Deletion of penicillin-binding protein 1b impairs biofilm formation and motility in *Escherichia coli*

Akash Kumar a, Sujoy K. Sarkar a, Dipankar Ghosh b, Anindya S. Ghosh a,*

a Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal 721302, India
b Special Center for Molecular Medicine, Jawaharlal Nehru University, New Delhi 110067, India

Received 23 November 2011; accepted 23 January 2012
Available online 7 February 2012

Abstract

The major bifunctional transpeptidases/transglycosylases of *Escherichia coli*, penicillin-binding proteins (PBPs) 1a and 1b, were evaluated for their influence on biofilm formation. While the PBP1a mutant was unaffected, the PBP1b mutant exhibited significantly decreased biofilm formation and motility. Interestingly, the extracellular indole concentration was higher in the PBP1b mutant, and similar phenotypic defects were replicated in the wild-type upon addition of exogenous indole. Expression of PBP1b in trans substantially decreased indole production and restored normal phenotypes. Results further suggest that *rpoS* deletion has a counteracting effect on the *mrcB* mutant. These findings indicate that PBP1b deletion influences biofilm formation and motility, possibly through indole.

© 2012 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: *Escherichia coli*; Penicillin-binding protein 1b; Biofilm; Indole

1. Introduction

The stress-bearing and shape-maintaining murein sacculus is essential for nearly all bacteria. Penicillin-binding proteins (PBPs) catalyze the final stages of murein biogenesis and are targets of β-lactam antibiotics (Ghosh et al., 2008). This tough polysaccharide layer is constructed from long glycan chains cross-linked to one another via peptide chains that form a continuous matrix to envelop the cell. Because bacteria are encased within this polymeric shell, their growth and morphogenesis are intimately linked to peptidoglycan (PG) synthesis and remodeling (Paradis-Bleau et al., 2010).

PBPs 1a and 1b are bifunctional enzymes with both transglycosylase and transpeptidase activity, and are required for insertion of new strands and cross-linking of the PG layer during both elongation and septation (Ghosh et al., 2008).

Deletion of one of the two genes, either *mrcA* (encoding PBP1a) or *mrcB* (encoding PBP1b), does not affect cell survival, as both have overlapping biochemical functions (Typas et al., 2010). However, a mutant lacking PBP1b is outcompeted by its parental strain in co-culture during stationary phase (Pepper et al., 2006), suggesting that PBP1b plays an important role in competitive stationary-phase survival of *Escherichia coli*. Biofilm formation is an important stationary-phase-associated phenotype, shown to increase bacterial survival (Corona-Izquierdo and Membrillo-Hernandez, 2002). Therefore, in the present study we examined whether this could be a reason behind the stationary phase-specific competition-defective (SPCD) phenotype of the PBP1b deletion mutant.

2. Materials and methods

2.1. Bacterial strains, materials and growth conditions

Bacterial strains used in this study were derived from *E. coli* 2443. PBP and *rpoS* mutants were constructed following
the P1 transduction procedure as described previously (Denome et al., 1999). BMM910 (ZK2686ΔproS::Kmr) was a gift from Prof. David J. Clarke (University College Cork) and the plasmid p588-1 from Prof. Kevin D. Young (University of Arkansas Medical School). The strains were grown in Luria Bertani (LB) broth and/or agar (Hi-Media). Chloramphenicol (20 μg ml⁻¹), kanamycin (50 μg ml⁻¹) and tetracycline (25 μg ml⁻¹) were added where necessary. Unless otherwise specified, chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Biofilm formation assays

Biofilm formation was assayed in microtiter plates as described previously (Gallant et al., 2005), with some modifications. In brief, cultures (A₆₀₀ ≈ 0.2) were inoculated in LB and incubated for 24 h at 37 °C. After incubation, planktonic cells were removed by washing with PBS. The formed biofilms were stained with 0.5% crystal violet (CV) for 15 min and excess CV was removed by washing with water. Finally, the retained CV was solubilized with absolute ethanol and absorbance was measured at 600 nm.

2.3. Microscopic analysis of biofilms

Biofilm development was observed through scanning electron microscopy (SEM). Cells were grown on glass coverslips. Cover-slips were removed after incubation, rinsed with PBS (pH 7.0) and fixed with 2% glutaraldehyde for 2 h at 4 °C. Finally, the biofilms were dehydrated in a graded series of ethanol (25, 50, 75, and 100%) and analyzed through SEM in 10,000× magnification. In case of fluorescence microscopy, biofilms were stained with BacLight Live/Dead dye (Invitrogen Inc., CA, USA) and viewed at 200× magnification through an Olympus IX 51 fluorescence microscope (Olympus Inc., Japan) fitted with an Evolution VF CCD Camera (Olympus Inc., Japan) and analyzed through Image Pro software (Media Cybernetics Inc., MD, USA).

2.4. Extracellular indole assay

The extracellular concentration of indole was measured with Kovac’s reagent (p-dimethylamino-benzaldehyde, 10 g; HCl, 50 ml; amyl alcohol, 150 ml) as reported previously (Lee et al., 2007). The optical density of 8 h culture was adjusted to A₆₀₀ ≈ 1.0, centrifuged and the supernatant (1.5 ml) was mixed with Kovac’s reagent (0.6 ml). The mixture was incubated at room temperature for 2 min to allow formation of two liquid phases. From the upper phase, 0.1 ml was aspirated and mixed with 0.9 ml of HCl–amyl alcohol mixture (30 ml of HCl and 90 ml of amyl alcohol), and absorbance was measured at 540 nm.

2.5. Motility assay

Swimming motility was assessed as described previously (Lee et al., 2007), with little changes. Swim plates were prepared by adding 0.25% agar to LB broth and inoculated with bacteria from an overnight culture. The plates were incubated at 37 °C and the diameters of bacterial migration halos were recorded after 14 h.

3. Results

3.1. Effect of PBP1b deletion on biofilm formation and motility

The deletion mutants of PBP1a (ΔmrcA) and PBP1b (ΔmrcB), constructed from wild-type strain E. coli 2443, were assessed quantitatively as well as qualitatively to understand their role in biofilm formation. While biofilm formation by the PBP1a mutant was unaffected, the PBP1b mutant produced significantly (~52%) less biofilm compared to the wild-type (Fig. 1). The biofilms visualized through scanning electron microscopy and fluorescence microscopy showed similar results (Fig. 3). In order to confirm that the difference in biofilm formation between the two strains did not arise from a difference in the growth rate of the strains, a growth assay was carried out. The PBP1b mutant did not show significant reduction in growth rate as compared to the wild-type (Supplementary Fig. 1). As motility plays an important role in the initial attachment process of biofilm formation by E. coli (Pratt and Kolter, 1998), we checked motility to investigate the mechanism influencing biofilm formation by the PBP1b mutant. The swimming motility assay revealed significantly reduced (~30%) motility for the PBP1b mutant as compared to the wild-type (Fig. 2).

3.2. Effect of PBP1b deletion on extracellular indole production

As both biofilm and motility phenotypes were affected, we compared the PBP1b mutant and the wild-type for production
of indole, which is an interspecies signal that can decrease biofilm formation by repressing motility (Lee et al., 2007). Indole concentrations measured at different time intervals showed that the concentration of indole initially increased with an increase in incubation time, reached a maximum value and then decreased (Supplementary Fig. 2). After 8 h, extracellular indole produced by the PBP1b mutant was significantly greater (~30%) compared to the wild-type (Fig. 2). To check whether the increased indole production could cause decreased biofilm formation by the wild-type, exogenous indole (110 mM), in a concentration equal to the difference between the mutant and wild-type levels, was added to the growth media. Interestingly, addition of exogenous indole decreased (~50%) biofilm formation by the wild-type to a level similar to that of the PBP1b deletion mutant, thus substantiating the crucial role of indole (Fig. 1).

3.3. Effect of expression of PBP1b in trans on extracellular indole production, biofilm formation and motility

To draw a direct correlation between PBP1b deletion and a defect in biofilm formation, cloned PBP1b was expressed in trans under the control of the arabinose-inducible promoter (pBAD) in the PBP1b mutant. Upon optimal expression (0.5 mM arabinose) of PBP1b from plasmid p588-1 in the PBP1b mutant, the extracellular indole concentration substantially reversed to parental levels (Fig. 2). Moreover, biofilm-forming ability (Fig. 1) and motility (Fig. 2) reverted back to near wild-type levels. The results obtained demonstrated the potentiating role of PBP1b in biofilm formation and motility, as PBP1b deletion decreased both phenotypes and its complementation rescued these defects.

3.4. Effect of rpoS deletion on biofilm formation and motility

The rpoS gene controls production of indole and its inactivation severely affects indole production (Lacour and Landini, 2004). Therefore, to further investigate the effect of indole on biofilm, the rpoS gene was deleted from the wild-type and the PBP1b mutant by moving the rpoS deletion from BMM910 (Ferrieres et al., 2009) through P1 transduction. Deletion of rpoS resulted in approximately equal levels of extracellular indole production in the double-mutant (ΔmrcBΔrpoS) as compared to the wild-type (i.e. for ΔrpoS) (Fig. 2). Likewise, deletion of rpoS led to a small increase (~18%) in biofilm formation by the wild-type (i.e. for ΔrpoS) strain, but rpoS deletion substantially increased (~130%) biofilm formation by the mrcB mutant (i.e. for ΔmrcBΔrpoS) (Fig. 1). Similarly, the
double-mutant (ΔmrcBΔrpoS) showed significantly increased (~54%) motility as compared to the mrcB mutant, while rpoS mutant had little effect (~13% increase) on motility compared to the wild-type (Fig. 2). These results apparently associated indole with the reduction in biofilm formation and motility against an mrcB mutant background.

4. Discussion

Considering the results obtained, it appears that the inhibitory effect upon biofilm formation by the mrcB mutant probably involves rpoS, which is stimulated in response to cell envelope stress (Laubacher and Ades, 2008) generated due to the PBP1b deletion. Stimulation of rpoS might promote the production of indole that acts as a signal molecule in the stationary phase (Lacour and Landini, 2004). Indole, thus produced because of envelope stress, possibly inhibits E. coli K-12 biofilm formation and expression of genes related to motility (Lee et al., 2007; Wang et al., 2001). It is interesting to note that, similarly, oxidative stress has also been shown to suppress formation of E. coli biofilm via indole signaling (Kuczynska-Wisnik et al., 2010). There could be many possible reasons behind increased levels of indole production by the mrcB mutant. Recently, it was proposed that proteins induced by indole are able to ensure survival of the bacterium under stress to a certain degree, and therefore biofilm (a relatively more expensive mechanism) need not be further developed (Hu et al., 2010). Evidently, indole has also been shown to enhance acid resistance in E. coli (Hirakawa et al., 2010) and to induce the Cpx, Bae and σE envelope stress response pathways involved in maintenance, adaptation and protection of the bacterial envelope in response to a variety of stressors in E. coli (Bury-Mone et al., 2009; Raivio, 2005).

Although further research might be required to more clearly elucidate this, the present study identifies a new role for PBP1b in regulating E. coli biofilms, and suggests diminished biofilm formation as being one of the reasons for the stationary phase-specific competition-defective (SPCD) phenotype of the PBP1b deletion mutant. In conclusion, these findings together with previous genetic evidence indicate that indole signaling plays a crucial role in the mechanism underlying biofilm suppression due to the PBP1b deletion.

Acknowledgments

We are grateful to Professor Kevin D. Young of the University of Arkansas Medical School for his valuable comments on the manuscript and constructive suggestions. This work was supported by a grant from the Council of Scientific and Industrial Research (CSIR), Govt. of India to ASG. AK was supported by a Research Fellowship from the University Grant Commission (UGC), Govt. of India.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.resmic.2012.01.006.

References


