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Mini Review

Synthesis and function of phospholipids in *Staphylococcus aureus*

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ABSTRACT

Phospholipids are the major components of bacterial membranes, and changes in phospholipid composition affect important cellular processes such as metabolism, stress response, antimicrobial resistance, and virulence. The most prominent phospholipids in *Staphylococcus aureus* are phosphatidylglycerol, lysyl-phosphatidylglycerol, and cardiolipin, whose biosynthesis is mediated by a complex protein machinery. Phospholipid composition of the staphylococcal membrane has to be continuously adjusted to changing external conditions, which is achieved by a series of transcriptional and biochemical regulatory mechanisms. This mini-review outlines the current state of knowledge concerning synthesis, regulation, and function of the major staphylococcal phospholipids.

Introduction

Phospholipids are the major components of bacterial membranes and their primary function is the formation of a semipermeable diffusion barrier which allows the intracellular accumulation of metabolites in physiologically relevant concentrations, selective uptake of nutrients, defined release of secretory molecules, and prevention of entry of harmful substances. Beyond that phospholipids are involved in several important cellular processes. Respiratory energy metabolism depends on the phospholipid bilayer, as its selective permeability allows the generation of the proton gradient necessary for ATP synthesis. Localization, folding, and function of a variety of membrane-integral or associated proteins are facilitated by interaction with phospholipids. The phospholipid composition of the membrane is crucial for bacteria to cope with environmental hazards such as extreme pH, high osmolarity, or exposure to surfactant molecules. Moreover phospholipids play an important role in bacterial infection as they represent both, target and barrier for antibiotics and host defense mechanisms such as cationic antimicrobial peptides and enzymes produced by phagocytes or epithelial cells (Kraus and Peschel, 2008). To fulfill these diverse functions membrane lipid composition is variable and continuously adjusted to changing external conditions (Zhang and Rock, 2008). Furthermore lipid composition varies profoundly between bacterial species. In *Staphylococcus aureus* the cytoplasmic membrane consists of a variety of lipids including glycolipids, apolar lipids, and lipid conjugates, but the great majority is comprised of the three polar phospholipids phosphatidylglycerol (PG), lysyl-phosphatidylglycerol (L-PG), and cardiolipin (CL) (Ratledge and Wilkinson, 1988) [Fig. 1]. Recent studies have shed new light on synthesis, regulation, and function of the major phospholipids, which is outlined in this mini review with specific emphasis on *S. aureus*.

Biosynthesis of staphylococcal phospholipids

Staphylococcal phospholipid synthesis begins with the stepwise acylation of glycerol-3-phosphate (G3P) with fatty acids (Lu et al., 2006). In a first step acyl–acyl carrier protein (acyl-ACP) derived from type II fatty acid synthesis (FASII), which is used by all bacteria except corynebacteria, mycobacteria, and nocardia, as well as plants (Kikuchi et al., 1992; Parsons and Rock, 2013). Acyl-ACP is converted to acyl-phosphate (acyl-PO_4) by the cytoplasmic enzyme PlsX [Fig. 2]. When *plsX* is deleted in *S. aureus*, the mutant becomes strictly fatty-acid auxotroph (Parsons et al., 2014b) [Table 1]. Acyl-PO_4 is used as substrate by the membrane-associated G3P acyltransferase PlsY that catalyzes the acylation of G3P to 1-acyl-G3P. PlsY is essential in *S. aureus* (Chaudhuri et al., 2009). PlsC, another membrane-bound acyltransferase, subsequently transfers a second fatty acid from acyl-ACP to the carbon 2

Abbreviations: Acyl-ACP, acyl–acyl carrier protein; Acyl-PO_4, acyl-phosphate; A-PG, alanine-PG; CAMP, cationic antimicrobial peptide; CDP-DAG, cytidine diphosphate-diacylglycerol; CL, cardiolipin; CTP, cytidine triphosphate; eFA, extracellular fatty acids; FASII, type II fatty acid synthesis; Glc_2-DAG, di-glucosyl-diacylglycerol; G3P, glycerol-3-phosphate; L-PG, lysyl-phosphatidylglycerol; LTA, lipoteichoic acid; PG, phosphatidylglycerol; PG-P, phosphatidylglycerolphosphate; PMNs, human polymorphonuclear leukocytes; PtdOH, phosphatidic acid.

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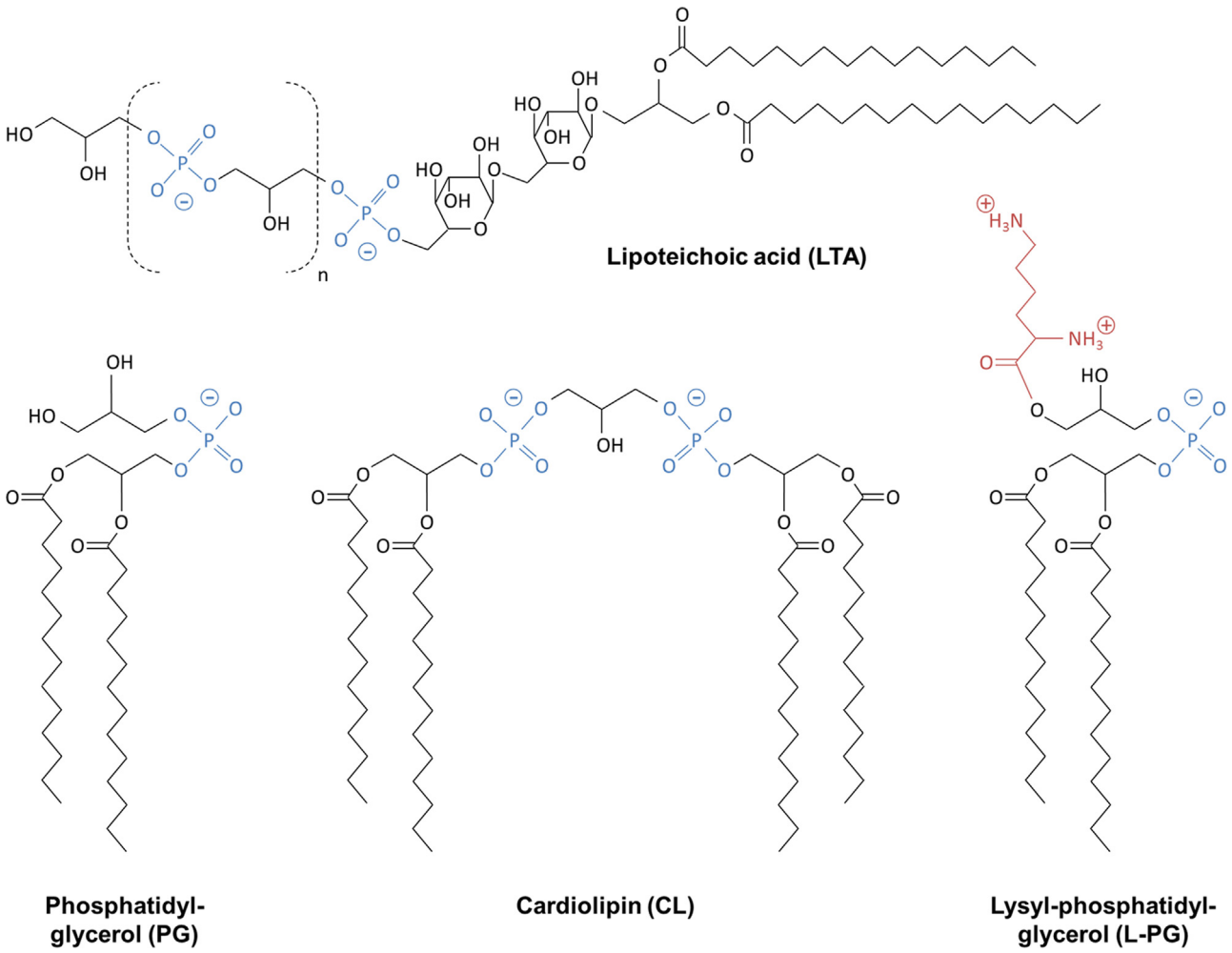

Fig. 1. Chemical structures of the three major staphylococcal phospholipids and of lipoteichoic acid (modified after Ernst and Peschel, 2011).

(*sn*-2) position of acyl-G3P, thereby generating phosphatidic acid (PtdOH), the universal bacterial phospholipid precursor. The PlsX/Y pathway is present in most known bacterial genomes including all staphylococcal genomes (Lu et al., 2006). The only bacterial group that lacks these genes is the Xanthomonadales in the class of gamma-proteobacteria. This order has the PlsB gene instead, which codes for an acyltransferase that uses acyl-ACP or acyl-CoA for the acylation of the carbon 1 position of G3P. In other bacterial genomes, mainly in gamma-proteobacteria, PlsB can be found in addition to PlsX/Y. PlsC is universally distributed in bacteria.

In addition to fatty acids synthesized by FASII, *S. aureus* can incorporate extracellular fatty acids (eFA) into PtdOH by a recently discovered fatty acid kinase-dependent pathway (Parsons et al., 2014b). In this pathway protonated fatty acids bind to one of two fatty-acid binding proteins (FakB1/FakB2) depending on their saturation state upon passive diffusion though the cytoplasmic membrane (Parsons et al., 2014a). Saturated fatty acids specifically bind to FakB1 whereas FakB2 preferentially binds unsaturated fatty acids. The ATP-binding protein FakA interacts with FakB and phosphorylates the bound fatty acid to yield acyl-PO_4, which in turn is deposited to the membrane in exchange for a new fatty acid. Depletion of FakA disables *S. aureus* to incorporate eFAs. Furthermore the mutant shows severely attenuated production of virulence factors, increased resistance to antimicrobial peptides (Li et al., 2009), and no α-hemolysin production (Bose et al., 2014) suggesting that FakA depletion leads to general stress response. Acyl-PO_4 is either used by PlsY to acylate G3P or converted to acyl-ACP by PlsX and subsequently elongated by FASII or used by PlsC to acylate the *sn*-2 position of G3P. This matches the observation that depletion of PlsX is lethal in *S. aureus* but can be compensated by addition of free fatty acids to the growth medium (Parsons et al., 2014b). Of note the *plsX* mutant remained sensitive to the FASII inhibitor AFN-1252, which means *S. aureus* cannot live exclusively on eFA but needs de novo fatty acid synthesis to produce phospholipids. Unlike other bacteria *S. aureus* is incapable of using fatty acids for energy generation by beta-oxidation (Parsons et al., 2011).

In the next step CDP-diacylglycerol (CDP-DAG) is synthesized from PtdOH and cytidine triphosphate (CTP) by the phosphatidate cytidylyltransferase Cds. The CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase PgsA then catalyzes the replacement of cytidine monophosphate with glycerolphosphate generating phosphatidylglycerolphosphate (PG-P). *pgsA* is essential in *S. aureus* (Martin et al., 1999) and mutations in this gene have been linked to daptomycin-nonsusceptibility (Peleg et al., 2012). Finally PG-P is dephosphorylated by a yet unidentified phosphatidylglycerophosphatase (PgpP) to yield PG the major phospholipid of *S. aureus* (Kanemasa et al., 1972).

Part of the PG pool is further processed to CL by the two CL synthases Cls1 and Cls2 (Koprivnjak et al., 2011). These proteins belong to the phospholipase D class of enzymes and catalyze the fusion of two PG molecules to CL accompanied by the release of glycerol (Short and White, 1972). Cls2 is the major CL synthase under normal growth conditions, whereas Cls1 is necessary for CL synthesis under acid stress (Tsai et al., 2011; Ohniwa et al., 2013). *cls* genes

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