DIVERSITY, PREVALENCE AND ROLE OF SUPEROXIDE DISMUTASE IN CYANOBACTERIA

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ABSTRACT

Superoxide dismutase (SOD), the antioxidant enzyme exists in four diverse forms in cyanobacteria i.e. FeSOD, MnSOD, Cu/ZnSOD and NiSOD. FeSOD has an ancestral origin from the GSB ancestor of PSI while MnSOD has protobacterial ancestor to PSII & mitochondria. (Cu/ZnSOD shows lateral gene transfer while NiSOD is rarely found. Fe & Mn forms share similarity while Cu/Zn form does not. SOD is prevalent in all subcellular locations where O²⁻ (superoxide) radicals are formed. SOD plays a defensive role at the time of environmental stress like chilling, dessication, light stress, metatoxicity etc and its activity is usually seen higher at the time of stress. Inactivation of SOD gene results in oxidative damage to the cell.

Key words: SOD, Antioxidant, Isoforms, Localization, Environmental stress.

1. INTRODUCTION

Superoxide dismutases (SOD, E.C. 1.15.1.1) are ubiquitous metalloenzymes with molecular weight 17 to 85 kDa that catalyze the disproportionation reaction of conversion of superoxide radicals to peroxide and molecular oxygen as shown in fig. 1. The metal ions are alternatively oxidized and reduced. There are four different metalloforms of SOD found in cyanobacteria but these are not equally distributed. The forms are FeSOD, MnSOD, CuZnSOD and NiSOD. A cambialistic form that uses Fe/Mn in its active site also exists.

Though oxygenic Photosynthetic Electron Transport (PET) and aerobic respiration have improved the efficiency of Carbon metabolism but the benefits are partially offset by their tendency to form reactive oxygen species (ROS). ROS can react with lipids, proteins, nucleic acids etc to cause irreversible damage to a cell. This necessitated the co-evolution of antioxidant systems with PET and aerobic respiration.

Cyanobacteria are the oldest oxygen evolving photosynthetic prokaryotes and possess plethora of bioactive compounds (Sudha et al, 2011). The name “cyanobacteria” comes from the Greek word kyanos, which means blue- green. These occupy a crucial position between prokaryotes and eukaryotes. They are considered as responsible for the conversion of primitive anaerobic reducing environment into the modern oxidizing one (Lukow et al, 2000). They have adapted to survive in almost every ecological niche including the extreme ones (Schopf, 2000). The protective role of cyanobacterial SOD was first studied in Anacystis nidulans (Herbert et al., 1992). Though the photosynthetic apparatus of cyanobacteria is similar to that of algae and plants but the antioxidant system is simpler as compared to plants. Also, it is easier to genetically manipulate it in respect to plants, like genes for antioxidant enzymes can be easily inactivated by insertional mutagenesis (Weixing et al., 2007). SOD possesses high therapeutic value and it was first commissioned in 1985 as a drug in USA for defence of donor organs against oxidative stress during periods of ischemia and reperfusion (Vellard, 2003).

2. THE DIVERSE FORMS OF SOD

Four different isoforms of SOD are found to be known till date in cyanobacteria. Among these Fe and Mn forms are the most prevalent.

Iron and Manganese SODs: According to Fink and Scandalias (2002), Fe and Mn SODs share 50% similarity based on amino acid alignments and suggest that they might have evolved from gene duplication from a common ancestor. The similarity in structure between the two forms is due to similar electrical properties of Fe & Mn. Both are homodimers or tetramers containing one metal atom per 200 to 220 amino acid residue subunit with molecular weight ranging from 14 to 30 kDa. Some Archea also possess cambialistic form of SOD. Inspite of high degree of similarity between these two forms, two specific amino acid residues i.e. 77 (glutamine in FeSOD and glycine in MnSOD) & 146 (alanine in FeSOD and glutamine or histidine in MnSOD) differentiate them (Weatherburn, 2001). However, it is possible to substitute Fe for Mn in the active site of MnSOD or vice versa but little or no catalytic activity is retained. An important difference between these two forms is their intracellular location. The FeSOD is found in the chloroplasts and cytoplasm (Fink and Scandalias, 2002) while MnSOD is mostly localized in mitochondria (Okamoto et al., 2001). An N terminal, hydrophobic, transmembrane helix tail on the MnSOD determines its location.
localization in cyanobacteria (Atzenhofer et al., 2002; Regelsberger et al., 2002).

**Copper-zinc SODs:** CuZnSOD exhibit structural as well as evolutionary dissimilarity from Fe and MnSOD because of different electrical properties of Cu/Zn from Fe & Mn. It is a homodimer with molecular weight between 31 to 33 kDa for each subunit and amino acid residues of length 150 to 160 per subunit (Fridovich, 1998). The enzyme is having two active sites one containing Cu and another Zn for each subunit. It is very stable as it can withstand multiple freeze-thaw cycles and prolonged refrigeration once purified. This may be attributed to high glycine content (13 to 17%) contributing to extensive β-pleated sheet conformation (Chen et al., 2001).

**Nickel SODs:** it is completely different from the other three forms of SOD. It was first discovered, cloned and characterized in the bacterial genus, *Streptomyces* (Wuerges et al., 2004). A survey of available genomes suggests that this form is also available in cyanobacteria as well (Palenik et al., 2003).

The reason behind the metal substitution during its evolutionary history may be related to the availability of soluble transition metal compounds in the biosphere with respect to the oxygen content of the atmosphere in different geographical eras (Bannister, 1991).

### 2.1 EVOLUTIONARY ASPECT AND LOCALIZATION OF SOD

The tree of life shows distribution of SOD in archaeabacteria, bacteria and eukaryote as shown in fig. 2. The ancestral history reflects origin of FeSOD from the GSB ancestor of PSI while MnSOD from protobacterial ancestor to PSI and mitochondria. The Cu/ZnSOD is believed to be originated by lateral gene transfers. SOD enzymes are compartmentalized in a cell. This may be due to the reason that O₂⁻ radicals cannot cross the cytoplasmic membranes and are imperative to be removed (Takahashi and Asada, 1988). All SODs are encoded by the nucleus and appear to have evolved by lateral gene transfer of three distinct genes to the nucleus after the endosymbiotic acquisition of mitochondria and plastids. FeSOD is localized in the chloroplasts and cytoplasm while MnSOD is found in the mitochondria. NiSOD is found to be associated with plastids. Cu-ZnSOD is found in chloroplast, cytoplasm & periplasm as shown in fig. 3.

### 2.2 PREVALENCE OF SOD FORMS IN DIFFERENT ORDERS OF CYANOBACTERIA

Below given is the prevalence of different forms of SOD in different orders of cyanobacteria based on analysis of Priya et al., (2007). It is cleared that the most prevalent forms are Fe and Mn.

**Fe-SOD:** *Anabaena variabilis*, *Cyanothecae sp.*, *Gloeobacter violaceus*, *Lyngbya sp.*, *Nostoc sp.*, *Plectonema boryanum*, *Thermosynechococcus elongatus*, *Trichodesmium erythraeum*

**Mn-SOD:** *Anabaena variabilis*, *Crocospheera watsonii*, *Gloeobacter violaceus*, *Leptolyngbya valderiana*, *Nostoc sp.*

Researchers have demonstrated that SOD plays a protective role against oxidative damage in cyanobacteria during different types of environmental stress. O₂⁻ radicals are produced inevitably in respiratory electron transport (Yankovskaya et al., 2003) and also in the chloroplasts by the reduction of oxygen at the site of PSI (Asada, 1999). SOD is found to protect the proton donating system in nitrogen fixation against ROS in heterocytes forming cyanobacterium *Anabaena cylindrica* (Henry et al., 1978). Several studies have been done using mutant strains of cyanobacteria with altered levels of SOD activity. A photooxidation resistant mutant strain of *Plectonema boryanum* with increased levels of MnSOD activity was isolated by Steinitz et al., (1979). The protective role of SOD during different environmental stress conditions is described below.

**Chilling stress:** Chilling stress is propounded to decrease membrane fluidity which is a primary symptom (Nishida and Murata, 1996). Chilling in presence of light increases production of ROS by creating reducing atmosphere which is further worsen by production of H₂O₂ and O₂ which inhibits enzymes of Calvin cycle (Kaiser, 1979). The activity of the Calvin Cycle and soluble enzymes has been found to decrease with low temperature without causing any significant decrease in light harvesting and electron transfer system. Recent studies offer that chilling suppress nitrate uptake in *Synechococcus sp.* PCC7002 (Sakamoto and Bryant, 1999) which resulted in chlorosis. Role of SODs in safeguarding chilling ravage in cyanobacterium *Synechococcus sp.* PCC79421 was done by David et al., 1999. Kwang et al., (2005) reported differential expression of MnSOD in near isogenic lines of wheat during cold acclimation. Punia and co-workers (2011) reported the role of SOD in combating chilling stress in *Arthospira* isolates. SOD activity was 5 times higher in tolerant strain and 2.5 times higher in sensitive strain at the time of chilling stress as compared to optimum temperature on native gel.

**Desiccation:** The role of SOD has been well studied in *Nostoc commune* after many years of desiccation to find out its function in detoxifying ROS and reducing stress (Shirkey et al., 2000). The study unveiled that FeSOD was the third most abundant soluble protein in *Nostoc commune* even after storage in desiccated state for long period. The FeSOD purified from *Nostoc commune* was purposed to be a 21kDa polypeptide. N-terminus of sodF was sequenced which was encoded by 200 codons. Upon rehydration, FeSOD was released in the cells and extra cellular fluid and suggested that this process helps in reducing oxidative stress offered by multiple cycles of desiccation. The retained activity of SodF after years of desiccation in *Nostoc commune* suggested that the enzyme might have structural features which allow it to...
remain in the native state despite of the removal of water from the cells or it might be sequestered in cell microenvironment (Shirkey et al., 2000). Similarly, considerable activity of SOD found in cell free extracts of dessication tolerant cyanobacteria *Lynghya arboricola* (Tripathi & Srivastava, 2001). Marked tolerance against high solar radiation along with dessication stress is reported in the cyanobacterium, *Toiyphothrix byssosidea*, occurring on the exposed rock surfaces of temples and monuments in various regions of India (Adhakary et al., 2000). The FeSOD gene was amplified and sequenced which was a 292 bp polypeptide encoding for 96 amino acids. The resulting gene found to be 49.2% similar with FeSOD of *Anabaena* sp. PCC7120. Another sequence of 358 bp was also sequenced and was supposed to be similar to sodF encoding for soluble FeSOD protein found in *Nostoc commune*. Efforts for transferring gene of desiccation tolerance in cyanobacterium *Chroococcidiopsis* have been made Daniela et al., 2001. *Chroococcidiops* is dominating community found in deserts. Plasmids from *Nostoc sp*. PCC7524 were transferred to various isolates of *Chroococcidiopsis* via conjugation and electroporation methods and offered as a suitable experimental strain for genetic studies.

**Visible light stress:** Light plays an important limiting factor for growth of photosynthetic organisms. Quail (1994) propounded that signal transduction and expression of protein is controlled by light. The impact of high irradiance on algae is drawing special attention (Cullen and Lewis, 1995). ROS are produced in a heavy amount by photochemical processes involved in photosynthesis. High light intensity reduces the rate of photosynthesis by harming photosynthetic apparatus (Critchley, 1994). Studies of photosystem expose that FeSOD is related with the protection of PSI (Thomas et al., 1998). SOD found to be selectively protective to heterocysts which can fix nitrogen and posses only PSI (Canini et al., 1998). Oxygen is reduced using light by PSI in cyanobacteria (Slain, 1991). SODs play crucial role in defending against ROS and its detoxification (Touati, 1997) and provide best tolerance to oxidative stress (Bhattacharya et al., 2004). MnSOD which is membrane associated in cyanobacteria are found to be protective to the photosynthetic apparatus in *Anabaena* sp. PCC7120 when exposed to high light intensity (Weixiang et al., 2007). The study conferred that lipid peroxidation occur at a high rate in absence of MnSOD under high light intensity. MnSOD was found to be critical in preserving both PSI and PSII in test sample. Earlier researches purported that superoxide ions are produced by PSII when light intensity is high (Navari-Izzo et al., 1999). So it was hypothesized that the major role of SOD is in shielding of PSII against photoinhibition. An intense study on *Synechocystis* sp. PCC6803 to understand light dependent expression of SOD was done by Jae and Kyong (2005). Results revealed that FeSOD was more active in cultures maintained in continuous light. Respiratory reaction largely contributes in activation of FeSOD as electron transfer system is a major site of \( \text{O}_2 \) generation (Imlay and Fridovich, 1991). *Microcystis aeruginos* is which is a bloom producing cyanobacteria when exposed to heavy irradiation for longer time, accounted for decrease in rate of photosynthesis (Abeliovich and Shilo, 1972). FeSOD is inactivated if *Microcystis aeruginos* is exposed to light stress for longer duration due to the production of excess of \( \text{H}_2\text{O}_2 \). There are records of about eightfold increase in amount of FeSOD when *Nodularia* collected at the depth of 10 m from Baltic sea was exposed to high irradiation (Canini et al., 1998).

**Ultraviolet radiation stress:** Ultraviolet radiation can cause oxidative damage depending upon its wavelength. UV-B (280-320 nm) can cause photosynthetic damage (Sinha and Hader, 2002). Another study stated that inactivation of cyanobacterial nitrogenase after exposure to UV-B (Kumar et al., 2003). UV-A also exhibits ravaging effect on the cell. UV-A stimulates genes in bold in negative phototactic responses in cyanobacteria when exposed for long time and high intensity. Photosensors such as cryptochrome Cry-DASH are involved in UV-A dependent signaling at low fluence rate. Ur5-UrrK and PCD are the important signalling component which have important role under oxidative stress condition in cyanobacteria. Study of SODs confirms its crucial role in maintaining UV-B level in algae (Rijstenbil, 2002) but there is no significant increase in SODs level when exposed to UV-A. Various nonenzymatic systems and SODs are suggested to have different target sites for UV-A and UV-B sabotage (Rijstenbil, 2003).

**Nutrient stress:** The rate of metabolism starts declining under nutrient stress condition which is a result of impairment caused to the cellular scaffolding. Due to lack of nutrients, catabolism of cellular protein starts in order to maintain photo synthetic activities (Falkowski and Raven, 1997). These conditions of respiratory degradation leads to the production of ROS. Punia and co-workers (2011) reported decreased activity of SOD in Pi starved strains of *Arthrosper*ia. Studies on role of SODs under nutrient limitation in cyanobacteria are getting more attention which will hopefully open up new perspectives in future.

**Metaltoxicity:** Presence of excess metal ions induces Fenton reactions. Cyanobacteria has been studied intensively and found to produce metal chelators and antioxidant buffers in order to absorb trace metals and different metals are found to be important for regulation of various metabolic processes (Mc Kay et al., 2001). Micronutrients settle the output of metabolic processes based on micronutrients uptake (Sunda 2000) and prevent Fenton reaction by acting as an antioxidant buffer (Martinez et al., 2000). *Synechocystis aquatilis* manifest decline of growth rate when concentration of copper is increased (Shavyrina et al., 2001). *Asparagopsis armata* also suffered reduction in growth in presence of metal (Segot et al., 1983). Copper is found to produce hydroxyl radical whereas zinc and lead disturb metabolic machinery via freezing antioxidant pool which results to increase load of ROS (Briet, 2002). Cu is mostly studied for understanding various SODs responses under presence of excess metal (Okamoto et al., 2001). A study on effect of heavy metal in *Anabaena variabilis* was done by Padmapiya and Anand (2010) to determine their effect on SODs. Copper was found to inhibit growth of *A. variabilis* to maximum amount. Production of peroxide was found to be less in Fe and Mn varied cultures whereas highest in Cu and Zn amended cultures. Increase in SOD activity was observed when amendments in micronutrients were done. Increase in
copper concentration in A. dolioiulum lead to 63% increase in SOD activity which also resulted in increase in lipid peroxidation (Mallick and Rai, 1999). A mutant strain of Synechococcus sp. PCC7942 has been constructed lacking detectable FeSOD activity and its photosynthetic electron transport was found to be more sensitive in the presence of methyl viologen and elevated O2 concentrations (Herbert et al., 1992; Thomas et al., 1998) but is not sensitive to oxidative stress induced with Norflurazon (David et al., 1999). The sodB strain had increased MnSOD activity as demonstrated by Herbert et al. (1992). It might be possible that the increased MnSOD activity was to compensate for the FeSOD and thus resists the effects of NF. SOD plays a significant role in combating heavy metal stress in the cyanobacterium Spirulina platensis-SS (Meenakshi et al., 2006).

Other stress: Some filamentous cyanobacteria possesses specialized cells known as heterocysts which are specialized chambers with thick lining involved in protection of nitrogenase enzyme from the poisonous effect of oxygen and heterocysts are formed under nitrogen deprived conditions (Zhang et al., 2002; Kaneko et al., 2001). Where FeSOD level increases when cell from nitrogen abundant conditions are transferred to nitrogen deprived conditions but MnSOD doesn’t show any up regulation. Nitrate uptake at low temperature slowdowns because at low temperature all three enzymes involved in nitrate assimilation are protected by membrane lipid unsaturation which reduces rate of nutrient uptake. Hence low temperature and nutrient stress both are involved in condition called nitrogen starvation.

3. CONCLUSION

SOD has gone under different metallic substitutions during its evolutionary history. This was due to the availability of different metals in context to oxygen in the biosphere in different geographical eras. Four different metalloforms exist in cyanobacteria with Fe & Mn as the most prevalent. Owing to its presence in all subcellular locations where superoxide radicals are produced it offers an effective defense system to the cell. Its gene inactivation results in oxidative damage to the cell. Cyanobacteria photosystem resembles to that of plants and thus can be extensively studied for stress mechanisms. Mass cultivation of cyanobacteria for SOD extraction can be promoted for use in therapeutics and clinical research.

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**Figure 1**: The Ascorbate-Glutathione cycle representing SOD as first line of defense to act (Source; Noctor and Foyer, 1998).

**Figure 2**: Synthetic distribution based on known evidence from biochemical and genetic data of the various SODs over the tree of life (figure modified after Baldauf et al 2004). Fe and MnSOD both having contrasting evolutionary histories, The
FeSOD is prevalent in all the major clades while MnSOD more in bacteria and Eukaryota. In cyanobacteria, FeSOD may have ancestral origin from the GSB ancestor of PSI while MnSOD may have the protobacterial ancestor to PSII and mitochondria. The CuZnSOD shows multiple lateral gene transfers and organisms often possess multiple copies of it in their genomes which are significantly phylogenetically distant. The NiSOD genes are found in genomes of four cyanobacteria. Branches are colored according to known SODs: magenta, FeSOD; cyan, MnSOD; cyan/magenta, Fe/MnSOD cambialistic); green, CuZnSOD; and gray, unknown form of SOD (Felisa et al, 2006).

**Figure 3:** Subcellular localization of SODs. This conceptual scheme represent all the possible locations of SODs in a eukaryotic cell (Felisa et al, 2006).