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THE CRYSTAL STRUCTURE OF THE RC/LH1 COMPLEX FROM *RHODOPSEUDOMONAS PALUSTRIS*

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A typical purple bacterial photosynthetic unit is composed of a reaction centre (RC) and two types of antenna complexes (LH1 and LH2). LH1 forms a stoichiometric complex with RC, called the RC/LH1 core complex, while LH2 antennas are arranged peripherally around the core. All of these pigment-protein complexes are integral membrane proteins. High-resolution structures have been determined for both the RC alone and the LH2. So far however the best structural data on LH1 has come from an 8.5 Å projection map obtained from electron diffraction of 2D-crystal of LH1 from *Rhodospirillum rubrum*. We report here the structure of RC/LH1 core complex from *R. palustris*, which was isolated from membranes following solubilization with the LDAO. The complex was purified by a combination of ion exchange and molecular sieve chromatography, then for crystallization exchanged into 1% sucrose monocholate. Crystals were grown by vapor diffusion in presence of 10 nM $MgCl_2$, 8% MMePEG2K and 1% spermidine. The 4.8 Å data were collected at 100 K (st.14.1, SRS Daresbury), and indexed in *P*1 (a = 76.04, b = 119.02, c = 130.43 Å, α = 69.3°, β = 72.7°, γ = 66.5°). 37283 reflections were measured with the average I/°s 7.5, completeness 97.4% and Rmerge 8.8%.

The structure of the RC/LH1 core complex was build by the combination of the MR method using RC from *Rhodopseudomonas spheroides* and by the modeling of LH1 components in subsequent electron density maps. The most interesting feature of this structure is the oval shape of the LH1 complex wrapping up the pseudodimeric structure of the RC, as it was expected to be circular.

Keywords: REACTION CENTRE, LIGHT-HARVESTING COMPLEX, INTEGRAL MEMBRANE PROTEINS

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ATOMIC STRUCTURE OF A CORE FRAGMENT OF HOOK PROTEIN, FlgE

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Bacterial flagellum is a helical propeller by means of which bacteria swim. The motor at its base rotates the flagellar filament. Between the flagellar filament and the motor there is a short, highly curved and flexible rod segment that transmits the torque of the motor to the filament. This short rod segment is called hook, and it functions as a universal joint. The hook is a helical assembly of a single protein, FlgE, which is also called hook protein. Similarly to the flagellar filament, the hook is a tubular structure composed of 11 protofilaments. In every revolution of the motor, each protofilament is thought to undergo a cycle of conformational changes from a state with a shorter repeat to the other with a longer repeat and from the longer to the shorter. To understand the universal joint mechanism of the hook, the high-resolution structure of hook is needed. Hook protein is very difficult to crystallize because of its high propensity to polymerize. Therefore, we expressed a 32 kDa fragment of hook protein, FlgE-32k, which lacks 70 and 30 residues of the N- and C-terminus, respectively. This fragment has no polymerization ability. We successfully crystallized FlgE-32k and the crystal structure has been solved at 1.8 Å resolution. FlgE-32k structure consists entirely of β-strands connected by short loops and turns. Two of the three domains of FlgE are clearly identified. The atomic structure of FlgE-32k helps us to understand the high flexibility of the hook.

Keywords: CRYSTALLIZATION, FLAGELLUM, MACROMOLECULAR ASSEMBLY

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INTERFACES IN LENS CRYSTALLIN STRUCTURES

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The long-lived lens β- and γ-crystallins are all derived from repeated duplications of an ancestral Greek-key motif that result in two domains with pseudo 2-fold pairing. A distant relative, the mycetezoan single-domain spherulin 3a, with its unique mode of domain pairing, is likely to be an evolutionary offshoot. Members of the fold family display a range of oligomeric assemblies, stabilities and solubilities. Our X-ray structures of β-crystallins indicate novel assembly rules that involve increasing the combinatorial diversity of these oligomers by interface selection. The resultant increase in polydispersity probably aids lens transparency. Our structures show how sequence extensions contribute to higher assembly by covering hydrophobic patches thus indicating how β-crystallin sequence truncations may affect solubility and size. We have defined the structural environment of certain amide sites in human γ-S-crystallin where deamidation could lead to altered backbone conformations and destabilisation. Lens α-crystallin, a member of the small heat shock family of proteins, is thought to bind non-native crystallins and thus prevent them from scattering light. Our X-ray structure of a dodecameric member of the small heat shock family shows how extended hydrophobic sequences can bind in grooves and crevices of unfinished β-sheet structures. This provides a view of how the α-crystallin domain might chaperone non-native substrate crystallins.

Keywords: OLIGOMERIC CRYSTALLIN ASSEMBLIES

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CRYSTALLOGRAPHIC STUDY OF A SUB-COMPLEX BETWEEN E2O AND E3 COMPONENTS OF 2-OXOGLUTARATE DEHYDROGENASE COMPLEX

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2-Oxoglutarate dehydrogenase complex consists of multiple copies of three different component enzymes E1o (2-oxoglutarate dehydrogenase), E2o (dihydrolipoamide succinyltransferase) and E3 (dihydrolipoamide dehydrogenase). To reveal the structural architecture, the sub-complex of E2o and E3 has been crystallized. Recombinant pig E2o and human E3, produced separately in *E. coli*, were highly purified and mixed with each other. The sub-complex with the two components was ultra-centrifuged and purified by gel-filtration. Plate crystals were obtained at 298K by the hanging-drop vapor-diffusion method; 15mg/ml sub-complex in 25mM potassium phosphate buffer (pH 7.0), and 2M NaCl and 10% PEG6000 reservoir solution. X-ray diffractions were recorded on imaging plates at 130K using synchrotron radiation at BL6B of PF (Tsukuba). Gel-filtration and fluorescent spectroscopy indicated that the crystal consists of the sub-complex with E2os and E3s. The crystallographic data were determined: space group *C*222 with a = 279.8, b = 283.6 and c = 129.0 Å. It has been proposed that E3 dimers are bound on the faces of the cubic core composed of 24 E2o subunits. Accordingly the sub-complex must occupy the center of 222, because E3 dimer has a two-fold symmetry. From the molecular weights of the two components, Vm is reasonably calculated to be 2.8 $Å^3$/Da (the solvent content 56%). The length of the c-axis suggests the size of the sub-complex, which is similar to a value estimated from the other axes. The small difference of the latter two axes indicates that molecular packing is deviated from a tetragonal symmetry, which is consistent to intensity distribution.

Keywords: 2-OXOGLUTARATE DEHYDROGENASE COMPLEX E2-E3 SUB-COMPLEX PROTEIN STRUCTURE