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Crystallization and preliminary X-ray analysis of a bacterial lysozyme produced by *Streptomyces globisporus*

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The extracellular bacteriolytic enzyme produced by *Streptomyces globisporus* shows a β-1,4-*N*,6-*O*-diacetylmuramidase activity as well as a β-1,4-*N*-acetylmuramidase activity. Crystals of this enzyme have been obtained by the hanging-drop vapour-diffusion method using polyethylene glycol as a precipitant. They belong to the tetragonal space group $P4_12_12$, with unit-cell parameters a = 63.11 (4), c = 121.1 (1) Å, diffract to at least 2.0 Å resolution and are suitable for high-resolution structure analysis. The crystal structure was solved by molecular replacement using lysozyme produced by *S. erythraeus* as a search model. The structure refinement is now in progress.

1. Introduction

Lysozyme, defined as β-1,4-*N*-acetylmuramidase, hydrolyzes the cell walls of bacteria such as *Micrococcus lysodeikticus* and exists ubiquitously in animals, plants and microorganisms. On the basis of amino-acid sequence homology, lysozymes found so far have been classified into four distinct types: (i) chicken (Jollés *et al.*, 1963; Canfield, 1963), (ii) phage (Tsugita & Inouye, 1968; Inouye & Tsugita, 1968), (iii) goose (Simpson & Morgan, 1983; Simpson *et al.*, 1980) and (iv) bacteria (Felch *et al.*, 1975; Lichenstein *et al.*, 1990). Although there is no obvious sequence homology between one class and another, the three-dimensional structures of hen egg-white lysozyme (Blake *et al.*, 1965), T4 phage (Remington *et al.*, 1978) and goose egg-white lysozyme (Grütter *et al.*, 1983) are topologically similar to one another. On the other hand, the bacterial lysozyme produced by *S. erythraeus* exhibits an entirely different three-dimensional structure (Harada *et al.*, 1981) from those of the other types. Moreover, bacterial lysozymes show β-1,4-*N*-acetylmuramidase and β-1,4-*N*,6-*O*-diacetylmuramidase activities. The latter activity accounts for their lytic action on *Staphylococcus aureus* (Ghuysen & Strominger, 1963), whose cell walls containing *N*,6-*O*-diacetylmuramic acid are not hydrolyzed by lysozymes of the chicken, phage and goose types.

The actinomycete *S. globisporus* produces two kinds of extracellular bacteriolytic enzymes called M-1 (M_r = 23 000) and M-2 (M_r = 11 000) lysozymes (Yokogawa *et al.*, 1975). The amino-acid sequence of M-1 lysozyme (Lichenstein *et al.*, 1990) indicates that it belongs to the bacterial type. In this study, we crystallized M-1 lysozyme in order to elucidate its unique function with β-1,4-*N*,6-*O*-diacetylmuramidase activity on the basis of the three-dimensional structure. The crystals obtained were stable to X-ray irradiation and diffracted to at least 2.0 Å resolution.

2. Materials and methods

The electrophoretically pure enzyme was isolated from mutanolysin, which was kindly supplied by Dainippon Pharmaceutical Corp. Ltd (Osaka, Japan), according to the method of Yokogawa *et al.* (1975) and was also purchased from Seikagaku Kogyo Corp. Ltd (Tokyo, Japan). Suitable crystallization conditions were screened at 293 K using the hanging-drop vapour-diffusion method. A 3 µl droplet containing 30 mg ml^{-1} protein dissolved in Milli-Q water was mixed on a siliconized cover glass with an equal volume of reservoir solution. Initial crystallization conditions were screened using the sparse-matrix method and the 'Crystal Screen' macromolecular crystallization reagent kit (Jancarik & Kim, 1991).

X-ray diffraction data were collected on a Rigaku rotating-anode generator equipped with a specially built imaging-plate camera. The crystal-to-detector distance was set to 108.1 mm. Cu $K\alpha$ radiation was Ni-filtered and doubly focused by a pair of Pt-coated mirrors. The diffraction images were collected at an oscillation range of 1.5° and the raw X-ray diffraction data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993).

The crystal structure of M-1 lysozyme was solved by the molecular-replacement method using the program *AMoRe* (Navaza, 1994) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). Because of the relatively high degree of amino-acid sequence homology (50%) between M-1 and *S. erythraeus* lysozymes, the structure of

S. erythraeus lysozyme refined at 1.7 Å resolution (Harada *et al.*, unpublished results) was used in the rotation and translation searches. All atomic coordinates of *S. erythraeus* lysozyme except for those of water molecules were retained in the search model.

3. Results and discussion

Of the 50 crystallization conditions in the Crystal Screen, the reagent containing 30% PEG 4000, 0.1 *M* Tris–HCl pH 8.5 and 0.2 *M* sodium acetate gave a shower of fine crystals. After optimization of pH and PEG 4000 concentration, tetragonal bipyramidal crystals grew to dimensions of 0.8 × 0.6 × 0.6 mm (Fig. 1) under the conditions 16–20%(*w*/*v*) PEG 4000, 0.1 *M* Tris–HCl pH 8.5 at 293 K. The crystals obtained belong to the tetragonal space group $P4_12_12$ (or its enantiomorph $P4_32_12$), with unit-cell parameters a = 63.11 (4), c = 121.1 (1) Å. Assuming one molecule of molecular mass 23.6 kDa per asymmetric unit, the V_M value is calculated to be 2.6 Å^3 Da^{-1} with an estimated solvent content of 53%; these values are within the range commonly observed for protein crystals (Matthews, 1968). Diffraction patterns were recorded from one crystal on 108 imaging plates at 277 K. A total of 191 462 observed reflections were merged into 15 417 unique reflections with an R_{merge} of 0.069 and a completeness of 95.8% in the 30.0–2.05 Å resolution range. The R_{merge} and completeness in the highest resolution shell (2.12–2.05 Å) were 0.144 and 81.8%, respectively.

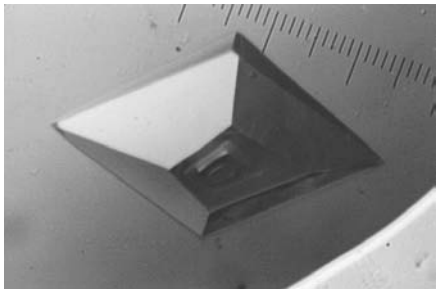

Figure 1
A crystal of M-1 lysozyme produced by *S. globisporus*.

A cross-rotation function calculated for the search model with a Patterson radius of 25 Å using diffraction data in the resolution range 8.0–4.0 Å gave a distinct solution with a correlation coefficient (CC) of 0.18, whereas the CCs of the second and third solutions were 0.14 and 0.12, respectively. A translation function calculated for the space group $P4_12_12$ gave a clear solution with a CC of 0.46 and an R factor of 0.44 after rigid-body refinement. The second solution of the translation function had a CC of 0.15 and an R factor of 0.53 and the third had a CC of 0.14 and an R factor of 0.54. A translation function calculated for the space group $P4_32_12$ resulted in a first solution with a CC of 0.29 and an R factor of 0.50. Using the best solution of the molecular replacement, the initial model of M-1 lysozyme was constructed from the structure of *S. erythraeus* lysozyme and refinement was undertaken using the program *X-PLOR* (Brünger, 1992). Amino-acid residues of the initial model that are different from *S. erythraeus* lysozyme were replaced with Ala. After iterative cycles of crystallographic refinement and manual model correction on a Silicon Graphics Iris workstation with the program *TURBO-FRODO* (Roussel & Cambillau, 1991), the model including all 1672 non-H atoms from 217 amino-acid residues of M-1 lysozyme and 127 water molecules was obtained. The current R factor for all 15 340 reflections in the resolution range 15.0–2.05 Å resolution is 0.186 (R_{free} = 0.238). Further steps of model correction and refinement are under way.

References

Blake, C. C. F., Koenig, D. F., Mair, G. A., North, A. C. T., Phillips, D. C. & Sarma, V. R. (1965). *Nature (London)*, **206**, 757–761.

Brünger, A. T. (1992). *X-PLOR Version* 3.1. *A System for X-ray Crystallography and NMR.* Yale University, New Haven, Connecticut, USA.

Canfield, R. E. (1963). *J. Biol. Chem.* **238**, 2698–2707.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Felch, J. W., Inagami, T. & Hash, J. H. (1975). *J. Biol. Chem.* **250**, 3713–3720.

Ghuysen, J. M. & Strominger, J. L. (1963). *Biochemistry*, **2**, 1119–1125.

Grütter, M. G., Weaver, L. H. & Matthews, B. W. (1983). *Nature (London)*, **303**, 828–831.

Harada, S., Sarma, R., Kakudo, M., Hara, S. & Ikenaka, T. (1981). *J. Biol. Chem.* **256**, 11600–11602.

Inouye, M. & Tsugita, A. (1968). *J. Mol. Biol.* **37**, 213–223.

Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Jollés, J., Jauregui-Adell, J., Bernier, I. & Jollés, P. (1963). *Biochim. Biophys. Acta*, **78**, 668–689.

Lichenstein, H. S., Hastings, A. E., Langley, K. E., Mendiaz, E. A., Rohde, M. F., Elmore, R. & Zukowski, M. M. (1990). *Gene*, **88**, 81–86.

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

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.

Remington, S. J., Anderson, W. F., Owen, J., Ten Eyck, L. F., Grainger, C. T. & Matthews, B. W. (1978). *J. Mol. Biol.* **118**, 81–98.

Roussel, A. & Cambillau, C. (1991). *TURBO-FRODO. Silicon Graphics Geometry Partners Directory*, p. 86. Silicon Graphics, Mountain View, California, USA.

Simpson, R. J., Begg, G. S., Dorow, D. S. & Morgan, F. J. (1980). *Biochemistry*, **19**, 1814–1819.

Simpson, R. J. & Morgan, F. J. (1983). *Biochim. Biophys. Acta*, **744**, 349–351.

Tsugita, A. & Inouye, M. (1968). *J. Mol. Biol.* **37**, 201–212.

Yokogawa, K., Kawata, S., Takemura, T. & Yohimura, Y. (1975). *Agric. Biol. Chem.* **39**, 1533–1543.