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Activation of the Cpx phosphorelay signal transduction system in acidic phospholipid-deficient *pgsA* mutant cells of *Escherichia coli*

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ABSTRACT

The *pgsA* gene encodes the enzyme for the committed step in the synthesis of acidic phospholipids in *Escherichia coli*, and the *pssA* gene does the same for zwitterionic phospholipid. It has been reported that the Rcs and Cpx phosphorelay signal transduction systems are activated in *pgsA*- and *pssA*-defective mutants, respectively. In this study, we show that the Cpx system is activated also in a *pgsA* mutant, whereas the Rcs system was not activated in a *pssA* mutant. Lack of phosphatidylglycerol in *pgsA* mutants causes inadequate modification of lipoproteins, resulting in poor localization to the outer membrane. The outer membrane lipoprotein RcsF is necessary for the response of the Rcs system to various stimuli, and Rcs activation in *pgsA* mutants involves inner membrane mislocalization of this lipoprotein. The outer membrane lipoprotein NlpE, however, while necessary for the surface adhesion-induced Cpx response, was not involved in Cpx activation in the *pgsA* mutant.

1. Introduction

The membranes of *Escherichia coli* (excluding the outer leaflet of the outer membrane, which is made up of lipopolysaccharide) are composed of acidic phospholipids (phosphatidylglycerol and cardiolipin) and zwitterionic phospholipid (phosphatidylethanolamine) [1] (Fig. 1). These are synthesized from a common intermediate, CDP-diacylglycerol. The committed steps to the synthesis of acidic and zwitterionic phospholipids are catalyzed by the gene products of *pgsA* and *pssA*, respectively (Fig. 1). A *pgsA* null mutant completely lacking phosphatidylglycerol and cardiolipin has been shown to be viable if it lacks the major outer membrane lipoprotein encoded by the *lpp* gene [2–4]. A *pssA* null mutant completely lacking phosphatidylethanolamine was found to be viable if grown in the presence of divalent metal ions at high concentrations [5,6].

It has been reported that in the *pgsA* null mutant the Rcs phosphorelay signal transduction system is activated, causing a thermosensitive growth defect [7,8], and that in the *pssA* null mutant the Cpx phosphorelay signal transduction system is activated [9]. The Rcs system is composed of the sensor kinase RcsC, the phosphotransmitter YojN (also called RcsD) and the response regulator RcsB [10]. The Cpx system is composed of the sensor kinase CpxA and the response regulator CpxR [11]. These systems respond to different kinds of envelope stresses, and both are implicated in biofilm formation. We wondered if the *pgsA* mutation would cause Cpx activation and if the *pssA* mutation might cause Rcs activation. We found that the former is the case, whereas the latter is not.

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture media

The *E. coli* strains and the plasmids used for this study are listed in Table 1. For the detailed strain construction procedure, see Supplementary Table S1. Primers used for this study are listed in Supplementary Table S2. For placement of the *pssA* gene under the control of the P_{BAD} promoter, the λ InCh system [12] was used. In pAI2, a NotI restriction site was created between the termination codon and the ρ-independent transcription terminator of the *cpxP* gene. The FLK2 cassette from pTOF30 [13] was introduced into the NotI site to construct pAI3. Replacement of the corresponding chromosomal region with the cloned fragment in pAI3 resulted in operon fusion, in which *lacZ* was fused downstream of *cpxP*. Genetic and recombinant DNA procedures were based on standard methods [14,15].

Luria–Bertani (LB) medium [14], buffered LB medium [7], and M9 medium [14] were used. For plates, media were solidified with 1.5% agar. When appropriate, antibiotics were included at the following concentrations (in μg ml^{-1}) for multicopy and single-copy resistance genes, respectively: ampicillin, 50 and 20; chloramphenicol, 50 and 10; kanamycin, 50 and 20; tetracycline, 3 (for single copy); spectinomycin, 50 and 10. L-Arabinose was used at a concentration of 0.2% as the inducer of the P_{BAD} promoter. Cell

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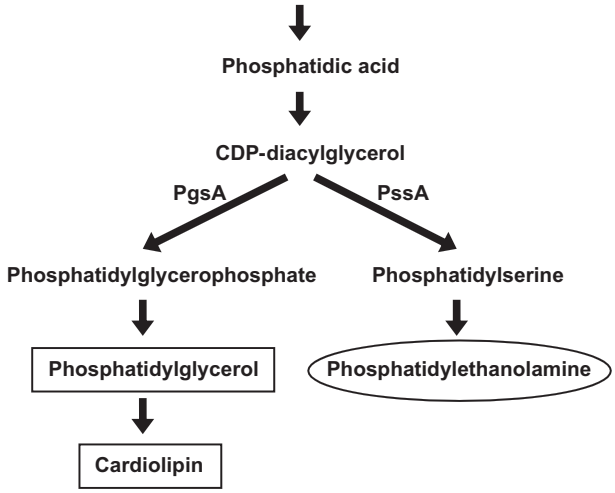

Fig. 1. The biosynthetic pathway for the major phospholipids in *E. coli*. Major acidic and zwitterionic phospholipids are enclosed in rectangles and an oval, respectively. Reactions catalyzed by *pgsA* and *pssA* gene products are indicated.

growth was monitored with a Klett-Summerson colorimeter equipped with a No. 54 filter.

2.2. Biochemical procedures

Cellular phospholipids were extracted and analyzed as described previously [16]. Densities of spots on thin layer chromatograms were quantified by High Speed TLC Scanner CS-920 (Shimadzu). The β-galactosidase assay method using *o*-nitrophenyl-β-D-galactoside as substrate and the unit definition were as described in Wang and Doi [17].

3. Results

3.1. Characterization of pgsA- and pssA-repressed cells

For this study we used strains in which *pgsA* or *pssA* is under the control of the L-arabinose-inducible P_{BAD} promoter, instead of using *pgsA* null or *pssA* null strains in which activation of the Rcs or Cpx system was first reported. The strain construction procedures were carried out in the presence of arabinose, and the activities of the signal transduction systems were tested in the absence of the inducer.

In the presence of arabinose, the *pgsA*-repressible strain UE107 and the *pssA*-repressible strain UE110 showed phospholipid composition similar to the wild type MG1655 (Table 2). In the absence of arabinose, phosphatidylglycerol and cardiolipin contents were greatly reduced in UE107, and the phosphatidylethanolamine content was significantly reduced in UE110. Although removal of the inducer did not lead to complete loss of phosphatidylglycerol in UE107 or of phosphatidylethanolamine in UE110, the growth phenotypes of these strains in the absence of arabinose were the same as those of *pgsA* null and *pssA* null strains: UE107 was thermosensitive at 42 °C, and UE110 was dependent on supplementation of a high concentration of Mg^{2+} for growth. Moreover, Rcs and Cpx signal transduction systems were activated in the *pgsA*- and *pssA*-repressed strains, respectively, (see below) just as in the corresponding null strains.

3.2. Cpx activation in the pgsA-repressed cells

The *cpxP* gene is positively regulated by the Cpx system [18]. In this study a *cpxP-lacZ* operon fusion was constructed and used for monitoring the activation of the system. Since CpxP is a regulatory component of the Cpx system [19], *cpxP* was kept intact and *lacZ*

Table 1
Bacterial strains and plasmids used for this study.

Strain or plasmid	Relevant genotype or description	Construction, source, or reference
Strains		
MG1655	Wild type	Laboratory collection
EDCM367	MG1655 Δ*lacZY*	[31]
UE107	EDCM367 *Δara714 lpp-2 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pgsA*) Δ*pgsA*::FRT[a]	This study
UE110	EDCM367 *Δara714 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pssA*) Δ*pssA10*::*cam*	This study
SG20781	MC4100 *cpsB10*::*lac*-Mu-*imm* λ	[21]
UE90	SG20781 Δ*ara714*	This study
UE46	SG20781 Δ*ara714 lpp-2 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pgsA*) *pgsA30*::*kan*	[7]
UE93	SG20781 Δ*ara714 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pssA*) Δ*pssA10*::*cam*	This study
AIT01	EDCM367 Φ(*cpxP-lacZ*)[b] Km^r Cm^s	pTOF procedure [13] using pAI3
AIT02	EDCM367 *Δara714 lpp-2 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pgsA*) Δ*pgsA*::FRT Φ (*cpxP-lacZ*) Km^r	P1(AIT01) × UE107
AIT03	EDCM367 *Δara714 Δ*(λ *attL-lom*)::(*bla araC* P_{BAD}-*pssA*) Δ*pssA10*::*cam* Φ (*cpxP-lacZ*) Km^r	P1(AIT01) × UE110
TR51	MC4100 *cpxR1*::*spc*	[19]
AIT04	AIT01 *cpxR1*::*spc*	P1(TR51) × AIT01
AIT05	AIT02 *cpxR1*::*spc*	P1(TR51) × AIT02
AIT06	AIT03*cpxR1*::*spc*	P1(TR51) × AIT03
S330A21	W3110 *lpp-2 pgsA30*::*kan rcsA330*::IS*5 rcsC*::mini-Tn*10 cam*	[7,8]
AIT07	AIT01 *rcsC*::mini-Tn*10 cam*	P1(S330A21) × AIT01
AIT08	AIT02 *rcsC*::mini-Tn*10 cam*	P1(S330A21) × AIT02
AIT09	AIT03 *rcsC*::mini-Tn*10 cam*	P1(S330A21) × AIT03
WBS262	MC4100 *nlpE*::*spc*	[29]
AIT10	AIT01 *nlpE*::*spc*	P1(WBS262) × AIT01
AIT11	AIT02 *nlpE*::*spc*	P1(WBS262) × AIT02
AIT12	AIT03 *nlpE*::*spc*	P1(WBS262) × AIT03
Plasmids		
pTOF24	pSC101 derivative; *repA*(Ts) *sacB* Cm^r Km^r	[13]
pAI2	pTOF24 ′*cpxP fieF*′; Cm^r Km^s	This study
pTOF30	pUC18 derivative; *lacZ aph* Ap^r Km^r	[13]
pAI3	pTOF24 ′*cpxP lacZ aph fieF*′; Km^r Cm^r	This study
pHR741	pGB2 $lacI^q$ P_{204}-*djlA*	[7]

[a] FRT, FLP recombinase recognition target.
[b] Φ(*cpxP-lacZ*), operon fusion in which *lacZ* is fused downstream of *cpxP*.