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Crystallization and preliminary X-ray analysis of bacteriophage lambda lysozyme in which all tryptophans have been replaced by aza-tryptophans

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Abstract

After many unsuccessful attempts to crystallize the bacteriophage lambda lysozyme, a mutant where all the tryptophan residues have been replaced by aza-tryptophans has been crystallized by the vapor-diffusion method. The crystals are orthorhombic and belong to space group $P2_12_12_1$ with cell dimensions $a = 73.01$, $b = 78.80$, $c = 82.31$ Å. Diffraction data were collected using synchrotron radiation sources. Crystals diffract to a resolution of 2.3 Å. Data from two different platinum derivatives were also recorded to 2.8 and 2.5 Å, respectively.

1. Introduction

The bacteriophage lambda lysozyme (λL) is a small protein of 158 amino acids. Like other known lysozymes, it is involved in the lysis of the cell walls of bacteria. Lysozymes are widespread in nature and have been isolated from a variety of organisms. Several classes of lysozymes have been defined on the basis of amino-acid sequence similarities. The crystal structures of lysozymes belonging to three different classes are known: type C (chicken), type G (goose) and type V (viral). No sequence homology is detected in lysozymes of different classes but nevertheless structural similarities are observed between them, suggesting the hypothesis of a diverging evolution from a remote common ancestor (Rossmann & Argos, 1976). In the three classes, the amino terminal contains the catalytic residues and consists of an α-helix followed by a three-stranded β-sheet. A precise structural alignment has been performed by Taylor & Orengo (1989): the alignment of residues in the β-sheets of T_4L (bacteriophage T_4 lysozyme) and HEWL (hen egg-white lysozyme) is given in Fig. 1.

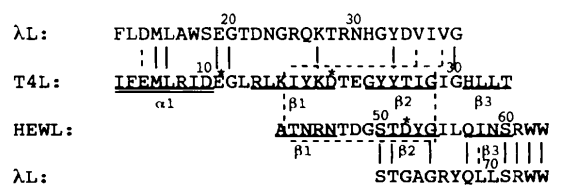


Fig. 1. Multiple alignment of the amino-terminal part of the sequence of λL with the sequence of T_4L and HEWL. The symbols | and ¦ indicate the presence of identical or similar residues in the homologous fragments. The two boxed sequences are aligned on the basis of structural homology as described by Taylor & Orengo (1989). The α-helixes and the β-strands of T_4L and HEWL are doubly or singly underlined. The catalytic residues of T_4L and HEWL are indicated by an asterisk.

An evolutionary relationship between λL and the V- and C-type lysozymes has been discussed in detail by Jespers, Sonveaux & Fastrez (1992) who aligned the amino-terminal sequences of λL and T_4L. This local alignment encompasses the first helix of T_4L and most of the β-sheet, suggesting a critical catalytic role for Glu19 of λL. The inactivation of the enzyme by mutation of Glu19 into Gln supports this hypothesis. The C-terminal sequences of λL and of T_4L can also be aligned. These observations indicate that λL is related to the V-type lysozymes. In attempts to align the sequence of λL with those of the C-type lysozymes, a fragment of 29 residues showing significant similarity was detected; it is located in the middle of the sequence of λL and aligns with the β-sheet part of the C-type lysozymes. Consequently, two regions in the sequence of λL align with a β-sheet structure if one considers, on the one hand, the alignment with T_4L and, on the other hand, the alignment with HEWL. These alignments suggest that there is a repetition of this motif in λL (Fig. 1). The distance between the two fragments (29 amino acids) coincides with the length of the high score alignment observed with C-type lysozymes.

All these observations suggest that λL could represent an evolutionary branch between C-type and V-type lysozymes, although the mechanism of action of λL differs. Lysozymes cleave the glycosidic bond between C1 of *N*-acetyl muramic acid (NAM) and C4 of *N*-acetyl glucosamine (NAG) of the peptidoglican of the bacterial wall. The mechanism of HEWL, GEWL (goose egg-white lysozyme) and T_4L is hydrolytic, while λL acts as a transglycosidase (Taylor, Das & van Heijenoort, 1975).

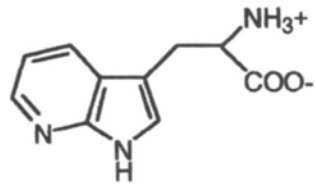
The determination of the three-dimensional structure of λL should be helpful in elucidating the difference in its enzymatic mechanism in comparison with other classes of lysozymes. It would also provide very useful information, regarding the mechanism of evolution of proteins.

2. Experimental

The bacteriophage lambda lysozyme was overexpressed in *Escherichia coli* and purified by a modification of a previously published protocol that included DEAE–cellulose, S-Sepharose Fast Flow and Sephadex G75 steps (Jespers, 1991; Jespers, Sonveaux, Fastrez, Phanapoulos & Davison, 1991; Soumillion & Fastrez, 1992). The only modification concerns the use of a phosphate buffer during the S-Sepharose Fast Flow step instead of a Hepes buffer. The purity of the enzyme (99%) was estimated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) and analytical chromatography on Pharmacia Mono S column. The lysozyme was concentrated by ultrafiltration (Millipore immersible CX-10 and Ultrafree-PF) to 20 mg ml^{-1} in 50 m*M* NaH_2PO_4/Na_2HPO_4 pH 6.7, NaN_3

0.02%. The final concentration was determined from absorbance at 280 nm using the molar absorption coefficient given by Soumillion, Jespers, Vervoort & Fastrez (1995).

In spite of numerous attempts, we were unable to grow crystals suitable for X-ray analysis from the native enzyme. A number of mutants were then used for screening the crystallization conditions (Jancarik & Kim, 1991) and the best results were obtained with a molecule in which the four tryptophan residues were replaced by aza-tryptophans (I) according to the experimental procedure described by Soumillion *et al.* (1995). The purification and concentration of this mutant follows the same protocol as the wild type.



(I)

This mutant was selected by NASA (National Aeronautics and Space Administration) for crystallization experiments under microgravity conditions during the Space Shuttle flights STS-63 and STS-67. The PCAM (Protein Crystallization Apparatus for Microgravity) experimental hardware (sitting-drop vapour diffusion) was used. Crystals were grown at 291 K: 5 µl of the protein solution (20 mg ml^{-1} in 50 m*M* NaH_2PO_4/Na_2HPO_4 pH 6.7, NaN_3 0.02%) was mixed with an aliquot of 5 µl of the reservoir solution [total volume 500 µl: 0.1 *M* sodium citrate pH 5.3, 15%(*v*/*v*) 2-propanol and 20%(*w*/*v*) polyethylene glycol 4000]. Needles of typical dimensions 0.10 × 0.12 × 1.00 mm (Fig. 2) were obtained at the end of flight STS-67 (16 d). Precession photographs using a rotating-anode generator showed that the crystals are orthorhombic, space group $P2_12_12_1$ with unit-cell dimensions $a = 73.01$, $b = 78.80$, $c = 82.31$ Å (values refined by the program *SCALEPACK* at the end of the data collection). Data were collected at room temperature using a synchrotron radiation source at the EMBL Hamburg outstation. Crystals diffracted to a resolution of 2.3 Å on the X31 beamline ($\lambda = 1.000$ Å), using a MAR Research imaging-plate detector (Fig. 3). Data were processed using *DENZO* (Otwinowski, 1993) then scaled by *SCALEPACK* (Otwinowski, 1993). The scale and the temperature factors between the successive images remained essentially constant, showing a total absence of crystal decay in the synchrotron X-ray beam during the time necessary for data collection. A total of 104 257 reflections were recorded among which 21 455 independent reflections with an $R_{merge}(I)$ of 9.0% (98.8% completeness). It is likely that the relatively high value of this index may be related to the high degree of redundancy (about 5) of the data. Average intensities in the highest resolution shell (2.34–2.30 Å) are more than three times their standard deviation.

3. Discussion

If we assume that two or three molecules are present in the asymmetric unit, the calculation of V_M (Matthews, 1968) leads to values of 3.4 and 2.2 Å^3 Da^{-1}, respectively, with associated solvent content of 62 and 44%. Both V_M values are consistent with those obtained for globular proteins though the situation in which the asymmetric unit contains three molecules seems more probable. In order to clarify the ambiguity, self-rotation function searches were attempted looking for non-crystallographic symmetry (program *X-PLOR*, Brünger, 1992). A systematic search through all rotation possibilities was conducted using several ranges of resolution. It was expected to find a twofold or threefold rotation axis but none of them was observed; in fact the self-rotation function was completely flat and no conclusive result was obtained. Moreover, no pure translation peak was found in the Patterson function and the unit-cell content remains undefined up to now.

As explained in the *Introduction*, the sequence of λL presents some similarities with those of T_4L and HEWL. Among them, T_4L presents the best percentage of amino-acid sequence

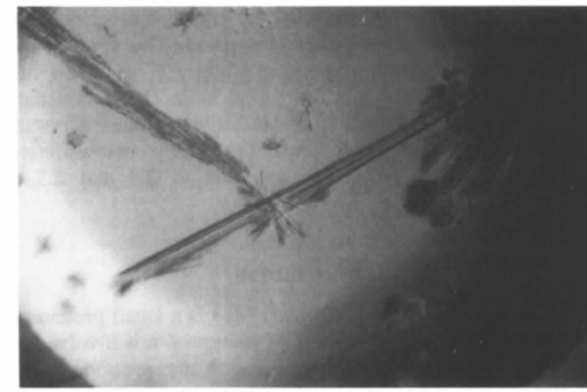


Fig. 2. Crystals of the aza-tryptophan mutant of bacteriophage lambda lysozyme grown in microgravity conditions during the Space Shuttle flight STS-67. The length of the largest needle is about 2 mm.

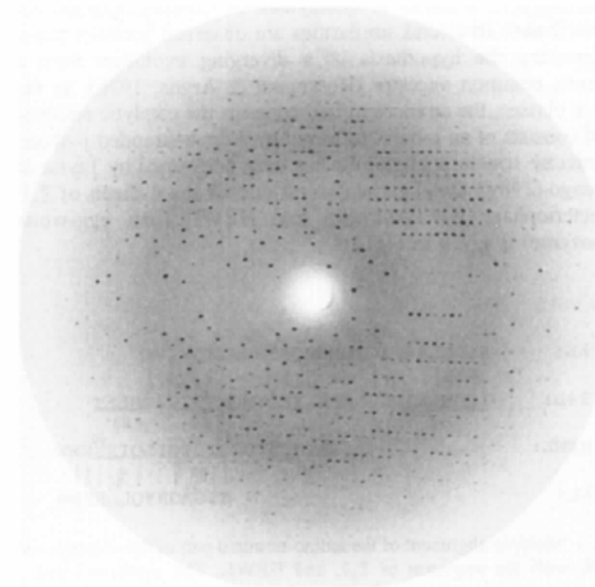


Fig. 3. A rotation image from a crystal of the aza-tryptophan mutant of bacteriophage lambda lysozyme recorded on a MAR Research imaging plate using the X31 beamline at EMBL Hamburg outstation. Rotation range 1.5°, crystal-to-film distance 190 mm, exposure time 360 s. The resolution at the edge of the photograph is 2.28 Å.

similarity but it is only 18%. Despite these low values the considerations presented in the *Introduction* suggest that it could eventually be possible to solve the phases problem by the molecular replacement method. According to Thunnissen *et al.* (1994), the C-terminal domain of the soluble lytic transglycosidase (SLT) from *E. coli* shows a folding very similar to those of HEWL and T_4L and this part of the enzyme was also considered as a possible model for molecular replacement. All the attempts were performed using *X-PLOR* (Brünger, 1992), *AMoRe* (Navaza, 1994), *POLARRFN* and *ALMN*, the latter two being part of the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994). The coordinates of the lysozyme models were obtained from the Protein Data Bank (Bernstein *et al.*, 1977): entries 3LZM (T_4L) and 2LZT (HEWL), those of SLT were kindly provided by the authors. We tried intensively to use the three full models as well as partial models from the two lysozyme molecules and also several combination of fragments, based on their sequence similarity with λL. Several resolution ranges were tested but all these trials were unsuccessful, even for the cross-rotation part of the problem. This fact strengthens the assumption that the structure of λL would be quite different from those of other classes of lysozymes and that the solution will require multiple isomorphous replacement.

Two platinum derivatives were prepared by soaking the crystals in the mother liquor with the addition of either 5 m*M* sodium tetrachloroplatinate (2 d) or 1 m*M* platinum terpyridine chloride (2–5 d). Derivative data including anomalous dispersion were collected to a resolution of 2.8 and 2.5 Å, respectively, on the wiggler beamline BW7B ($\lambda = 0.859$ Å) at the EMBL Hamburg outstation. A total of 22 714 independent reflections out of 60 359 reflections were collected for the first derivative with an $R_{\rm merge}(I)$ value of 7.4% (98.9% of completeness) and 31 791 independent reflections out of 77 184 reflections for the second derivative with an $R_{\rm merge}(I)$ value of 10.2% (98.9% completeness). The scaling R factors between the native and the two derivative crystals are 25.4 and 21.6%, respectively. The analysis of these derivatives is now under way.

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References

Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F. Jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977). *J. Mol. Biol.* **112**, 535-542.

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

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

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

Jespers, L. (1991). PhD thesis, University of Louvain, Belgium.

Jespers, L., Sonveaux, E. & Fastrez, J. (1992). *J. Mol. Biol.* **228**, 529–538.

Jespers, L., Sonveaux, E., Fastrez, J., Phanapoulos, A. & Davison, J. (1991). *Protein Eng.* **4**, 485–492.

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

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

Otwinowski, Z. (1993). *Data Collection and Processing. Proceedings of the CCP4 Study Weekend*, pp 56–62. Warrington, England: Daresbury Laboratory.

Rossmann, M. G. & Argos, P. (1976). *J. Mol. Biol.* **105**, 75–95.

Soumillion, P. & Fastrez, J. (1992). *J. Biochem.* **286**, 187–191.

Soumillion, P., Jespers, L., Vervoort, J. & Fastrez, J. (1995). *Protein Eng.* **5**, 451 456.

Taylor, A., Das, B. C. & van Heijenoort, J. (1975). *Eur. J. Biochem.* **53**, 47–54.

Taylor, W. R. & Orengo, C. A. (1989). *J. Mol. Biol.* **208**, 1–22.

Thunnissen, A. W. H., Dijkstra, A. J., Kalk, K. H., Rozeboom, H. J., Engel, H., Keck, W. & Dijkstra, B. W. (1994). *Nature (London)*, **367**, 750–753.