



ROLE OF PHOTOSYNTHETIC PIGMENTS IN PROTECTION AGAINST OXIDATIVE DAMAGE

Praveena, B. and Murthy, S.D.S.*

Department of Biochemistry, Sri Venkateswara University, Tirupathi-517502.India.

Email: sdsmurthy@rediffmail.com

ABSTRACT: The process of photosynthesis always begins with the absorption of light energy. This is done by certain pigments called photosynthetic pigments in plants and cyanobacteria. These pigments are of various types like chlorophyll, phycobilins, carotenoids and certain sunscreen pigments like mycosporine like amino acids (MAAs) and scytonemin. These pigments are mainly involved in the absorption of light energy and also they have certain defence mechanisms like antioxidant property which is helpful in protection of plant or cell against the environmental stress. In this review, the role of photosynthetic pigments and their protection against oxidative damage by antioxidant activity is discussed.

Keywords: Antioxidant property, photosynthetic pigment, sunscreen pigments.

INTRODUCTION

Photosynthesis is a process which is used by plants and other organisms to convert light energy into chemical energy. Photosynthesis is performed differently by different species but the process always begins with absorbing energy from light by proteins called reaction centres which contain pigments. These pigments are of various types in plants and cyanobacteria, they are chlorophyll, phycobilins, carotenoids and certain sunscreen pigments like mycosporine like amino acids (MAAs) and scytonemin. These pigments play a vital role in the protection of plants or organisms from oxidative damage.

Photosynthetic pigments

Chlorophylls

Chlorophyll is a green colour pigment present in plants and cyanobacteria. It is a photosynthetic pigment present in photosystems function as light-harvesting antenna. Chlorophylls are classified into seven isoforms *a* through *f* depending on the difference in ring substituents. Chlorophyll *e* is not used as a technical term because it is considered as artificial product. The most common isoform found in cyanobacteria is chlorophyll *a* but in recent years it has been found that *Acaryochloris marina*, a cyanobacterium contain isoforms *d* and *f* [1]. Chlorophylls have a specific absorption spectrum with red and blue absorption bands in visible light region which is due to the wide π -conjugated plane[2]. For example chlorophyll *a* has two absorption bands at 660nm and 430nm in diethyl ether. The blue absorption band is called Soret band. These bands further split into two more bands because of their asymmetrically substituted side groups.

Phycobilins

Phycobilins are the unique light harvesting complex present in Cyanobacteria which is associated with photosystem II to serve as light energy transfer. Phycobilins are classified on the basis of their colour into two large groups: the phycoerythrobilin (red) and the phycocyanobilin (blue) and a minor group phycourobilin. These phycobilins absorb their specific wavelength of light between 650 nm and 470 nm which are shorter wavelength regions than red absorption band (Q-band) around 660 nm of chlorophylls. It consists mainly of two kinds of phycobiliproteins: phycocyanin as a rod and allophycocyanin as a core and in some bacteria it consists of phycoerythrin as an additional rod protein. Phycoerythrin is sensitive to pH, salt concentration, temperature change, desiccation and light stress [3]. The size and protein composition of this complex varies in response to light and nutrition available. There are certain differences in spectral properties of phycobiliproteins and these differences are because of the different types of apoproteins attached with chromophore rather than the structural differences in proteins of phycobiliproteins.

Antioxidant activity of chlorophylls and phycobilins

As the antioxidant properties of the chlorophylls have been elucidated, *invitro* studies show that various chlorophyll derivatives react with the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), one of the stable organic radicals. Earlier studies of experiments discovered that using DPPH and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals whose radical scavenging activity by chlorophyll *a* is much stronger than that of chlorophyll *b* [4]. Chlorophylls are widely known as sensitizers for the protection of singlet O₂ and it is still difficult to say that chlorophylls act as antioxidants *in vivo*. Chlorophylls which are metal free, i.e. derivatives which lose metal ion center, such as chlorins, pheophytin and pyropheophytins have significantly lower radical scavenging activity when compared to that of metal ion containing chlorophylls like Mg-Chlorophyll, Zn-pheophytins, Zn-pyropheophytins, Cu-pheophytins and Cu-chlorophyllins.

Phycobilins do not have a central metal ion as chlorophyll rather they have a tetrapyrrole structure. This structure is similar to that of biliverdin and bilirubin which are formed from heme. As these have strong antioxidant properties phycobilins are also expected to have similar antioxidant properties as the structure is the same as theirs. Phycocyanin exhibits radical scavenging activity in dark environments though it generates hydroxyl radicals in light environments. The denaturation of phycocyanin proteins by urea, surfactants and alkaline can completely diminish hydroxyl radical production while the radical scavenging activity still remains [5], so it is possible that phycobilins function as antioxidants.

Carotenoids and xanthophylls

All cyanobacteria contain yellow and orange colour pigments called carotenoids and xanthophylls respectively which act as accessory pigments in photosynthesis. Carotenoids are organic pigments that are naturally occurring in chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria. There are over 600 known carotenoids; they split into two classes xanthophylls and carotenes. They absorb blue light. Carotenoids serve two key roles in plants and algae: they absorb light energy for use in photosynthesis and they protect chlorophyll from photo damage. The common structure of carotenoids is a long π -conjugated polyene structure with several methyl substituents. These carotenoids have three absorption bands in the region between 400-500 nm. They are localized and function in biomembranes as they are usually strongly hydrophobic which is due to the presence of a long unsaturated alkene chain. The major carotenoids in cyanobacteria are β -carotene, zeaxanthin and nostoxanthin as hydroxyl derivatives and echinenone and canthaxanthin as ketoderivatives. Orange carotenoid protein (OCP) a water soluble 35 kDa protein was identified in three genera cyanobacteria. This OCP crystal structure analysis in *Anthrospira maxima* revealed that carotenoid, 3'-hydroxyechinenone binds to the protein non-covalently between N and C terminal domains. The light absorption by OCP induced structural changes in the carotenoid and protein which results in conversion of a stable orange form into red which is unstable and active form. The red form accumulated under the condition in which photo-protection is required [6, 7].

Antioxidant activity of carotenoids

Carotenoids' main function is thought to protect photosynthetic machinery from oxidative damage by acting as sunscreen pigments and antioxidants and these are not directly involved in photochemical reactions. The amounts of carotenoids increased slightly by exposure to UV-A in aquatic cyanobacterium *Oscillatoria* species [8] and the amounts of echinenone and myxoxanthophyll increase on exposure to UV-B light in terrestrial cyanobacterium *Nostoc commune* [9]. These results help in suggesting that both carotenoids and their glycosides function as photoprotectants. Antioxidant mechanisms of carotenoids are divided into four categories they are sun screening, singlet oxygen quenching, releasing excessive light energy through xanthophyll cycle and radical scavenging. Carotenoids can also inhibit photosensitized oxidation, so that they can act as an efficient quencher of singlet oxygen. The principal quenching mechanism is electron exchange energy transfer so called Dexter-type energy transfer. The electron exchange between singlet oxygen and carotenoids (which are in singlet state) forms oxygen which is in triplet state and excited carotenoids of triplet state. These formed excited carotenoids release the energy as heat by skeletal vibration.

Sunscreen pigments

Cyanobacteria have two types of sunscreen pigments scytonemin and mycosporine like amino acids (MAAs). These are also called as secondary metabolites. These secondary metabolites are thought to play multiple roles against several environmental stresses such as UV-radiation and desiccation.

Mycosporine like amino acids

MAAs are ultraviolet absorbing molecules having absorption maxima between 320-360 nm. These are one of the pigment molecules produced in cyanobacteria and algae. It is believed that these MAAs are derived from parasitic microorganisms or microorganisms taken in through ingestion.

MAAs are small molecules which are <400 Da, these are colourless water-soluble compounds composed of cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acids or its imino alcohol. Some MAAs also contain sulphate esters or glycosidic linkages through the imine substituent's. MAAs are highly hydrophilic in nature this hydrophilic nature is due to their zwitter ionic form when compared to other UV absorbing antioxidants present in cyanobacteria like chlorophylls, carotenoids and scytonemin. The hydrophilic nature of MAAs is further increases due to modification by sulfonic acids [10] and sugar molecules [11].

Sunscreen properties of MAAs

The function that mycosporines play as nature's sunscreen compounds has been the subject of a number of review articles. However in recent years evidences are accumulating that mycosporines play additional roles, for example they serve as antioxidant molecules scavenging toxic oxygen radicals, they also function as compatible solutes to protect the cells against salt stress. They are also involved in protection against desiccation or thermal stress in certain organisms and they may serve as an intercellular nitrogen reservoir. By all these functions mycosporines are mentioned as multi response secondary metabolites. The finding that UV light is generally the strongest inducer for the biosynthesis of MAAs agrees well with their function as sunscreen components MAAs synthesis is promoted with UV light. MAAs synthesis in cyanobacteria enhanced by photosynthetically active radiation (PAR), UV-A(315-400 nm) UV-B (280-315 nm) light radiation. UV-B light is the highest of three light regimes in enhancement of MAAs. UV-B light can also enhance the ROS production because of their higher energy when compared to UV-A. The accumulated ROS can function as signal transfer molecules which effect antioxidant genes to turn on signal transduction for the biosynthesis of various antioxidants and antioxidant enzymes. Therefore MAAs biosynthesis can be activated in an indirect manner via ROS accumulation which is a primary event caused by UV-B radiation. MAAs functions as UV-A absorbing agent and can be release light energy as heat without ROS production [12, 13, 14]. This is because the ranges of absorption bands of MAAs overlap well with the UV-A range and their molar extinction coefficients are very high.

Radical Scavenging activity of MAAs

It is well known that MAAs react with several radical species, such as 2,2 azobis(2-amidino propane) dihydro chloride (AAPH) radical and the ABTS radical which is a carbon centered and nitrogen centered radical respectively. These scavenging activities mainly depend on the skeletal structure of the MAAs. The radical scavenging activity of MAAs change in pH dependent manner and is specially prominent in aminocyclohexene imine structures [15]. The radical scavenging activity of carbonyl- type MAAs is much greater than that of imine-type MAAs. Imine-type MAAs decreases reactivity against radical species at a low PH because they cannot be widely delocalised compared with carbonyl type MAAs. The radical electron is delocalised by resonance over the double bonds in cyclohexene groups, this delocalisation sterilises the radical species formed. Thus, because of the basicity imine-type of MAAs present as protonated form at low pH. Therefore, MAAs containing amino cyclohexenone structure plays an important role in the antioxidant mechanism of MAAs. Biosynthesis of MAAs is triggered by oxidative stress caused due to the exposure of light and heat suggesting antioxidant function of MAAs. Most of the MAAs discovered until now have an aminocyclohexene imine structure, i.e., imine-type MAA, during stress MAAs with aminocyclohexene in a structure may be rapidly converted to MAAs with an aminocyclohexenone structure under oxidative stress to enhance the antioxidant capacity and this type of mechanism has not been identified yet.

Scytonemin

Scytonemin is a biological pigment synthesised by many strains of cyanobacteria. It is believed to be a bacterial sunscreen pigment which has the absorption from 325-425 nm and a separate maxima at 250 nm. The biosynthesis of scytonemin is triggered by UV light. It is hydrophobic pigment whereas MAAs are hydrophilic pigments. The structure of this pigment is found by the dimerization of two polycyclic chromophores made by the condensation of cyclopentanone and indole rings[16]. These two chromophores are connected by a single bond in an oxidised state. Therefore these can be rotate freely and this can prevent steric repulsions. In living Cyanobacteria, scytonemin exists in an oxidised state, because of this, their presence is easily recognised by their brown colour which can be observed in a bright field optical microscope. Almost no scytonemin derivatives have been discovered unlike MAAs, except for methoxy substituted scytonemin derivatives. These are isolated from *Scytonema* sp. and their structures were determined by NMR and MS spectrometers [17].

Sunscreen properties of scytonemin

Scytonemin biosynthesis is activated during the exposure of light to the cyanobacteria to UVA/B (especially UV-A)[18,19]. By this it is expected that Scytonemin functions as a photo protective compound. It is also known that repeated desiccation activities in some anhydrobiotic cyanobacteria activates the synthesis of scytonemin under UV-A exposure [20,21]. It is clearly reported that molar extinction co-efficient of Scytonemin is large (250 l/g•cm) at wavelength 384 nm [22].

Therefore Scytonemin is an efficient photoprotective compound due to its large extinction co-efficient. The wide absorption range of Scytonemin also helps for photoprotection. This is because it can cover not only the UV-B range, but also UV-A range. Scytonemin can protect the cells by preventing 90% of UV-A light penetrating into cell [23].

Radical scavenging activity of Scytonemin

Scytonemin has strong radical scavenging activity similar to that of MAAs species. Matsui *et.al.*, (2012) studied the radical scavenging activity of scytonemin using electron spin resonance spectroscopy[24]. These findings indicate that scytonemin is a multifunctional molecule that can absorb UV light and prevent generation of ROS as well as scavenging ROS.

Future Prospective

The main photosynthetic pigments in cyanobacteria and their antioxidant properties are outlined in this review. These pigments inhibit ROS and prevent the excess light energy penetrating into the cells by absorbing UV light. However these pigments also suppress the cell damage directly by scavenging ROS, which is produced under environmental stress conditions such as UV-radiation. The antioxidant properties of these pigments is the main defence mechanism occur against the stress condition. Further studies are required to know the molecular mechanisms of pigments in minimising oxidative damage.

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