Structural And Functional Diversity Of Microbial Globins

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ABSTRACT

Hemoglobin and myoglobin are heme proteins that can bind oxygen reversibly and were first reported in vertebrates. Later on, occurrence of hemoglobin was found in all forms of life ranging from prokaryotes to eukaryotes. Myxobacteria are Gram-negative, rod-shaped eubacteria and that belong to delta proteobacteria. They predominantly thrive in soil and have very large genome size with high G+C content in comparison to other eubacteria. It is evident that the globins have been characterized predominantly from bacteria involved in photosynthesis, nitrogen fixation andpathogenicity, where these are implicated in and the protection of photosynthetic machinery from the oxidative damage,protection of nitrogen fixing enzyme from nitrosative and oxidative stress and the virulence of the host, respectively.
Keywords: Myxobacteria, hemoglobin, myoglobin, phytoglobin, androglobin.

1. INTRODUCTION

Globins are heme containing proteins which comprise five to eight α-helices that are connected by short intervening loops and have a common characteristic of binding oxygen reversibly [1-4]. These are widely reported in all the three domains of life forms such as Archaebacteria, eubacteria and eukaryotes [1]. In addition to their well-known function of oxygenstorage and transport, they are also involved in nitric oxide detoxification, oxygensensing or act as oxidation-reduction sensors [2]. Globins containhighly conserved structural folds, which build the protein crevice to hold the heme group [5, 6]. The proximal heme binding site in the hemoglobins of different organisms is perpetually occupied by His-F8, which forms a co-ordinate covalent bond with the iron of heme.

2. GLOBIN DIVERSITY

Hemoglobinand myoglobin are the well characterized globins present in vertebrates, and also among the first proteins, the crystal structure of which was determined [7-9]. Myoglobinis found in the cytoplasm of metabolically active cells such as cardiac and skeletal muscle cells as a monomer [10, 11]. Myoglobin has 8 α-helices and they are designated as A to H from N- to C-terminus [6]. This type of globin fold is referred to as 3-on-3 α-helical sandwich. Hemoglobin is a heterotetramer of 2α and 2β polypeptide chains, each with an associated heme group. The heme binding pocket of the vertebrate globins is characterized by His-F8 at proximal heme site and PheCD1, HisE7 and ValE11 at the distal heme site [12]. Hemoglobin functions as an oxygen transporter from the lungs to the respiratory peripheral tissues and cells, and myoglobin is involved in storage and diffusion of oxygen molecule to the mitochondria of cardiac and skeletal muscles cells [13]. Myoglobin has also been proposed to have nitric oxide dioxygenase and nitrate reductase activity [14]. Several other hemoglobins have also been reported in vertebrates such as neuroglobin, cytoglobin, globin X, globin E, globin Y and chimeric androglobin [15-19].Heme based oxygen carriers,such as hemoglobin, myoglobin and erythrocruorin are widespread but are sporadic in non-vertebrates [20]. The non-vertebrate globins exist as monomers or as multi-subunit assemblies which are present either in the cytoplasm of specific tissues or freely dissolved in body fluids. The structural diversity of non-vertebrate globins also reflects in their functions. Besides their role in oxygen transport and storage, the non-vertebrates globins are implicated in various functions, such as sulphide binding and its transport in molluscs, nitric oxide regulation and metabolism in nematodes, maintenance of acid-base equilibrium, oxygen sensing and scavenging, oxidase or peroxidase like activities and regulation of buoyancy of aquatic backswimmer insects [21-23]. The non-vertebrate globins have been studied from Aschelminthes (*Ascaris*), Annelida (*Pheretimalumbricus*, *Arenicola marina*), Arthropoda (*Chironomus thummithummi*), Mollusca (*Artemia* sp.) and Echinodermata (*Caudinaarenicola*). *Ascarissuum*hemoglobin is the best characterized globin among the invertebrates.

2.1. Phytoglobins

Plants produce oxygen through photosynthesis and also consume it for cellular respiration to produce energy for survival. Plant globinsare known as phytoglobins and widely occur in primitive plants (bryophytes) to higher plants such as monocots and dicots. Leghemoglobin was the first plant globin reported from the root nodules of legume plants and it facilitates the transport of oxygento the symbiotic bacteria of the root nodules. Leghemoglobin is also acts as oxygen scavenger to protect the nitrogenase complex of the symbiotic bacteria [24]. Generally, phytoglobins are categorized into symbiotic, non-symbiotic and truncated phytoglobins. Symbiotic phytoglobins are localized in the root nodules of nitrogen fixing plants whereas non-symbiotic and truncated phytoglobins are localized in the diverse organs throughout the plants [25, 26]. On the basis of oxygen affinity and sequence similarity,non-symbiotic phytoglobins can be further divided in to type 1 and type 2phytoglobins[27]. Type 1 non-symbiotic phytoglobins have a high oxygen affinity and it has been proposed that these globins are involved in the scavenging of nitric oxide at low oxygenconditions [28]. Type 2 non-symbiotic phytoglobins have moderate affinity with oxygen and are related with the facilitated oxygentransport [29]. The truncated phytoglobinshave been identified to participate in nitric oxide metabolism [30].

2.2 Microbial globins

The truncated globins (2/2globins) are 20-40 amino acid residues shorter than the classical globins and they adopt a 2-on-2 α-helical sandwich fold. The first report of globin like proteins in microbes was given by Keilin in 1925, who demonstrated a distinctive redox-dependent absorbance band of a yeast cell suspension using his hand spectroscope [31]. Later on, biochemical studies on the yeast cell suspension demonstrated that the characteristic absorbance bands are those of yeast protein, which consists not only of a heme domain but also a flavin chromophore [32-35]. Further, Wakabayashi and co-workers (1986) discovered hemoglobin from a Gram-negative bacterium *Vitreoscilla*sp. C1 and changed the existing notion thatglobins are exclusive to the higher forms of life [36]. Thereafter, mining of the large number of microbial genomes revealed the ubiquitous occurrence of globins in almost all life forms.

3. CLASSIFICATION OFMICROBIAL GLOBINS AND THEIR ASSOCIATED FUNCTIONS

Prokaryotic globins have been categorized into three families: “M-family” which contains myoglobin like globin domain, “S-family” which contains sensor globins and “T-family” which consists truncated 2/2 globins. The M-family comprises of flavohemoglobin and single domain globin while the S-family comprises of Globin coupled sensor, Protoglobin and single domain sensor globins. Both M- and S-family globins display a canonical 3/3- α helical globin fold characteristic of the metazoan globins while T-familyglobins display a 2/2 α-helical fold and are further classified into HbN, HbO and HbP [37].

3.1 M-family globins

M-family consist of the single domain globins as well as the flavohemoglobins. *Vitreoscilla*hemoglobin is a single domain globin which adopts a 3/3 canonical globin fold and it is the best characterized globin among the prokaryotes. *Vitreoscilla*hemoglobin, in addition to the oxygen transport to terminal oxidases, is involved in detoxification of nitric oxide [38], delivery of oxygen to oxygenases [39], oxygen sensing and regulation of transcription factors [40]. Flavohemoglobinsare chimeric proteins, consisting of an N-terminal globin domain (3/3 myoglobin like) fused with a C-terminal Ferredoxin NADP+ reductase (FNR) domain and they are widely distributed within bacteria and eukaryotic microbes. Flavohemoglobin from *Escherichia coli* (Hmp) was the first protein to be identified from this subfamily and its physiological role has been investigated in detail [41]. The expression of *hmp*is up-regulated in response to nitrosative stress and Hmp shows nitric oxide dioxygenase activity [42, 43].

3.2 S-family globins

S-family comprises of globin coupled sensors (GCSs), protoglobins and single domain sensor globins. GCSs are heme based sensors, comprised of an N-terminal myoglobin like sensor domain and variable C-terminal transducer domain. GCSs are widely distributed in archaea and bacteria; however, they have not been reported from eukaryotes. Pgbs are single domain globins and together with GCSs form a single lineage of globin superfamily. Pgbs are characterized by the presence of pre A-helix known as Z-helix [44]. 2/2 globins are small heme proteins, generally 20-40 amino acid residues shorter than vertebrate globin and are widely distributed in bacteria, microbial eukaryotes and plants. 2/2 globins host the heme group in 2-over-2 helical sandwich built by four helices corresponding to the B, E, G and H helices.

3.3 T-family globins

T-family is further divided into three groups: I, II and III (HbN, HbO and HbP respectively). It has been found that 2/2 globins from different groups co-exist in the same organism indicating their functional divergence [44]. Generally, 2/2 globins are either monomeric or dimeric form and have medium to very high oxygen affinities. Three dimensional structures of 2/2 globins from *Paramecium caudatum, Chlamydomonas eugametos, Mycobacterium tuberculosis, Bacillus subtilis* etc. have been determined [45-48]. Based on their biophysical properties, the physiological role of these globins has been suggested in nitric oxide detoxification, oxygen delivery under hypoxic condition, long term ligand storage, oxygen sensing and as redox sensor [44, 49, 50]. 2/2 globin from *M. tuberculosis* is the most characterized globin among the T-familyglobins. 2/2HbN from *M. tuberculosis* has a very potent oxygen dependent nitric oxide dioxygenase activity and relieves the heterologous hosts from nitrosative stress [51]. 2/2HbO from *M. tuberculosis* has been implicated in oxygen transport and delivery, but strong oxygen affinity and low oxygen dissociation rate constant of this globin suggests its role as a redox sensor [52, 53].

Myxobacteria and their globins

Myxobacteria exhibit an unusual feature of coordinated movement on solid surfaces, known as swarming, and formation of multicellular fruiting bodies when nutrient exhausted. The secondary metabolites produced by these bacteria are of great pharmaceutical interest. *Myxococcusxanthus* is a mesophilic obligate aerobe, which usually grows in soil rich in organic matter. *M. xanthus*DK1622 is a model organism of myxobacteria to study the genetics and biochemistry of the unusual and unique characters. Myxobacteria have very large genome size up to 16 Mb with very high G+C content and have complex life cycle. In the genomes of myxobacteria, globins have been identified and some genomes (e.g. *Sorangiumcellulosum*) have up to 7 globins from different subfamilies [54]. However, till date, there is no report of the structure-function studies on the globins from myxobacteria. The presence of such a large number of diverse globins in myxobacteria indicates their diverse role in the complex life cycle of these bacteria.

4. CONCLUSION

There is a huge structural and functional diversity in the hemoglobin superfamily present in Microorganisms, plants and animals. Most of the functional studies of hemoglobins are based on their ligand binding characteristics which largely depend on the residues present in the heme binding pocket. Therefore, any structural change in the globin fold alters the ligand binding properties and their proposed function.

Conflict of interest

The author declares no conflict of interest.

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