biofilm or colony. In cyanobacteria, MAA content correlates with a moderate
physiological amelioration of photodamage, which remains even under conditions of physiological inactivity, as would be expected for a sunscreen (Garcia-Pichel and Castenholz 1993). In most MAA-synthesizing organisms, the accumulation of MAA is induced or enhanced by exposure to intense solar radiation, although the exact action spectrum for the response varies (Shick and Dunlap, 2002). The role of MAAs as sunscreens has been elegantly shown in invertebrate eggs (Adams and Shick, 1996) and dinoflagellates (Neale et al., 1998). Mycosporines synthesized by primary producers in nature can be passed along the food chain: after being taken up by a range of grazers (from copepod crustaceans to fish; Sommaruga and Garcia-Pichel, 1999), they are selectively preserved and then accumulate in UV-light-sensitive tissues (such as the epidermis of holothurians or the eye lenses of fish). MAAs produced by symbiotic protists (zoanthellae) are the source of the large mycosporine pools found in scleractinian corals.

Alternative roles of MAAs

Mycosporine-glycine and porphyra-334 are efficient antioxidant. Tao et al. (2009) reported that porphyra-334 efficiently suppressed the lipid peroxidation induced by singlet oxygen although the antioxidative effect observed was relatively moderate at the initial stage of oxidation and can scavenge ROS produced during UV exposure (or otherwise), but other MAAs, such as shinorine, does not share this antioxidant property (Dunlap and Yamamoto, 1995). Because in some strains MAA biosynthesis can be induced by osmotic shock, and because certain halophilic cyanobacteria and fungi accumulate unusually large concentrations of MAA, a role as compatible solutes has been proposed for these compounds (Oren, 1997; Kogej et al., 2006). However, quantitative assessments reveal that the contribution of MAA to the overall intracellular osmotic pressure is negligible when compared to that of co-occurring, bona fide compatible solutes accumulated by the same cells (Portwich and Garcia-Pichel, 1999). In fungi, some mycosporines seem to be effective in eliciting morphogenetic changes: conidia germination at high conidial density is inhibited by the presence of extracellular mycosporine-Ala (Leite and Nicholson, 1993). Some study shows that MAAs act as nitrogen reservoir but experimental proofs are needed in this context. It is found that MAAs effectively block thymine dimer formation by UVR in vitro, however, in vivo experiments are needed to prove the role of MAAs in protecting DNA of organisms (Rastogi et al., 2010). MAAs also have a role in sunscreen for skin protection as these protects skin from premature skin-aging (Schmid et al., 2003).

Biosynthesis

Preliminary genomic studies in fungi and cyanobacteria (Favre-Bonvin et al., 1987; Portwich and Garcia-Pichel, 2003) suggested that the biosynthesis of MAAs originates from the first part of the shikimate pathway, possibly at the level of 3-dehydroquinate synthase (DHQ synthase, encoded by aroB) (Portwich and Garcia-Pichel, 2003) (Fig. 2). Tracer experiments in Chlorogloeopsis sp. revealed that the amino acid condensed directly on the cyclohexenone ring and resulted in the origin of the variable substituents. Most probably mycosporine-gly was the first MAA synthesized and acted as a precursor for other bi-substituted imino-mycosporines, such as shinorine (Portwich and Garcia-Pichel, 2003). Recently, comparative genomics of cyanobacteria has helped to identify a region of potential interest as the locus for MAA biosynthesis (Singh et al., 2010a). This was based on the presence of a DHQ synthase homologue (ORF Ava_3858) flanked by a putative O-methyltransferase (O-MT), which might potentially lead to the cyclohexenone core. The gene products of N. punctiforme also formed 4-deoxyxagudosul (4-DG) when expressed in tandem in vitro if supplied with sedohetulose -6-phosphate (SHP) as substrate, but not when supplied with 3-DHQ. Details of biosynthetic pathway of MAA and tentative organization of genes involved in its synthesis are well described in the review of Gao and Garcia-Pichel (2011). However, much work is needed to decipher the biosynthetic pathway especially the role of various genes in synthesis of MAA in different species of cyanobacteria (Balskus and Walsh, 2010).

Regulation

MAA synthesis in cyanobacteria and fungi is typically induced or enhanced by exposure to UV-B radiation (Portwich and Garcia-Pichel, 1999; Rozema et al., 2002; Sinha et al., 2003; Portwich and Garcia-Pichel, 2000). The process of UV photoreception has been studied in Chlorogloeopsis sp. (Portwich and Garcia-Pichel, 2000), in which the UV-dependency of synthesis could be traced to a true photosensory process mediated by a UV-B photoreceptor. The action spectrum for MAA induction and the sensitivity of the photosensory process to specific biosynthesis inhibitors and antagonists suggest that an asset-unidentified pterin acts as the chromophore in the photoreceptor. Nothing is known about signal transduction or how this links to gene expression. Most probably a range of sensory inputs is likely to contribute to MAA regulation in an organism-specific manner. For example, in A. variabilis, as in algae, visible light can induce MAA synthesis (Singh et al., 2008), and osmotic stress differentially regulates mono- and bi-substituted MAAs in Chlorogloeopsis spp. (Portwich and Garcia-Pichel, 1999). Nitrogen and sulphur availability may also influence the allocation of resources for synthesizing MAAs (Singh et al., 2010b).
Fig. 2. Putative biosynthetic pathways for mycosporine-like amino acids.

**Future prospects**

Many key aspects of the scytonemin and MAAs biology are yet to revealed. The predicted localization for the route of biosynthesis of scytonemin remains the most exciting open question because of its uniqueness in cyanobacterial bacterial biology. A combination of high-resolution bioimaging techniques and molecular tagging of the enzymes involved will be needed to test it. Exact role of a large number of genes that are associated with the scytonemin operon is still unknown, there is a need resolve this issue. Lastly, the molecular mechanism governing the regulation of scytonemin synthesis is still a black box and
one has to study this process from cyanobacteria growing in diverse habitat but favorable for bulk synthesis of scytonemin.

Considering the large number of MAA variations, perhaps one of the most daunting future tasks will be to establish the molecular basis for this diversity. Equally important is the aspects of MAAs regulation, how it relates to environmental sensory processes, signal transduction, gene expression and post-transcriptional regulation clearly represent the next frontier in MAAs research. During the last two decades a vast array of data related to UV-B effects on cyanobacteria have emerged but there is a need to expedite research in this emerging area with renewed interest involving researchers from diverse field of modern biology. Studies conducted may surely lead to the discovery of many exciting facts which are otherwise hidden till date.

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REFERENCES


Mandalka, A. Studies on Scytonemin Synthesis in Cyanobacteria Univ. of Bremen, Germany, 1999


