Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of the acyl carrier protein synthase (AcpS) from *Staphylococcus aureus*

Dayna L. Daubaras, Eileen M. Wilson, Todd Black, Corey Strickland, Brian M. Beyer and Peter Orth*

Schering–Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, USA

Correspondence e-mail: peter.orth@spcorp.com

Received 19 December 2003
Accepted 10 February 2004

Acyl carrier protein synthase (AcpS) catalyzes the transfer of 4′-phophopantetheine from coenzyme A to the acyl carrier protein (ACP) to activate it for fatty-acid biosynthesis. Two crystal forms of *Staphylococcus aureus* AcpS have been generated at 277 K using either NaCl or PEG 6000 as a precipitant. The diffraction patterns of the crystals extend to 1.65 and 1.8 Å, respectively. Full sets of X-ray diffraction data were collected from native crystals and the crystal structures were solved by molecular replacement.

© 2004 International Union of Crystallography
Printed in Denmark – all rights reserved

1. Introduction

In response to an increasing occurrence of bacterial pathogens that are resistant to current antimicrobial therapies, there is a need for novel chemical agents that are effective against target enzymes of biological pathways (Miesel *et al.*, 2003). In addition to target enzymes of current therapeutic agents such as those involved in cell-wall biosynthesis and DNA replication (Green, 2002), fatty-acid biosynthetic enzymes are acceptable targets since fatty acids are required for bacterial growth: they are major components of bacterial membranes (Campbell & Cronan, 2001). Bacterial fatty-acid biosynthesis involves repeat cycles of condensation, reduction, dehydration and isomerization reactions catalyzed by various enzymes encoded by individual genes (Magnuson *et al.*, 1993). In these reactions, the 8.8 kDa acidic acyl carrier protein (ACP) plays an essential role as the carrier of fatty-acid precursors and growing acyl intermediates. ACP is synthesized as inactive apo-ACP and converted to active holo-ACP. The enzyme holo acyl carrier protein synthase (AcpS) activates apo-ACP by catalyzing the covalent attachment of the 4′-phosphopantetheinyl moiety of coenzyme A (CoA) to a conserved serine residue (Ser36 of *Saccharomyces aureus* ACP).

The AcpS enzyme, first identified in *Escherichia coli*, has been found to be essential for bacterial growth (Takiff *et al.*, 1992). It has been included in the list of 192 essential genes reported for *Bacillus subtilis* (Kobayashi *et al.*, 2003). The *B. subtilis* AcpS (PDB code 1f7t; Parris *et al.*, 2000) and the *Streptococcus pneumoniae* AcpS (PDB code 1fth; Chirgadze *et al.*, 2000) crystal structures have been resolved at 1.5 and 1.9 Å, respectively. The overall protein sequence identity of *S. aureus* AcpS to *B. subtilis* AcpS and *S. pneumoniae* AcpS are 49 and 37%, respectively. The human 4′-phosphopantetheinyl transferase has recently been identified and shows virtually no protein sequence identity to the bacterial AcpS family (Joshi *et al.*, 2003). Within the Gram-positive and Gram-negative bacterial AcpS enzymes, the active site is highly conserved and therefore the crystal structures will be useful in the design of broad-spectrum antibiotics. Here, we report the purification and crystallization of two crystal forms of *S. aureus* AcpS diffracting to 1.65 and 1.8 Å.

2. Expression and purification

For increased protein production, the *acpS* gene from *S. aureus* was amplified by PCR and cloned into the T7-RNA polymerase-based expression vector pET22b from Novagen (Studier & Moffatt, 1986). *E. coli* strain BL21 (DE3) harboring the recombinant *acpS* was incubated in yeast-tryptone medium supplemented with 50 m*M* carbenicillin for selection. At an optical density (600 nm) of 1.0, protein expression was induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1.0 m*M*. After 16 h of continued incubation at 289 K, cells were harvested by centrifugation.

Cell pellets were lysed in 50 m*M* Tris pH 8.0 buffer containing B-PER (Bacterial Protein Extraction Reagent, Pierce), 5.0 m*M* $MgSO_4$, 2.0 m*M* DTT, 12 500 units l^{-1} benzonase and 5 ml l^{-1} protease inhibitor cocktail (Roche). The cell extract was clarified by centrifugation at 19 000 rev min^{-1} prior to incubation with Q-Sepharose Fast Flow (Amersham Biosciences) for 1 h. The resin was removed by centrifugation and the supernatant was brought to pH 6.5 using 0.5 *M* MES buffer. The supernatant containing AcpS was loaded onto a SP-Sepharose Fast Flow column equilibrated with 50 m*M* MES pH 6.5 buffer containing 2.0 m*M* DTT. AcpS was eluted with the same buffer containing 250 m*M* NaCl. The protein was further purified by size-exclusion chro-

matography using a Superdex 200 gel-filtration column equilibrated with 75 mM potassium phosphate pH 7.5 containing 5 mM DTT. The fractions containing AcpS were pooled and concentrated to 15.0 mg ml^{-1}.

3. Crystallization

The hanging-drop vapor-diffusion method at 277 K using 24-well plates was used for crystallization screening (McPherson, 1982). Conditions were screened using Crystal Screen from Hampton Research. Typically, 2.0 µl droplets were prepared on siliconized cover slips by mixing equal amounts of protein solution and reservoir solution and subsequently vapor-equilibrating against 1.0 ml reservoir solution. AcpS crystallized in two different crystal forms: rod-shaped and plate-shaped crystals. Rod-shaped crystals formed when using 2 M NaCl and 100 mM sodium citrate buffer pH 4.6 (crystal form I; Fig. 1*a*). Plate-shaped crystals appeared when using 10% PEG 4000 and 100 mM $CaCl_2$ in 100 mM HEPES buffer pH 7.1 (crystal form II; Fig. 1*b*). Both crystal forms appeared after one week.

4. Data collection and analysis

Cryogenic data collection was performed at 100 K by transferring crystals into reservoir solution containing an additional 20% glycerol and then flash-freezing in liquid nitrogen. X-ray diffraction data were collected using the synchrotron-radiation source at the IMCA-CAT beamline BM17 at Argonne National Laboratory (Argonne, IL, USA). The *HKL*2000 (Otwinowski & Minor, 1997) and *CCP*4 (Collaborative Computational Project, Number 4, 1994) packages were used for data reduction and the results are summarized in Table 1.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the last shell.

	Form I	Form II
Space group	$I4$	$P2_1$
Unit-cell parameters (Å, °)	a = 103.5, c = 71.0	a = 46.71, b = 106.17, c = 67.78, β = 92.6
Resolution (Å)	50–1.65 (1.71–1.65)	50–1.8 (1.86–1.80)
Observed reflections	153468	222400
Independent reflections	44440	59477
Completeness	98.4 (94.6)	98.5 (90.1)
Redundancy	3.4 (3.0)	3.7 (3.1)
$R_{\rm merge}(I)$†	3.2 (38.8)	3.4 (49.2)
$I/\sigma(I)$	20.3 (3.1)	19.2 (2.2)

† $R_{\rm merge} = \sum |I_h - \langle I \rangle| / \sum I_h$, where I_h is the measured and $\langle I \rangle$ is the average intensity of reflection hkl.

5. X-ray analysis

The solvent content in the AcpS crystals was calculated as 46.5 and 39.4% assuming one and two trimers in the asymmetric unit for crystal forms I and II, respectively, with a density of 1.30 g ml^{-1}. The Matthews coefficients (Matthews, 1968) were 2.3 Å^3 Da^{-1} (crystal form I) and 2.0 Å^3 Da^{-1} (crystal form II) based on a molecular weight of 41 kDa per subunit for AcpS. The structures were solved by molecular replacement using the program *AMoRe* (Navaza, 1994) and *B. subtilis* AcpS (PDB code 1f7t) as the search model. The protein sequence identity between *B. subtilis* and *S. aureus* AcpS is about 49%. Since the AcpS trimer contains a threefold rotational symmetry, rotation searches provided sets of three related solutions. Three significant unique rotation-search solutions of the $I4$ data set in the resolution range 4–12 Å had correlation coefficients in the range 10.2–11.4 (background 7.7). The best solution was used in the translation function, resulting in a correlation coefficient of 22.8 (background 11.3). Using the $P2_1$ data set, nine solutions with correlation coefficients in the range 11.7–14.7 (background 8.5) were observed, of which the best solution resulted in a translation-search correlation coefficient of 15.4 (background 11.8). The second AcpS trimer was identified based on the first translation-function solution. The alternate space groups $I4_1$ and $I4_3$ as well as $P2$ were eliminated, as their translation function resulted in values in the range of the background obtained for the correct space groups. In addition, systematic absences along 0k0 in the second crystal form verified the choice of space group. In both crystal forms, a reasonable crystal packing was observed. The initial molecular-replacement solution was subjected to molecular-dynamics refinement in *CNX* (Molecular Simulations, Inc.) and rebuilding with *QUANTA*.

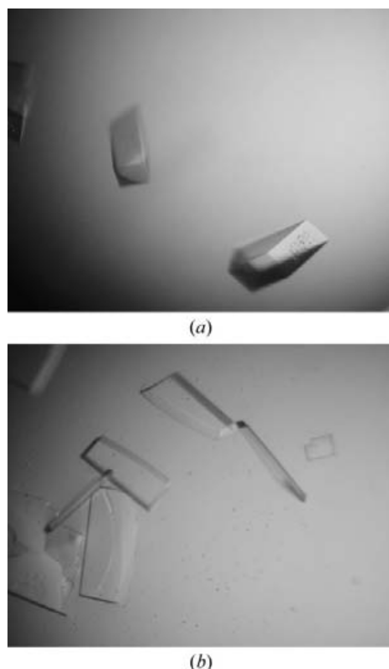

Figure 1
Two crystal forms of Acps. (*a*) Crystals of form I with average crystal dimensions 0.7 × 0.2 × 2 mm. (*b*) Plates of crystal form II grew to 0.5 × 0.4 × 0.1 mm.

We thank the IMCA-CAT staff for assistance with synchrotron data collection. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. W-31-109-Eng-38.

References

Campbell, J. W. & Cronan, J. E. (2001). *Annu. Rev. Microbiol.* **55**, 305–332.
Chirgadze, N. Y., Briggs, S. L., McAllister, K. A., Fischl, A. S. & Zhao, G. (2000). *EMBO J.* **19**, 5281–5287.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Green, D. W. (2002). *Expert Opin. Ther. Targets*, **6**, 1–19.
Joshi, A. K., Zhang, L., Rangan, V. S. & Smith, S. (2003). *J. Biol. Chem.* **278**, 33142–33149.
Kobayashi, K. *et al.* (2003). *Proc. Natl Acad. Sci. USA*, **100**, 4678–4683.
McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*, pp. 82–127. New York: Wiley.
Magnuson, K., Jackowski, S., Rock, C. O. & Cronan, J. E. (1993). *Microbiol. Rev.* **57**, 522–542.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Miesel, L., Greene, J. & Black, T. A. (2003). *Nature Rev. Genet.* **4**, 442–456.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Parris, K. D., Lin, L., Tam, A., Mathew, R., Hixon, J., Stahl, M., Fritz, C. C., Seehra, J. & Somers, W. S. (2000). *Structure*, **8**, 883–895.
Studier, F. W. & Moffatt, B. A. (1986). *J. Mol. Biol.* **189**, 113–130.
Takiff, H. E., Baker, T., Copeland, T., Chen, S. M. & Court, D. L. (1992). *J. Bacteriol.* **174**, 1544–1553.