

Overexpression, purification and biochemical characterization of a class A high-molecular-mass penicillin-binding protein (PBP), PBP1* and its soluble derivative from *Mycobacterium tuberculosis*

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The product of the gene *ponA* present in cosmid MTCY21D4, one of the collection of clones representing the genome of *Mycobacterium tuberculosis*, has been named penicillin-binding protein 1* (PBP1*), by analogy to the previously characterized PBP1* of *M. leprae*. This gene has been overexpressed in *Escherichia coli*. His₆-tagged PBP1* localizes to the membranes of induced *E. coli* cells. Its susceptibility to degradation upon proteinase K digestion of spheroplasts from *E. coli* expressing the protein supports the view that the majority of the protein translocates to the periplasmic side of the membrane. Recombinant PBP1* binds benzylpenicillin and several other β -lactams, notably cefotaxime, with high affinity. Truncation of the N-terminal 64 amino acid residues results in an expressed protein

present exclusively in inclusion bodies and unable to associate with the membrane. The C-terminal module encompassing amino acids 272–663 can be extracted from inclusion bodies under denaturing conditions using guanidine/HCl and refolded to give a protein fully competent in penicillin-binding. Deletion of Gly⁹⁵–Gln¹⁴³ results in the expression of a protein, which is localized in the cytosol. The soluble derivative of PBP1* binds benzylpenicillin with the same efficiency as the full-length protein. This is the first report of a soluble derivative of a class A high-molecular-mass PBP.

Key words: cell wall biosynthesis, β -lactam, transpeptidase.

INTRODUCTION

The advent of multidrug-resistant *Mycobacterium tuberculosis* strains has necessitated a search for new antimycobacterial agents as well as a re-evaluation of the potential of existing antimicrobials. In spite of reports that some β -lactam antibiotics appear to be active *in vitro* [1–3] and *in vivo* [4], *M. tuberculosis* historically has been regarded as intrinsically resistant, due to the presence of one or more β -lactamases [5,6] and its lipid-rich outer cell wall [7]. However, the targets of the β -lactams, the penicillin-binding (PB) proteins (PBPs), remain to be characterized. In order for a β -lactam to be effective, it is a prerequisite that its targets should bind with high affinities to the β -lactams. Previously, it has been reported [8] that the PBPs of *M. tuberculosis* bind β -lactams at clinically relevant antibiotic concentrations, making it important to undertake a serious evaluation of the potential of β -lactam antibiotics through the characterization of these targets.

PBPs are a set of membrane-bound proteins that catalyse the final steps of bacterial cell wall peptidoglycan synthesis. The low-molecular-mass PBPs are single catalytic entities possessing DD-peptidase, esterase and thiol esterase activities [9]. The high-molecular-mass PBPs of classes A and B are involved in cell wall peptidoglycan assembly and cell morphogenesis. These are multidomain proteins consisting of an N-terminal module fused to a C-terminal module harbouring the transpeptidase activity and capable of binding penicillin [10]. Transglycosylase activity has been attributed to the N-terminal non-PB (n-PB) module of class A high-molecular-mass PBPs, although this has

been clearly identified only in *Escherichia coli* PBPs 1a and 1b [11]. The completion of the genome sequence of *M. tuberculosis* [12] has revealed the presence of two open reading frames (ORFs), Rv3682 and Rv0050, encoding two putative class A high-molecular-mass PBPs. Since the high-molecular-mass PBPs are essential enzymes in the bacterial cell wall peptidoglycan synthesizing machinery, it was reasoned that, as a step towards evaluating potential drugs directed against these targets, the first phase would be to characterize the proteins encoded by these genes, and attempt to generate soluble forms of these enzymes to facilitate structural studies. In the present study, we report the overexpression and characterization of the *M. tuberculosis* high-molecular-mass PBP1* encoded by the ORF Rv0050 [12] and its soluble derivative, soluble PBP-1* (s-PBP1*).

MATERIALS AND METHODS

Materials

[¹⁴C]Benzylpenicillin was purchased from Amersham Biosciences (Little Chalfont, Bucks., U.K.). The vector pET-28a(+) was from Novagen (Madison, WI, U.S.A.) and oligonucleotides were purchased from Life Technologies (Rockville, MD, U.S.A.). Transformations were routinely carried out in *E. coli* DH5 α . The expression of recombinant PBP1* and its derivatives was carried out in *E. coli* BL21(DE3) or *E. coli* Top10. *N*-dodecyl-*N,N*-dimethyl-3-ammonio-1-propanesulphonate (Zwittergent 3–12), Nonidet P40, cetyltrimethylammonium bromide (CTAB), CHAPS, Triton X-100, arabinose, benzylpenicillin, cefuroxime,

Abbreviations used: CTAB, cetyltrimethylammonium bromide; IPTG, isopropyl β -D-thiogalactoside; NTA, nitrilotriacetate; ORF, open reading frame; PB, penicillin-binding; PBP, PB protein; n-PB module, non-PB module; PB-PBP1*, truncated PBP1* lacking residues Val²–Gln²⁷¹; s-PBP1*, soluble PBP1* (truncated PBP1* lacking residues Gly⁹⁵–Gln¹⁴³); Zwittergent 3–12, *N*-dodecyl-*N,N*-dimethyl-3-ammonio-1-propanesulphonate.

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cefotaxime, cefoxitin, ceftriaxone, ampicillin and amoxicillin were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.); isopropyl β -D-thiogalactoside (IPTG) and kanamycin were from Amersham Biosciences.

Construction of the expression systems

The cosmid MTCY21D4 (a gift from Dr Stewart Cole, Institut Pasteur, Paris, France) was amplified in *E. coli* DH5 α and digested with *Bam*HI. A 6.6-kb fragment containing the gene *ponA* was cloned in the vector pK19 [13] to give the plasmid pJS251. The gene *ponA* was amplified from pJS251 using the primers 5'-TTTAAGCTTCATATGGTACCTTCACG-3' (sense) and 5'-ATGAATTCGTGGGAGTCTCACCG-3' (antisense; primer **a**). The bold letters in these primer sequences and those below represent the restriction enzyme sites indicated. The *ponA* gene was ligated into the vector pET28a between the *Nde*I and *Eco*RI sites to give pJS253 (encoding [His]₆-PBP1*). All recombinant proteins described below carried the His₆-tag at the N-terminus. The product obtained by PCR using the primer pair 5'-CGGATCCATATGGAAGACCGCAATTTCTATTCG-3' (sense) and primer **a** (antisense), and pJS253 as template, was cloned between the *Bgl*II and *Eco*RI sites of the pBAD-HisA vector (Invitrogen, Carlsbad, CA, U.S.A.) to give pJS255 [encoding (Δ V2-A64)-PBP1*; PBP1* lacking Val²-Ala⁶⁴]. The product obtained using the primer pair 5'-TTTAAGCTTCATATGGGCGGATCGACGATTACCC-3' (sense) and primer **a** (antisense) was cloned between the *Nde*I and *Eco*RI sites (indicated in bold) in pET28a to give pJS257 [encoding (Δ V2-Q94)PBP1*; PBP1* lacking Val²-Gln⁹⁴]. pJS259 [encoding (Δ V2-S137)PBP1*; PBP1* lacking Val²-Ser¹³⁷] was obtained by PCR using the primer pair 5'-ATGGATCCATATGAAAGACGATGTGCTGCAG-3' (sense) and primer **a** (antisense), followed by cloning between the *Nde*I and *Eco*RI sites of pET28a. pJS255 was digested with *Xho*I to remove the portion of the gene encoding the first 258 amino acid residues of PBP1*. The overhangs were religated to yield pJS261 encoding (Δ V2-S258)PBP1*. pJS262 [encoding (Δ V2-Q271)PBP1*; PBP1* lacking Val²-Gln²⁷¹] was generated by PCR using the primer pair 5'-TTGGATCCATATGGGGCTGTGGTCAACCAC-3' (sense) and primer **a** (antisense), followed by cloning between the *Nde*I and *Eco*RI sites of pET28a. pJS253 was digested to completion with *Pst*I to remove a 144-bp fragment, followed by religation to give pJS263 [encoding (Δ G95-Q143)PBP1*; PBP1* lacking Gly⁹⁵-Gln¹⁴³]. All constructs were checked by DNA sequencing.

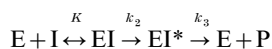
Expression and protein purification

Genes cloned in the vector pET28a were expressed in *E. coli* BL21(DE3). Cells harbouring the various plasmids were grown overnight with shaking at 37 °C in Luria-Bertani broth supplemented with 50 μ g/ml kanamycin. *E. coli* transformants, grown to a D_{600} of 0.6, were induced with 100 μ M IPTG for 3 h at 30 °C. Genes cloned in the pBAD-HisA vector were expressed in *E. coli* Top10 by induction with 0.0002% arabinose for 4 h at 37 °C. pBAD was chosen as the *ara*BAD promoter of *E. coli* allows regulated expression, thus facilitating optimum expression of soluble protein. Cells were harvested, resuspended in a solution containing 10 mM Tris/HCl (pH 7.4), 1 mM MgCl₂ and 1 μ g/ml DNase and sonicated at 200 W for 2 min. Unbroken cells and debris were removed by centrifugation at 600 *g* for 10 min. Inclusion bodies were obtained by centrifugation at 5000 *g* for 10 min, and plasma membranes by centrifugation at 100 000 *g* for 30 min. Both inclusion bodies and plasma membranes were washed twice and stored at -20 °C until further use.

In order to purify the full-length recombinant PBP1*, membranes (10 mg of protein) were suspended in 5 ml of 10 mM Tris/HCl (pH 8) containing 1% (w/v) Zwittergent 3-12 and maintained at 4 °C for 40 min. After centrifugation at 100 000 *g* for 30 min, the supernatant was removed and NaCl was added to give a final concentration of 0.5 M. The supernatant was loaded on to a 1 ml Ni²⁺-nitrilotriacetate (NTA)-agarose column (Qiagen GmbH, Hilden, Germany) equilibrated with buffer A [50 mM sodium phosphate buffer (pH 7.4) and 0.5 M NaCl] and the column was washed with buffer A containing 100 mM imidazole. PBP1* was eluted with a gradient of 100–200 mM imidazole/1% (w/v) Zwittergent 3-12 in buffer A. All steps were carried out at 4 °C.

Interaction of PBP1* with β -lactam antibiotics

This was interpreted on the basis of the following three-step scheme [14,15]:



where E is the active enzyme, I is the β -lactam compound, EI is the Henri-Michaelis complex, EI* is the acyl-enzyme, P is the biologically inactive product, K is the dissociation constant of the Henri-Michaelis complex, k_2 is the first-order rate constant for acyl-enzyme formation and k_3 is the first-order rate constant for the deacylation step.

The kinetic parameters k_2/K (the second-order rate constant for the acylation step) and k_3 of interaction with [¹⁴C]-benzylpenicillin were determined as described previously [15,16]. Knowing these values, the k_2/K value for non-radioactive β -lactams was determined in a one-step competition with [¹⁴C]-benzylpenicillin, as described by Frere et al. [17,18] from the equation

$$(k_2/K)_1 = (k_2/K)_2 \frac{[E - D_1^*][D_2]}{[E - D_2^*][D_1]}$$

The amount of radioactive acyl-enzyme formed with β -lactam 2 ([¹⁴C]benzylpenicillin) in the absence ($[E - D_2^*]_0$; where D_2^* is the concentration of [¹⁴C]benzylpenicillin) or presence ($[E - D_2^*]$) of β -lactam 1 (non-radioactive β -lactam), was measured by densitometric scanning of the fluorograms and using the equation $[E - D_1^*] = [E - D_2^*]_0 - [E - D_2^*]$ (where D_1^* is the concentration of non-radioactive β -lactam); $[D_1]$ and $[D_2] \geq [E_0]$. Incubation of the enzyme with antibiotics was performed for three different periods, with the time of contact between the enzyme and the antibiotic being short in each case compared with the lifetime of the E-D* adducts. Under these conditions the ratio of $[E - D_1^*]/[E - D_2^*]$ was independent of the time of contact.

[¹⁴C]Benzylpenicillin (0.1 mM) binding assays were carried out at 25 °C for 2 min.

The values of k_2/K given in the Results section are means \pm S.D. of three independent determinations.

Preparation and protease treatment of spheroplasts from *E. coli* transformants

E. coli cells were pelleted and resuspended at 2×10^{10} cells/ml in 15 mM Tris/HCl (pH 8) containing 12.5% (w/v) sucrose. The cell suspensions were then treated with the same buffer containing 2 mg/ml lysozyme and 5 mM EDTA for 25 min at 30 °C. Under these conditions all the cells were converted into spheroplasts. Where necessary, spheroplasts were pelleted and the supernatant was used to detect periplasmic proteins. Spheroplasts were lysed by osmotic shock and the membranes were pelleted by centri-

fugation (600 g for 10 min at 25 °C). The supernatant contained the cytosolic proteins.

Western blotting

Western blotting was performed on periplasmic and cytosolic proteins using either an anti-His antibody (1:2500 dilution) or an antibody against *M. leprae* PBP1* (1:1000 dilution; the antibody is also able to detect full-length *M. tuberculosis* PBP1*). A horseradish peroxidase-conjugated anti-(rabbit IgG) antibody was used as the secondary antibody, and colour development was performed using 4-chloro-1-naphthol.

RESULTS

***M. tuberculosis* PBP1* sequence analysis**

From sequence alignments, the ORF Rv0050 (*ponA*) was predicted to encode a class A high-molecular-mass PBP from *M. tuberculosis*, which we have named *M. tuberculosis* PBP1* by analogy with the previously reported PBP1* from *M. leprae* [19]. A genome-based search also identified Rv3682 as the ORF encoding the putative PBP1 from *M. tuberculosis*, which appears to be the counterpart of PBP1 from *M. leprae* [20]. A similarity search of the amino acid sequence using the BLAST algorithm [21] showed that *M. tuberculosis* PBP1* shared 83% identity

with *M. leprae* PBP1*. *M. tuberculosis* PBP1* was characterized by the presence of the conserved modules of the class A high-molecular-mass PBPs, as shown in Figure 1. The n-PB module, containing the characteristic conserved motifs 1–6, is fused to a C-terminal PB module, containing motifs 7–9, which is connected to a C-terminal extension of 127 amino acid residues. From the hydropathy plot, Met¹–Asn²² was identified as the pseudo-signal peptide. Ser³³⁹ of the PB module was assumed to be the active-site serine.

Expression of *pon A* in *E. coli* and properties of recombinant PBP1*

Expression of recombinant PBP1* in *E. coli* BL21(DE3)/pJS253 was inducible by IPTG and under the control of the T7 promoter and *lac* operator. The overexpressed PBP1* localized to the membrane (Figure 2A, lane a).

Experiments were performed to confirm that the majority of the polypeptide chain of the membrane-bound His-tagged PBP1* that had translocated to the periplasmic side of the membrane adopted the predicted membrane topology. *E. coli* transformants were grown, induced with IPTG and converted into spheroplasts. Spheroplasts were treated with varying concentrations of protease K for 25 min at 30 °C. The amount of PBP1* left intact in the protease-treated spheroplasts was evaluated by SDS/

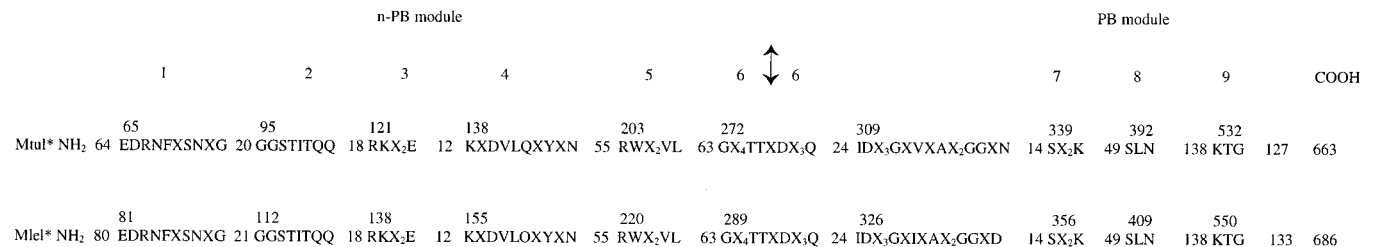


Figure 1 Modular design of the class A PBP1* from *M. tuberculosis* (Mtu1*) and *M. leprae* (Mle1*)

The conserved motifs along the amino acid sequence have been designated as described by Goffin and Ghuysen [10]. The numbers between the amino acid residues indicate the number of amino acids between each sequence. X represents any amino acid, and ↓ depicts the intermodule junction. The EMBL DNA databank accession numbers for Mtu1* and Mle1* are Z80775 and L39923 respectively.

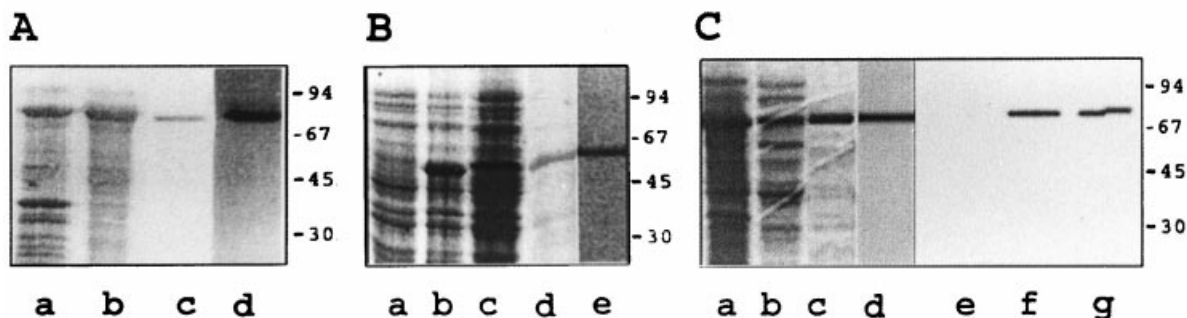


Figure 2 Overexpression, purification and binding of recombinant PBP1* to [¹⁴C]benzylpenicillin

(A) Coomassie-Blue-stained gels of membranes fractions of induced *E. coli* cells expressing PBP1* (lane a), Zwittergent 3–12-solubilized membrane proteins (lane b), purified PBP1* (lane c) and purified PBP1* labelled with [¹⁴C]benzylpenicillin (lane d). (B) Coomassie-Blue-stained gels of uninduced cells (lane a), induced cells (lane b), inclusion bodies from cells expressing PB-PBP1* (lane c), purified PB-PBP1* (lane d) and purified PB-PBP1* labelled with [¹⁴C]benzylpenicillin (lane e). (C) Coomassie-Blue-stained gels of induced cells expressing s-PBP1* (lane a), cell-free supernatant of the same cells (lane b), purified s-PBP1* (lane c) and purified s-PBP1* labelled with [¹⁴C]benzylpenicillin (lane d, e, f, g). Western blotting of the periplasmic (lane e) and cytosolic (lane f) extracts from cells over expressing s-PBP1* and crude extract from induced cells expressing s-PBP1* (lane g) probed with the anti-His antibody. The amount of protein loaded in each lane was derived from an equal number of cells. Molecular-mass markers are indicated (in kDa) on the right.

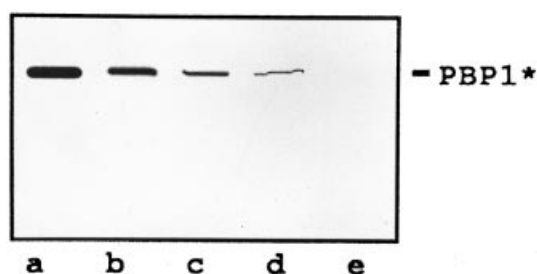


Figure 3 Protease digestion of PBP1* in spheroplasts

Spheroplasts of cells expressing full-length PBP1* were incubated with proteinase K at 0 (lane a), 40 (lane b), 80 (lane c), 200 (lane d) and 1000 (lane e) $\mu\text{g/ml}$, as described in the Materials and methods section. Samples were fractionated by SDS/PAGE, transferred on to nitrocellulose membranes and probed with antibodies against *M. leprae* PBP1*.

PAGE and Western blotting using an antibody against *M. leprae* PBP1*. PBP1* was totally degraded in spheroplasts at 1000 $\mu\text{g/ml}$ proteinase K (Figure 3), supporting the view that PBP1* adopts the expected membrane topology.

Purification of PBP1*

Extraction of PBP1* from the membranes was attempted using various detergents [1% (v/v) Triton X-100, 1% (w/v) CTAB, 1% (w/v) Zwittergent 3-12, 1% (v/v) Nonidet P40 and 1% (w/v) CHAPS], incubation temperatures of 25 and 4 °C and different incubation times. PBP1* was not solubilized with Nonidet P40, and was poorly extracted with Triton X-100 and CHAPS. Extraction was most efficient with CTAB and Zwittergent 3-12 at 4 °C. The Zwittergent 3-12-solubilized PBP1* was adsorbed on to a Ni²⁺-NTA-agarose column and subsequently eluted with a gradient of 100–200 mM imidazole/Zwittergent 3-12 in buffer A. The purified PBP1* (Figure 2A, lane c) was > 95% homogeneous, as determined by SDS/PAGE, and was capable of binding [¹⁴C]benzylpenicillin (Figure 2A, lane d). Purified PBP1* was thermolabile, similar to its *M. leprae* PBP1* counterpart [19]. Incubation for 5 min at 37 °C led to a loss of PB activity. The half-life of the purified protein was 15 min at 25 °C in buffer A containing 1% (w/v) Zwittergent 3-12.

Interaction of PBP1* with radioactive and non-radioactive β -lactams

At 25 °C, the k_2/K value of benzylpenicillin was $110000 \pm 11000 \text{ M}^{-1} \cdot \text{s}^{-1}$, in comparison with values of 5–10, > 50000, 800 and $150 \text{ M}^{-1} \cdot \text{s}^{-1}$ for *M. leprae* PBP1 and PBP1*, *E. coli* PBP1a and 1b respectively [19,20]. The k_3 value was $< 10^{-4} \text{ s}^{-1}$. The k_2/K values for interaction of the membrane-bound PBP1* with ampicillin, amoxicillin, cefoxitin, cefotaxime, ceftriaxone and cefuroxime were 10000 ± 800 , 20000 ± 2100 , 3400 ± 200 , 100000 ± 11000 , 11000 ± 1000 and $76000 \pm 8000 \text{ M}^{-1} \cdot \text{s}^{-1}$ respectively. The effectiveness of ampicillin and amoxicillin in acylating PBP1* is consistent with previous observations, demonstrating the *in vitro* activity of ampicillin [2] and amoxicillin [1,3] against *M. tuberculosis*. Temocillin, piperacillin, methicillin and moxalactam were ineffective in acylating PBP1* at concentrations up to 1 mM. The purified protein interacted with [¹⁴C]benzylpenicillin and non-radioactive β -lactams with the same affinity as the membrane-bound PBP.

Truncation of the N-terminal n-PB module of PBP1*

In the case of *E. coli* PBP1b, the prototype class A high-molecular-mass PBP, the n-PB module has been shown to be required both for the proper folding of the PBP as well as for the transpeptidation function of the PB module [22]. Thus we attempted to evaluate the role of the n-PB module in these functions of *M. tuberculosis* PBP1*. In order to do so, successive deletions of the conserved motifs of the n-PB module were carried out by genetic manipulation. Deletion of the first conserved motif of the N-terminal module was performed in the vector pBAD-HisA at 37 °C, and the expression of the plasmid induced at 30 °C using various arabinose concentrations [0.0002–0.2 (w/v)]. The lowest concentration of arabinose required for expression was found to be 0.0002%. Even under these conditions, ($\Delta\text{V2-A64}$)PBP1* was present exclusively in the inclusion bodies (results not shown). Consistent with this observations, successive truncated versions of PBP1*, namely ($\Delta\text{V2-Q94}$)PBP1*, ($\Delta\text{V2-S137}$)PBP1*, ($\Delta\text{V2-L258}$)PBP1* and ($\Delta\text{V2-Q271}$)PBP1*, were localized to the inclusion bodies (results not shown).

Purification of the PB module

Attempts to generate a functional PB module of PBP1b, the *E. coli* class A high-molecular-mass PBP, have been unsuccessful [22]. Thus we tested whether the PB module of PBP1* could be obtained in a conformation competent to bind penicillin. The smallest truncated derivative, ($\Delta\text{V2-Q271}$)PBP1* (hereafter designated PB-PBP1*), representing the PB module fused to the N-terminal end of motif 6 of the n-PB module, localized to inclusion bodies when expressed in *E. coli*. The expressed protein was extracted from the inclusion bodies using 5 M guanidine/HCl in a solution containing 150 mM Tris/HCl (pH 8), 1 mM dithiothreitol and 1 mM EDTA, and dialysed against different buffers in order to obtain the ideal conditions for obtaining a refolded protein, as judged by its stability and its ability to bind penicillin. The protein was optimally refolded when dialysed against 400 mM Tris/HCl (pH 8) containing 20% (v/v) glycerol and 1 mM EDTA at 20 °C for 16 h. The yield of the solubilized recombinant protein was 20% of that present in the inclusion bodies. PB-PBP1* was purified by affinity chromatography on Ni²⁺-NTA-agarose (Figure 2B), as described above, and judged to be 90% pure by SDS/PAGE. PB-PBP1* was able to bind penicillin with the same efficiency as the full-length PBP1*. The k_2/K for benzylpenicillin binding was determined to be $100000 \pm 10000 \text{ M}^{-1} \cdot \text{s}^{-1}$.

Construction of s-PBP1*

The SecB protein binds to the first third of the precursor of the maltose-binding protein, preventing its premature folding and loss of competence for export [23]. To investigate the possibility of a similar interaction with PBP1*, in-frame deletions of the N-terminal module were generated. ($\Delta\text{G95-Q143}$)PBP1* (hereafter referred to as s-PBP1*) was obtained in the cell-free supernatant of *E. coli* in a soluble, translocation-defective form (Figure 2C, lane b). Chou-Fassman analysis suggested that the region encompassed by residues Gly⁹⁵–Gln¹⁴³ adopted a β -sheet structure. In order to localize s-PBP1*, periplasmic proteins obtained after the conversion of cells expressing s-PBP1* into spheroplasts and the cytosolic extract obtained after lysis of spheroplasts were separated by SDS/PAGE, electroblotted on to nitrocellulose and subjected to Western blotting using anti-His antibodies. It was observed that $90 \pm 5\%$ of s-PBP1* was retained in the cytosol. Under conditions in which 5% or more of the

expressed protein would be detectable if present in any fraction, no s-PBP1* was detected in the periplasm by Western blotting using anti-His antibodies (Figure 2C, lanes e–g). s-PBP1* was purified by affinity chromatography on Ni²⁺-NTA-agarose and judged to be 95% pure by SDS/PAGE (Figure 2C, lane c). The protein was capable of binding penicillin with the same efficiency ($k_2/K = 105000 \pm 9000 \text{ M}^{-1} \cdot \text{s}^{-1}$) as the full-length protein (Figure 2C, lane d). Its thermostability was also comparable with that of full-length PBP1*.

DISCUSSION

Analysis of the core-based clustering of the multimodular PBPs by sequence alignments indicates that the class A PBPs of *M. tuberculosis* and *M. leprae* form a distinct cluster [22]. *M. tuberculosis* PBP1* described in the present study is most similar in sequence to *M. leprae* PBP1* (Figure 1). The sequence similarity and sensitivity of *M. tuberculosis* PBP1* to β -lactam antibiotics suggest that it is the counterpart of *M. leprae* PBP1* [19]. It also makes it an attractive target for antimycobacterial chemotherapy. We demonstrate that the PB module retains its ability to bind penicillin even in the absence of the majority of the n-PB module. Deletion of the N-terminal 64 amino acid residues gives rise to a protein that is exclusively localized in inclusion bodies. The region encompassed by amino acid residues Gly⁹⁵–Gln¹⁴³ was found to be necessary for transport of PBP1* to the membrane. s-PBP1* is a soluble derivative of the protein probably representing a folded state, since it binds benzylpenicillin with the same catalytic efficiency as the full-length protein. This is the first report of the *in vivo* overexpression of a soluble derivative of a class A high-molecular-mass PBP capable of binding penicillin. It promises to be an important step towards the elucidation of the structure of the protein.

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