Indian J.Sci.Res. 4(2) : 31-35, 2013

ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

COMPUTATIONAL ANALYSIS FOR SMALL SUBUNIT OF RuBisCo IN CYANOBACTERIA

S. MEENAKSHI[a1] AND S. SRISUDHA[b]

[ab]Research Centre in Botany and Microbiology, Lady Doak College, Madurai, Tamilnadu, India

ABSTRACT

The key enzyme of the CBB pathway is ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo). RuBisCo-based CO_2 fixation occurs in all cyanobacteria. The availability of internet based tools and servers provide an excellent opportunity to characterize the physicochemical properties of Cyanobacterial RuBisCo as well as their primary, secondary and three-dimensional structural properties. The aim of the study was to determine the physicochemical charecteristics of cyanobacterial RuBisCo and to develop the 3D models of selected cyanobacterial RuBisCo. Molecular weight, isoelectric point, aliphatic index, instability, number of residues, Grand Average Hydropathicity (GRAVY) and disulfide bond were computed. Secondary structure of α-helix and β-sheet of cyanobacterial small subunit of RuBisCo has more predicted value than plant small subunit of RuBisCo. Putative phosphorylation sites also have high range from 69%-96%. The small subunit of RuBisCo can act as an inducer to large subunit but it doesn't have any function separately. These organisms are homologues and may provide useful information in the study of small-subunit function.

KEYWORDS : Ribulose-1,5-bisphosphate carboxylase/oxygenase, photosynthetic efficiency, computational analysis, Small subunit

Ribulose-1, 5-bisphosphate carboxylase/ oxygenase (RuBisCo, EC 4.1.1.39) catalyses the addition of CO_2 to ribulose1, 5-bisphosphate (RuBP) resulting in the formation of unstable six-carbon compound, cleaved later into two molecules of 3-phosphoglyceric acid. Three dimensional high resolution-structures of RuBisCo are now known for a number of species, allowing insights of the enzyme with bound substrate, product and transition-state analogues (Mizohata et al., 2002). Since we have no direct experimental evidence on carbon sequestration in *Synechococcus* WH8102 or on the marine *Synechococcus/Prochlorococcus* group, most of what we know at present is inferred from other cyanobacteria or derived from analysis of the genome sequence data analyzed in the two sequenced *Prochlorococcus* sp. in the context of the assimilation of inorganic carbon (Hess et al., 2001).

The proteomics study revealed several novel proteins, apart from the well characterized proteins involved in carbon concentrating mechanisms (CCMs), that were upregulated upon shift of the cells from high CO_2 concentration (3%) to that in air level (0.039%) (Carmel et al., 2011). The sequence diversity in small-subunit structure may account for the differences in carboxylation efficiency and CO_2/O_2 specificity that are observed among Rubisco enzymes from various plant species (Robert et al., 2000). The main focus of small subunit of RuBisCo is the organisms are evolutionary homologues and may provide useful information in the study of small-subunit function and diversity in cyanobacteria and their role in carbon fixation.

METERIALS AND METHODS

Ten RuBisCo protein sequences of Cyanobacterial strains and three Plant RuBisCo protein sequences were selected. The selected Cyanobacterial and Plant sequences were converted to FASTA format [www.ncbi.nlm.nih.gov] (Gilbert, 2003). The Amino acid composition of the cyanobacterial and plant RuBisCo sequences was computed using the pepstats analysis tool (Rice et al., 2000). The physico-chemical parameters such as the molecular weight, isoelectric point (pI), instability index (II), aliphatic index (Ai), and grand average of hydropathicity (GRAVY), -R: No. of negative residues and +R: No. of positive residues was computed using the ProtParam. Secondary structure prediction was conducted using double prediction method for cyanobacterial and plant RuBisCo sequences. This enables the prediction of secondary structure such as helix, sheet and coil (Deleage and Roux, 1987). CysRec program was used for prediction of S-S-bonding states of cysteines and location of disulfide bridges in proteins, matrix of pair scores results of S-S-bonding states and the most probable pattern of pairs. Protscale was used for computing and representation in the form of two-dimensional plot of the

[1]**Corresponding author**

profile produced by any amino acid scale on a selected protein. The Hydrophobicity and Hydrophilicity analysis of the amino acid sequences of Cyanobacteria RuBisCo and Plant RuBisCo was fulfilled with Protscale program (Kyce and Doolittle, 1982). Multiple sequence alignments were performed with ClustalW (Larkin et al., 2007). Phylogenetic analysis of protein sequences was generated using the alignment obtained with ClustalW. The selected amino acid sequences were converted into FASTA format. These set of sequences was submitted for alignment with ClustalW for both Cyanobacterial and Plant RuBisCo sequence. SWISSMODEL was used for the three-dimensional structure construction of RuBisCo protein sequences (Arnold et al., 2006). Swiss PDB viewer molecular graphics program was applied for the visualization of Cyanobacterial and Plant RuBisCo proteins. The loaded molecule was observed for various shapes, stand and surface representations. The modeled 3D structures were evaluated and validated with the RAMPAGE programs (Lovell et al., 2003)

RESULTS AND DISCUSION

Ten small subunit of cyanobacterial RuBisCo sequences and three small subunit of plant RuBisCo sequences were selected from NCBI databases. The sequences were converted into FASTA format. The primary structure analysis of small subunit of Cyanobacterial RuBisCo. The percentage of tryptophan residues in small subunit was less than 1 to 2% in cyanobacterial RuBisCo. Leucine and glutamine constituted 5-10%. In plant RuBisCo, alanine comprised 7-8% of primary structure.

Molecular weight of RuBisCo was slightly larger in cyanobacterial small subunit ie., 58977.4g mol^{-1} and conversely, plant RuBisCo's had an average molecular weight of 19630.6g mol^{-1}. The computed isoelectric point (pI) of all cyanobacterial RuBisCo's was below 7 indicating that the proteins will precipitate in acidic buffers but plant RuBisCo have pI above 7 indicating that the proteins are soluble in basic buffers. According to the ProtParam server, a protein whose Instability Index (II) is larger than 40 may be unstable and smaller than 40 predicts that the proteins are stable in nature in solution. The small subunit of four cyanobacterial RuBisCo's namely *Cylindrospermopsis*, *Fischerella*, *Lyngbya* and *Thermosynechococcus* are smaller than 40 hence it is stable in solution. *Geitlerinema* sp. PCC 8501, *Microcystis aeruginosa* PCC 7806, *Planktothrix rubescens*, *Trichodesmium erythraeum* IMS101, *Raphidiopsis brookii* D9 and *Arthrospira platensis* NIES-39 Ii index of small subunit are larger than 40 indicating the unstable nature of protein in solution. The aliphatic index which gives a measure of the relative volume occupied by alanine, valine, isoleucine and leucine. It ranged from 67.52 to 89.11 for small subunit of cyanobacterial RuBisCo's. The Grand Average Hydropathy (GRAVY) values ranged from -0.324 to -0.580, indicate that the proteins will interact favorably with water where as in plant RuBisCo protein ranged from -0.198 to -0.221. A GRAVY score indicated the relative value for the hydrophobic residues of the protein. Although no positional or interaction effects for adjacent residues are taken into consideration by the GRAVY score, it still provides some indication of the physical state of the protein (Smith et al., 2008).

Despite the low cysteine content, the disulfide bridge prediction tool CYS-REC computed disulfide bonds in most cyanobacterial and plant RuBisCo's. The secondary structure of α-helix predicted a range from 10 to 39% for small subunit of cyanobacterial RuBisCo's but, in plant RuBisCo of small subunit has a range of 9 to 12%. The secondary structure of β-sheet predicted ranged from 4 to 11% for small subunit of cyanobacterial RuBisCo's. The results of the consensus indicate that cyanobacterial RuBisCo's are largely alpha helical with less than 10% beta structures. Coil comprised 48-50% in both cyanobacteria and plant RuBisCo sequences. In *Planktothrix rubescens*, no α, β helical structure were detected and 100% of the secondary structure was represented by coils.

The secondary structure consensus prediction program of the Protein Sequence Analysis Server generated a secondary consensus where the most predicted conformational state is reported for each amino acid. The Hydrophobicity represent the maximum score value in Protscale server. The small subunit of cyanobacterial RuBisCo was located at 32^{th} position with a top score of 2.686 but plant RuBisCo was located at 159^{th} position with a top score of 2.214. Hydrophilicity denotes the minimum

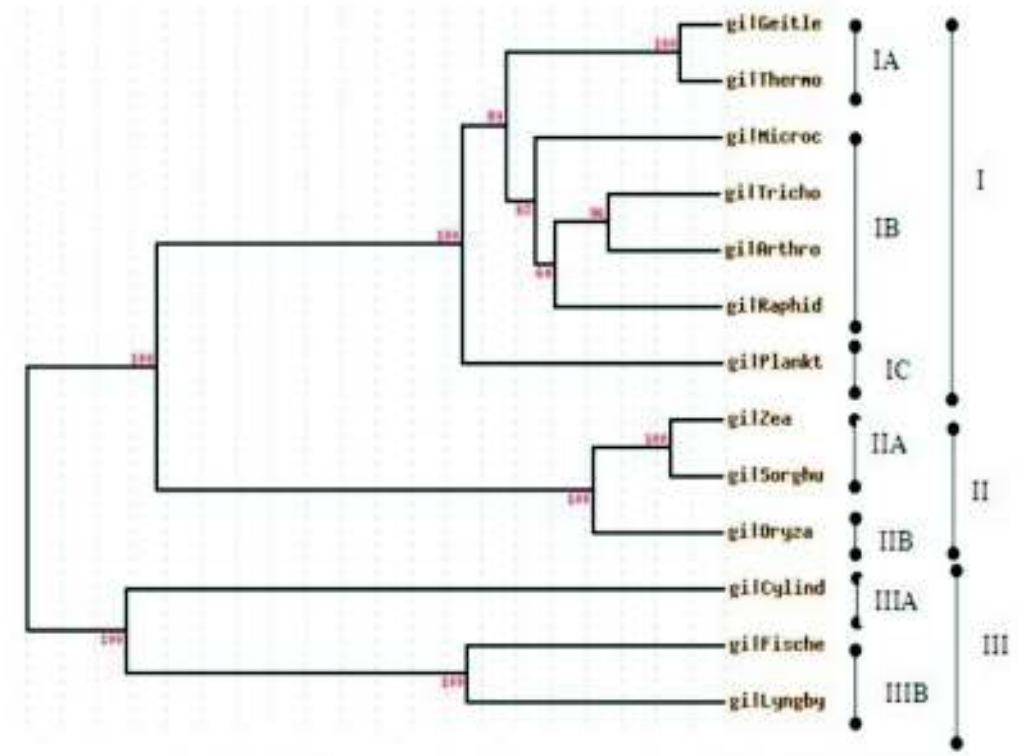

Figure 1 : Phylogram of various cyanobacterial RuBisCo (SSU) sequences. RuBisCo seems to share a higher degree of homology with RuBisCo from Cyanobacteria than Plant RuBisCo.

score, the small subunit of cyanobacterial subunit contain -2.929, situated at 80th position but plant RuBisCo protein showed a score of -2.200 at 101th position.

Computational analysis helps us in identifying the phosphorylation site for enhancing the function of RuBisCo. The server predicts phosphorylation sites for all the three amino acids serine, threonine and tyrosine. Protein phosphorylation at serine, threonine or tyrosine residues affects a multitude of cellular signaling processes. Artificial neural network method predicted phosphorylation sites in independent sequences with sensitivity in the range from 69% to 96%. The small subunit of *Cylindrospermopsis raciborskii* CS-505 has maximum 31 amino acid residues but small subunit of plant *Sorghum bicolor* contain 11 amino acid residues.

Leucine residues are found as dimers in cyanobacterial and plant RuBisCo. Most significantly, the sequences WKLPLF are found in a few cyanobacteria and plant RuBisCo's. The sequences YLLR and GFVYREN are found in plant RuBisCo sequences indicating that these sequences are conserved. These sequences represent mostly alpha helix.

Phylogenetic and bioinformatic analysis of RuBisCo (Figure, 1) provide a useful framework to understand the relationship of the different forms and how they have evolved from a common ancestor. Inclusion of short sequences presents a major difficulty in reconstruction of phylogenetic relationships as they limit the number of informative positions in an alignment on RuBisCo. For small subunit, three different clades were observed. Clade I was found to be intermixed and was further divided into four different sub clades (IA, IB, IC and ID). The subclade IA consists of *Geitlerinema* sp. PCC 8501 and *Thermosynechococcus vulcanus* with maximum boot strap value of 100%. Microcystis alone occupied the second sub clade (IB). *Trichodesmium erythraeum* IMS10, *Raphidiitopsis brookii* I and *Arthrospira* formed the sub clade (IC). Planktothrix was found to be distinct and it

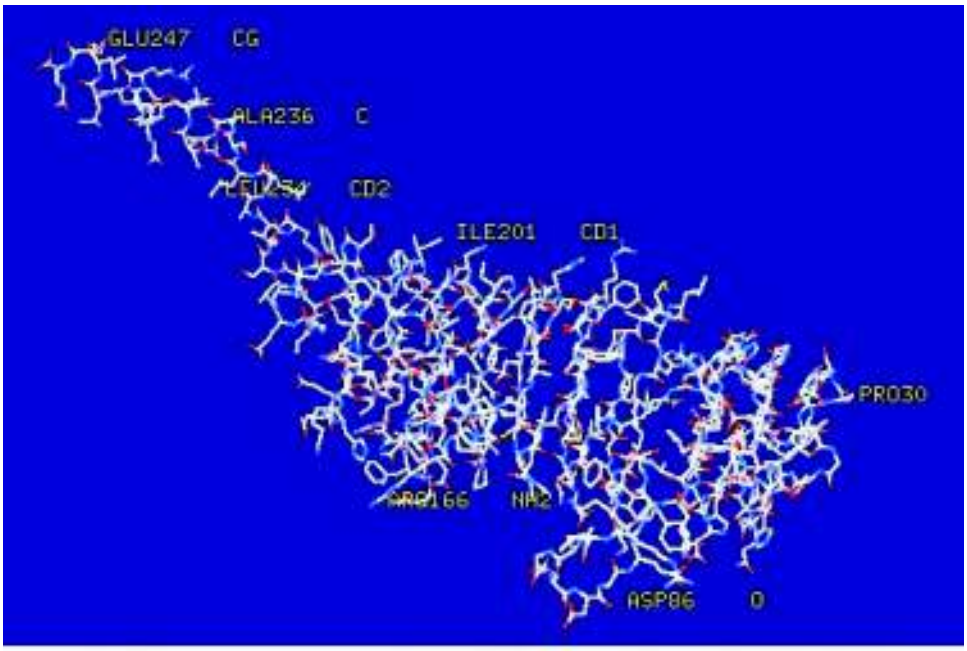

Figure 2 : Supplementary strands of *Cylindrospermopsis raciorskii* CS-505 was generated with SWISS MODEL and viewed with swiss PDB viewer

formed a separate sub clade ID. All three plant RuBisCo small subunit comprised the clade two. Zea mays and *Sorghum bicolor* exhibited the high sequence similarity when compared to *Oryza sativa*. Clade III was further divided into two sub clade, *Fischerella* and *Lyngbya* constituted the second sub clade IIIB while *Cylindrospermopsis* formed the sub clade IIIA. The predicted phylogenetic tree based on small subunit revealed high sequence similarity among *Geitlerinema sp.* PCC 8501 and *Thermosynechococcus vulcanus.* From the predicted phylogenetic trees, *Zea mays* and *Sorghum bicolor* were found to have highly similar sequences when compared to *Oryza sativa*.

The homology models of RuBisCo from cyanobacteria and plant species were predicted using SWISS-MODEL and viewed in Swiss PDB viewer (Figure, 2). The stereochemical and energetic properties of the models were evaluated with the RAMPAGE servers. As per the Ramachandran plot, 80 to 94.3% residues were in the most favored regions in cyanobacterial small subunit but plant small subunit has 95.8% of the residues in favored region. These results indicate that the models were geometrically viable.

Molecular weight, isoelectric point, aliphatic index, instability, number of negative residues both positive and negative, Grand Average of Hydropathicity (GRAVY) and disulfide bond were computed. Physicochemical parameters provided useful data for the purification of cyanobacterial RuBisCo. Small subunits of *Cylindrospermopsis* are highly effective and efficient. The small subunit of *Cylindrospermopsis* has high molecular weight, aliphatic index, GRAVY, +R and R values, random coil and disulfide bond prediction. The 3D model of *Cylindrospermopsis* RuBisCo suggests that the molecule contain more beta-sheet and alpha helix. Detailed understanding of RuBisCo catalysis has taken advantage of an exponential rise in computer power and the increasing accuracy of theoretical protein models. RuBisCo small subunit is more important to enhance the activity for better fixation of CO_2 but it does not have any active site for fixation of CO_2. It is mainly involved as an inducer for effective fixation. Bioinformatics analyses indicated that the *Cylindrospermopsis* RuBisCo is more efficient than other cyanobacterial RuBisCo as well as Plant RuBisCo's.

ACKNOWLEDGEMENTS

This work was financially supported, by Women Scientist Scheme (Research in Basic Sciences) from Department of Science and Technology (DST), New Delhi.

REFERENCES

Arnold K., Bordoli L., Kopp J. and Schwede T., 2006. The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. Bioinformatics, **22**: 195-201.

Carmel D., Mulo P., Battchikova N. and Aro E.M., 2011. Membrane attachment of Slr0006 in Synechocystis PCC 6803 is determined by divalent ions. Photosynthsis Research, **108**: 241-245.

Deleage G. and Roux B., 1987. An algorithm for protein secondary structure prediction based on class prediction. Protein Eng., **1**: 289-294.

Gilbert D., 2003. Sequence files format conversion with command-line readseq, in Current Protocols in Bioinformatics, Baxevanis A. D., Petsko G. A., Stein L. D., and Stormo G. D., Eds., John Wiley & Sons, Hoboken, NJ, USA.

Hess H.R., Rocap G., Ting C.S., Larimer F., Stilwagen S., Lamerdin J. and Chisholm S.W., 2001. The photosynthetic apparatus of Prochlorococcus: Insights through comparative genomics. Photosynthesis Res., **70**:53-71

Kyce J. and Doolittle R.F., 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol, **157**(6): 105-132.

Larkin M.A., Blackshields G., Brown N.P., Chenna R. and McGettigan P.A., 2007 ClustalW and ClustalX version 2.0. Bioinformatic, **23**: 2947-2948.

Lovell S.C., Davis I.W., Arendall W.B., De Bakker P.I., Word J.M. et al., 2003. Structure validation by Cα geometry: CΨ and Cβ deviation. Proteins, **50**: 437-450.

Mizohata E., Matsumura H., Okano Y., 2002. Crystal structure of activated ribulose-1,5-bisphosphate carboxylase/oxygenase from green alga Chlamydomonas reinhardtii complexed with 2-carboxyarabinitol-1,5-bisphosphate. Journal of Molecular Biology, **316**: 679-691.

Rice P., Longden I. and Bleasby A., 2000. Emboss: The European molecular biology open software suite. Trends Genet, **16**: 276-277.

Robert J.S., Maria E.G., Du Yu-Chun., nwaruzzaman Md., Patrick M.D., 2000. Structure-function relationships of the RuBisCo small subunit. American Society of Plant Biologists.

Smith A.A., Coomes M.W. and Smith T.E., 2008. Isolation and sequence of the phosphoenolpyruvate carboxylase gene of the marine cyanobacterium Synechococcus PCC 7002. J. Boil. Sci., **8**: 1261-1270.