PS04.07.12 CRYSTALLOGRAPHIC STUDY OF THE TETRAMERIZATION DOMAIN OF A SHAKEr-TYPE POTASSIUM CHANNEL

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Functional diversity of ion channels arises not only from multiple genes and gene splicing, but also from diverse formation of heterotetramers. Potassium channels are grouped into four subfamilies: Shaker, Shab, Shaw; and Shal. Members of the same family share high sequence homology and can assemble, whereas members from different subfamilies do not. The N-terminal domains of Shaker-type K+-channels has been shown to be crucial for subunit assembly into functional channels and are able to tetramerize in solution when isolated.

Studies by Shen et al. (1995) identified the core region responsible for the tetramerization of the Aplysia potassium channel protein. We purified and crystallized this tetramerization domain derived from Aplysia. Crystals have been obtained by hanging drop method and belong to the space group P41 with a=b=51.034, c=65.605 Å, with one molecule per asymmetric unit. These crystals diffract to beyond 2.0 Å resolution at room temperature. A complete native data set to 2.0 Å has been collected at SSRL with an Rmerge of 6.3% and an overall completeness of 99%. The search for heavy atom derivatives and structure determination are in progress. Three-dimensional structure can reveal the structural basis for subfamily-specific recognition motifs underlying the formation of diverse channel properties.


PS04.07.13 CRYSTAL STRUCTURE OF THYMIDYLATE KINASE FROM SACCHAROMYCES CEREVISIAE WITH SUBSTRATE BOUND

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The three-dimensional X-ray structure of Thymidylate Kinase (TmPK) from Saccharomyces cerevisiae with TDP bound has been solved at 2.5Å resolution. TmPK plays a crucial role in cell proliferation catalyzing the phosphorylation of TMP to TDP, which is then further phosphorylated by Nucleoside Diphosphate Kinase to TTP. It is this activated form of thymidine which is the substrate for DNA polymerase, thus making TmPK an essential enzyme for DNA synthesis. The substrate is seen bound in the monophosphate binding interface is composed of an hydrophobic core made up by 6 leucine residues from all three domains. TmPK plays a crucial role in cell DNA synthesis. It is the primary enzyme responsible for the tetramerization of the Shaker-type K+-channel even though there is very low sequence homology between AK and TmPK. TmPK, which is a dimer in solution, crystallizes as tetramers. The crystals have been obtained, and native data sets collected. The refinement of these structures is in progress, and together they should provide a clue to the different conformations of TmPK upon substrate binding, in analogy to the different states found for AK.

PS04.07.14 CRYSTAL STRUCTURE OF SAICAR SYNTASE FROM SACCHAROMYCES CEREVISIAE

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The three-dimensional crystal structure of SAICAR synthase of Saccharomyces cerevisiae has been solved by multiple isomorphous replacement and refined at 1.9 Å resolution to an R=0.154. SAICAR synthase is a monomeric enzyme comprising a single polypeptide chain of 306 amino acids arranged into three domains. The cores of the first two domains comprise antiparallel β-sheets and the third is composed of two long α-helices. There is a long deep cleft in the middle of the molecule which is made up of residues from all three domains. The positions of two sulphate ions bound in the cleft and comparison of SAICAR synthase structure with known structures of other nucleotide binding proteins indicate the most probable binding sites of the phosphate moieties of ATP and phosphoribosylpyrophosphate and the enzyme.

Sequence alignment of SAICAR synthases from different organisms reveals 26% conserved amino acid residues, 14 of which are charged. Almost all of them are located at the surface of the interdomain cleft. Some of them are presumably implicated in substrate binding.

Comparison of the SAICAR synthase with other nucleotide binding proteins shows that this protein does not belong to the protein families with classical di- and mononucleotide-binding fold. However SAICAR synthase structure has some resemblance with glutathione synthetase, D-alanine:D-alanine ligase and cyclic AMP-dependent protein kinase. The probable ATP phosphate anchor in the structure of SAICAR synthase made up of a β-loop-β-motif typical for the actin and heat-shock cognate protein.

Studies of the enzyme-substrate complexes is in progress.

PS04.07.15 STRUCTURE OF THE BACTERIOCHLOROPHYLL a PROTEIN FROM CHLOROBIM TEPIDUM

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The bacteriochlorophyll (BChl) a protein accepts energy from the chlorosome antenna complex and then transfers the excitation energy to the reaction center in green photosynthetic bacteria. Studies on the antenna system from Chlorobium tepidum have shown the unusual property that energy transfer efficiency could be modulated by the redox potential. The nearly 100% efficiency of energy transfer in reducing conditions is reduced to 10% or less under oxidizing conditions due to unknown changes of the BChla protein (Blankenship et al., 1993, Photosynth. Res. 37, 57-107).

The BChla protein from C. tepidum has been crystallized using the sitting drop method of vapor diffusion. These crystals belong to the cubic space group P41,32 with cell dimensions of a = b = c = 169.5 Å. A native data set has been collected to a resolution of 2.0 Å. An initial solution has been determined by using the molecular replacement with X-PLOOR. The search model was the structure of the BChla protein from Prosthecochloris aestuarii (Tromsdorf, D. E. & Matthews, B. W., 1995, The Photosynthetic Reaction Centers. Norris, J. & Deisenhofer, J., eds., pp. 13-51, Academic Press, NY). The model was refined to R=0.21 for the 2.0 Å data set.

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