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The crystal structure of the β-lactamase of *Streptomyces albus* G at 0.3 nm resolution

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The crystal structure of the β-lactamase of *Streptomyces albus* G has been solved at 0.3 nm resolution by X-ray-diffraction methods. The enzyme is a typical two-domain protein. One domain consists of five α-helices, and the other is five-stranded β-sheet with α-helices on both sides of the sheet. The active-site serine residue (Ser-48) is within a cleft located between the two domains.

INTRODUCTION

Three classes, A, B and C, of β-lactamases (β-lactam hydrolases, EC 3.5.2.6) have been identified on the basis of their substrate specificity, amino acid sequence and mechanistic properties. The β-lactamases of class B possess a zinc cofactor; those of class A and C operate by an acyl-enzyme mechanism involving an active-site serine residue. Several β-lactamases of class A and C have been studied by X-ray-crystallographic methods (Knox *et al.*, 1976; Aschaffenburg *et al.*, 1978; Charlier *et al.*, 1983; Dideberg *et al.*, 1985; Moult *et al.*, 1985; Kelly *et al.*, 1986; Samraoui *et al.*, 1986). As reported by Kelly *et al.* (1986) and Samraoui *et al.* (1986), the class A β-lactamases of *Bacillus licheniformis* and *B. cereus* on the one hand and the DD-peptidase of *Streptomyces* R61 on the other are similar in the extent and distribution of secondary structures (though lacking relatedness in primary structure). In no case, however, have these studies disclosed the complete tracing of the polypeptide chain. Recently, the gene encoding the class A β-lactamase of *Streptomyces albus* G has been cloned and sequenced, thus giving access to the primary structure of the enzyme (Dehottay *et al.*, 1987), and the active-site serine residue has been identified at position 48 (i.e. position 89 in the precursor) after β-iodopenicillanate derivatization of the protein (De Meester *et al.*, 1987). In turn, we here present the three-dimensional structure of this *Streptomyces albus* G β-lactamase, solved at 0.3 nm resolution.

MATERIALS AND METHODS

The enzyme (purified to protein homogeneity as described by Duez *et al.*, 1981) was crystallized using the vapour-diffusion technique (McPherson, 1976). The protein [21 mg·ml^{-1} in 25 mM-sodium phosphate buffer, pH 7.0, containing 8% (v/v) glycerol and 8% ethylene glycol] was dialysed against 50 mM-Tris/HCl, pH 7.0, containing 10 mM-NaN_3. After centrifugation, 10 μl drops of the protein solution were (i) mixed with 3 μl of a 10% solution of 10000-M_r poly(ethylene glycol) made in the above buffer and (ii) suspended over 1 ml wells containing the same poly(ethylene glycol) solution. After 1 week of equilibration at 12 °C, well-formed crystals were obtained which grew up to 1.2 mm in length and 0.5 mm in width. When exposed to nitrocefin (0.1 mM in the mother liquor), the crystal and the surrounding mixture became red after 3 min at 18 °C, as a result of the catalysed hydrolysis of the β-lactam amide bond. The space group was $p2_12_12_1$ and the unit cell parameters were $a = 4.067$ nm, $b = 4.353$ nm and $c = 13.843$ nm. Assuming one 28500-M_r protein molecule per asymmetric unit, a specific value of 0.00216 nm^3 (2.16 Å^3)/Da

Table 1. Data-collection and phasing parameters

Abbreviation: *p*CMB, *p*-chloromercuribenzoate.

Enzyme derivative	Soaking conditions		R_{iso}*(%)	No. of sites	Phasing power (F_H/E)	Resolution (nm)
	Concn. (mM)	Period (days)				
Native	–	–	–	–	–	0.23
$K_2Pt(C_2O_4)_2$	5	5	12	–	–	0.4
$K_3UO_2F_5$	5	2	26	–	–	0.4
*p*CMB	1	2	18	1	2.4	0.3
$SmCl_3$	0.5	2	12	2	2.0	0.4
$UO_2(NO_3)_2$	1	1	15	3	1.7	0.3
K_2PtCl_4	5	7	9	–	–	0.4

$$*R_{iso} = \frac{\sum_{hkl} |F_{PH} - F_P|}{\sum_{hkl} |F_P|}$$

was calculated. This value was within the range found for protein crystals (Matthews, 1968). Three native data sets were collected to a resolution of 0.23 nm.

Six heavy-atom derivatives [$K_2Pt(C_2O_4)_2$, $K_3UO_2F_5$, $SmCl_3$, $UO_2(NO_3)_2$, K_2PtCl_4 and *p*-chloromercuribenzoate] of the enzyme were studied by collecting the intensity data on a Stoe–Siemens diffractometer with graphite monochromated CuK_α radiation. Two equivalent reflections were measured with the native enzyme and, for the derivatives, the Bijvoet mate of each reflection was collected at -2θ. Decay was monitored by using two standard reflections for every 100 reflections. Lorentz, polarization and absorption (North *et al.*, 1968) corrections were applied. The main data-collection and phasing parameters are shown in Table 1.

The difference Patterson functions with coefficients $(|F_{PH}|-|F_P|)^2$ or $(|F^+|-|F^-|)^2$ for the *p*-chloromercuribenzoate derivative were easily interpretable. The heavy-atom positions and occupancies were refined by using the lowest estimate of the heavy-atom structure amplitude, F_{HLE}. The protein phases thus derived were then used to calculate a cross-phased difference Fourier map and to locate the heavy-atom-binding sites of the other derivatives. Each solution was compared with the two Patterson functions. Only two derivatives [$SmCl_3$ and $UO_2(NO_3)_2$] gave a consistent set of heavy-atom sites (so that three derivatives were used for the phasing procedure).

Positional, thermal and occupancy parameters were varied in the phase refinement. Minor sites were found by inspecting the difference Fourier map. The overall figure-of-merit was 0.79 for 4462 reflections. A mini-map (scale 0.4 nm/cm) was calculated on a grid of $32 \times 32 \times 100$ using the phase obtained and the amplitude weighted by the individual figure-of-merit. Protein and solvent regions appeared well in the map. Approximate C_α positions were directly read off the map using a grid chart, and the C_α positions served as guide points to construct the polypeptide backbone. The polyalanine model was fitted to the 0.3 nm electron-density map with a PS300 Evans and Sutherland colour graphics display system using the program FRODO (Jones, 1985).

RESULTS AND DISCUSSION

The residue numbering used below refers to the mature enzyme (whose *N*-terminus is Gly-40 of the precursor protein). Except for the residues-77–91 amino-acid stretch, which lacked continuous density, the tracing of the chain as derived from the electron-density map was unambiguous (Fig. 1). The two-domain protein has an overall dimension of 5.5 nm × 4.3 nm × 3.8 nm. One of the domains has a central structural core consisting of a five-stranded β-sheet with three α-helices on one face and one α-helix on the other. The strands S_3, S_4 and S_5 form a β-meander, a very stable structure that is also found in the active-site serine proteinases of the trypsin family (Schulz & Schirmer, 1979). The strands S_1 and S_2 have a hairpin connection, and the β-sheet has the usual left-handed twist with a rather small Ω angle. The second domain consists of five α-helices. Stabilizing salt bridges occur throughout the structure. In particular, Glu-16 of helix H_1 interacts with Arg-39 of strand S_2.

The active site (*, Fig. 1), identified as the cleft possessing the serine residue at position 48 and known to be labelled by β-iodopenicillanate derivatization of the protein (see the Introduction), is topologically defined by the *N*-terminal portion of helix H_3 on one side, strand S_3 on the other side, helix H_2 at the back, a loop connecting helices H_7 and H_8 on the top and a loop connecting helices H_5 and H_6 at the bottom (Fig. 2). The active-site Ser-48 is at the *N*-terminus of helix H_2, a position that may facilitate catalysis (Hol, 1985). Other residues which, in concert with Ser-48, may play important roles in catalysis and/or substrate binding are Lys-51 (on H_2), Glu-150 and Asn-154 (on a loop between H_5 and H_6), and Lys-218

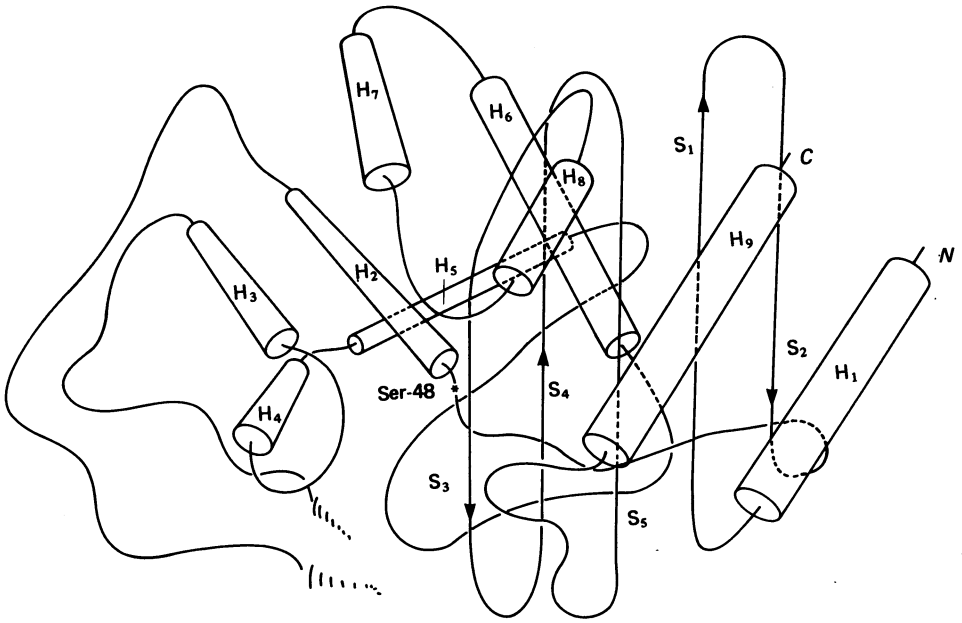

Fig. 1. Schematic representation of the three-dimensional structure of the *Streptomyces albus* G β-lactamase

β-Strands (S_i) are represented by arrows, and helices (H_i) by cylinders. *N* is the *N*-terminus; *C* is the *C*-terminus.

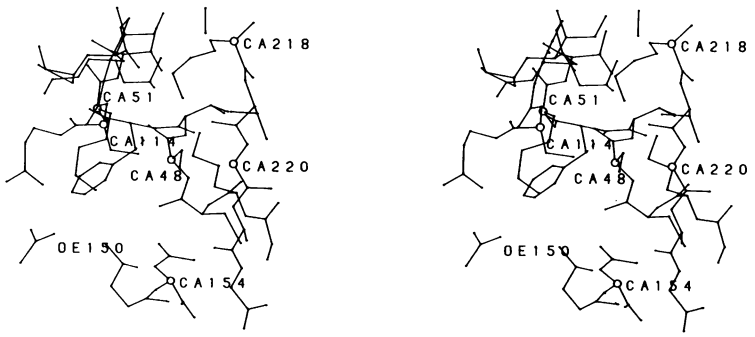

Fig. 2. A stereo view showing potentially important residues in the active site of the *Streptomyces albus* G β-lactamase

The orientation of the molecule is similar to that Fig. 1. The labelled residues are: Ser-48, Lys-51, Ser-114, Glu-150, Asn-154, Lys-218 and Gly-220. ○, C_α(CA) atom.

and Gly-220 (on S_3). Lys-51, Lys-218 and Gly-220 are conserved in the β-lactamases of class A and the DD-peptidases/penicillin-binding proteins of known primary structure.

The arrangement of secondary-structure elements in the *Streptomyces albus* G β-lactamase is comparable with those found in *B. licheniformis* and *B. cereus* I enzymes (Kelly *et al.*, 1986; Samraoui *et al.*, 1986), except that one helix is missing in the two latter structures (H_8 in *B. licheniformis* enzyme and H5 in the *B. cereus* enzyme).

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REFERENCES

Aschaffenburg, R., Phillips, D. C., Sutton, B. J., Baldwin, G., Kiener, P. A. & Waley, S. G. (1978) J. Mol. Biol. **120**, 447–449

Charlier, P., Dideberg, O., Frère, J. M., Moews, P. C. & Knox, J. R. (1983) J. Mol. Biol. **171**, 237–238

Dehottay, P., Dusart, J., De Meester, F., Joris, B., Van Beeumen, J., Erpicum, T., Frère, J. M. & Ghuysen, J. M. (1987) Eur. J. Biochem., in the press

De Meester, F., Joris, B., Lenzini, M. V., Dehottay, P., Erpicum, T., Dusart, J., Klein, J. M., Ghuysen, J. M., Frère, J. M. & Van Beeumen, J. (1987) Biochem. J. **244**, 427–432

Dideberg, O., Libert, M., Frère, J. M., Charlier, P., Zhao, H. & Knox, J. R. (1985) J. Mol. Biol. **181**, 145–146

Duez, C., Frère, J. M., Klein, D., Noël, M., Ghuysen, J. M., Delcambe, L. & Dierickx, L. (1981) Biochem. J. **193**, 75–82

Hol, W. G. J. (1985) Prog. Biophys. Mol. Biol. **45**, 149–195

Jones, T. A. (1985) Methods Enzymol. **115**, 157–171

Kelly, J. A., Dideberg, O., Charlier, P., Wéry, J. P., Libert, M., Moews, P. C. Knox, J. R., Frère, J. M. & Ghuysen, J. M. (1986) Science **231**, 1429–1431

Knox, J. R., Kelly, J. A., Moews, P. C. & Murthy, N. S. (1976) J. Mol. Biol. **104**, 865–875

Matthews, B. W. (1968) J. Mol. Biol. **33**, 491–497

McPherson, A. (1976) Methods Biochem. Anal. **23**, 249–345

Moult, J., Sawyer, L., Hertzberg, O., Jones, C. L., Coulson, A. F. W., Green, D. W., Harding, M. M. & Ambler, R. P. (1985) Biochem. J. **225**, 167–176

North, A. C. T., Phillips, D. C. & Matthews, F. S. (1968) Acta Crystallogr. **A24**, 351–359

Samraoui, B., Sutton, B. J., Todd, R. J., Artymiuk, P. J., Waley, S. G. & Phillips D. C. (1986) Nature (London) **320**, 378–380

Schulz, G. E. & Schirmer, R. M. (1979) Principles of Protein Structure (Cantor, C. R., ed.), p. 83, Springer-Verlag, New York

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