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Characterization of the diversity of lipoprotein-targeting mechanisms in proteobacteria

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Research aims

Bacterial lipoproteins constitute a subset of membrane proteins with their N-terminal cysteine residues modified with a lipid moiety. Lipoproteins are synthesized in the cytoplasm as precursors, translocated across the inner membrane and modified with lipids at periplasmic surface of the inner membrane[1]. In Gram-negative bacteria, lipoproteins destined for the outer membrane are escorted to their final destination by the function of the Lol system that is composed of a lipoprotein-specific ABC transporter, a periplasmic carrier protein and an outer membrane receptor protein, whereas inner membrane lipoproteins are not transported by the Lol system and remain in the inner membrane[2]. Molecular mechanisms involved in outer-membrane targeting of lipoproteins have been studied using *Eschericiha coli* as a model organism. Other Gram-negative bacteria also posses homologous genes to *lol* genes of *E. coli*, and therefore it is assumed that Lol system-mediated transport is the common mechanism for outer-membrane localization of lipoproteins in Gram-negative bacteria. However, while β,δ,γ-proteobacteria have complete set of genes for Lol proteins, α-proteobacteria lack homologous gene for the outer membrane receptor protein, LolB. To investigate the role of Lol proteins in α-proteobacteria, genes for Lol proteins were cloned from the nitrogen-fixing rhizobia *Mesorhizobium loti*.

Methods

Two orthologous genes for *lolA* (mll4242 and mll8064) were amplified by PCR from the chromosomal DNA of *M. loti* MAFF 303099, and cloned into plasmids so that each DNA sequence for the mature region of LolA is inserted between those for the signal peptide of *E. coli* OmpF and C-terminal hexahistidine tag. The chromosomal region containing two orthologous genes for *lolC* and *lolD* was also amplified from the *M. loti* genome and cloned into a plasmid with a sequence that encodes hexahistidine tag attached at the C-terminus of LolD. Lol proteins were expressed in *E. coli* C43 and purified with immobilized metal affinity chromatography using TALON Metal Affinity Resin (Clontech). Lipoprotein release assay from *E. coli* spheroplasts was performed according to the method described by Matsuyama *et al*[3].

Results

The chromosome of *M. loti* contains two orthologues of *lolA* (mll4242 and mll8064, which are referred here as *lolA1* and *lolA2*, respectively), *lolC* and *lolD* but not *lolB* nor *lolE*[4]. In *E. coli*, the lipoprotein-specific ABC transporter is composed of two molecules of nucleotide-binding protein LolD and one molecule each of integral membrane protein LolC and LolE, which share 26% sequence identity each other[5]. In contrast, the lipoprotein-specific ABC transporter of *M. loti* may consist of two molecules each of LolC and LolD.

LolA1 and LolA2 were synthesized in *E. coli* as fusion proteins with signal peptides of *E. coli* OmpF. Periplasmic fractions were prepared from each culture and mature LolA1 and LolA2 were purified by utilizing hexahistidine tag attached at the C-terminus of each protein. Although both proteins could be purified, LolA2 became insoluble immediately after purification and therefore was not further characterized. Genes encoding LolC and hexahistidine-tagged LolD were cloned as an operon into a single plasmid and these proteins were synthesized in *E. coli*. Membrane fraction was prepared and proteins were solubilized by *n*-dodecyl-β-D-maltopyranoside, and subjected to an immobilized metal affinity chromatography. LolC was co-purified with hexahistidine-tagged LolD with a stoichiometry of 1 : 1, supporting the assumption that the lipoprotein-specific ABC transporter of *M. loti* is composed of two molecules each of LolC and LolD.

Lipoprotein-releasing activity of LolA and LolCDE can be monitored by using *E. coli* spheroplasts[6]. *E. coli* cells expressing both LolC and LolD were converted into spheroplasts, and release of an endogenous outer membrane lipoprotein Lpp was monitored. Upon addition of LolA1, Lpp appeared in the supernatant fraction of spheroplasts, indicating that Lpp was released from the inner membrane by forming a soluble complex with LolA1. On the contrary, Lpp was not detected in the supernatant fraction even in the presence of LolA1 when spheroplasts were prepared from *E. coli* cells that was transformed with a vector. This results indicates that the LolCD complex is responsible for LolA1-dependent release of Lpp from spheroplasts.

Conclusion

Lol system is characterized by formation of soluble intermediate of lipoprotein and LolA in the periplasmic space.

In this study, lipoprotein-releasing reaction mediated by LolA1 and LolCD of *M. loti* was reconstructed in *E. coli*. From these results, it was suggested that Lol system is responsible for outer membrane localization of lipoproteins in α-proteobacteria, even though they lack orthologous gene for lolB. Although it has become clear that lipoproteins are released as soluble complexes with LolA1 from the inner membrane into the periplasmic space, how they are incorporated into the outer membrane remain unknown. Further analysis on the interaction of lipoprotein-LolA1 complex and the outer membrane of *M. loti* is needed to understand the evolution of lipoprotein-targeting pathway in Gram-negative bacteria.

References

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