Studies of lysophosphatidic acid acyltransferases generating membrane lipid diversity in bacteria

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Lysophosphatidic acid acyltransferase (LPAAT) produces a phosphatidic acid that serves as a precursor for the synthesis of various glycerophospholipids, major components of bacterial membrane. Some bacteria, such as *Neisseria meningitidis*, *Pseudomonas fluorescens*, and *Rhodobacter capsulatus*, have multiple LPAAT homologs. These LPAAT homologs possibly play different roles *in vivo* and contribute to the generation of the diversity of membrane phospholipids (PLs). However, enzymatic properties of these LPAAT homologs, in particular their substrate specificities, and the physiological significance of the occurrence of multiple LPAAT homologs are poorly understood, partly due to difficulty in purification of these membrane proteins in their active form. In Chapter I, I characterized one of five putative LPAAT homologs of *Shewanella livingstonensis* Ac10 named PlsC4. In Chapter II, I investigated the physiological roles of PLs containing branched-chain fatty acyl groups synthesized by PlsC4 in *S. livingstonensis* Ac10 at low temperatures. In Chapter III, I found that *Escherichia coli* has an uncharacterized LPAAT homolog named YihG. I conducted the *in vivo* characterization of YihG. Overall, this study contributes to understanding of molecular mechanism and physiological significance of generating membrane lipid diversity in bacteria.

CHAPTER I

In vivo substrate specificity of a novel lysophosphatidic acid acyltransferase homolog from *Shewanella livingstonensis* Ac10

S. livingstonensis Ac10 is a psychrotrophic bacterium and produces eicosapentaenoic acid (EPA) at low temperatures. This bacterium has five putative LPAAT homologs (PlsC1 to 5). It was previously reported that PlsC1 is responsible for the production of PLs containing EPA. In this chapter, I characterized another putative LPAAT homolog of this bacterium named PlsC4. I revealed that PLs containing 13:0 found in the parental strain were almost completely absent in the *plsC4*-disrupted mutant. The loss of these PLs was suppressed by introduction of a *plsC4*-expression plasmid. PLs containing 15:0 were also drastically decreased by the *plsC4* disruption. Gas chromatography-mass spectrometry analysis of fatty acyl methyl esters derived from PLs of the parental strain showed that the 13:0 and 15:0 groups were an 11-methyllauroyl group and a 13-methylmyristoyl group, respectively. Phospholipase A2 treatment revealed that these fatty acyl groups were linked to the *sn*-2 position of PLs. Thus, PlsC4 is a new type of LPAAT homolog that is responsible for the synthesis of PLs containing a branched-chain fatty acyl group at the *sn*-2 position and plays a clearly different role from that of PlsC1 *in vivo*.

CHAPTER II

Phenotypic analysis of a mutant of *Shewanella livingstonensis* Ac10 that lacks lysophosphatidic acid acyltransferase for the synthesis of phospholipids with a branched-chain fatty acyl group

In this chapter, I investigated the physiological functions of PLs containing branched-chain fatty acyl groups of *S. livingstonensis* Ac10. To compare the functions of branched-chain fatty acyl groups and an eicosapentaenoyl group, I analyzed the phenotypes of the *plsC4*- and *plsC1*-disrupted mutants. I used a cultivation medium of the mineral composition mimicking seawater (MB), considering that *S. livingstonensis* Ac10 was isolated from seawater. As the results, the lack of PlsC1 did not affect the growth rate and morphology in MB at low temperatures. The lack of PlsC4 also did not affect the growth rate and morphology but uniquely induced abnormal cell flocculation and deficiency of swimming motility in MB at low temperatures. The number of viable *plsC4*-disrupted mutant cells was significantly lower than that of the parental cells in the late stationary phase. Scanning electron microscopic observation revealed that the flocculated *plsC4*-disrupted mutant cells formed biofilm-like structures. Thus, PLs containing branched-chain fatty acyl groups synthesized by PlsC4 may affect the structure and function of some membrane proteins regulating the transition between the biofilm and motile states of *S. livingstonensis* Ac10 at low temperatures. These results suggested that PLs containing branched-chain fatty acyl groups and PLs containing an eicosapentaenoyl group have distinct roles from each other.

CHAPTER III

In vivo characterization of a novel lysophosphatidic acid acyltransferase homolog from *Escherichia coli*

It has long been believed that *E. coli* has only one essential LPAAT homolog named PlsC, and the deletion of *E. coli* PlsC is lethal. However, I found that *E. coli* possesses a PlsC4 ortholog named YihG, showing 39.1% sequence identity to PlsC4. YihG is also conserved in some γ-proteobacteria such as *Salmonella typhimurium* and *Vibrio cholerae*. I characterized *in vivo* function of YihG from *E. coli* and investigated its physiological roles. I found that overexpression of YihG in *E. coli* JC201 carrying a temperature-sensitive mutation in *plsC* allowed its growth at non-permissive temperature. Analysis of the fatty acyl composition of PLs from the JC201 cells overexpressing YihG and PlsC revealed that YihG facilitates the synthesis of PLs containing 14:0 and 18:1 at the *sn*-2 position and 18:1 at the *sn*-1 position, whereas PlsC facilitates the synthesis of PLs containing 16:1 and 16:0 at the *sn*-2 position and 16:0 at the *sn*-1 position, demonstrating that YihG has different substrate specificity from PlsC. Analysis of the fatty acyl composition of PLs from the *yihG*-deletion mutant revealed that the endogenous YihG introduces 18:1 into the *sn*-2 position of membrane PLs. Phenotypic analysis revealed that the lack of YihG causes high expression of FliC and enhanced swimming motility but does not affect the cell growth and morphology. These results suggested that PlsC is responsible for the synthesis of the majority of membrane PLs, whereas YihG has more specific functions related to the swimming motility by modulating fatty acyl composition of membrane PLs.