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ABSTRACT

Biliproteins (BPs) are water-soluble, colored light harvesting pigment proteins commonly found in cyanobacteria, red algae, glaucophytes and cryptophytes. The different subunits of BPs along with closely associated chromophores are linked with each other through linker polypeptides and assembled in supramolecular complex phycobilisome (PBS) forms. The energy transfer in the organism proceeds successively in the direction from PE $\rightarrow$ PC $\rightarrow$ APC $\rightarrow$ chlorophyll *a*, with an efficiency of more than 98%. Different BPs have distinct regions of absorption maxima (λ_{max}) and it varies from species to species. Each BP is made up of alpha and beta subunits.

FL-αC-PE (19 kDa), the full length alpha subunit of cyanobacterial phycoerythrin, and its naturally truncated Tr-αC-PE (14 kDa) which is devoid of 31 N-terminal residues, were successfully isolated and purified. Tr-αC-PE was obtained from culture of more than 45 days grown under starved conditions but with similar three-dimensional structure and optical properties as compared to that of FL-αC-PE. However, several differences in their pattern of absorbance, structure and stability were observed.

Covalently linked tetrapyrrole chromophores and Trp108 intrinsic probes are profoundly influenced in presence of different denaturants and change in pH. Thus, GdmCl-, urea-, weak salts- and pH were used and different intrinsic and extrinsic probes viz. $\Delta\epsilon_{565}$, F_{340}, F_{350}, F_{573}, Soret CD and far UV-CD at 222 nm were applied. This revealed the importance of 31 N-terminal residues and factors responsible in stability and folding of the two natural variants and thus compared. In addition, various results may were approved and confirmed through MD simulation studies. Values of ΔG_D^0 (protein stability) obtained from the analysis of FL-αC-PE and Tr-αC-PE denaturation curves are accurate, and the stability of the full length and truncated proteins in terms of ΔG_D^0 are not very different.

After denaturation studies, it was concluded that: The 31-residue long N-terminal segment of the full length phycoerythrin does not contribute significantly to the overall stability of the protein, for the full length protein is only ~10% more stable than the truncated protein. The N-terminal truncated segment in the form of two α-helices consists of 12% of the total α-helical content. Transition curves of both proteins follow a two-state denaturation N ↔ D. The 31 N-terminal residues have no effect on the mechanism of folding of the protein fragment (32-164 residues), for both proteins undergo a two-state transition between N and D states. This has also been confirmed from the reversibility feature obtained from chemical-induced denaturation.. Reversibility of Tr-αC-PE suggests that truncation may occur earlier than that of the folding, as the 31 N-terminal sequences is not required for reversibility. Moreover, the non-covalent interactions due to 31 N-terminal residues in the FL-αC-PE monomeric protein provide structural rigidity, not significant stability. Since, deletion of the N-terminal segment does not perturb the protein-PEBs interaction, thus both FL-αC-PE and Tr-αC-PE have same absorbance capability and thus optical functions.The MD simulation with respect to results of urea-induced denaturation shows that the stable nature of both variants supports the experimental observations that the biological activity is retained in absence of these residues.

Denaturation of FL-αC-PE and Tr-αC-PE by altering the pH of their microenvironment and plotting the transition curves also revealed that both proteins undergo two-state mechanism of denaturation with three sequential regions: N, N → D (transition) and D. Both proteins found to be optimally active and stable at a pH range 4.75 – 9.25. The experimental results were further supported with the help of MD simulations performed on both the proteins. The MD results indicated the unstable nature of both proteins both in the acidic and basic environment probably due to distortion of the α-helical bundles. It can also be concluded from the refolding ability of Tr-αC-PE after pH-induced denaturation that truncation of 31 N-terminal residues may take place before the folding process and at the transcription level during gene expression. Thus, we have established the role of 31 N-terminal residues in the folding and stability of C-PE.

This type of folding ↔ unfolding, structure, optical function and stability based studies on FL-αC-PE and its naturally truncated Tr-αC-PE will be helpful for a better understanding about the mechanism of energy transfer in autotrophic blue-green algae and other photosynthetic lower organisms especially in stress conditions like nutrition deficiency and alkaline environments.