the pore of the β-barrel. This structure supports the model of passenger domain transport through the narrow β-barrel pore and is inconsistent with a model for transport through a central channel formed by an oligomer of translocator domains.

PagL is an enzyme that removes the 3-*O*-acyl chain of LPS, resulting in less toxic LPS. The crystal structure of PagL from *Pseudomonas aeruginosa* reveals a Ser-His-Glu catalytic triad. The surface hydrophobicity and the positions of aromatic residues, strongly suggest an unusual tilt of the β-barrel axis with respect to the membrane. We have modeled a substrate to the active site of PagL, providing insight into the specificity of PagL towards its substrate.

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Keywords: membrane protein structures, membrane channel transport, hydrolase

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X-ray Structures of Phospholipids in Bovine Heart Cytochrome *c* Oxidase

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Cytochrome c oxidase is the terminal oxidase of cell respiration which reduces molecular oxygen to water coupled with proton pumping. The structural and functional investigations of this enzyme have been greatly stimulated by the X-ray structural determinations of the bovine and bacterial enzymes in 1995. However, the composition and conformations of phospholipids have been yet to be determined. Phosphatidylethanolamines(PE), phosphatidylglycerols(PG), phosphatidylcholines(PC) and cardiolipins(CL) were identified in the crystalline bovine heart cytochrome c oxidase preparation by chemical analysis, showing unique fatty acid structures for each phospholipid except PC. All these phospholipids were detectable in the X-ray structure of bovine heart cytochrome c oxidase in the oxidized state at 1.8 Å resolution. The head groups of these phospholipids were located at either one of the surfaces of the transmembrane protein region facing the protein regions protruding to the aqueous phases. A CL located in the intermembrane side bridged the two monomers in the dimeric structure to stabilize the dimer state. The temperature factors of the three head groups of phospholipids (2PG and 1PE) as well as their tail portions in subunit III are unusually low as those of the phospholipids found in X-ray structures of proteins determined thus far, suggesting some physiologically important functions of subunit III, such as the O_2 storage.

Keywords: cytochrome oxidase, phospholipids protein interactions, X-ray crystallography of biological macromolecules

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Structure of Rhodopsin as a G Protein-Coupled Receptor

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In rhodopsin, light-induced isomerization of the 11-*cis*-retinal to its all-*trans* form initiates conformational changes within the transmembrane protein that enable the activated rhodopsin to bind and activate the heterotrimeric G protein transducin on the cytoplasmic surface. The structure of rhodopsin (PDB code 1GZM) was recently determined at 2.65Å resolution [1, 2] in a $P3_1$ crystal form where the packing retains an amphipathic molecular environment similar to that in a membrane, so the transducin-interaction segments from the cytoplasmic interface are likely to represent their native conformations. By docking this crystal structure into a cryo-EM map of 2D crystals [3] we consider the mechanistically important details in the membrane context.

The kinked transmembrane helices enfold the retinal in a tight binding pocket. In the membrane interior, ordered water molecules mediate key interactions. Glu113 with a water molecule hydrogen bonded between its main chain and side chain oxygen atoms forms a complex counterion for the protonated Schiff base linking the retinal to helix H7, which may then stabilise the salt bridge with the protonated Schiff and help to stabilise the dark state. Other water molecules extend the inter-helical hydrogen bonding networks, linking Trp265 in the retinal (ligand) binding pocket to the NPxxY motif on the cytoplasmic side, important for G protein-coupling, and the Glu113 counterion to the extracellular surface. Modulation of these networks is likely to be involved in the activation process.

The G protein interaction sites mapped to the cytoplasmic ends of H5 and H6 and a spiral extension of H5 are elevated above the bilayer. The conserved Glu134-Arg135 ion pair in H3 is sequestered in the walls of a surface cavity. The highest temperature factors in the cytoplasmic loops suggest that they are quite flexible when not interacting with G protein or regulatory proteins. A tightly bound detergent molecule wraps around the kink in H6, stabilizing the structure around the potential hinge in H6. These findings support an activation mechanism that involves pivoting movements of kinked helices, which, while maintaining hydrophobic contacts in the membrane interior, can be coupled to amplified translation of the helix ends near the membrane surfaces.

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Keywords: membrane protein structures, G protein-coupled receptors, ligand binding pocket

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Dephosphorylation of the Calcium Pump Coupled to Conterion Occlusion

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We have crystallized rabbit sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a) in complex with aluminium fluoride (E2-AlF) and solved its structure at 3.0 Å resolution using PHASER. We find the planar aluminium fluoride group to be located between the conserved Asp351 side chain and a water molecule positioned for hydrolysis. Further, we find the cation-conducting pathway to be in an occluded and protonated state. Further supported by biochemical data we conclude that the transition state of hydrolysis of the phosphoenzyme intermediate couples with occlusion of bound H+ counter-cations[1]. The mechanism is then similar to that of phosphorylation from ATP, which couples to the occlusion of Ca2+ ions[2]. A regulatory K+ site, identified by difference Fourier analysis using crystals prepared in $RbCl^3$, is observed to stabilize a helix cluster which positions the conserved TGES motif to activate the hydrolysis at the phosphorylation site. Initial identification of crystallization conditions was based on a screen of 48 combinations of PEG, salt, pH and alcohol additives, which we have found to be of general value for the crystallization of Ca2+-ATPase in various functional states.

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