The Bimodular G57-V577 Polypeptide Chain of the Class B Penicillin-Binding Protein 3 of Escherichia coli Catalyzes Peptide Bond Formation from Thiolesters and Does Not Catalyze Glycan Chain Polymerization from the Lipid II Intermediate

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Abstract

Because the specificity profile of the membrane anchor-free G57-V577 penicillin-binding protein 3 (PBP3) of Escherichia coli for a large series of β-lactam antibiotics is similar to that of the full-size membrane-bound PBP, the truncated PBP is expected to adopt the native folded conformation. The truncated PBP3 functions as a thiolesterase. In aqueous media and in the presence of millimolar concentrations of a properly structured amino compound, it catalyzes the aminolysis of the thiolester until completion, suggesting that the penicillin-binding module of PBP3 is designed to catalyze transpeptidation reactions. In contrast, the truncated PBP3 is devoid of glycan polymerization activity on the E. coli lipid II intermediate, suggesting that the non-penicillin-binding module of PBP3 is not a transglycosylase.

The multimodular class B penicillin-binding protein 3 (PBP3) is a key element of the cell septation network in Escherichia coli (30). This tripartite protein consists of an M1-E56 membrane anchor-containing module that is fused to a G57-1237 non-penicillin-binding (n-PB) module that is fused to the D238-V577 PB module (7, 12, 16). The membrane anchor-free G57-V577 polypeptide chain of PBP3 which comprises the n-PB and PB modules has been overproduced in the periplasm of E. coli as an autonomous folding entity (9, 16). With "p" denoting "periplasmic," this truncated PBP3 is called PBP3p.

Indirect experimental evidence suggests that the PB module of PBP3 is involved, one way or another, in peptide cross-linking during synthesis of the septal peptidoglycan (3, 26). In contrast, the role of the n-PB module of PBP3 is a matter of controversy. According to Ishino and Matsushashi (19), this module is a transglycosylase catalyzing glycan chain elongation from disaccharide (peptide) lipid II intermediates. According to van Heijenoort et al. (29), it is not.

Because the thermostability and affinity for benzylpenicillin and cephalaxin of the folded, membrane anchor-free polypeptide are unchanged in comparison with those of the membrane-bound PBP3, one can reasonably postulate that the conformation adopted by the truncated PBP3 reflects faithfully that of the native-state structure (9). β-Lactams and thiolester carbonyl donors and the disaccharide (peptide) lipid II intermediate were used to probe the enzymatic activities of the G57-V577 PBP3 and thus shed light on the possible functions of the protein in cell septation. The results are presented below.

(Some of the work described in this paper is part of a dissertation presented by M.A. in partial fulfilment of a Ph.D. degree at the University of Liège.)

MATERIALS AND METHODS

PBP3p and carbonyl donor substrates. PBP3p was purified as described previously (9). It was stored frozen at -20°C in 10 mM Tris-HCl (pH 8.0)-10% (vol/vol) glycerol-10% (vol/vol) ethylene glycol-0.5 M NaCl. The esters and thioesters were described previously (1, 2). [14C]benzylpenicillin (54 Ci/mol) was from Amersham International (Buckinghamshire, United Kingdom). 5'-Fluoresceyl-glycyl-6-aminopenicillanate (5'-Flu-Gly-6-
APAp), 5′-fluoresceyl-ampicillin, 6′-fluoresceyl-ampicillin, and dansyl-6-APA were described previously (4, 23). Nonlabelled β-lactams were gifts from pharmaceutical companies.

Kinetic parameters. The interactions between PBP3p (E, for enzyme) and the thiolester and β-lactam compounds (D, for carbonyl donor) were interpreted on the basis of the following three-step reaction:

\[
E + D \xrightleftharpoons[k_{-1}]{k_1} E\cdot D \xrightarrow{k_{s3}} E\cdot HY + P1 \xrightleftharpoons[k_{-2}]{k_2} E + P2
\]

\(K\) is the dissociation constant of the Henri-Michaelis complex \(E\cdot D\). \(k_{s3}/K\) (M\(^{-1}\) s\(^{-1}\)) is the second-order rate constant of acyl enzyme (E - D\(^*\)) formation, \(k_{s3}\) (s\(^{-1}\)) is the first-order rate constant of acyl enzyme breakdown, \(P1\) is the leaving group, HY is the attacking nucleophile (H\(_2\)O or amino compound), and \(P2\) is the second reaction product.

Hydrolysis of properly structured thioesters proceeded to completion. D-Alanine and the tetrapeptide L-Ala-γ-D-Glu(amide)-(L)mApm(L)-D-alanine (where mApm is meso-diaminopimelic acid [15]) were used as amino acceptors (HY) of the transfer reactions. With the β-lactam antibiotics, the leaving group, \(P1\), remained part of the acyl enzyme, E - D\(^*\), and the reaction stopped at this level, at least for a long time (3 to 5 h).

Inactivation of PBP3p by β-lactam antibiotics, (i) Determination of the \(k_{s3}\) values. PBP3p samples were quantitatively inactivated by the β-lactam, the β-lactam in excess was hydrolyzed by treatment with the β-lactamase of Bacillus licheniformis (or that of the Actinomadura sp. strain R39 in the case of aztreonam), the β-lactamase was inactivated by treatment with 5 μM β-iodopenicillanate, and the samples were maintained at 37°C for increasing time intervals. The PBP3p released in an active form during the latter incubation was labelled with 2 × 10\(^{3}\) M \([1^{3}C]\)benzylpenicillin. The acyl PBP3p was precipitated with trichloroacetic acid as described previously (25), the pellet was dissolved in 1% sodium dodecyl sulfate, and the radioactivity was measured. One may note that the \(k_{s3}\) values are related to the half-lives of the acyl PBP3ps by the equation 0.693/k\(_{s3}\) = half-life.

(ii) Determination of the \(k_{s2}/K\) values. PBP3p samples were incubated with increasing β-lactam concentrations during a given time at 37°C, and the extents of PBP labelling were estimated. The \(k_{s2}/K\) value for cephalixin was determined with the thiolester benzyl-D-alanyl-thioglycolate as the reporter substrate (6). On the basis of this value (45 M\(^{-1}\) s\(^{-1}\)), the \(k_{s2}/K\) value for benzylpenicillin was determined by competition experiments between cephalixin and \([1^{4}C]\)benzylpenicillin (10). On the basis of this value (4,000 M\(^{-1}\) s\(^{-1}\)), the \(k_{s2}/K\) values for the other β-lactams were determined by competition with \([1^{4}C]\)benzylpenicillin. The \(k_{s2}/K\) values for cephalixin and benzylpenicillin were confirmed by competition with dansyl-6-APA. Acylation of PBP3p by dansyl-6-APA caused fluorescence transfer to the dansyl group (excitation at 280 nm and maximum emission at 535 nm).

PBP3p-catalyzed acyl transfer reactions on esters and thioesters. PBP3p samples were dialyzed against 10 mM Tris-HCl (pH 7.8) containing 10% (vol/vol) dimethyl sulfoxide and 0.5 M NaCl (to eliminate the glycerol and ethylene glycol present in the enzyme preparation, which were susceptible to function as nucleophiles in transfer reactions). PBP3p and the esters and thioesters were incubated at 37°C in 0.5 mM Tris-HCl-10 mM sodium phosphate (pH 7.4) containing 10% (vol/vol) dimethyl sulfoxide and 0.5 M NaCl. The \(k_{s2}/K\) values (equivalent to the \(k_{s2}/V\) values) were determined under first-order conditions at substrate concentrations lower than the \(K_{s}\) values. The increase of \(A_{254}\) (Δε = 500 M\(^{-1}\) cm\(^{-1}\) [esterase activity]) and the decrease of \(A_{260}\) (Δε = 2,200 M\(^{-1}\) cm\(^{-1}\) [thiolesterase activity]) were monitored with a UVIKON 860 spectrophotometer coupled to a computerized data acquisition system via an RJ322 interface (20-22). In those experiments in which D-alanine and L-Ala-γ-D-Glu(NH\(_2\))-mApm-L-Ala were used as alternative acceptors of the transfer reactions, the carbonyl donor (D) and the hydrolysis (H) and aminolysis (T [transpeptidation]) products were separated by high-performance liquid chromatography with an ET 250/8/4 Nucleosil 5C\(_{18}\) column (Macherey and Nagel) under the conditions used previously (2). With the cocrystals benzyl-D-Ala-thioglycolate and tetrapeptide, the retention times for D, H, and T were 14.89, 12.94, and 11.3 min, respectively (solvent A, 10 mM sodium acetate [pH 3.5] solvent B, acetonitrile gradient [0 to 100%] for 30 min). The T/H ratios were measured as described previously (2, 20). The product of the transfer reaction to the tetrapeptide was identified by electrospray mass spectrometry (8).

Polymerization of disaccharide-peptide units by transglycosylation from the lipid II intermediate. The E. coli lipid II intermediate N-acetylglucosaminyl-N-acetylmuramyl [L-Ala-γ-D-Glu-(L)mApm-(L)-D-Ala-D-Ala] pyrophosphate-undecaprenol labelled with D-[\(^{13}C\)]alanine residues in its peptide moiety was prepared as described previously (29). Lipid II was used in two different in vitro polymerization assays (19-29). PBP3p was used at a final protein concentration of 5.9 μM in both assays.
RESULTS

Inactivation of PBP3p by β-lactam antibiotics. In all cases tested, the acyl enzymes were very stable. Very low $k_{+3}$ values (at 37°C) were obtained with cephalexin ($1.5 \times 10^{-6}$ s$^{-1}$), benzylpenicillin ($4 \times 10^{-5}$ s$^{-1}$), carbenicillin, ampicillin, mezlocillin, and aztreonam (from $3 \times 10^{-6}$ s$^{-1}$ to $5 \times 10^{-6}$ s$^{-1}$). Hence, variations in the inactivation potency of the β-lactams for PBP3p depended on their efficacy as acylating agents.

The data shown in Fig. 1 led to the following conclusions, (i) The $k_{+3}/K$ values (at 37°C) varied greatly, depending on the structure of the exocyclic substituents of the β-lactams, from 45 M$^{-1}$ s$^{-1}$ for cephalexin to 230,000 M$^{-1}$ s$^{-1}$ for mezlocillin. (ii) Mezlocillin, piperacillin, cefmenoxime, cefotaxime, cefoperazone, and aztreonam, which each cause filamentation of the E. coli cells at low concentrations, were the most potent...
acylating agents of PBP3p (kₐ/K values of ≥80,000 M⁻¹ s⁻¹). (iii) Four groups of structurally related β-lactams highlighted the effects of the side chains on the rate of protein acylation, namely, the group benzylpenicillin < ampicillin < piperacillin < mezlocillin; the group benzylpenicillin > carbenicillin < ticarcillin > temocillin; the diad piperacillin ≥ cefoperazone; and the triad ceftezoxime, cefotaxime, and cefmenoxime.

The kₐ/K values obtained with PBP3p (at 37°C) were compared to the corresponding kₐ/K values for the membrane-bound PBP3 (at 30°C). The latter values were calculated from the published ID₅₀ (or ID₉₀) values, i.e., the antibiotic concentrations which inactivate the PBP by 50% or 90% after a given time of incubation (5, 11), by the equation [ID₅₀] · t = 0.69 k/Kₐ or [ID₉₀] · t = 4.6 k/Kₐ (14). As shown by the data in Table 1, all of the β-lactam antibiotics tested fell in the same order of decreasing acylating potency toward the two PBP forms showing unambiguously that PBP3p adopted the same folded conformation as the native PBP3. Given the different temperatures used in these experiments, PBP3p was expected to be more susceptible to inactivation (at 37°C) than the membrane-bound PBP3 (at 30°C). However, the observed ratios of kₐ/K (for PBP3p) versus kₐ/K (for PBP3) varied from 3 to 11, depending on the antibiotics, except for cephalaxin, which was a very weak acylating agent. Note that binding to isolated membranes involves a competition between multiple PBPs. Moreover, not all of the ID₅₀ (ID₉₀) values may have been determined under the conditions under which the use of the aforementioned conversion equations was fully justified.

### TABLE 1. Second-order rate constant (kₐ/K) of acylation of E. coli PBP3p and membrane-bound PBP3 by β-lactam antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>kₐ/K (M⁻¹ s⁻¹)</th>
<th>A/B ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mezlocillin</td>
<td>230,000 ± 20,000</td>
<td>26,700</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>100,000 ± 10,000</td>
<td>33,000</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>80,000 ± 10,000</td>
<td>15,000</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>80,000 ± 5,000</td>
<td>&gt;11,500</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>16,000 ± 2,000</td>
<td>5,750</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4,800 ± 200</td>
<td>480</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>4,000 ± 200</td>
<td>450</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>1,500 ± 200</td>
<td>230</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1,000 ± 200</td>
<td>90</td>
</tr>
<tr>
<td>Cephalaxin</td>
<td>45 ± 5</td>
<td>50</td>
</tr>
</tbody>
</table>

The kₐ/K values for the wild-type PBP3 were derived from the ID₅₀ value for aztreonam (10); in all the other cases, they were derived from ID₉₀ values (5).

**PBP3p-catalyzed transfer reactions on acyclic carbonyl donors.** PBP3p hydrolyzed properly structured thiolesters of the type C₆H₅-CO-NH-CH(R)-COS-CH(R')COOH (Table 2) but not the corresponding esters. Hydrolysis proceeded to completion with release of the leaving group, HS-CH(R')COOH. The Kₐ values were high, and only the kₛ/Kₐ values were computed by analysis of the complete time courses (6). The kₛ/Kₐ values are equivalent to the second-order rate constant (kₛ/K) values of protein acylation. Table 2 also compares the kₛ/Kₐ values obtained with PBP3p with those obtained with a soluble form of the Streptococcus pneumoniae class B PBP2x (21), the Streptomyces sp. strain K15 monofunctional DD-transpeptidase/PBP (17), and the Streptomyces sp. strain R61 monofunctional DD-carboxypeptidase/PBP (22). With this limited series of thiolesters, the activity of PBP3p was comparable to that of the Streptomyces sp. strain K15 transerase (17).

In the presence of D-alanine, the PBP3p-catalyzed conversion of the thiolester benzoyl-d-alanyl-thiolglycolate (thiolester 1 in Table 2) underwent partitioning into hydrolysis (H reaction products, benzoyl-d-alanine plus thiolglycolate) and aminolysis (T [transpeptidation] reaction products, benzoyl-d-alanyl-d-alanine plus thiolglycolate). Because the presence of D-alanine did not modify the kₛ/Kₐ values of the catalyzed reactions, it was concluded that D-alanine did not influence the rate of enzyme acylation by the thiolester. D-Alanine behaved as simple alternative nucleophile at the level of the acyl enzyme leading to the formation of a ternary acyl enzyme-D-alanine complex that was productive only toward the formation of a peptide bond. The data in Table 3 show that (i) at a fixed 1 mM concentration of D-alanine, the T/H ratio was independent of the thiolester concentration from 50 to 200 μM; (ii) at a fixed 1.1 mM concentration of thiolester, the T/H ratios increased proportionally to the thiolester concentration from 0.5 to 4 mM; and (iii) at D-alanine concentrations larger than 4 mM, the thiolester was quantitatively converted into benzoyl-d-alanyl-d-alanine. One may note that this...
reaction product was not a substrate of PBP3p.

PBP3p also utilized the tetrapeptide L-Ala-γ-D-Glu(NH₂)-mA₂pm-D-Ala (a close analog of the tetrapeptide unit of the *E. coli* peptidoglycan) as an amino acceptor for the catalyzed aminolysis of benzoyl-D-alanylthioglycolate. The amino group on the D-center of the diaminopimelic acid residue presumably served as the required nucleophile. Electrospray mass spectrometry showed that the reaction product had the expected *M*ₐ of 636.

**Transglycosylation.** The possible transglycosylation activity of PBP3p was tested with radiolabelled lipid II as a substrate according to the assay developed for PBP1b (29). Under these conditions, purified PBP1b catalyzed glycan polymerization (Fig. 2), whereas none was detectable with PBP3p as previously observed with complete PBP3 (29). Moreover, when the assays were carried out under the conditions initially proposed for PBP3 (18), no glycan polymerization was detectable with PBP3p, whereas PBP1b was fully active.

**TABLE 2.** Second-order rate constant (*k*₉/K₉ [equivalent to *k*₉/K₉]) of acylation of the *E. coli* PBP3p, *S. pneumoniae* PBP2x, *Streptomyces* sp. strain K15 DD-transpeptidase-PBP, and *Streptomyces* sp. strain R61 DD-carboxypeptidase-PBP by thiolesters C₆H₅-CO-NH-CH(R)-CO-S-CH(R')COOH in the hydrolysis pathway

<table>
<thead>
<tr>
<th>Thiolester[a]</th>
<th>R</th>
<th>R'</th>
<th><em>k</em>₉/K₉ (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PBP3p</td>
</tr>
<tr>
<td>1</td>
<td>CH₃</td>
<td>H</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>H</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>CH₃</td>
<td>CH₃</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>CH₃</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>5</td>
<td>(CH₃)₂-CH</td>
<td>H</td>
<td>NA</td>
</tr>
</tbody>
</table>

[a] In thiolesters 1, 2, and 3, the asymmetric carbon atoms have the D-configuration. Thiolesters 4 and 5 are racemic mixtures.

[b] ND, not determined; NA, no detectable activity. For details about PBP2x, *Streptomyces* sp. strain K15 PBP, and *Streptomyces* species strain R61 PBP, see references 20, 16, and 21, respectively.

**TABLE 3.** *E. coli* PBP3p-catalyzed hydrolysis (H) and aminolysis (T) of the thiolester benzoyl-D-alanyl-thioglycolate

<table>
<thead>
<tr>
<th>Reaction condition</th>
<th>Concq (mM) of:</th>
<th>T/H ratio[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thiolester 3</td>
<td>D-Ala</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>1</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>0.10</td>
<td>1</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>0.15</td>
<td>1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>0.20</td>
<td>1</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.10</td>
<td>0.5</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>1.10</td>
<td>1</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>1.10</td>
<td>2</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>1.10</td>
<td>4</td>
<td>10.3 ± 0.8</td>
</tr>
</tbody>
</table>

[a] Thiolester 1 in Table 2. Data represent the effects of increasing concentrations of thiolester at a fixed concentration of D-alanine (reaction condition A) and of increasing concentrations of D-alanine at a fixed concentration of thiolester (reaction condition B). The enzyme concentration was 1.56 µM. Incubation was for 20 min at 37°C.

[b] Reaction products: benzoyl-D-alanine (H) or benzoyl-D-alanyl-D-alanine (T) plus thioglycolate.
FIG. 2. Separation of the products of the polymerization reaction by paper chromatography. The in vitro glycan polymerization was assayed as described previously (29). Reaction products were separated by chromatography on Whatman no. 1 filter paper run overnight in isobutyric acid-1 M ammonia (5:3). Radioactive compounds (polymerized peptidoglycan material at the start, and lipid II at an Rf of 0.85) were detected with a Berthold scanner model LB283. a, assay without any enzyme; b, assay with 8 µg of PBP3p; c, assay with 8 µg of PBP1b.

DISCUSSION

The essential feature of the lipid II precursor is that the lipid-transported disaccharide peptide units can undergo polymerization into peptidoglycan at extracellular sites without any input of energy. Two enzymatic activities, a transglycosylase and a transpeptidase, are required. Assay systems leading to the in vitro PBP-catalyzed conversion of lipid II into polymeric peptidoglycan have been developed, and on this basis, bifunctionality with transglycosylase (the n-PB module) and transpeptidase (the PB module) activities has been assigned to the class A PBP1a and PBP1b of E. coli.

Serine-assisted peptidoglycan cross-linking implies that an amino acceptor prevents water from performing a nucleophilic attack on the peptidyl enzyme formed by reaction of the transpeptidase with the D-alanyl-D-alanine-terminated carbonyl donor. The 262-amino-acid residue monofunctional DD-transpeptidase-PBP of Streptomyces sp. strain K15 fulfills this criterion (26). The enzyme catalyzes the rupture of the carboxy-terminal peptide bond of (acetyl)2-L-lysyl-D-alanyl-D-alanine. However, in aqueous media at 55.5 M H2O, the enzyme is seemingly inert, because D-alanine that is released during the enzyme acylation step is reutilized as an amino acceptor for the ensuing enzyme deacylation step, so that the original carbonyl donor is continuously regenerated. Glycyl-glycine has a much greater acceptor activity than water. Millimolar concentrations of this dipeptide led to the enzyme-catalyzed conversion of the tripeptide into the tetrapeptide (acetyl)2-L-lysyl-D-alanyl-glycyl-glycine (26).

PBP3p is a truncated, membrane anchor-free form of the class B PBP3 of E. coli. Its specificity profile for a large series of β-lactam antibiotics is identical, within the limits of experimental errors, to that of the wild-type PBP. As a corollary, the folded conformation adopted by PBP3p is expected to be identical or at least very similar to that of the native structure, and the enzymatic properties of PBP3p on acyclic carbonyl donors should reflect faithfully those for the membrane-bound PBP.

PBP3p has no detectable activity on the tripeptide (acetyl)2-L-lysyl-D-alanyl-D-alanine, but in analogy with the Streptomyces sp. strain K15 DD-transpeptidase-PBP, aqueous assay systems containing a millimolar
concentration of D-alanine or a peptide related to the E. coli wall peptidoglycan led to the PBP3p-catalyzed conversion of properly structured thiolesters into the corresponding aminolytic products, showing that the acyl serine transferase-PB module of PBP3 can catalyze the formation of a new peptide bond at the expense of a preformed thiolester bond. Because the substrate requirements conferring optimal substrate activity to a carbonyl donor are not the same for a β-lactam, peptide, ester, or thiolester, PBP3 may well be capable of catalyzing the formation of a new peptide bond at the expense of a preformed peptide bond. Arising from this view, the peptide carbonyl donor and amino acceptor that PBP3 utilizes for the synthesis of the septal peptidoglycan remain to be characterized biochemically.

PBP3p has no detectable glycan chain polymerization activity on the E. coli lipid II intermediate under conditions under which the class A PBP1b functions as a transglycosylase. That the n-PB module of the class B PBP3 is not a transglycosylase is consistent with the fact that it has a different amino acid signature from that of the n-PB module of the bienzymatic (transglycosylase-transpeptidase) PBP1a and PBP1b of class A and of the monofunctional transglycosylases (12, 13, 23). In addition to its assisted folding function (16), the n-PB module of PBP3 may serve as a "wiring" element connecting the associated acyl serine transferase module to the transglycosylase module of the class A PBP1a and/or PBP1b and to other cell cycle proteins. Experimental evidence suggests that PBP3, PBP1b, the monofunctional (endopeptidase) PBP7, and the lytic transglycosylase Slt70 interact with each other (18). Also note that the M1-E56 membrane-spanning module of PBP3, which is not involved in polypeptide folding, is essential for the cell septation activity of the protein in exponentially growing cells (16). Positioning of PBP3 at the septum site might be the result of interactions between the intracellular segment of its membrane-anchoring module and the FtsZ ring (24).

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REFERENCES


