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Short crystallization paper

Crystallization of AcpA, a respiratory burst-inhibiting acid phosphatase from *Francisella tularensis*

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Abstract

Francisella tularensis is a highly infectious bacterial pathogen that is classified as a Category A Pathogen by the Centers for Disease Control and Prevention. Here, we report crystallization of a recombinant form of *F. tularensis* AcpA, a unique and highly expressed acid phosphatase that is thought to play a role in intracellular survival by inhibiting the host respiratory burst. Three crystal forms have been obtained, with form III being the most suitable for high-resolution structure determination. Form III crystals were grown in the presence of PEG 1500 and the competitive inhibitor sodium orthovanadate (5 mM). The space group is C222(1) with unit cell parameters $a = 112.1$ Å, $b = 144.4$ Å, $c = 123.9$ Å. The asymmetric unit is predicted to contain two protein molecules and 43% solvent. A 1.75-Å native data set was recorded at beamline 8.3.1 of the Advanced Light Source. To our knowledge, this is the first report of high-resolution crystals of any *F. tularensis* protein.

Keywords: *Francisella tularensis*; Acid phosphatase; AcpA; Biodefense; Crystallization; X-ray diffraction

Francisella tularensis is a highly infectious, intracellular bacterial pathogen and it is the causative agent of the zoonotic disease tularemia [1]. The CDC (Centers for Disease Control and Prevention) considers *F. tularensis* to be a potential bioterrorism agent, in part, because of its very high infectivity after aerosolization and its presence in former biowarfare stockpiles [2–4]. Accordingly, the CDC has classified *F. tularensis* as a Category A Pathogen. Understanding the biochemistry and biology underlying *F. tularensis* virulence is necessary for the development of improved vaccines and antimicrobial agents that will provide protection and treatment in the event of a *F. tularensis*-based attack. Thus, much effort is currently being directed at identifying proteins that play roles in virulence and intracellular survival mechanisms [5–10]. The recent release of the complete genome sequence of a highly virulent strain of *F. tularensis* is expected to greatly facilitate this effort [11].

As part of our research program aimed at understanding the roles of phosphatases in *F. tularensis* survival and virulence, we report the crystallization of a recombinant form of AcpA, an acid phosphatase that is highly expressed by *F. tularensis*. AcpA is one of the best-characterized *F. tularensis* proteins to date. The enzyme has been studied, in part, because it appears to play a role in facilitating the intracellular lifestyle of *F. tularensis* [12] by inhibiting the host respiratory burst response during entry of the pathogen into macrophages [13].

AcpA is a 57-kDa enzyme that hydrolyzes a wide variety of phosphomonoesters including nucleotides, *p*-nitrophenyl phosphate, 4-methylumbelliferyl-phosphate and amino acid phosphates [13,14]. The enzyme is most active at pH 6.0, consistent with its designation as an acid phosphatase [13,14]. AcpA also displays low-level phospholipase C activity [13]. This aspect of the enzyme has prompted speculation that AcpA might help degrade the phagosomal membrane and thus facilitate escape of the bacterium into the cytosol of host cells [11].

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AcpA appears to be a unique bacterial acid phosphatase. The amino acid sequence does not contain any of the short conserved sequence motifs that would allow classification into the A/B/C scheme that has been proposed by Rossolini and coworkers [15] for bacterial acid phosphatases. Nor does the AcpA sequence share any significant similarity with any protein in the Protein Data Bank (PDB, [16]). Thus, AcpA potentially represents the prototype of a new family of bacterial acid phosphatase.

Recombinant AcpA (rAcpA) was expressed in *E. coli* and purified using methods described elsewhere [14]. The purified enzyme was dialyzed against 50 mM sodium acetate, 150 mM NaCl, pH 6.0, and concentrated to 2–20 mg/mL. The protein concentration was measured with the Coomassie Plus assay (Pierce). Purified rAcpA was judged to be dimeric in solution and highly monodisperse based on gel filtration chromatography, dynamic light scattering, and analytical ultracentrifugation [14].

All crystallization experiments were performed at 295 K using the sitting drop method of vapor diffusion with drops formed by mixing equal volumes of the reservoir and protein solution. The Hampton Index Screen was used to identify initial crystallization conditions. Several conditions in the screen yielded crystals of various size and quantity (Hampton Index Formulations 37, 63, 68, 73, 77, 85, 86, 87, 88, 89, 91, 92, 94). Polyethylene glycol (PEG) in the molecular weight range 1500–5000 was common to all of the positive conditions.

Optimization studies using PEGs with different molecular weights, variation of the protein concentration, and inclusion of an inhibitor in the crystallization setup resulted in the growth of three crystal forms of rAcpA (Table 1). Crystal form I was grown with a 2- to 4-mg/mL protein stock solution and reservoir consisting of 5.0% Tacsimate, 0.1 M HEPES pH 7.0, 10% (w/v) PEG monomethyl ether (PEGMME) 5000. These crystals displayed a coffin-shaped plate-like morphology (Fig. 1a) and diffracted to 3.2 Å resolution when analyzed on a Cu rotating anode X-ray diffraction system equipped with an Raxis-IV image plate detector and Osmic confocal optics. Autoindexing calculations performed with HKL [17] indicated a primitive orthorhombic lattice with unit cell dimensions $a=85$ Å, $b=262$ Å, $c=296$ Å. Analysis of the solvent content using the method of Matthews [18] suggested the presence of 10–16 rAcpA molecules per asymmetric unit and a solvent content of 32–57%. Clearly, form I is not optimal for high-resolution structure determination.

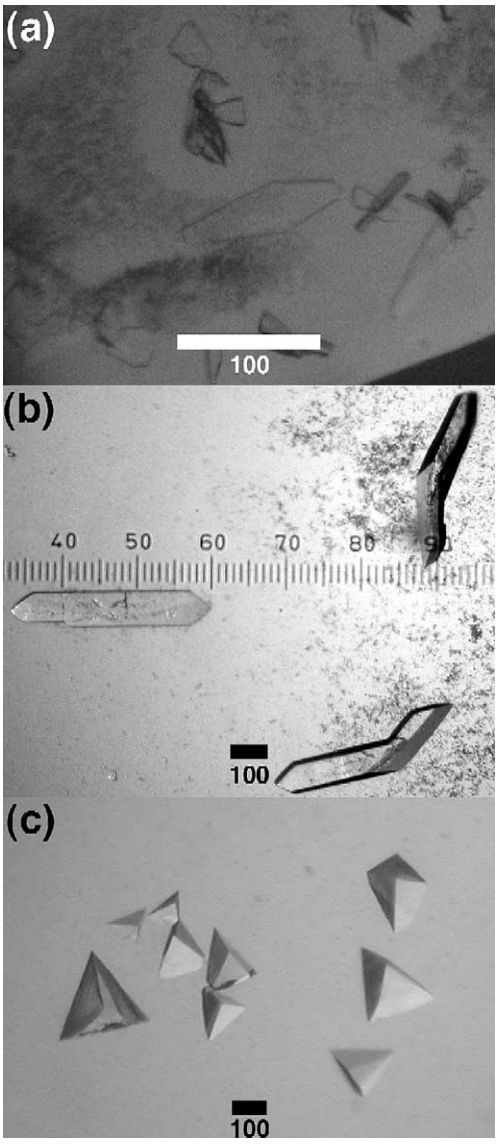

Fig. 1. Three crystal forms of recombinant *F. tularensis* AcpA. Forms I, II, and III are shown in panels (a), (b), and (c), respectively. The bar in each panel represents approximately 100 μm.

Table 1
Summary of three crystal forms of recombinant AcpA

	Form I	Form II	Form III
Precipitating agent	PEGMME 5000	PEG 1500	PEG 1500
Protein concentration (mg/mL)	2–4	2–4	17–20
Inhibitor	None	Na_3VO_4	Na_3VO_4
Bravais lattice or space group	*Pmmm*	*Pmmm*	$C222_1$
Unit-cell dimensions (Å)	$a=85$ $b=262$ $c=296$	$a=110$ $b=126$ $c=144$	$a=112$ $b=144$ $c=124$
Diffraction limit (Å)	3.2	2.2	1.75
Molecules in asymmetric unit	10–16	3–5	2
V_M (Å^3/Da)	1.8–2.9	1.8–2.9	2.2
Solvent content (%)	32–57	30–58	43

Crystal form II was obtained by incubating the enzyme with the competitive inhibitor sodium orthovanadate (5 mM, Na_3VO_4) prior to crystallization. Monomeric vanadate is a structural and electronic analogue of phosphate and thus it is a potent inhibitor of many phosphatases [19], including AcpA [13,14]. A protein stock solution of 2–4

Table 2
Data-collection and processing statistics (crystal form III)

Beamline	ALS 8.3.1
Wavelength (Å)	1.127129
Space group	$C222_1$
Unit-cell dimensions (Å)	$a=112.1$, $b=144.4$, $c=123.9$
Diffraction resolution (Å)	50–1.75 (1.81–1.75)
No. of observations	397516
No. of unique reflections	98137
Redundancy	4.1 (4.0)
Refined mosaicity (°)	0.3
Completeness (%)	97.2 (94.9)
Average $I/\sigma(I)$	26.6 (3.8)
R_{merge}	0.047 (0.357)

Values for the outer resolution shell of data are given in parentheses.

mg/mL was used and the optimal reservoir condition was 16–18% (w/v) PEG 1500. These crystals grew within 3 days after setup and took the form of long, rectangular solids with pointed ends (Fig. 1b). Diffraction to 2.2 Å resolution was observed, and autoindexing suggested a primitive orthorhombic lattice with unit cell dimensions $a=110$ Å, $b=126$ Å, $c=144$ Å. The asymmetric unit of crystal form II is expected to contain 3–5 rAcpA molecules and 30–58% solvent. Since AcpA is expected to be dimeric [14], the most likely content of the asymmetric unit for form II crystals would be 4 molecules (2 dimers).

As with form II, crystal form III grew in the presence of 5 mM Na_3VO_4 and PEG 1500, however, the protein concentration was approximately 5–10 times higher (17–20 mg/mL) than that used to crystallize forms I and II (Table 1). The optimal reservoir condition was 19.5% (w/v) PEG 1500. Tetrahedron-shaped crystals typically grew to a maximum dimension of 0.4 mm within 36 h (Fig. 1c). In preparation for low temperature data collection, the crystals were cryoprotected by exchanging the mother liquor with a solution containing 25% (w/v) PEG 1500 and 25% (v/v) PEG 200. After a few minutes, the crystals were picked up with Hampton mounting loops and plunged into liquid nitrogen.

Form III crystals have space group $C222_1$ with unit cell dimensions $a=112$ Å, $b=144$ Å, $c=124$ Å. The asymmetric unit contains only two rAcpA molecules, with a Matthews coefficient of 2.2 $Å^3$/Da and a solvent content of 43%. These crystals diffracted well beyond 2 Å when analyzed using the rotating anode system.

Form III has been chosen for structure determination because of its exceptional diffraction characteristics, low mosaicity, and amenable unit cell dimensions. A 1.75-Å resolution native data set was collected at beamline 8.3.1 of the Advanced Light Source, and the data were processed with HKL2000 [17]. The data set consisted of 100 frames with an oscillation angle of 1°, exposure time of 1.2 s per frame, and detector distance of 100 mm. See Table 2 for data processing statistics. AcpA is sufficiently novel that suitable molecular replacement search models are not available from the PDB. Structure determination by the method of multiple isomorphous replacement is currently in progress.

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References

[1] R.W. Titball, A. Johansson, M. Forsman, Will the enigma of *Francisella tularensis* virulence soon be solved? Trends Microbiol. 11 (2003) 118–123.
[2] D.T. Dennis, T.V. Inglesby, D.A. Henderson, J.G. Bartlett, M.S. Ascher, E. Eitzen, A.D. Fine, A.M. Friedlander, J. Hauer, M. Layton, S.R. Lillibridge, J.E. McDade, M.T. Osterholm, T. O'Toole, G. Parker, T.M. Perl, P.K. Russell, K. Tonat, Tularemia as a biological weapon: medical and public health management, JAMA 285 (2001) 2763–2773.
[3] A.F. Kaufmann, M.I. Meltzer, G.P. Schmid, The economic impact of a bioterrorist attack: are prevention and postattack intervention programs justifiable? Emerg. Infect. Dis. 3 (1997) 83–94.
[4] P.C. Oyston, A. Sjostedt, R.W. Titball, Tularaemia: bioterrorism defence renews interest in *Francisella tularensis*, Nat. Rev., Microbiol. 2 (2004) 967–978.
[5] I. Golovliov, M. Ericsson, G. Sandstrom, A. Tarnvik, A. Sjostedt, Identification of proteins of *Francisella tularensis* induced during growth in macrophages and cloning of the gene encoding a prominently induced 23-kilodalton protein, Infect. Immun. 65 (1997) 2183–2189.
[6] F.E. Nano, N. Zhang, S.C. Cowley, K.E. Klose, K.K. Cheung, M.J. Roberts, J.S. Ludu, G.W. Letendre, A.I. Meierovics, G. Stephens, K.L. Elkins, A *Francisella tularensis* pathogenicity island required for intramacrophage growth, J. Bacteriol. 186 (2004) 6430–6436.
[7] C.M. Lauriano, J.R. Barker, S.S. Yoon, F.E. Nano, B.P. Arulanandam, D.J. Hassett, K.E. Klose, MglA regulates transcription of virulence factors necessary for *Francisella tularensis* intraamoebae and intramacrophage survival, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4246–4249.
[8] M. Hubalek, L. Hernychova, M. Brychta, J. Lenco, J. Zechovska, J. Stulik, Comparative proteome analysis of cellular proteins extracted from highly virulent *Francisella tularensis* ssp. tularensis and less virulent *F. tularensis* ssp. holarctica and *F. tularensis* ssp. mediaasiatica, Proteomics 4 (2004) 3048–3060.
[9] X.H. Lai, I. Golovliov, A. Sjostedt, Expression of IglC is necessary for intracellular growth and induction of apoptosis in murine macrophages by *Francisella tularensis*, Microb. Pathog. 37 (2004) 225–230.
[10] A. Sjostedt, Virulence determinants and protective antigens of *Francisella tularensis*, Curr. Opin. Microbiol. 6 (2003) 66–71.

[11] P. Larsson, P.C. Oyston, P. Chain, M.C. Chu, M. Duffield, H.H. Fuxelius, E. Garcia, G. Halltorp, D. Johansson, K.E. Isherwood, P.D. Karp, E. Larsson, Y. Liu, S. Michell, J. Prior, R. Prior, S. Malfatti, A. Sjostedt, K. Svensson, N. Thompson, L. Vergez, J.K. Wagg, B.W. Wren, L.E. Lindler, S.G. Andersson, M. Forsman, R.W. Titball, The complete genome sequence of *Francisella tularensis*, the causative agent of tularemia, Nat. Genet. 37 (2005) 153–159.

[12] A.H. Fortier, S.J. Green, T. Polsinelli, T.R. Jones, R.M. Crawford, D.A. Leiby, K.L. Elkins, M.S. Meltzer, C.A. Nacy, Life and death of an intracellular pathogen: *Francisella tularensis* and the macrophage, Immunol. Ser. 60 (1994) 349–361.

[13] T.J. Reilly, G.S. Baron, F.E. Nano, M.S. Kuhlenschmidt, Characterization and sequencing of a respiratory burst-inhibiting acid phosphatase from *Francisella tularensis*, J. Biol. Chem. 271 (1996) 10973–10983.

[14] T.J. Reilly, R.L. Felts, M.T. Henzl, M.J. Calcutt, J.J. Tanner, Characterization of recombinant *Francisella tularensis* acid phosphatase, Prot. Exp. Purif. (in press).

[15] G.M. Rossolini, S. Schippa, M.L. Riccio, F. Berlutti, L.E. Macaskie, M.C. Thaller, Bacterial nonspecific acid phosphohydrolases: physiology, evolution and use as tools in microbial biotechnology, Cell. Mol. Life Sci. 54 (1998) 833–850.

[16] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The protein data bank, Nucleic Acids. Res. 28 (2000) 235–242.

[17] Z. Otwinowski, W. Minor, Processing of X-ray diffraction data collected in oscillation mode, Methods Enzymol. 276 (1997) 307–326.

[18] B.W. Matthews, Solvent content of protein crystals, J. Mol. Biol. 33 (1968) 491–497.

[19] D.C. Crans, J.J. Smee, E. Gaidamauskas, L. Yang, The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds, Chem. Rev. 104 (2004) 849–902.