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LPXTGase of *S. pyogenes* is Internally Cross-Linked Through the ε-Amino Groups of Lysines and the Cross-Linking is Catalyzed by Penicillin-Binding Proteins

Sung G Lee, Assaf Raz, Vincent A Fischetti*

Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, USA

***Corresponding author:** Vincent A. Fischetti, Laboratory of Bacterial Pathogenesis and Immunology, the Rockefeller University, New York, USA. Tel: +1-2123278166; Fax: +1-2123277584; Email: vaf@rockefeller.edu

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Abstract

LPXTGase of *S. pyogenes* contains 7 lysine residues. However, trypsin digestion of the enzyme yields only two tryptic fragments. Exhaustive dinitrophenylation of the enzyme and subsequent hydrolysis of the dinitrophenylated enzyme by 6N HCl revealed that, of the 7 lysine residues present in the enzyme, only two lysine residue are dinitrophenylated, while five are not, indicating that both α-amino group and Ɛ-aminogroups of five lysine residues are linked internally to uncharacterized amino acids in the enzyme. When *S. pyogenes* was grown in a sub-lethal dose of penicillin, LPXTGase synthesis in the organism is greatly reduced, suggesting that the cross-linking in the LPXTGase molecule is mediated by penicillin binding proteins. A knockout of the transpeptidase domains of penicillin binding proteins 1a and 1b produced less LPXTGase, but a transpeptidase knockout mutant of a single penicillin binding protein did not completely abolish LPXTGase production, indicating that more than one penicillin binding protein cooperatively participate in the internal cross-linking of LPXTGase.

Keywords: ε-Amino Groups; Cross-Linked; Penicillin Binding Proteins; Peptidoglycan, *S. aureus;* Sortase; *S. pyogenes*

Introduction

Precursors of cell-surface proteins in gram-positive bacteria contain a consensus LPXTG motif near their C-termini [1]. The proproteins are cleaved within this motif during transport of the proproteins across cell membrane and the newly created C-termini become covalently linked to the cell wall peptidoglycan. Schneewind and his 3 colleagues found an enzyme, sortase, which cleaves after threonine of LPXTG motif of pro-Protein A in *S. aureus* and showed further that the same enzyme catalyzes the covalent linking of the carboxyl group of threonine to the glycine of the cross-bridge that links muramyl pentapeptides of the peptidoglycan super structure [2-4]. Soon afterward we discovered a different enzyme from both *S. pyogenes* and *S. aureus* that also cleaves the LPXTG sequence [5,6]. We found that this enzyme, which we named LPXTGase, cleaves the LPXTG sequence several hundred times faster than sortase [5]. The importance of LPXTGase in bacterial survival is unclear currently. Because of the roughly 30-minute division time of streptococci and staphylococci and the large number of surface proteins that require anchoring to the cell wall peptidoglycan through an LPXTG motif in this short time period, the relatively slow activity of sortase [5] may not be sufficient for proper anchoring of all surface proteins and LPXTGase may be mobilized during active logarithmic growth. LPXTGases are unusual enzymes in many respects. For example, LPXTGase from both *S. pyogenes* and *S. aureus* are highly glycosylated, which is essential for enzyme activity [5,6]. Moreover, these enzymes contain D-alanine and unconventional amino acids [6,7], indicating that they are not synthesized through the conventional ribosomal process. We also noted that the amino acid composition of LPXTGases from staphylococci and streptococci are very similar to the cell wall peptidoglycan of their respective organisms; the *S. pyogenes* enzyme having more alanine and the *S. aureus* enzyme more glycine at the same ratios found in their respective peptidoglycans [6]. Unfortunately, since the synthesis of LPXTGase is not gene dependent, determining a

phenotypic change through gene knockout experiments is not an option. In our previous publication [5] we discovered that while LPXTGase from *S. pyogenes* contained 7 lysines, only 2 peptide fragments (one small and one large) could be released after trypsin digestion. We therefore suspected that the inability of trypsin to cleave LPXTGases after every lysine may be the result of linkage of the Ɛ-amino groups of lysines to carboxyl groups within the enzyme just as the Ɛ-amino group of lysine in the muramyl pentapeptide is cross-linked internally to the carboxyl group of the cross-bridge amino acid in the peptidoglycan. Here we show that when we dinitrophenylated the *S. pyogenes* LPXTGase, and acid hydrolyzed it to individual amino acids, we find that of seven lysine residues present in the enzyme, five were not dinitrophenylated at the Ɛ-amino group, indicating that the Ɛ-amino groups of five lysines are involved in internal cross-linking of the enzyme. We also show evidence that penicillin binding proteins (PBP) are responsible for this cross-linking.

Materials and Methods

Enzyme

LPXTGase was purified from *S. pyogenes* to near homogeneity as described previously [5].

Substrates for LPXTGase

Two different substrates were used for our experiments. The oligopeptide of the first substrate, KRQLPSTGETANPFY, was synthesized by the Proteomic Center of the Rockefeller University. The tyrosine of the peptide was iodinated with ^{125}I using iodobeads (Sigma) and the labeled peptide was linked to carboxymethyl-glass beads (Sigma). The linking was catalyzed by a carbodiimide, N-(3-5dimethylaminopropyl)-N-ethylcarbodiimide (Sigma), as described previously [5]. The oligopeptide of the second substrate, KRQLPSTGETANPWSSWC was synthesized by the Proteomic Center of the Rockefeller University. Coumarin-maleimide conjugate (Invitrogen) was linked to the carboxy terminal cysteine by the Proteomic Center. Usually 200 mg carboxymethyl glass beads (50 µM) were suspended in 2 ml of 100 mM MES buffer, pH 4.8, in a 15 ml conical tube, 20 mg of the second peptide linked to coumarin-maleimide (10 µM) in 1 ml of the same buffer was added to the tube, and then 19.2 mg of carbodiimide (100 µM) powder was added. The mixture was gently shaken at 37° C for 4 hours. The reaction mixture was introduced into a small column and unreacted peptide-coumarin-maleimide was washed away by passing >10 column volumes of water through the column. The bead-bound substrate was suspended in 20 ml of 20 mM Tris-HCl buffer, pH 7.5, containing 0.1% Brij 35.

Enzyme Assay

10 µl suspension of bead-bound substrate and 40µl enzyme in 20mM Tris-HCl buffer, pH 7.5, containing 0.1% Brij 35 were introduced into microfuge tubes and the mixture was shaken vigorously for one hour at 37° C. When radioactive iodine labeled substrate was used, the reaction mixture was centrifuged and 40µl supernatant was withdrawn and radioactivity counted. With second substrate, 0.75 ml of water was added to reaction mixture and centrifuged. From the tube 0.7 ml was withdrawn, and fluorescence was measured at emission wavelength of 465 nm and excitation wavelength of 385 nm.

Other Materials

1-Fluoro-2,4-Dinitrobenzene (FDNB) and N_ε-dinitrophenyl-lysine (N_ε-DNP-lysine) were purchased from Sigma. Silica gel plates were purchased from EMD.

Dinitrophenylation of Free Amino Groups in LPXTGase

About 320 µg of highly purified LPXTGase of *S. pyogenes* [5], was incubated in a 300 µl reaction volume of 30 mM borate buffer, pH 10 [8], with 80-fold molar excess of FDNB for 42 hours at room temperature with vigorous shaking to ensure that all primary amine groups in the enzyme become dinitrophenylated. The reaction mixtures were then lyophilized to dry residues, and several washings of the residues with acetone removed unreached FDNB.

Dinitrophenylation of Lysine

In a 500 µl volume of 30 mM borate buffer, 0.2 mM lysine was incubated for 8 hours with 0.2 mM FDNB at room temperature with vigorous shaking.

Acid Hydrolysis of Dinitrophenylated LPXTGase and Separation of Components

DNP-LPXTGase was dissolved in 1.2 ml of 6N HCl, placed in a glass tube, and after sealing the tube the enzyme was hydrolyzed in an oven at 110° C for 4 days. After removing HCl from the hydrolysate by a Speed-Vac, the hydrolysis products were separated by silica gel thin layer chromatography using water/isopropanol/ethyl acetate (1:3:1) as running solvent. Lysine, which remains at the origin (Figure 1, see Figure 2 for standards), and the two UV absorbing bands at Rf of 0.5 to 0.65 (DNP-lysines) (Figure 1) were scraped off the thin layer plate and placed in microfuge tubes. To determine the free lysine content in the sample from the origin, it was mixed with 3.3 mg of ninhydrin in 1 ml of water, and heated for 30 minutes at 100^0 C, and absorbance was measured at 480 nm. A similar reaction was performed with lysine standards. To determine DNP-lysine content of the two UV absorbing bands in (Figure 1), (which were combined in one tube) they were dissolved in 1 ml of 50% methanol, and absorbance at 360 nm was measured. A similar reaction was performed with N_ε-DNP-lysine standards at various concentrations.

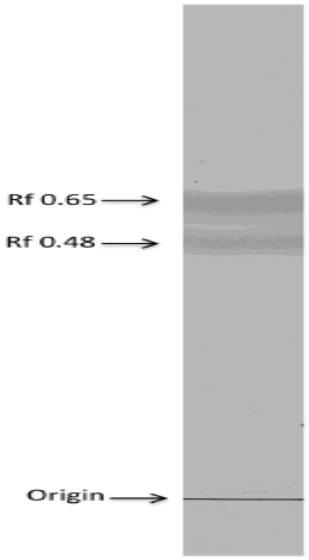

Figure 1: Silica gel thin layer chromatography of acid hydrolysate of dinitrophenylated LPXTGase. LPXTGase was dinitrophenylated and hydrolyzed with 6N HCl as described in methods. Running solvent was water/isopropanol/ethyl acetate (1:3:1).

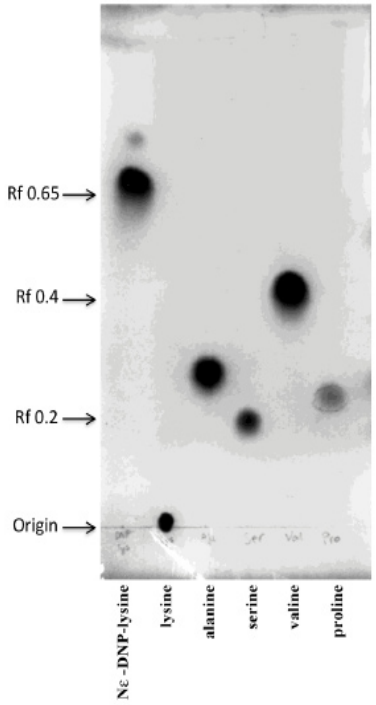

Figure 2: Silica gel thin layer chromatography of N_{ε}-2,4-DNP-lysine and free amino acids present in *S. pyogenes* LPXTGase. Running solvent was water/isopropanol/ethyl acetate (1:3:1).

Effect of Penicillin G on LPXTGase Synthesis

S. pyogenes was grown in 1-liter Todd-Hewitt broth supplemented with 1% yeast extract. When the $O.D._{650nm}$ reached 0.2 (early-exponential growth phase), 40 μg of penicillin was added to the culture, and the cells were harvested when the $O.D._{650nm}$ reached 1.3 (late-exponential growth phase). Identification of LPXTGase was as previously described [6]. Briefly, cells were suspended in 40 ml of 20 mM phosphate, pH 6.5, and the cells were lysed with PlyC, a lysin from group C streptococcus phage [9], for one hour. The lysate was centrifuged at 8,000 rpm for 20 minutes, and supernatant was collected. The supernatant was passed through DEAE-cellulose column (10 cm x 2.5 cm) and flow through material was collected. After reducing its volume to about 2 ml by ultrafiltration, the material was subjected to gel filtration using a Sephadex G50 column (45 cm x 1.5 cm). Enzyme activity was measured with radioactive iodine labeled bead-bound substrate. For comparison, a control sample was grown without penicillin, harvested, and enzyme activity was determined as above.

Construction of Transpeptidase Domain Knockout Within Penicillin Binding Proteins 1a, 1b

For the deletion of PBP1a transpeptidase domain, an 858bp DNA fragment encompassing amino acids 111-389 of this protein (homologous to M6_Spy1401 on the sequenced MGAS10394 genome), was amplified from *S. pyogenes* D471 genomic DNA using primers _5PBP1a_int (5'-cgcgtcgacggtgctgcctttcataatctaacgagtc-3'), and 3PBP1a_int (5'-cccagatctctaaacaccagattcaatagcaggagcatag-3'). A stop codon was introduced on the 3'primer, following amino acid 389. The resulting PCR fragment was 151 inserted between the SalI and BglII sites of pFW8 [10], yielding pAR240-PBP1a. For the deletion of PBP1b transpeptidase domain, a 1198 bp DNA fragment encompassing amino acids 27-418 of this protein (homologous to M6_Spy0130 on the sequenced MGAS10394 genome), was amplified from *S. pyogenes* D471 DNA using primers 5PBP1b_int (5'-ccggtcgacgccggtgctattacgtacgttgagac-3') and 3PBP1b_int (5'- cccagatctctaaccaacaaaacctaacacagcaccagtc-3'). A stop codon was introduced on the 3' primer, following amino acid 418. The resulting PCR fragment was inserted between the SalI and BglII sites of pFW8 [10], yielding pAR241-PBP1b. Plasmids pAR240-PBP1a and pAR241-PBP1b were transformed into *S. pyogenes* strain D471, and chloramphenicol resistant colonies were screened by PCR for correct integration. The resulting strains were termed D471Ω pAR240-PBP1a, and D471ΩpAR241-PBP1b.

LPXTGase Activity in Penicillin Binding Protein Knockout Mutants

Penicillin binding protein 1a and 1b transpeptidase domain knockout mutants were grown, harvested, and enzyme activities

were measured as above using coumarin-labeled substrate and measuring fluorescence of the cleaved substrate fragment.

Results

Identification of Free Amino Groups in LPXTGase

In earlier experiments, amino acid composition analysis showed that LPXTGase from *S. pyogenes* and *S. aureus* contain 7 and 6 lysine residues respectively. Nevertheless, exhaustive trypsin digestion of deglycosylated LPXTGase of *S. pyogenes* yielded only two peptides fragments [5], while trypsin was unable to cleave the *S. aureus* enzyme [6]. These results raise the possibility that most of the ε-amino groups of LPXTGases are not exposed but modified in some way in these organisms. One intriguing prospect is that the ε-amino groups may form a peptide bond with amino acids within the enzyme, in a manner like the ε-amino group of lysine in the muramyl pentapeptide during cross-bridge construction in the peptidoglycan. To examine whether the ε-amino group of some lysine residues are indeed tied up in internal cross-linking, highly purified LPXTGase from *S. pyogenes* was incubated with 80-fold molar excess of FDNB for 42 hours to ensure that all primary amines become dinitrophenylated. The resulting DNP-LPXTGase was totally unreactive to ninhydrin, indicating complete dinitrophenylation of all primary amino groups in the enzyme (data not shown).

To determine which amino groups were dinitrophenylated, the reacted DNP LPXTGase was acid hydrolyzed at 110 C for 4 days. The hydrolysis products were then separated by silica gel Thin Layer Chromatography (TLC) using water/isopropanol/ ethyl acetate (1:3:1) as the running solvent. Examination of the chromatogram of the acid hydrolysate under UV light (Figure 1) showed two UV-absorbing bands with Rf 0.48 and 0.65. In this TLC system, non-DNP modified free lysine remains at the origin whereas Nε-DNP-lysine migrates to a position of Rf 0.65 (Figure 2), indicating that the faster moving band in the DNP-LPXTGase hydrolysate is Nε-DNP-lysine (Figure 1). We also found that the other amino acids present in LPXTGase migrate between Rf 0.2 to 0.4 and away from both lysine derivatives, a useful feature when quantifying the lysines (see below). We suspected that the slower moving (Rf 0.48) band was Nα-DNP-lysine. To verify this, free lysine was dinitrophenylated with an equimolar amount of FDNB, which would yield Nα-DNP-lysine, Nε-DNP-lysine and Nα, ε-di-DNP-lysine. The resulting mixture was separated by silica gel TLC, alongside a standard Nε-DNP-lysine, and FDNB alone (Figure 3). The DNP-lysine mixture was resolved into species with Rf 0.48 and Rf 0.65, corresponding to Nα-DNP-lysine and Nε-DNP-lysine respectively based on the Nε-DNP-lysine standard, as well as a DNP-lysine species near the solvent front, corresponding to Nα,ε-di-DNP-lysine.

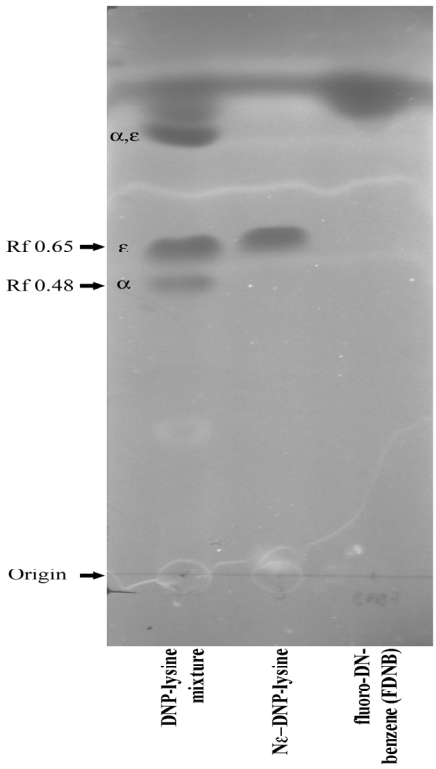

Figure 3: Separation of Nα-DNP-283 lysine, N_{ε}-DNP-lysine and $N\alpha,_{\varepsilon}$-di-DNP-lysine by silica gel thin layer chromatography. DNP-lysin mixture, standard N_{ε}-DNP-lysine, and FDNB alone, were separated using water/ isopropanol/ ethyl acetate (1:3:1) as running solvent. DNP-lysine species were prepared as described in Methods.

Quantitation of Free Lysines and DNP-Lysines

To determine the free lysine content of the DNP-LPXTGase hydrolysate, the free lysine removed from the origin of the TLC plate (Figure1) and a series of lysine standards were analyzed by ninhydrin. Samples in separate microfuge tubes were mixed with 3.3 mg of ninhydrin in 1 ml of water, heated for 30 min at 100 C, and the absorption of the ninhydrin reaction products was measured at 480 nm. Conversely, to determine DNP-lysine content of the 2 bands from the TLC plate (Figure 1), the combined bands and a series of Nε-DNP-lysine standards were dissolved in 1 ml of 50% methanol, and the absorbance was measured at 360 nm. The results shown in (Figure 4) indicate that the enzyme hydrolysate contained 0.175 μM of free lysines (Figure 4A), which originated from lysine residues that were cross-linked, and 0.069 μM DNP-lysines (Figure 4B), which were those with unblocked ε-amino groups. The ratio of lysine residues with cross-linked ε-amino groups to lysine residues with free ε-amino groups (0.175 to 0.069) was 5 to 2. Therefore, it may be concluded that of the 7 lysine residues present in the LPXTGase enzyme, 5 are internally cross-linked through their ε-amino groups. Furthermore, of the two free

lysines, one has a free α-amino group represented by the Nα-DNP-lysine and the other a free ε-amino group represented by Nε-DNP-lysine.

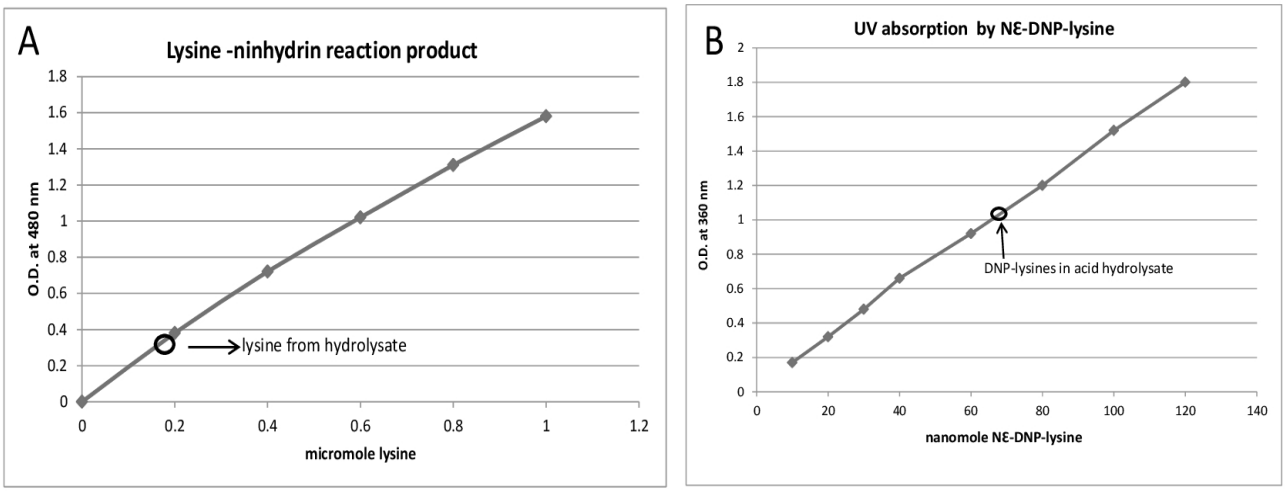

Figure 4A and 4B: Determination of free lysine content in the acid hydrolysate of LPXTGase. The free lysine at the origin of the TLC plate (Figure 1) was reacted with ninhydrin as were lysine standards. Absorbance at 480 nm was used to determine the quantity of the ninhydrin reaction products. B. Determination of DNP-lysine content in the acid hydrolysate of LPXTGase. The DNP-lysine in the Rf 0.65 and Rf 0.48 bands of the TLC plate (Figure1), and DNP-lysine standards were suspended in 50% methanol and absorbance at 360 nm was used to determine the quantity of product.

Effect of Penicillin on LPXTGase Synthesis in the Organism

To determine if the inhibitory activity of penicillin on penicillin binding proteins affected the synthesis of LPXTGase, streptococci were grown in sublethal doses of penicillin. 40 μg of penicillin G was added to a 1-liter culture of *S. pyogenes* when the $O.D._{650nm}$ reached 0.2 (40 ng/ml final concentration) and cells were then allowed to grow to $O.D._{650nm}$ 1.3, at which time the cells were harvested. We found that the cells grew normally and showed normal cell morphology at this concentration of penicillin. However, when LPXTGase was isolated from these cells we found that its cleavage activity was virtually eliminated (Figure 5), suggesting that penicillin-binding proteins are involved in some way with the assembly of LPXTGase.

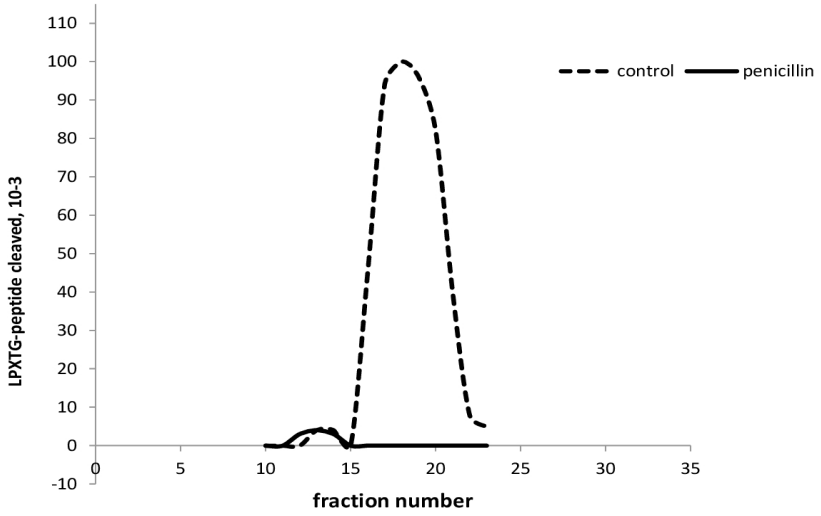

Figure 5: Inhibition of LPXTGase synthesis with low dose penicillin. *S. pyogenes* was grown in 1 liter of medium. When OD_{650} reached 0.2, 40 μg of penicillin was added to the culture, and cells were harvested at $O.D._{650nm}$ 1.3. Cells were suspended phosphate buffer and lysed with PlyC lysin for one hour. The lysate was centrifuged at 8,000 rpm for 20 minutes, and supernatant collected. LPXTGase was purified by DEAE-cellulose and Sephadex G50 chromatography. LPXTGase activity was measured as previously described [6]. For comparison, a control sample was grown without penicillin, harvested, and enzyme activity was determined.

Role of PBP in LPXTGase Assembly

S. pyogenes PBP1a and PBP1b are anchored to the membrane through an N-terminal transmembrane helix and have two major domains: An N-terminal transglycosylase domain, and a C-terminal transpeptidase domain. To test the possible role of these two enzymes in the assembly of LPXTGase, we introduced a stop codon downstream of the transglycosylase domain, thereby preventing the expression of the transpeptidase domain. This was achieved through integration of a pAR240-PBP1a and pAR241-PBP1b into the Pbp1a and Pbp1b genes of *S. pyogenes* D471, respectively. Both plasmids are derivatives of pFW8 [10]. As seen in (Figure: 6A, 6B and 6C), the knockout mutants of PBP 1a and 1b produced considerably less LPXTGase.

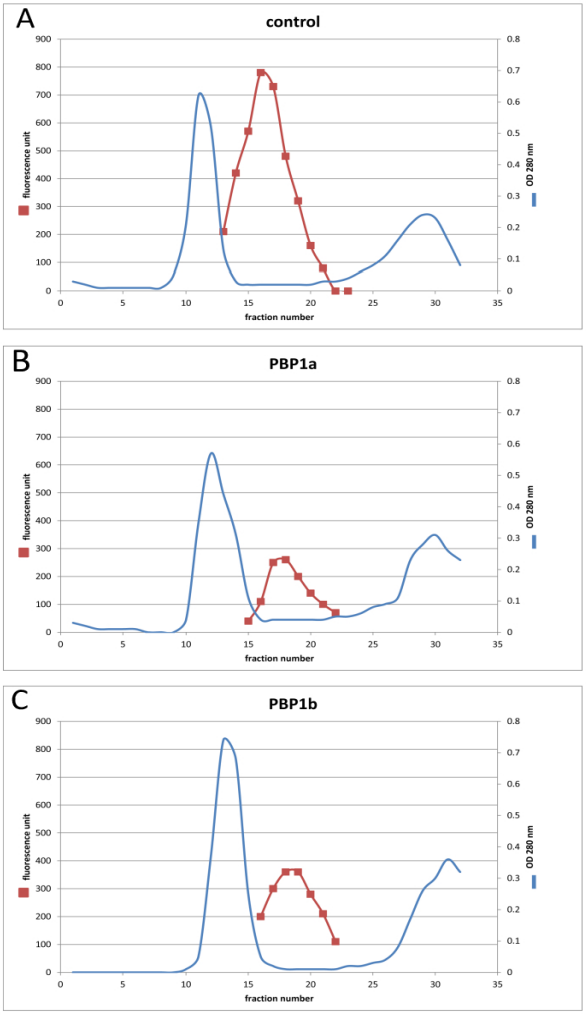

Figure 6A, B and C: LPXTGase syntheses in PBP 1a and 1b knockout mutants. PBP mutants were grown to an OD650 of 1.3. The cells were then processed and tested for LPXTGase as described in (Figure 5).

Discussion

Gram-positive bacteria have two enzymes that cleave the LPXTG anchor motif of surface-displayed proteins, sortase and LPXTGase. However, cleaving the LPXTG motif in surface proteins is the only similarity between these enzymes. While sortase is a transpeptidase [11], LPXTGase exhibits only endopeptidase activity [5]. Sortase, a conventional enzyme, differs radically from LPXTGase, an unconventional enzyme whose endopeptidase activity is dependent on considerable glycosylation [6]. Furthermore, LPXTGase is the first enzyme described containing both conventional and unconventional amino acids including D-alanine [5,7], making its synthesis independent from ribosomal machinery. Since streptococci, staphylococci and other non-soil organisms do not contain Non-Ribosomal Peptide Synthetases (NRPS) capable of synthesizing short peptides composed of both conventional and unconventional amino acids, it is unclear how such an enzyme is produced. A clue to the synthesis of LPXTGase came in our previous publication [6] where we found that the ratio of those amino acids found in the peptidoglycan of streptococci (Ala, Glu, Lys) and staphylococci (Ala, Glu, Lys, Gly) are the same as those found in their respective LPXTGase enzymes. This strongly suggested to us that the same enzymes used in the assembly of the peptidoglycan (i.e., PBPs) were used to assemble LPXTGase. In support of this, we show here that like in the peptidoglycan, the lysines in LPXTGase are cross-linked through their ε-amino-groups. In addition, we find that PBP 1a and 1b play some role in the proper assembly of the enzyme, since deletion of either PBP 1a or 1b significantly affects the yield of active LPXTGase in these PBP mutants. These results support our idea that perhaps cell wall precursors on lipid II after being translocated to the outer surface of the cytoplasmic membrane in some way become incorporated with unconventional amino acids and cross-linked by PBP 1a and 1b, and finally glycosylated. Additionally, we show that streptococci grown in the presence of very low doses of penicillin do not contain active LPXTGase. This latter result suggests that like sortase A, LPXTGase may not be an essential enzyme for growth and viability, but since its catalytic activity is orders of magnitude greater than sortase [5], it may be important in other cellular aspects, such as the display of certain protein molecules on the cell surface during rapid cell growth.

Acknowledgement

Supported by USPHS grant AI11822 to VAF. All the data that is available is contained in this manuscript. Sung G. Lee passed away suddenly after completing this manuscript.

References

1. Fischetti VA, Pancholi V, Schneewind O (1990) Conservation of a hexapeptide sequence in the anchor region of surface proteins of Gram-positive cocci. MolecMicrobiol 4: 1603-1605.

2. Navarre WW, Schneewind O (1994) Proteolytic cleavage and cell wall anchoring at the LPXTG motif of surface proteins in gram-positive bacteria. MolecMicrobiol 14: 115-121.

3. Navarre WW, Schneewind O (1999) Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. MicrobiolMolecBiolReviews 63: 174-229.

4. Mazmanian SK, Liu G, Ton-That H, Schneewind O (1999) *Staphylococcus aureus* Sortase, an enzyme that anchors surface proteins to the cell wall. Science 285: 760-762.

5. Lee SG, Pancholi V, Fischetti VA (2002) Characterization of a unique glycosylated anchor endopeptidase that cleaves the LPXTG sequence motif of cell surface proteins of Gram-positive bacteria. J Biol Chem 277: 46912-46922.

6. Lee SG, Fischetti VA (2006) Purification and characterization of LPXTGase from *Staphylococcus aureus* : the amino acid composition mirrors that found in the peptidoglycan. Journal of Bacteriology 188: 389-398.

7. Lee SG, Fischetti VA (2003) Presence of D-Alanine in an Endopeptidase from *Streptococcus pyogenes*. J Biol Chem 278: 46649-46653.

8. Yamada H, Matsunaga N, Domoto H, Imoto T (1986) Dinitrophenylation as a probe for the determination of environments of amino groups in protein. Reactivates of individual amino groups in lysozyme. Journal of biochemistry 100: 233-241.

9. Nelson D, Loomis L, Fischetti VA (2001) Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. ProcNatlAcadSciUSA 98: 4107-4112.

10. Podbielski A, Spellerberg B, Woischnik M, Pohl B, Lutticken R (1996) Novel series of plasmid vectors for gene inactivation and expression analysis in group A streptococci (GAS). Gene 177: 137-147.

11. Mazmanian SK, Ton-That H, Schneewind O (2001) Sortase-catalysed anchoring of surface proteins to the cell wall of *Staphylococcus aureus*. MolMicrobiol 40: 1049-1057.