Crystal Structures of Bacterial Lipoprotein Localization Factors, LolA and LolB

Bacterial lipoproteins possessing a lipid-modified cysteine residue at the N-terminus are anchored on membranes because of their hydrophobic nature. Lipoproteins matured on the inner membrane are sorted and localized to their respective membranes. The transportation of such hydrophobic lipoproteins from the inner membrane to the outer membrane through the aqueous periplasm is mediated by the Lol system, which is widely distributed in Gram-negative bacteria. The LolCDE complex in the inner membrane belongs to the ATP-binding cassette (ABC) transporter superfamily and releases outer-membrane-specific lipoproteins using the ATP energy [1]. The released lipoproteins form a water-soluble complex with a periplasmic lipoprotein carrier, LolA [2]. The LolA-lipoprotein complex crosses the periplasm to the outer membrane, where a lipoprotein receptor, LolB, is present [3]. Upon the interaction of the LolA-lipoprotein complex with LolB, lipoproteins are spontaneously transferred from LolA to LolB, and finally localized on the outer membrane. To elucidate the molecular mechanism of lipoprotein transport by the Lol system, the crystal structures of LolA and LolB from *Escherichia coli* were determined using diffraction data collected at beamlines **BL38B1** and **BL44B2** [4].

The structure of LolA is characterized by an eleven-strand antiparallel β-sheet forming an unclosed β-barrel and three α-helices (α1-α3) plugging the β-sheet (Fig. 1) [4]. The long loop is located outside the β-sheet. An additional strand forms a parallel β-sheet with the barrel. The side chain of Arg43 is oriented toward the interior of the molecule due to the unusual *cis* peptide bond, and is hydrogen bonded to the main chain of residues in the α1- and α2-helices, thereby causing the tight fixation of the helices to the β-sheet. The inner surfaces of the β-sheet and three α-helices consist of aromatic residues and form a hydrophobic hollow cavity (Fig. 1). The cavity of LolA is a possible binding site of the lipid moiety of the lipoprotein. The plugging α-helices are expected to control opening and closing upon the accommodation and release of lipoproteins, respectively.

The structure of LolB also comprises an antiparallel β-sheet covered by three α-helices (α1-α3) as shown in Fig. 2 [4]. Surprisingly, the molecular structure of LolB is very similar to that of LolA, despite the low sequence identity of 8% between these two proteins. However, the position and orientation of the three α-helices are largely different from those of LolA. The cavity of LolB is also hydrophobic, but it opens outside

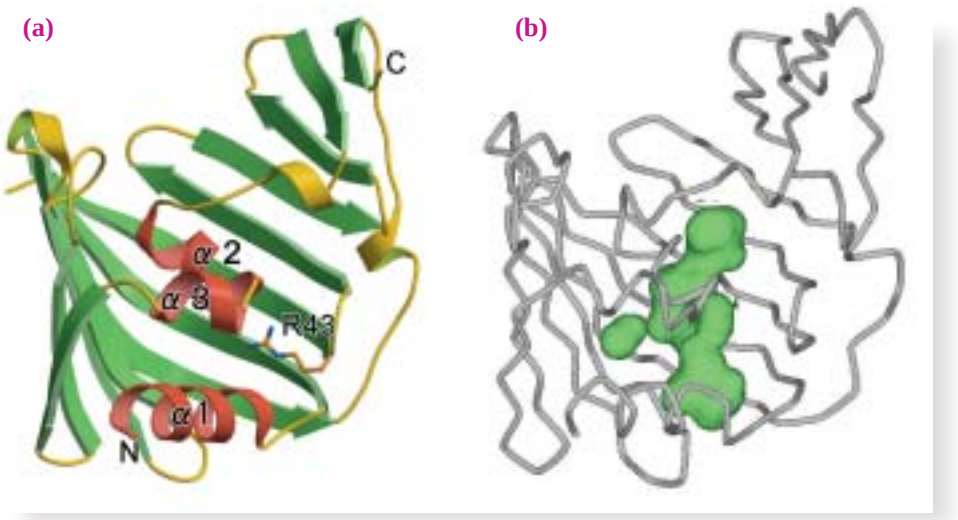

Fig. 1. Crystal structure of LolA. Ribbon presentation of the overall structure with α-helices (α1-α3) in red and β-strands in green **(a)** and the hydrophobic cavity in green **(b)**.

to contact with the solvent region. Polyethylene glycol monomethyl ether (PEGMME) used for crystallization is observed in this cavity, which might show a plausible binding mode of the lipid moiety of the lipoprotein.

These structural differences between two proteins cause the affinity difference for lipoproteins. Lipoproteins are energy-independently transferred from LolA to LolB by this affinity difference in the periplasmic space where ATP is not consumed. This result leads us to further insights on how the ATP-energy controls trafficking events beyond lipid membranes and how these insights can be generalized for other biological processes.

The localization of lipoproteins between membranes is a very important event in pathogenic bacteria which have various lipoproteins and Lol proteins, since lipoproteins induce the immunoresponse of host cells. Lol proteins are indispensable for the growth of all Gram-negative bacteria, and lack of any members is lethal for bacteria. Therefore, the insight on the structures of LolA and LolB is useful for designing effective antibiotics targeting to the proteins.

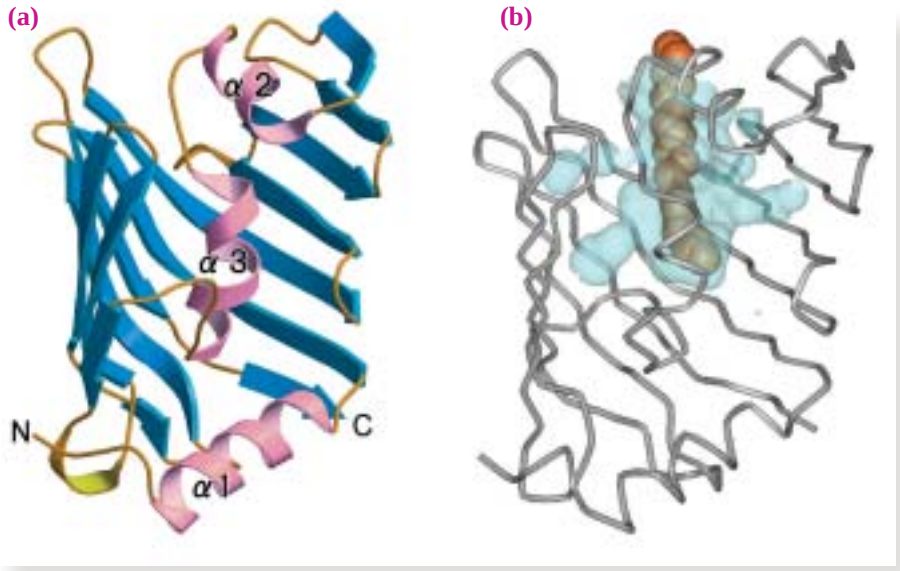

Fig. 2. Crystal structure of LolB. Ribbon presentation of the overall structure with α-helices (α1-α3) in pink and β-strands in blue **(a)** and the hydrophobic cavity in light blue **(b)** in which the PEGMME molecule bound to the cavity is shown as a CPK model in orange.

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