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DsbB is an *E. coli* membrane protein that oxidizes DsbA, the primary protein disulfide donor present in the periplasm. To understand how disulfide bonds are generated and introduced into secreted proteins, we determined the crystal structure of DsbB in a complex with DsbA and endogenous ubiquinone at 3.7 Å resolution. The first structure of DsbB revealed that DsbB contains the four-helix bundle scaffold in the transmembrane region and one short membrane-parallel α-helix in the long periplasmic loop. Strikingly, the disulfide-generating reaction center composed of Cys41, Cys44, Arg48 and ubiquinone is located near the N-terminus of the transmembrane helix 2, where oxidizing equivalents of ubiquinone are converted to a protein disulfide bond de novo. Whereas DsbB in the resting state contains a Cys104-Cys130 disulfide, Cys104 in the ternary complex is engaged in the intermolecular disulfide bond and captured by the hydrophobic groove of DsbA, resulting in its separation from Cys130. This DsbA-induced conformational change in DsbB seems to prevent the backward resolution of the complex and thereby promote the physiological electron flow from DsbA to DsbB. Recently, I examined functional roles of the membrane-parallel α-helix with strong amphiphilicity by systematic mutation analyses. Introduction of charged or helix-breaking residues into this region not only disrupted the peripheral membrane-association of this helix but also impaired DsbA oxidation activity of DsbB. On the basis of structural and biochemical data so far obtained, I propose the "cysteine relocation mechanism", by which DsbB oxidizes the extremely oxidizing (reduction-prone) dithiol oxidase, DsbA, efficiently.

Keywords: membrane proteins, redox enzymes, X-ray crystal structure determination

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Crystal structure of the plasma membrane proton pump

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A prerequisite for life is the ability to maintain electrochemical imbalances across biomembranes. In all eukaryotes the plasma membrane potential and secondary transport systems are energized by the activity of P-type ATPase membrane proteins: H^+-ATPase (the proton pump) in plants and fungi, and Na^+,K^+-ATPase (the sodium-potassium pump) in animals. Electron microscopy has revealed the overall shape of proton pumps, however, an atomic structure has been lacking. We present the first structure of a P-type proton pump determined by X-ray crystallography[1]. The structure was solved to a resolution of 3.6 Å based on heavy-atom derivatives and density modification by inter-crystal averaging. Ten transmembrane helices and three cytoplasmic domains define the functional unit of ATP-coupled proton transport across the plasma membrane, and the structure is locked in a functional state not previously observed in P-type ATPases. The transmembrane domain reveals a large cavity, which is likely to be filled with water, located near the middle of the membrane plane where it is lined by conserved hydrophilic and charged residues. Proton transport against a high membrane potential is readily explained by this structural arrangement. We will also address some of the challenges of the low-resolution structure determination, such as generally high anisotropy, low phasing power of derivatives, and how to deal with poor electron density maps when building and refining a model.

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Keywords: membrane protein structure, membrane protein crystal structure determination, biological macromolecular crystallography

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Bacterial multi drug efflux transporter AcrB, - The pumping mechanism

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The emergence of bacterial multi-drug resistance is an increasing problem in the treatment of infectious diseases. AcrB and its homologues are the major multi-drug efflux transporter in gram-negative bacteria, which confer intrinsic drug tolerance and multi-drug resistance when they are overproduced. AcrB exports a wide variety of toxic compounds including anionic, cationic, zwitterionic, and neutral compounds directly out of the cells bypassing the periplasm driven by proton motive force. To understand molecular mechanism of multidrug recognition and active transport by multidrug transporter, we performed X-ray crystallographic analysis of this transporter. In 2002, we successfully solved the crystal structure of AcrB at 3.5 angstrom resolution[1]. In 2006, we solve the crystal structures of AcrB with and without substrates in the new crystal form at 2.8 angstrom resolution[2]. The new crystal structure solved with new crystal form is asymmetric. The AcrB-drug complex consists of asymmetric three protomers, each of which has different conformation corresponding to one of the three functional states of the transport cycle. Bound substrate was found in the periplasmic domain of one of the three protomers. The voluminous binding pocket is aromatic and allows multi-site binding. The structures show that drugs are presumably exported by a three-step functionally rotating mechanism in which drugs undergo ordered binding change.

[1] Murakami, S. et al., Nature, 1997, 419, 587.
[2] Murakami, S. et al., Nature, 2006, 443, 173.

Keywords: membrane protein X-ray crystal structure determina, membrane transport, drug-protein interactions

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New Opportunities in Synchrotron Data Collection with the Pilatus Detectors

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A novel type of x-ray detector has been developed at the Paul Scherrer Institut at the Swiss Light Source (SLS). The PILATUS