J. Mol. Biol. (1979) **129**, 677–679

Crystallographic Data for the DD-Carboxypeptidase-Endopeptidase of Low Penicillin Sensitivity Excreted by *Streptomyces albus* G

The DD-carboxypeptidase-endopeptidase of low penicillin sensitivity that is excreted by *Streptomyces albus* G has been crystallized from a polyethylene glycol (M_r 6000 to 7500) solution at pH 8·0. X-ray examination of the prismatic crystals shows that the space group is $P2_1$, with unit cell dimensions $a = 51{\cdot}1$ Å, $b = 49{\cdot}7$ Å, $c = 38{\cdot}7$ Å, $\beta = 100{\cdot}6°$ and one molecule in the asymmetric unit. A crystal suspension made in 50 mM-Tris·HCl buffer (pH 8·0) supplemented with 5 mM-$MgCl_2$ and 16% (w/v) polyethylene glycol exhibits enzyme activity on the substrate Ac_2-L-Lys-D-Ala-D-Ala.

The bacterial wall peptidoglycan synthesizing system, a membrane-bound enzyme complex performing antagonistic DD-transpeptidase, carboxypeptidase and endopeptidase activities, catalyses attachment of the nascent peptidoglycan strands to the pre-existing wall peptidoglycan and controls the final extent of crosslinkages. As a whole, this enzyme complex is the cell target that is specifically attacked by penicillin and other β-lactam antibiotics. Depending upon the enzyme under consideration and the bacterial species, the penicillin sensitivity may vary to a great extent (Ghuysen *et al.*, 1979). Actinomycetes, during growth, excrete extracellular enzymes which catalyse *in vitro* reactions that are identical to those performed by the membrane-bound enzymes. The DD-carboxypeptidase-transpeptidase excreted by *Streptomyces* strain R61, a highly penicillin-sensitive enzyme, has been crystallized (Knox *et al.*, 1979). We report here the crystallization of the DD-carboxypeptidase-endopeptidase excreted by *Streptomyces albus* G. This latter enzyme functions solely as a hydrolase, it requires Mg^{2+} ions for activity and it exhibits a low sensitivity to penicillins (Leyh-Bouille *et al.*, 1970; Frère *et al.*, 1978). Study of the structures and active sites of the R61 and G enzymes may open the possibility of pharmaceutical chemists being able to devise antibacterial β-lactams on a rational basis.

The G enzyme was purified to 98·5% purity; only form II (M_r = 18,500) was used in this study (Duez *et al.*, 1978). As revealed by sodium dodecyl sulphate (0·1%)/polyacrylamide (12%) gel electrophoresis (Laemmli & Favre, 1973), the contaminating material (1·5%) consisted of minor amounts of a protein of M_r 25,000. The G enzyme (isoelectric point at pH 8·5) was crystallized from polyethylene glycol (M_r 6000 to 7500) using the vapour diffusion technique. Droplets (10 μl) of the enzyme preparation (2% protein, final concentration) in 50 mM-Tris·HCl buffer (pH 8·0) containing 5 mM-$MgCl_2$ and 10 mM-NaN_3 (TMN buffer) were deposited on a siliconized slide and supplemented with 10 μl of a 12% (w/v) polyethylene glycol solution in TMN buffer. The slide with the droplets on it was placed in a plastic box containing 10 ml of the 12% polyethylene glycol solution in TMN buffer. The box was sealed and maintained at 4°C for 6 weeks after which time prismatic crystals (1·2 mm × 0·4 mm × 0·4 mm) were obtained.

Crystals were washed with the 16% polyethylene glycol solution in TMN buffer and

0022–2836/79/120677–03 $02.00/0

then dissolved in the TMN buffer alone. The specific enzyme activity of the protein solution thus obtained (measured as the rate of hydrolysis of Ac_2-L-Lys-D-Ala-D-Ala) was 96±5% of that of the initial preparation. Analysis of a large sample of this enzyme solution (60 μg protein) by sodium dodecyl sulphate/polyacrylamide gel electrophoresis showed that crystallization had resulted in the complete removal of the minor protein contaminant. A single crystal, whose protein content was estimated on the basis of the crystal sizes and a 50% hydration (see below), was washed with the 16% polyethylene glycol solution in TMN buffer and resuspended in 200 μl of the same medium supplemented with 150 nmoles Ac_2-L-Lys-D-Ala-D-Ala.Hydrolysis of the substrate occurred. A time-course experiment (up to 15 min at 37°C) showed no sign of acceleration of the reaction rate as a function of time. The observed enzyme activity was thus attributable to the crystal itself and the specific activity of the latter was estimated to be approximately 2 to 4% of that of an equivalent protein solution.

For X-ray analysis, crystals were sealed in glass capillaries containing a small amount of mother liquor. Precession photographs indicated that the space group was $P2_1$. Unit cell dimensions, least-squares refined using a Hilger-Watts single crystal diffractometer, are $a = 51{\cdot}1(1)$ Å, $b = 49{\cdot}7(1)$ Å, $c = 38{\cdot}7(1)$ Å and $\beta = 100{\cdot}6(2)°$ at 20°C (estimated errors are given in parenthesis). Crystal density, as measured by flotation in a solution of *m*-xylene/carbon tetrachloride is 1·28±0·01 g/cm³. Assuming one molecule of protein of M_r 18,500 per asymmetric unit, the fractional volume of the unit cell occupied by the solvent is 0·5; this value is well in the range of those given by Matthews (1968) for protein crystals (0·28 to 0·60). Measurable intensities can be observed to 1·5 Å resolution and the crystals exhibit good resistance to radiation damage. At present, native data have been collected to 3·5 Å resolution.

Dialysis replacement of the Mg^{2+} by Ba^{2+} ions did not alter the specific activity of the enzyme on the tripeptide substrate. Precession photographs of the Ba^{2+} derivative showed changes in the intensities of the spots of the diffraction pattern when compared with those of the native crystal. Search for other heavy-atom derivatives is under way.

We thank Professors J. Toussaint (Laboratory of Crystallography, University of Liège) and T. Blundell (Laboratory of Molecular Biology, Department of Crystallography, Birkbeck College, University of London) for their interest and M. Vermeire and D. Klein for technical assistance. This work was supported in part by grants to one of the authors (O. D.) from the Fonds National de la Recherche Scientifique, Brussels, Belgium, and to another of the authors (J. M. G.) from the National Institutes of Health, Washington, D.C. (contract 1 RO1 AI 13364 01 MBC).

Laboratoire de Cristallographie
Institut de Physique B5
Université de Liège au Sart Tilman
B-4000 Liège, Belgique

OTTO DIDEBERG

Service de Microbiologie, Faculté de Médecine
Université de Liège au Sart Tilman
Institut de Botanique B22
B-4000 Liège, Belgique

JEAN-MARIE FRÈRE
JEAN-MARIE GHUYSEN

Received 12 December 1978

REFERENCES

Duez, C., Frère, J.-M., Geurts, F., Ghuysen, J.-M., Dierickx, L. & Delcambe, L. (1978). *Biochem. J.* **173**, 793–800.

Frère, J.-M., Geurts, F. & Ghuysen, J.-M. (1978). *Biochem. J.* **173**, 801–805.

Ghuysen, J.-M., Frère, J.-M., Leyh-Bouille, M., Coyette, J., Dusart, J. & Nguyen-Distèche, M. (1979). *Ann. Rev. Biochem.* in the press.

Knox, J. R., DeLucia, M. L., Murthy, N. S., Kelly, J. A., Moews, P. C., Frère, J.-M. & Ghuysen, J.-M. (1979). *J. Mol. Biol.* **127**, 217–224.

Laemmli, U. K. & Favre, M. (1973). *J. Mol. Biol.* **80**, 575–599.

Leyh-Bouille, M., Ghuysen, J.-M., Bonaly, R., Nieto, M., Perkins, H. R., Schleifer, K. H. & Kandler, O. (1970). *Biochemistry*, **9**, 2961–2971.

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