Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction studies of glycerol 3-phosphate cytidylyltransferase from *Staphylococcus aureus*

Veronica C. N. Yim, Michela Zolli, David S. Badurina, Laura Rossi, Eric D. Brown and Albert M. Berghuis*

Antimicrobial Research Centre and Department of Biochemistry, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada

Correspondence e-mail: berghuis@mcmaster.ca

Received 2 February 2001
Accepted 23 March 2001

Glycerol 3-phosphate cytidylyltransferase from *Staphylococcus aureus* ($TarD_{Sa}$) has been expressed in *Escherichia coli*, purified to homogeneity and crystallized. The strategy used for determining crystallization conditions employed hanging-drop sparse-matrix screens and required a combination of three different optimization approaches. Specifically, the presence or absence of cofactors needed to be surveyed, the effects of small-molecule additives required exploration and the rate of vapour-diffusion had to be varied in order to obtain crystals of $TarD_{Sa}$ suitable for diffraction studies. Crystals thus obtained belong to the space group $P3_121$, with unit-cell parameters $a = b = 92.2$, $c = 156.1$ Å, and contain four $TarD_{Sa}$ molecules per asymmetric unit. The resolution limit observed for these crystals using synchrotron radiation is 3.0 Å.

1. Introduction

The human pathogen *S. aureus* is one of the most common causes of hospital- and community-acquired infections such as infection of surgical wounds, toxic shock syndrome and food poisoning (Lowy, 1998). The properties of *S. aureus* that can perhaps explain its virulence are its ability to produce a variety of toxins and its ability to resist the onslaught of antibiotics (Archer, 1998; Lowy, 1998). To illustrate this last point, within 3 y of the introduction of penicillin in 1940 clinical resistance to this antibiotic had already developed in *S. aureus* (Infectious Diseases and Immunization Committee, 1999). The latest development of antibiotic resistance in *S. aureus* is methicillin resistance. As a consequence of this dubious achievement, the only viable antibiotic currently available for treatment of resistant *S. aureus* infections is vancomycin (Lowy, 1998). However, it is questionable if this treatment option will remain available, since incidences of reduced vancomycin susceptibility in *S. aureus* have recently been reported in Japan and the United States (see, for example, Marchese *et al.*, 2000; Fridkin, 2001). Thus, there is a pressing need for the development of antibiotics that can combat infections caused by multidrug-resistant strains of the human pathogen *S. aureus*.

One of the most successful targets for antibiotics is bacterial cell-wall synthesis (*e.g.* penicillins and vancomycin; Walsh, 2000). In *S. aureus* and other Gram-positive bacteria, one of the major components of the cell wall is a teichoic acid polymer (Shockman & Barrett, 1983; Lowy, 1998). Studies in *Bacillus subtilis* have shown that synthesis of this polymer is essential for survival (Mauel *et al.*, 1989). We have initiated crystallographic studies of one of the enzymes from *S. aureus* involved in teichoic acid biosynthesis ($TarD_{Sa}$), with the objective of using the structural information in the development of novel antibiotics to Gram-positive pathogenic bacteria such as *S. aureus*. $TarD_{Sa}$, which is also referred to as glycerol 3-phosphate cytidylyltransferase, is a 132 amino-acid soluble protein. It catalyzes the formation of CDP-glycerol from CTP and glycerol-3-phosphate. The CDP-glycerol product is subsequently used as a building block for synthesis of the teichoic acid polymer. Note that the structure of the homologue of this enzyme from *B. subtilis* has recently been determined (Weber *et al.*, 1999).

2. Experimental procedures

2.1. Cloning and plasmid construction

All plasmids were propagated in *E. coli* Novablue cells (Novagen). The gene $tarD_{Sa}$ was amplified from *S. aureus* genomic DNA using the following primers: 5′-CCGGGC-**CATATG**AAACGTGTAATAACCTATGGC-ACCTATGACTTACTTC-3′ and 5′-CGCGC-**GGATCC**TTATTTAGCATCTTTACCA-3′. The bold sequences represent *Nde*I and *Bam*HI restriction-enzyme sites, respectively. The first primer incorporated two silent mutations in *tarD* so as to eliminate two *Nde*I sites present near the start of the gene. The blunt-ended product was inserted at the *Eco*RV restriction site of pBluescript SK (−/+)

(Stratagene). Subsequently, *tarD* was excized from pBluescript using *Nde*I and *Hin*dIII and inserted into the backbone of pBF-9, a pKK233 expression-vector derivative described in Daigle *et al.* (1999). This construct was confirmed by sequencing (MOBIX, McMaster University). Competent *E. coli* W3110 cells were transformed using established methods and ultimately used for the overexpression of $TarD_{Sa}$.

2.2. Overexpression and purification

Single colonies of *E. coli* W3110 containing the overexpression vector were used to inoculate 25 ml of LB-Amp (50 µg ml^{-1}) and grown overnight at 310 K. This starter culture was then used to grow 1 l LB-Amp (50 µg ml^{-1}) until an OD_{600} of approximately 0.8. Following this, the culture was induced with IPTG and allowed to grow for an additional 5 h. Cells were harvested by centrifugation at 5000*g* for 10 min, washed with 0.85% NaCl and pelleted by centrifugation at 5000*g* for an additional 10 min. The cell pellet was then resuspended in 25 m*M* HEPES pH 8.0, 5 m*M* EDTA, 1 m*M* DTT and lysed by two passes through a French press. To remove cell debris, lysed cells were centrifuged for 1 h at 15 000*g*. The supernatant was loaded onto a 50 ml Q-Sepharose anion-exchange column and eluted with a linear gradient of 1 *M* NaCl. Fractions containing $TarD_{Sa}$ were dialyzed overnight against 25 m*M* HEPES pH 8.0, 10 m*M* $MgCl_2$, 1 m*M* DTT and loaded onto a 30 ml Reactive blue 4 affinity column. $TarD_{Sa}$ was eluted from the Reactive blue 4 column with 25 m*M* HEPES pH 8.0, 10 m*M* $MgCl_2$, 1 m*M* DDT, 1 m*M* CTP. Pooled fractions containing $TarD_{Sa}$ were concentrated by precipitation with 80% ammonium sulfate followed by passage over a 125 ml Sephadex 200 size-exclusion column. The Sephadex 200 column was equilibrated with 25 m*M* HEPES pH 8.0 and 1 m*M* DTT. The progress of the purification was monitored by Coomassie- and silver-stained SDS–PAGE gels and the final concentration of pure protein was determined using the Bradford Assay (Bradford, 1976). Using this protein-purification procedure, 15 mg of $TarD_{Sa}$ could be obtained from a 1 l culture.

2.3. Crystallization

Prior to crystallization, the concentration of purified protein was adjusted to 10 mg ml^{-1} in 25 m*M* HEPES pH 8.0, 1 m*M* DTT. The buffer condition was selected based on light-scattering studies (Zulauf & D'Arcy, 1992). Crystallization was carried out using the hanging-drop vapour-diffusion method, in which the well contained 600 µl of reservoir solution and the drop contained 2 µl of reservoir solution and 2 µl of protein solution (McPherson, 1976). Crystallization experiments were conducted at two different temperatures: 277 and 295 K. Sparse-matrix screening was performed using 290 conditions obtained from commercial suppliers (Crystal Screen I and II from Hampton Research, Wizard I and II and Cryo I and II from Emerald BioStructures; Jancarik & Kim, 1991). Refinements of the preliminary conditions producing crystals are described in §3.

2.4. Data collection and processing

Data were collected from a single crystal at the X8-C beamline of the National Synchrotron Light Source at Brookhaven National Laboratory equipped with an ADSC Quantum 4R CCD detector. The crystal was mounted in a cryoloop and placed in a stream of cold nitrogen at 100 K. Prior to mounting, the crystal was briefly transferred to a 25% ethylene glycol solution. The wavelength used for data collection was 1.072 Å and the crystal-to-detector distance was 280 mm, with an oscillation angle of 1.5° per frame. The crystallographic data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

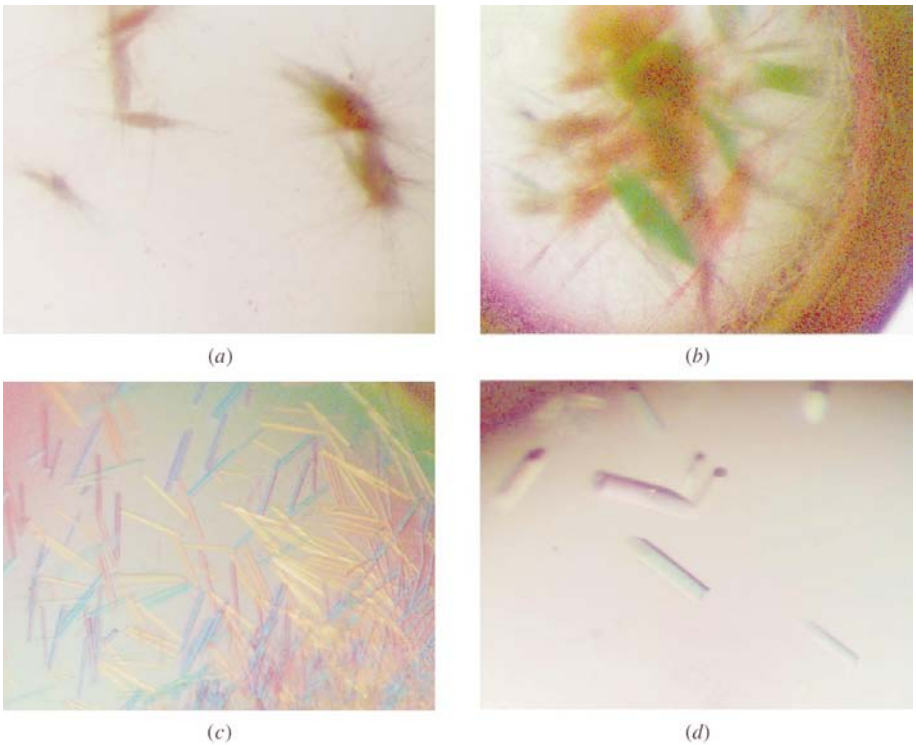

Figure 1
Crystals of $TarD_{Sa}$ obtained during optimization of growth conditions. (*a*) Crystals of $TarD_{Sa}$ grown in the presence of CTP and $MgCl_2$ obtained from a sparse-matrix screen. (*b*) Crystals obtained after refining precipitant and protein concentrations. (*c*) Results of adding D(+) glucose to the crystallization conditions. (*d*) Final optimized crystals of $TarD_{Sa}$ obtained by reducing the rate of vapour diffusion. Photographs were taken under polarized light.

3. Results and discussion

Initial sparse-matrix screening of crystallization conditions for $TarD_{Sa}$ yielded rosette-shaped crystalline material under several conditions. However, an exhaustive survey of similar conditions in which protein, precipitant and salt concentrations as well as pH and temperature were varied failed to improve the quality of these quasi-crystals. For this reason, sparse-matrix screening was repeated with a protein solution that also contained a five-molar excess of CTP and $MgCl_2$. Long thin hair-like crystalline material with a maximal length of approximately 0.4 mm was obtained using 25% ethylene glycol as a well solution at 295 K (Fig. 1*a*). Varying the precipitant and protein concentrations of this preliminary condition did not significantly improve the quality of the crystalline material, but 8% ethylene glycol produced the best results (Fig. 1*b*). The approach for improving the quality of these $TarD_{Sa}$ quasi-crystals was an

Table 1
Data-collection statistics.

Space group	$P3_121$
Unit-cell parameters (Å,°)	$a = b = 92.2$, $c = 156.12$, $\alpha = \beta = 90$, $\gamma = 120$
Resolution (Å)	50–3.0
No. of observed reflections	106644
No. of unique reflections	15217
Completeness (%)	95.2
$\langle I/\sigma(I)\rangle$	7.0
R_{sym}†	0.135

† R_{sym} value based on intensities.

examination of the effects of small molecules on crystal growth (Sousa, 1995). Addition of 0.5 µl of 30% D(+) glucose to the drop proved the most effective in that this resulted in rod-shaped crystals with approximate dimensions of 0.2 × 0.02 × 0.02 mm (Fig. 1*c*). However, excessive nucleation was observed under these crystallization conditions, which could also account for the relatively small size of $TarD_{Sa}$ crystals. It was speculated that this excessive amount of nucleation could be remedied by reducing the rate of vapour diffusion through the use of oils (Chayen, 1997). Crystallization experiments using the partially optimized conditions were repeated with the modification that a mixture of paraffin and silicon oil in various ratios and volumes was placed on top of the reservoir solution. The most successful procedure proved to be the application of a 250 µl mixture of silicon and paraffin oil in a 2:1 ratio. In about two weeks, crystals grew to 0.25 mm in length and 0.08 mm in diameter (Fig. 1*d*). Note that the same condition at 277 K did not produce any crystals, stressing the importance of temperature as a variable in crystallization of $TarD_{Sa}$.

Examination of diffraction data from a $TarD_{Sa}$ crystal revealed that these crystals diffract to just beyond 3.0 Å and are trigonal, with unit-cell parameters $a = b = 92.2$, $c = 156.12$ Å, $\alpha = \beta = 90$, $\gamma = 120°$ (see Table 1). Analysis of symmetry-related reflections and systematic absences indicated that the space group was either $P3_121$ or its enantiomorph $P3_221$. These results suggested that there are four $TarD_{Sa}$ molecules present in the asymmetric unit, giving the crystals a solvent content of 60% (Matthews, 1968). The presence of four $TarD_{Sa}$ molecules is not surprising in that gel-filtration and ultracentrifugation studies indicate that $TarD_{Sa}$ exists as a tetramer in solution. Using molecular-replacement methods, we have now established the space group to be $P3_121$ and confirmed the presence of four $TarD_{Sa}$ molecules per asymmetric unit.

We thank members of the Berghuis and Brown groups for their assistance. This work was supported by a grant from the Canadian Institutes of Health Research to AMB (MT-13107). Use of the X8-C beamline at the National Synchrotron Light Source was made possible through a multi-user maintenance grant from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada. VCNY was supported through a Premier's Research Excellence Award given to AMB. AMB is the recipient of a CIHR/PMAC-HRF Research Career Award in the Health Sciences and EDB is the recipient of a CIHR Scholarship.

References

Archer, G. L. (1998). *Clin. Infect. Dis.* **26**, 1179–1181.
Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.
Chayen, N. E. (1997). *J. Appl. Cryst.* **30**, 198–202.
Daigle, D., Hughes, D. W. & Wright, G. D. (1999). *Chem. Biol.* **6**, 99–110.
Fridkin, S. K. (2001). *Clin. Infect. Dis.* **32**, 108–115.
Infectious Diseases & Immunization Committee (1999). *Paediatr. Health*, **4**, 337–341.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Lowy, F. D. (1998). *N. Engl. J. Med.* **339**, 520–532.
McPherson, A. (1976). *Methods Biochem. Anal.* **23**, 249–345.
Marchese, A., Schito, G. C. & Debbia, E. A. (2000). *J. Chemother.* **12**, 459–462.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mauel, C., Young, M., Margot, P. & Karamata, D. (1989). *Mol. Gen. Genet.* **215**, 388–394.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Shockman, G. D. & Barrett, J. F. (1983). *Annu. Rev. Microbiol.* **37**, 501–527.
Sousa, R. (1995). *Acta Cryst.* D**51**, 271–277.
Walsh, C. (2000). *Nature (London)*, **406**, 775–781.
Weber, C. H., Park, Y. S., Sanker, S., Kent, C. & Ludwig, M. L. (1999). *Structure*, **7**, 1113–1124.
Zulauf, M. & D'Arcy, A. (1992). *J. Cryst. Growth*, **122**, 102–106.