Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Expression, purification and crystallization of *Enterococcus faecium* streptogramin A acetyltransferase

Michele Sugantino and Steven L. Roderick*

Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA

Correspondence e-mail: roderick@aecom.yu.edu

Received 9 November 1999
Accepted 7 February 2000

The streptogramin A acetyltransferase from *Enterococcus faecium* (SWISS-PROT P50870) has been overexpressed in *Escherichia coli*, purified and crystallized. Crystallization conditions were screened using the hanging-drop vapor-diffusion method and resulted in two distinct crystal forms. Form I crystals diffract to 2.5 Å and belong to space group $P2_12_12_1$, with unit-cell parameters a = 68.6, b = 102.6, c = 107.5 Å. Form II crystals diffract to 2.7 Å and belong to space group $F222$, with unit-cell parameters a = 185.8, b = 185.8, c = 186.5 Å. Rotation-function and packing analyses for both crystal forms indicate that the asymmetric unit may contain one and two copies of the trimeric enzyme for crystal forms I and II, respectively.

1. Introduction

Antibiotics of the streptogramin class are produced by many members of the *Streptomyces* genus and are classified according to their covalent structure as belonging to either an A or B group (Cocito, 1979). Group A compounds are polyunsaturated macrolactones of approximate molecular weight 500 Da and include streptogramin A (also termed pristinamycin II_A, mikamycin A, vernamycin A, osteogrycin A or virginiamycin M_1). Group B compounds are cyclic hexadepsipeptides with molecular weights of about 800 Da and include pristinamycin I_A and virginiamycin S_1. Compounds of the A and B groups act synergistically both *in vivo* and *in vitro* to inactivate bacterial protein synthesis by binding to ribosomes, where they interfere with peptidyltransferase and peptidyl-tRNA binding, respectively (Bycroft, 1977; Pechère, 1996).

The prevalence of drug-resistant bacterial strains has intensified efforts to identify and characterize the enzymes responsible for conferring various resistance phenotypes. Analysis of clinical isolates has identified several genes from resistance plasmids that confer bacterial resistance to streptogramins. These include putative transporters such as *vga* (Allignet *et al.*, 1992) and *vgaB* (Allignet & El Solh, 1997) from *Staphylococcus aureus*, as well as acetyltransferases such as *satA* from *E. faecium* BM4145 (Rende-Fouriner *et al.*, 1993), *vat* from *S. aureus* BM3093 (Allignet *et al.*, 1993) and *vatB* from *S. aureus* BM3385 (Allignet & El Solh, 1995). Sequence analysis of *satA*, *vat* and *vatB* suggests that these enzymes are homologous and that they belong to the xenobiotic acetyltransferase family of enzymes that covalently modify a variety of natural acceptors by means of coenzyme-A-dependent acetylation (Murray & Shaw, 1997). All enzymes of this family belong to a larger superfamily of trimeric enzymes termed hexapeptide acyltransferases, whose signature structural domain is a left-handed parallel β-helix encoded by imperfect tandem repeats of a hexapeptide amino-acid sequence (Dicker & Seetharam, 1992; Vaara, 1992; Raetz & Roderick, 1995). Structural representatives of this superfamily studied by X-ray crystallography include UDP-*N*-acetylglucosamine acetyltransferase (Raetz & Roderick, 1995), tetrahydrodipicolinate *N*-succinyltransferase (Beaman *et al.*, 1997), a xenobiotic acetyltransferase capable of low-affinity chloramphenicol acetylation (Beaman *et al.*, 1998) and an *N*-acetylglucosamine 1-phosphate uridyltransferase (Brown *et al.*, 1999).

The product of the *satA* gene from *E. faecium* has been identified as a streptogramin A acetyltransferase (SatA) based on its ability to acetylate and inactivate members of the streptogramin A group of antibiotics in an acetyl-CoA-dependent reaction (Rende-Fouriner *et al.*, 1993). Structural analysis of *E. faecium* SatA is expected to reveal the means by which this enzyme recognizes and acetylates group A streptogramins and will also place this enzyme in structural context with the growing superfamily of hexapeptide acyltransferases.

2. Results and discussion

2.1. Construction of the overexpression plasmid pET-11a/*satA*

In order to obtain a gene encoding SatA for heterologous overexpression in *E. coli*, a gene corresponding to the amino-acid sequence of SatA was synthesized using codons selected for

optimal expression in *E. coli* (Operon Technologies). This gene was subcloned into a pET-11a expression vector (Novagen) using *Nde*I and *Bam*HI restriction sites and transformed into *E. coli* BL21(DE3) host cells for overexpression.

2.2. Overexpression and purification

4 l of Luria–Bertani medium containing 50 µg ml^{-1} carbenicillin was inoculated with 120 ml of stationary phase BL21(DE3)/[pET-11a/*satA*] culture. Fermentation at 310 K was continued to an OD_{600} of approximately 0.5 and was followed by induction with 1 m*M* isopropyl β-D-1-thio-galactopyranoside (IPTG). The cells were harvested by centrifugation after 4 h and the cell paste was resuspended in 50 m*M* triethanolamine (TEA) pH 8.0 prior to storage at 203 K.

Frozen cells were thawed and lysed by sonication. The crude extract was recovered by centrifugation at 48 000*g* for 1 h at 277 K. The resulting supernatant was passed through a 0.2 µm syringe filter and applied to a 375 ml Q-Sepharose Fast Flow column equilibrated with 25 m*M* TEA pH 7.8 (buffer *A*) and eluted with a 0–1 *M* linear NaCl gradient. Fractions were analyzed by SDS–PAGE and those containing SatA were concentrated to 30 mg ml^{-1} and applied to a 300 ml Superdex-200 gel-filtration column also equilibrated with buffer *A*. Isocratic elution of this column resulted in 128 mg of purified SatA, which was pooled and stored at 203 K. The mass of the purified enzyme was measured by electrospray mass spectrometry to be 23 650 Da, within error of the predicted 23 649 Da mass of the covalently unmodified polypeptide (data not shown).

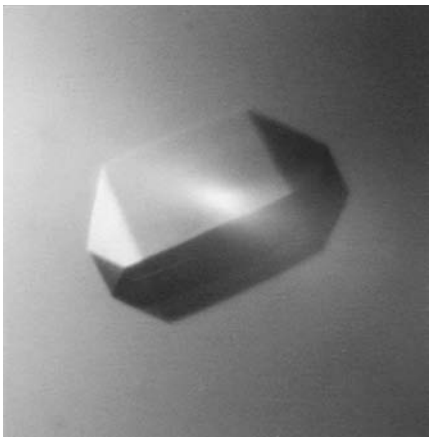

Figure 1
Photograph of a form II crystal of *E. faecium* SatA. The size of this crystal is approximately 0.6 × 0.4 × 0.4 mm.

2.3. Crystallization and X-ray data measurement

Crystallization conditions were screened with the hanging-drop vapor-diffusion method at ambient temperature, initially using commercially available kits (Hampton Research). Two distinct crystal forms were identified, both appearing in drops produced by combining 4 µl of 20 mg ml^{-1} protein solution and 4 µl of 50–150 m*M* sodium citrate, 25–35%(*w*/*v*) polyethyleneglycol 400, 5–8%(*v*/*v*) ethanol and 0.2 *M* Tris–HCl pH 8.0. Both form I and form II crystals appeared after about 5 d and grew to maximum dimensions of approximately 0.6 × 0.4 × 0.4 mm.

Table 1
X-ray data-measurement statistics.

	Form I	Form II
Space group	$P2_12_12_1$	$F222$
Unit-cell parameters (Å)		
a	68.5	185.8
b	102.6	185.8
c	107.5	186.5
Resolution (Å)	20–2.5	20–2.7
No. of observations	106003	207126
No. unique	26649	39580
$R_{\rm merge}$† (%)	8.2	6.8
$R_{\rm merge}$ (last shell)‡ (%)	27.0	19.2
Completeness (%)	99.0	99.7
Completeness (last shell)	96.4	98.2

† $R_{\rm merge}$ (%) = $(\sum|I_i - \langle I\rangle|/\sum I_i) \times 100$, where I_i is an individual intensity observation, $\langle I\rangle$ is the mean intensity for that reflection and the summation is over all reflections. ‡ The last shell is the 2.60–2.50 Å resolution bin for form I and 2.80–2.70 Å for form II.

X-ray intensity data were collected at ambient temperature with a Siemens X1000 area detector, using Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode X-ray generator operating at 50 kV and 80 mA. The X-ray data were reduced with *XDS* and *XSCALE* (Kabsch, 1988). Form I crystals were observed only in crystallization experiments conducted with the first protein preparation, later judged to be the least pure of several ensuing preparations. These crystals belong to space group $P2_12_12_1$ and supported measurement of a data set to

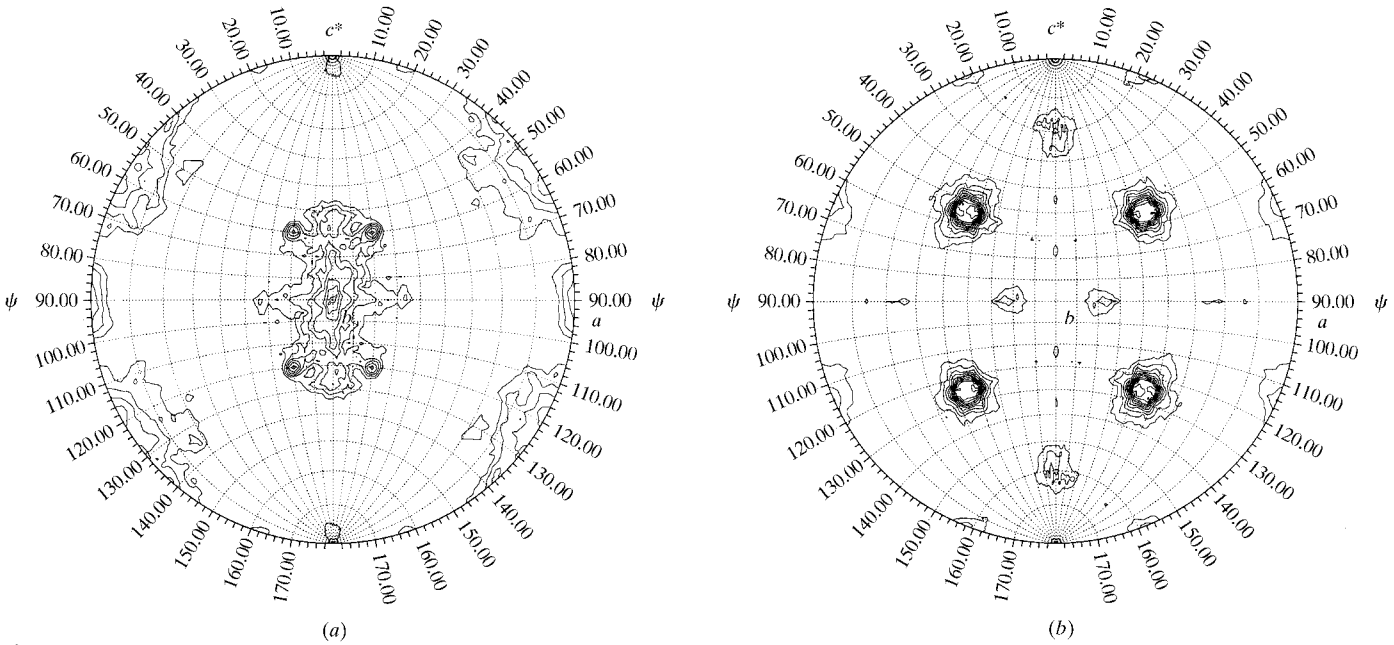

Figure 2
Self-rotation function for the rotation angle $\kappa = 120°$. The circumference of the figure corresponds to $\varphi = 0°$. (*a*) Form I crystal. (*b*) Form II crystal.

2.5 Å resolution (Table 1). This crystal form could not be reproduced using protein from ensuing preparations. Form II crystals (Fig. 1), however, could be reproduced from all preparations and allowed measurement of a 2.7 Å resolution data set.

The similarity in the unit-cell edge lengths for the form II crystals initially suggested that the space group could be the cubic group *F*23. However, calculation of merging *R* factors for various crystal symmetries led to the surprising conclusion that the observed Laue group lacked crystallographic threefold symmetry along [111] or fourfold crystallographic symmetry along any axial direction, but possessed only approximate threefold symmetry in the [111] direction relating *hkl*, *klh* and *lhk* reflections. As a result, the space group *F*222 was assigned. This departure from true crystallographic threefold symmetry is a reproducible feature of all form II crystals studied to date.

2.4. Self-rotation function and crystal packing

All structurally characterized left-handed parallel β-helical proteins are trimers and gel-filtration data for SatA is consistent with this assignment of quaternary structure (data not shown). In order to identify the direction of prospective molecular threefold rotation axes, self-rotation functions were calculated for the $\kappa = 120^\circ$ rotation angle using *GLRF* (Tong & Rossmann, 1990) using data in the 20–4 Å resolution range for both crystal forms (Fig. 2). The presence and direction of non-crystallographic threefold symmetry axes is indicated by the spherical polar angles $(\varphi, \psi) = (70, 60^\circ)$ and $(45, 55^\circ)$ for the form I and form II crystals, respectively. The direction of the prospective threefold axis in the form II crystals is not surprising, as the diffraction pattern indicated approximate threefold symmetry in the [111] direction of a cell of nearly equal cell-edge lengths. The spherical polar angles for a crystallographic threefold in the [111] direction of a cubic space group would have been $(\varphi, \psi) = (45.0, 54.7^\circ)$. It is therefore possible that the molecular packing of the orthorhombic cell of form II crystals is similar to space group *F*23.

Assuming that SatA is a trimeric enzyme (70 947 Da per trimer), ordinary protein crystal packing density ranges suggest that one trimeric molecule could be present in the asymmetric unit of the form I crystals, corresponding to a V_m value of 2.67 $Å^3$ Da^{-1} and an estimated solvent content of 54% (Matthews, 1974). A V_m value for the form II crystals of 2.87 $Å^3$ Da^{-1} is obtained on the assumption of two trimers in the asymmetric unit and would suggest a resultant solvent content of approximately 57%. Such a packing arrangement would be consistent with a single peak on the $\kappa = 120^\circ$ self-rotation function if the molecular threefold rotation axis of each trimer shared the same direction or perhaps were coincident. The structure determination of SatA by the molecular-replacement method is now in progress.

This work was supported by grants AI38328 and AI42154 from the National Institutes of Health.

References

Allignet, J. & El Solh, N. (1995). *Antimicrob. Agents Chemother.* **39**, 2027–2036.
Allignet, J. & El Solh, N. (1997). *Gene*, **202**, 133–138.
Allignet, J., Loncle, V. & El Solh, N. (1992). *Gene*, **117**, 45–51.
Allignet, J., Loncle, V., Simenel, C., Delepierre, M. & El Solh, N. (1993). *Gene*, **130**, 91–98.
Beaman, T. W., Binder, D. A., Blanchard, J. S. & Roderick, S. L. (1997). *Biochemistry*, **36**, 489–494.
Beaman, T. W., Sugantino, M. & Roderick, S. L. (1998). *Biochemistry*, **37**, 6689–6696.
Brown, K., Pompeo, F., Dixon, S., Mengin-Lecreulx, D., Cambillau, C. & Bourne, Y. (1999). *EMBO J.* **18**, 4096–4107.
Bycroft, B. (1977). *J. Chem. Soc.* **1**, 2464–2470.
Cocito, C. (1979). *Microbiol. Rev.* **43**, 145–198.
Dicker, I. B. & Seetharam, S. (1992). *Mol. Microbiol.* **6**, 817–823.
Kabsch, W. (1988). *J. Appl. Cryst.* **21**, 916–924.
Matthews, B. W. (1974). *J. Mol. Biol.* **82**, 513–526.
Murray, I. A. & Shaw, W. V. (1997). *Antimicrob. Agents Chemother.* **41**, 1–6.
Pechère, J.-C. (1996). *Drugs*, **51**, 13–19.
Raetz, C. R. H. & Roderick, S. L. (1995). *Science*, **270**, 997–1000.
Rende-Fouriner, R., Leclercq, R., Galimand, M., Duval, J. & Courvalin, P. (1993). *Antimicrob. Agents Chemother.* **37**, 2119–2125.
Tong, L. & Rossmann, M. G. (1990). *Acta Cryst.* A**46**, 783–792.
Vaara, M. (1992). *FEMS Microbiol. Lett.* **97**, 249–254.