FOR THE RECORD

Crystallization and preliminary X-ray crystallographic analysis of the 38-kDa immunodominant antigen of *Mycobacterium tuberculosis*

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Abstract: The 38-kDa lipoprotein is one of the most potent cell surface immunogens of *Mycobacterium tuberculosis* in antibody- and T cell-mediated reactions. Using a pure recombinant form of the protein, we have recently shown that it binds phosphate much like that of the phosphate-binding protein ($M_r = 34.4$ kDa) that is localized in the periplasm of *Escherichia coli* and is involved as an initial receptor for active transport of phosphate. The purified 38-kDa protein has been crystallized in 2 forms that are suitable for high-resolution structural analyses. One form belongs to the monoclinic space group $P2_1$ with unit cell dimensions of $a = 67.42$ Å, $b = 113.38$ Å, $c = 42.68$ Å, and $\beta = 108.53^\circ$. The other is of the orthorhombic space group $P2_12_12$ with $a = 125.46$ Å, $b = 72.27$ Å, and $c = 73.43$ Å. Both crystal forms diffract to about 2 Å resolution on a fine focus rotating anode.

Keywords: antigen; *Mycobacterium tuberculosis*; phosphate-binding protein; X-ray crystallography

Development of highly specific serodiagnostic reagents for and potential vaccines against tuberculosis continues to receive intense effort. In light of the resurgence of tuberculosis and its link with AIDS, meningitis, or arthritis (Lan & Wu, 1992; Cacho et al., 1993; Gomez et al., 1993; Israel, 1993; Rom & Zhang, 1993; Uttley & Poznaniak, 1993), this effort has received considerable urgency. Toward this end, several antigens of *Mycobacterium tuberculosis* have been identified. Of these antigens, the 38-kDa protein is one of the most interesting and also one of the most immunoreactive. It has been extensively studied by several independent groups who have given it different designations—antigen 5, antigen 78, 38K protein, and protein antigen b (for review, see Harboe & Wiker, 1992). There are strong indi-

cations that the 38-kDa protein is a lipoprotein with the lipoyl moiety associated with the surface cell wall or cytoplasmic membrane (Young & Garbe, 1991). This location is consistent with the finding that the protein is somehow involved in phosphate metabolism (Andersen et al., 1990). The amino acid sequence of the 38-kDa protein also indicates the presence of a signal peptide at the N-terminal end (Andersen et al., 1989).

In our effort to study the structure and function of the 38-kDa protein and its interaction with a variety of monoclonal antibodies, we have recently overexpressed the gene encoding the protein in *Escherichia coli* and developed a protein purification procedure (Chang et al., 1994). The mature recombinant 38-kDa protein is missing the signal sequence (residues –1 to –23), and the Cys residue that immediately follows the signal sequence and is believed to be the site of lipoyl attachment. With the availability of a large quantity of pure 38-kDa protein, we were able to demonstrate that the protein binds phosphate in a manner very similar to that of the well-characterized PhoS (also known as Pst) phosphate-binding protein (PBP) from *E. coli* (Chang et al., 1994). PBP, which is smaller ($M_r = 34.4$ kDa) and found in the periplasmic space, is the primary high-affinity receptor for the osmotic shock-sensitive high-affinity active transport of phosphate. This demonstration has prompted us to designate the 38-kDa protein from *M. tuberculosis* also as a PBP. Comparison of the amino acid sequence of the *M. tuberculosis* PBP with that of the *E. coli* PBP shows about 50% homology and 30% identity (Andersen et al., 1989; Chang et al., 1994). Based on the crystal structure of the *E. coli* PBP complexed with phosphate (Luecke & Quicho, 1990), a much higher conservation is observed for the residues making hydrogen bonds to the bound phosphate (Chang et al., 1994). The lack of a complete conservation of the hydrogen bonding residues could account for the observation that the *M. tuberculosis* PBP exhibits 2–6-fold higher phosphate-binding activities than the *E. coli* PBP over a pH range of 4.5–8.45 (Wang et al., 1994). Direct comparison between the 2 proteins will require the structure determination of the *M. tuberculosis* PBP.

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Table 1. Crystal data

Crystal	Space group	Unit cell dimensions						Molecules per asymmetric unit	V_m^a (Å ³ /Da)	Resolution (Å)
		<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)	α (deg)	β (deg)	γ (deg)			
I	P2 ₁	67.4	113.4	42.7	90.0	108.5	90.0	2	2.04	2.1
II	P2 ₁ 2 ₁ 2	125.5	72.3	73.4	90.0	90.0	90.0	2	2.18	2.3

^a Matthews (1968).

The purified recombinant *M. tuberculosis* PBP (Chang et al., 1994) was crystallized by the vapor diffusion method at room temperature. Using crystal screen 1 (Hampton Research), conditions #9, 15, and 40 yielded initial crystals with #9 showing the most promise. Two extensive modifications of #9 were found that yielded monoclinic and orthorhombic crystal forms that are suitable for structural analysis.

The monoclinic crystals were obtained by seeding a 5- μ L drop containing 10 mg/mL protein, 14.4% polyethylene glycol (PEG)-4000, 96 mM ammonium acetate, and 48 mM sodium citrate, pH 5.6, and hanging over a well containing 1 mL of 24% PEG-4000, 160 mM ammonium acetate, and 80 mM sodium citrate, pH 5.6. The orthorhombic crystals were obtained by seeding 5- μ L drops of 10 mg/mL protein, 18% PEG-4000, 120 mM ammonium chloride, and 10 mM magnesium acetate, pH 7, and hanging over a well of 1 mL 20% PEG-4000, 0.2 M ammonium chloride, and 0.1 M magnesium acetate, pH 7.

Initial examination of the 2 crystal forms was performed using a 2-multiwire SDMS area detector mounted on a Rigaku RU-200 rotating anode with fine focused CuK α radiation operated at 40 kV and 110 mA. The results are collected in Table 1. The monoclinic and orthorhombic forms exhibit space groups P2₁ and P2₁2₁2, respectively. Both crystal forms diffract to about 2 Å resolution. Two molecules per asymmetric unit were assumed in both the monoclinic and orthorhombic crystals with corresponding V_m values shown in Table 1. These V_m values are within the range of 1.68–3.53 Å³ normally found for over a hundred crystalline proteins (Matthews, 1968).

Because the use of molecular replacement technique, with the known crystal structure of the *E. coli* PBP with bound phosphate as a search model, to determine the structure of the *M. tuberculosis* PBP has thus far proven unsuccessful, we have re-

sorted to the use of the multiple isomorphous replacement technique.

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