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RESEARCH ARTICLE

AN *IN SILICO* APPROACH TO STUDY THE INTERACTION OF PHYCOBILISOMES AND CHLOROPHYLL IN *ANABAENA CYLINDRICA PCC7122*

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ABSTRACT

Cyanobacterial species are ubiquitous in nature and the pigments present are capable of trapping the energy from the sunlight for the process of photosynthesis. All cyanobacteria which photosynthesize contain a core green pigment chlorophyll *a* which are supported by the other ancillary pigments like the Phycobilisome/phycobilins. The combination of phycobilins and chlorophyll produces the characteristic blue green colour from which these organisms derive their name. Chlorophyll which portrays a vital function of absorbing light energy for photosynthesis. It is a porphyrin containing magnesium and exists in several forms which have different side chains. Phycocyanin and allophycocyanin are light harvesting accessory pigments to chlorophyll. To understand the role of these pigments homology modelling of phycocyanin and allophycocyanin with chlorophyll in *Anabaena cylindrica* PCC7122 was investigated in the present study. To study the interaction of chlorophyll in phycocyanin and allophycocyanin, docking analysis was performed. Two docking models each corresponding to phycocyanin and allophycocyanin with their energy minimization scores were obtained.Their interaction establishes the relationship between chlorophyll and the light harvesting components.

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INTRODUCTION

Cyanobacteria are the simplest and the oldest prokaryotic organisms to have evolved on earth. They are classified under gram negative bacterial phyla and occupy the diverse range of habitats. They show a wide range of morphological diversity ranging from unicellular to colonial and filamentous. Cyanobacteria are photosynthetic microorganisms by which they are capable to grow photo-autotrophically in a manner similar to those of eukaryotic algae and plants. They can be used as experimental and model strains for studying the diversification of prokaryotic cells and the physiological processes occurring within the cell (Berrendero *et al.,* 2011).

Life on Earth is based on the energy of solar radiation, which is captured by higher plants, algae, and photosynthetic bacteria. These organisms contain photosynthetic pigments such as chlorophylls, phycobilins, and carotenoids, which absorb light in a wide range of wavelengths, covering the whole visible region and extending even to the near infrared region. Figure 1 shows the spectrum of light absorbed by various photosynthetic pigments. By means of the so-called antenna system, the photosynthetic organisms can harvest light quanta efficiently and funnel the excitation energy to the reaction centers, where the captured light energy is converted with a high quantum yield into chemical energy. Finally, the energy is stored in the form of carbohydrates and other hydrogen-containing organic compounds. It has been estimated that photosynthesis produces annually about 5 × 1010 tons of organic carbon, which means liberation of 13 × 1010 tons of oxygen into the air and fixation of about 20 × 1011 tons of carbon dioxide (CO_2) from the air and the oceans (Deisenhofer *et al.,* 1985). Chlorophyll *a* contains a magnesium ion encased in a large ring structure known as a chlorin. The chlorin ring is a heterocyclic compound derived from pyrrole. Four nitrogen atoms from the chlorin surround and bind the magnesium atom. The magnesium center uniquely defines the structure as a chlorophyll molecule (Zeiger *et al.,* 2006). Light harvesting antennas or complex are essential to efficiently entrap solar energy for the process of photosynthesis. These antennas are specifically associated with photosystem I or II (PSI or PSII respectively) (Neilson *et al.,* 2010). In cyanobacteria, the Phycobilisome serves as major antenna for PSII. No specific antenna has been isolated for PS1 in cyanobacteria, although PBS transfers light to PS1 under condition of state transition (Mullineaux, 2008). Going deeper and deeper under water, light quality gets modified by a selective impoverishment in red photons. Since Chlorophyll*a* has major absorption bands on the red edge of the spectrum, it becomes less effective for light harvesting in deep water.

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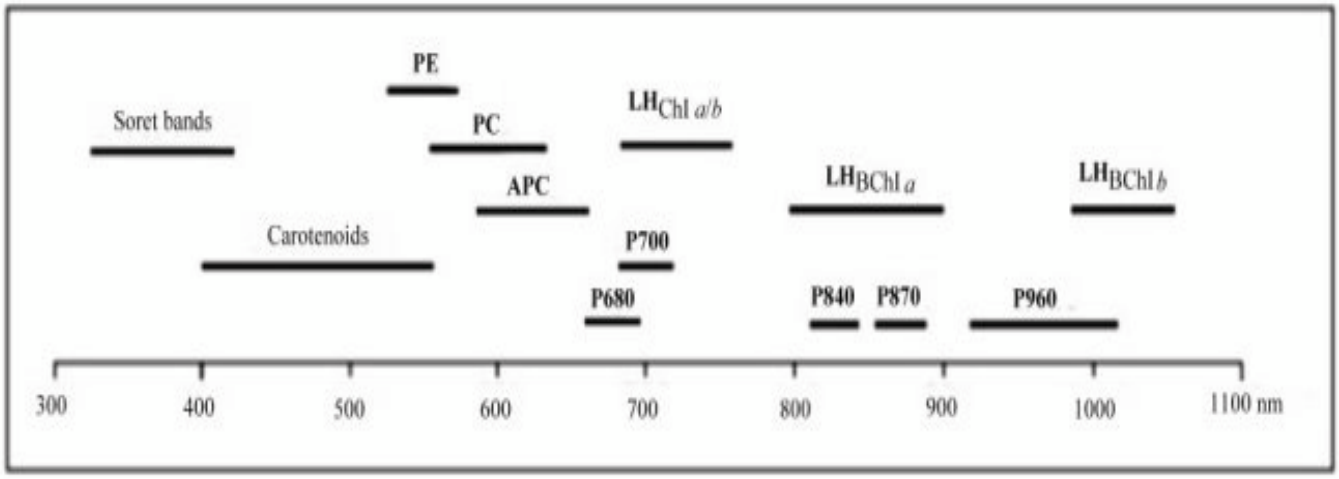


Fig.1. Light absorption of Photosynthetic pigments [Source:Paavo H. Hynninen and Tuomo S. Leppäkases; The Functions of Chlorophylls in Photosynthesis - *Physiology & Maintenance*– Vol. V

Photosynthetic organisms living in aquatic media often have developed accessory antennae, containing different kind of pigments that allow using the whole wavelength range available. Most cyanobacteria employ a special class of complexes called phycobilisomes for that purpose. Early studies from the 19th century revealed that some cyanobacteria produce soluble pigments, blue or red in color, emitting strong pink fluorescence (Marsac, 2003). These pigments were shown to form gigantic granules visible by electron microscopy (EM) on thylakoid membranes and accordingly named phycobilisomes (PBs) (Gantt E., Conti S.F. 1966). Phycobilisomes contain chromophorylated proteins called phycobiliproteins that represent up to 80% of their mass (Marsac and de, Cohen-bazire, 1977). Phycobiliproteins are classified into 4 families according to the nature of their bilins, i.e, Allophycocyanin (APC), Phycocyanin (PC), Phycoerythrin (PE) and Phycoerythrocyanin(PEC) (adapted from Bryant, 1982). PC and APC are common components of all cyanobacterial PBs, whereas Phycoerythrin and Phycoerythrocyanin production occurs only in certain species (MacColl, 1998).

All cyanobacteria andrhodophytes contain allophycocyanin and phycocyanin, sometimes accompanied by phycoerythrin. Most of the cellular phycobiliprotein absorbance is attributable to phycocyanin, phycoerythrin, or a mixture of the two pigments; and action spectra show that both are effective in harvesting light. However, allophycocyanin accounts on a weight basis for 10% or less of the total cellular phycobiliprotein. Its absorption maximum (about 650 nm) in whole cells or crude extracts is largely masked by the much greater absorbancy of phycocyanin and of chlorophyll holochromes in this region. A few published photosynthetic action spectra of cyanobacteria and rhodophytes show a slight inflection, or a very minor peak, at (or near) 650 nm, which is possibly attributable to allophycocyanin, as Halldal (Halldal P. 1970) has suggested. The best evidence for its light-harvesting role was obtained by Blinks (Blinks, 1950) during a study of the action spectra for chromatic transient activity (one manifestation of the Emerson effect) in marine algae (Lemasson *et al.,* 1973).The present paper envisaged on the role of these accessories pigments called Phycobillisomes- PC, and APC with the chlorophyll molecule using bioinformatics based tools.

MATERIALS AND METHODS

Retrieval of sequence

The sequence of the two proteins, phycocyanin and allophycocyanin of *Anabaena cylindrical* were retrieved from NCBI database. Phycocyanin with 172 amino acids having a linear structure with Accession Nos AFZ57513 and Allophycocyanin with 169 amino acids with the Accession No ALB42390 were retrieved for the present study.

Structural characterization

The sequence of phycocyanin and allophycocyanin selected from FASTA were analyzed for Domain architecture of *Anabaena cylindrica* PCC 7122 using NCBI domain database CDART (Geer *et al.,* 2002) in order to determine the structurally conserved region and also analyzed with MotifScan (Naughton *et al.,* 2006).

Homology modeling

Using Swiss-Model server, protein homology modelling was carried out for both phycocyanin and allophycocyanin sequences taken from FASTA retrieved from NCBI (Schwede *et al.,* 2003). Evaluation of the 3D structures modeled was carried out using MOLPROBITY (Chen *et al.,* 2010). The energy minimization of the modelled structures was done with the QMEAN server (Benkert *et al.,* 2009). The structures were subsequently visualized by Chimera software (Pettersen *et al.,* 2004).

Molecular Docking studies

Molecular docking studies were carried out using the PATCHDOCK server (Duhovny *et al.,* 2002, Schneidman-Duhovny *et al.,* 2005) and the energy minimization was carried out with QMEAN server and the molecular docking results were further refined with the help of FIREDOCK (Andrusier *et al.,* 2007, Mashiach e*t al.,* 2008).

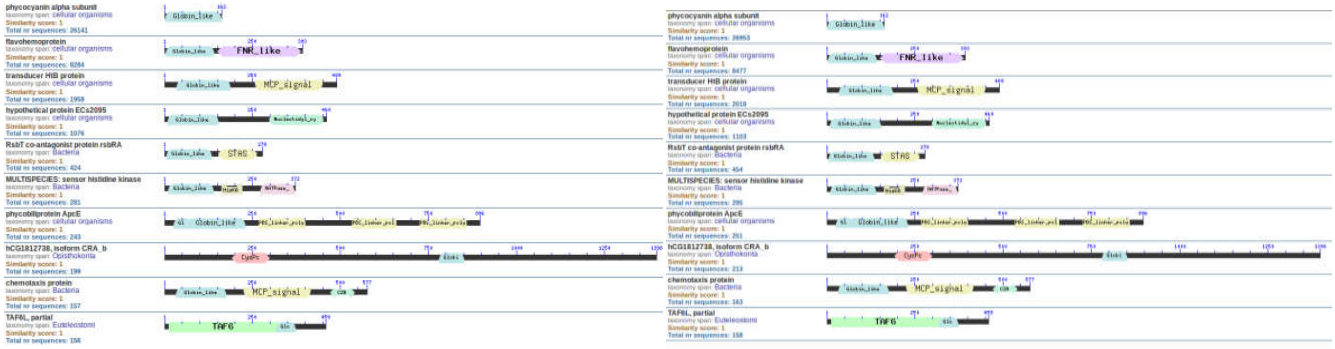
RESULTS

The analysis on the presence of functional domains of phycocyanin and allophycocyanin revealed that both pigments

differ in the non-redundant sequences. The total number of non-redundant sequences in phycocyanin ranges from 26141 to 1 and in allophycocyanin the sequences ranges from 26953 to 1 which are also widely spread in the taxonomy span including cellular organisms, bacteria, Opisthokonta, Euteleostomi etc. (Fig 2). Furthermore beside the difference in the non-redundant sequences the other domains present in both phycocyanin and allophycocyanin are the same taxonomic span with concomitant presence of globin like sensors, flavohemoprotein, transducer HtB protein, RscT co-antagonist protein rsbRA, sensor histidine kinase, phycobilliproteinApcE, isoform CRA_b, a chemotaxis protein, TAF6L (partial) etc..

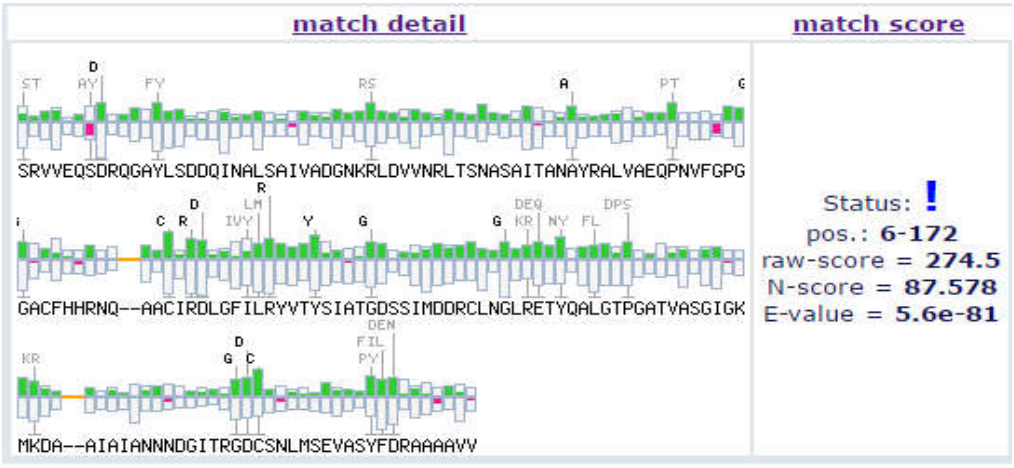
Motif Scan

Motif Scan results reveals that the motif present both phycocyanin (172 amino acid) and allophycocyanin (169 amino acid) are the same, i.e. Phycobillisome protein. The position of the motif in phycocyanin is between 6-172 amino acid and in allophycocyanin it ranges from 1-165. The raw score of the motif in phycocyanin is lesser than the raw score in allophycocyanin for the same (274.5 in phycocyanin and 297.2 in allophycocyanin). The N-Score and E-value of phycocyanin and allophycocyanin are 87.578 and 5.6e-81, and 94.423 and 8e-88 respectively (Fig 3).

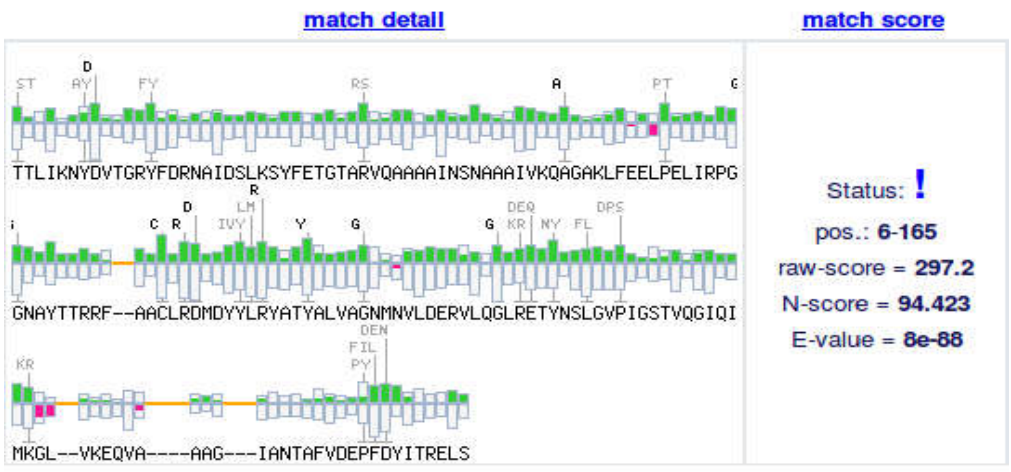


(a) (b)

Fig. 2. The functional and the putative domains present in (a) phycocyanin and (b) allophycocyanin



(a)



(b)

Fig. 3. The presence of phycobillisome protein as a motif in both (a) phycocyanin and (b) allophycocyanin

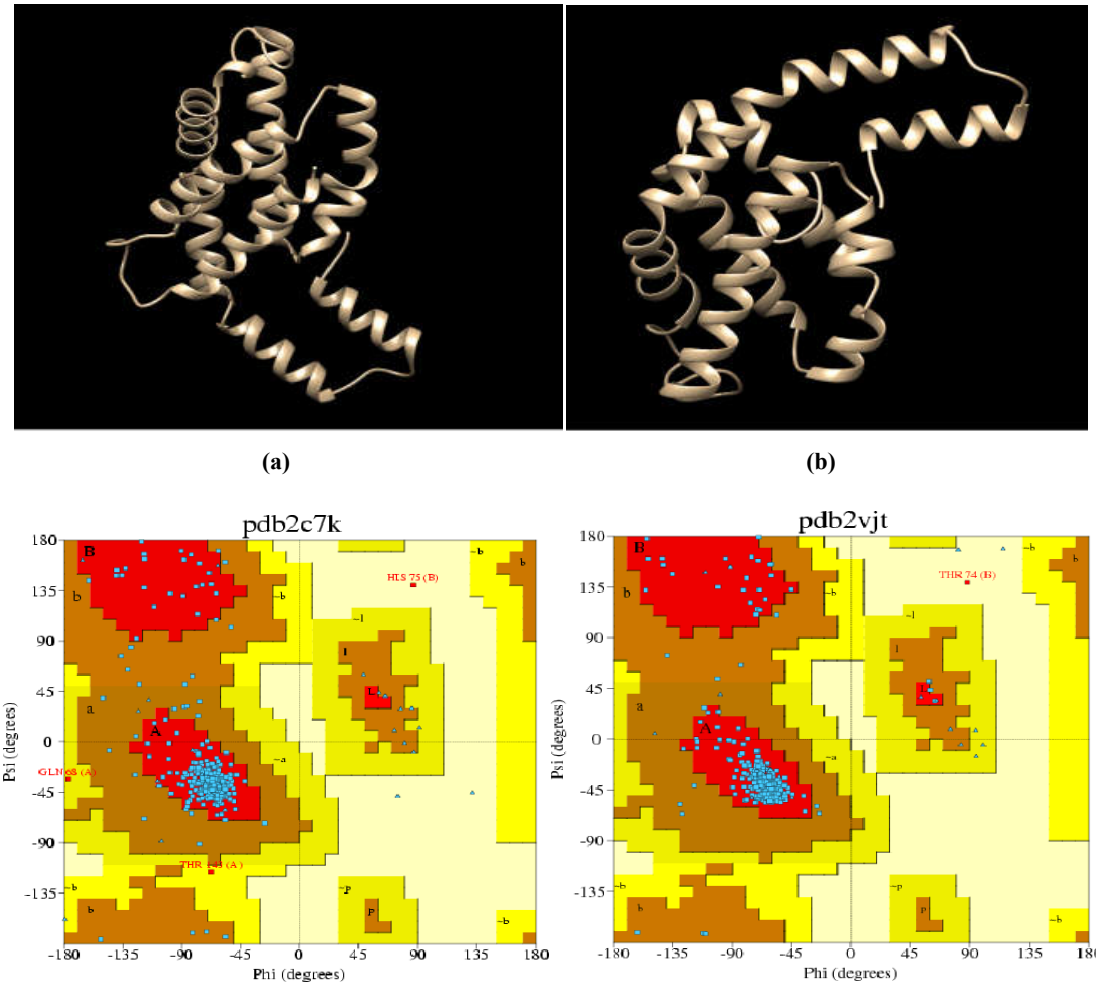


Fig. 4. The modelled structures of (a) Phycocyanin and (b) Allophycocyanin and their corresponding Ramachandran Plots respectively

Homology Modelling

The Swiss Model server was used to generate the homology modelled structure of both phycocyanin and allophycocyanin. 2CK7 corresponds to phycocyanin with a sequence similarity of 82.46% showing 89.1% amino acids in the most favored regions followed by 0.7% in generously allowed region and 0.3% in the disallowed region respectively, with 25 Glycine residues and 11 Proline residues. 2VJT corresponds to allophycocyanin with a sequence similarity of 59.38% showing 94.3% amino acids in the most favored regions followed by 0.0% in the generously favored regions and 0.4% in the disallowed region respectively, with 28 Glycine residues and 10 Proline residues. This has been confirmed by the Ramachandran plots and energy minimized using QMean server generated by ProCheck server (Fig 4).

Molecular Docking

Molecular docking studies for phycocyanin and allophycocyanin models with Chlorophyll molecule were successfully carried out with the help of online e docking server PatchDock and the resulting complexes were viewed under Chimera 1.9 software. Out of 10 conformations the best complex with high global energy, docking score, area, Atomic Contact energy, attractive Vanderwaalforces, repulsive Vanderwaal forces and Hydrogen bonds were taken into consideration (Table 1). Although there wasn't showing any noticeable hydrogen bonding yet the attractive Van der Waals forces and repulsive Van der Waals forces confirms the binding of chlorophyll with both the phycocyanin and allophycocyanin (Fig 4)

DISCUSSION

Phycocyanin is a blue pigment with photosynthetic function found in most cyanobacteria as the major phycobiliprotein with an absorption peak of 618 nm and absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~650 nm. Its molecular weight is between 70,000 and 110,000 Daltons. The pigment is composed of two subunits, α and β, which occur in equal numbers, but the exact number of α and β pairs which make up the molecule may differ from species to species. Both α and β subunits contain only the phycocyanobilin (PCB) chromophore. In addition to absorbing light directly, this intensely blue pigment accepts quanta from phycoerythrin by fluorescent energy transfer in organisms in which PE is present. The red fluorescence of C-PC is transferred to allophycocyanin. Allophycocyanin (APC) is an intensely bright phycobiliprotein that exhibits far-red fluorescence with high quantum yields, found in the phycobilisome (PBS) core complex. It is found in various species of red or blue-green algae having a molecular weight of 105,000 Daltons. APC has an absorption maximum of 652 nm and additional absorption peak of 625 nm and an emission maximum of 657.5nm. Composed of two subunits, α and β, each unit has one PCB chromophore. The interaction between Chlorophyll and both phycocyanin and allophycocyanin shows

that the light harvesting components are helping Chlorophyll to utilize the light and make the necessary arrangents for the plants for the survival and most importantly food production via the process of photosynthesis. Henceforth, both phycocyanin and allophycocyanin are very important for the chlorophyll for harvesting light and use it for the plant.

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Conflict of Interests

The authors hereby declare that we have no conflict of interest regarding this research paper.

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