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Crystallization and preliminary X-ray analysis of thiaminase I from *Bacillus thiaminolyticus*: space group change upon freezing of crystals

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Abstract

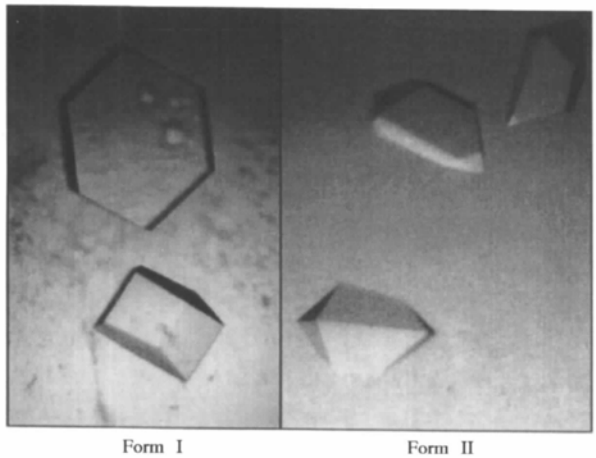
Thiaminase I (M_r = 42 100) from *B. thiaminolyticus*, expressed in *E. coli*, has been crystallized by the vapor-diffusion method. Three crystal forms, two of which grew from 0.1 *M* sodium acetate (pH = 4.6), 0.2 *M* ammonium sulfate and 30%(*w*/*v*) PEG 2000, have been examined by X-ray analysis. One crystal form diffracted to 2.5 Å at room temperature, was orthorhombic, and had unit-cell edges of a = 87.7, b = 120.5 and c = 76.7 Å with space group $P2_12_12_1$. A self-Patterson map showed a strong peak indicating noncrystallographic translational pseudosymmetry with (u, v, w) = (0.03, 0.0, 0.5). When these crystals were frozen at liquid-nitrogen temperatures, a second crystal form was observed which had unit-cell dimensions a = 85.5, b = 117.5 and c = 36.6 Å with space group $P2_12_12$. A third crystal form grew from 0.1 *M* Tris (pH = 8.5), 0.2 *M* sodium acetate trihydrate and 28%(*w*/*v*) PEG 6000 to produce orthorhombic crystals of space group $P2_12_12_1$ with cell edges of a = 114.4, b = 123.1 and c = 92.5 Å.

1. Introduction

Thiaminases catalyze the degradation of thiamin (vitamin B_1). This activity has been detected in bacteria, marine organisms and plants (Fujita, 1954, 1972; Evans, 1975; Murata, 1982), and two classes of thiamin-degrading enzymes have been identified. Thiaminase I (E.C. 2.5.1.2) catalyzes the reaction in which the thiazole group of thiamin is replaced by a variety of organic nucleophiles, *e.g.* aniline, quinoline, pyridine and veratylamine. Thiaminase II is specific for the use of water as the nucleophile. Although the biological function of thiaminase I is unknown, it has been demonstrated that animals and humans who ingest thiaminase-I-containing foods may develop symptoms of thiamin deficiency (Hayashi, 1957; Duffy *et al.*, 1981; Earl & McCleary, 1994; Evans, 1975).

Thiaminase I has been purified from *B. thiaminolyticus* (Douthit & Airth, 1966) and is a better characterized system than thiaminase II (Lienhard, 1970; Pearson & Lipman, 1988). Thiaminase I is an extracellular, 42 kDa protein. Its gene has been cloned, sequenced and overexpressed in *E. coli*. (Abe *et al.*, 1987; Costello *et al.*, 1996). Amino-acid sequence analysis of thiaminase I has been confirmed by electrospray ionization Fourier-transform mass spectrometry (ESI/FT-MS) (Kelleher *et al.*, 1995). Amino-acid sequence analysis showed no sequence similarity to other proteins in the protein sequence database.

To obtain a detailed understanding of the reaction mechanism of thiaminase I, we have initiated a crystallographic study of this enzyme. We report here the crystallization of recombinant thiaminase I and X-ray diffraction analysis of three crystal forms, two of which grew from the same precipitating conditions but depend on the temperature at which data were collected. X-ray diffraction data collected at room temperature showed pseudo-translational symmetry; whereas data collected at liquid-nitrogen temperatures showed no pseudo-translational symmetry and half the length of the c axis, indicating a phase change. Other examples of pseudo-translational symmetry and phase change have been seen in crystals of α-bungarotoxin (Love & Stroud, 1986), peroxidase from *Coprinus cinereus* (Petersen *et al.*, 1994) and scorpion toxin (Ealick *et al.*, 1984). In addition to the mechanistic details of thiamin degradation, the structure determination of the multiple crystal forms may provide insight into protein packing and the nature of the phase transition between the temperature-dependent crystal forms of thiaminase I.



Form I Form II

Fig. 1. Thiaminase I crystals. Form I crystals grew as six-sided parallelepipeds. The larger form I crystal has dimensions 0.3 × 0.8 × 0.6 mm. Form II crystals grew as extended octahedra and tend to be smaller.

2. Crystallization and X-ray diffraction analysis

Thiaminase I from *B. thiaminolyticus* was overexpressed and purified from an *E. coli* expression system, as described previously (Costello *et al.*, 1996). From a 20 l culture, 15 mg of pure protein was purified. The stock solution of thiaminase I, which is stored at 193 K, is composed of 15 mg ml^{-1} protein, 10%(*v*/*v*) glycerol, 50 m*M* sodium phosphate, (pH = 7.0), 2 m*M* dithiothreitol (DTT) and 2 m*M* ethylenediamine tetraacetic acid (EDTA). Before each crystallization trial, the glycerol was removed by diluting and concentrating the

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protein solution in a Centricon-10YM concentrator (Amicon) with the above buffer minus the glycerol.

The initial screening for crystallization was performed by the hanging-drop method over Linbro plates by using the Crystal Screen kits I and II (Hampton Research) (Cudney *et al.*, 1994; Jancarik & Kim, 1991). The drop was made by mixing 2 µl of protein solution and 2 µl of the precipitating solution. Each well contained 750 µl of a precipitating solution. Condition 13 of the Crystal Screen II kit [30%(*w*/*v*) PEG 2000, 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate (pH = 4.6)] and condition 22 of Crystal Screen II [28%(*w*/*v*) PEG 6000, 0.2 *M* sodium acetate trihydrate, 0.1 *M* Tris (pH = 8.5)] yielded single crystals at room temperature. These crystals will be referred to as form I and form II, respectively. Crystals of form I were six-sided parallelepipeds that grew to a maximum of 1.0 × 0.8 × 0.5 mm in 10–14 d (Fig. 1) and diffracted to 2.5 Å resolution at room temperature from X-rays generated from a rotating anode. Attempts to optimize the growth of form I crystals found successful crystallization conditions at 0.1–0.2 *M* ammonium sulfate, 26–32%(*w*/*v*) PEG 2000 and 0.1 *M* sodium acetate (pH = 4.0–5.0). The best conditions for crystal growth were at 30%(*w*/*v*) PEG 2000, 0.2 *M* ammonium sulfate and 0.1 *M* sodium acetate (pH = 4.6). This condition also acted as a stabilization buffer for crystal storage, heavy-atom soaks, or crystal freezing. Form II crystals were elongated octahedrons that grew to a maximum of 0.8 × 0.3 × 0.3 mm in 20–30 d and diffracted to 2.7 Å resolution. Attempts to optimize crystal growth found that protein concentration was a critical variable for producing single crystals. Higher concentrations produced needles and thin plates. Microseeding and lower protein concentration (10 mg ml^{-1}) aided in producing single crystals within 14 d. Unfortunately, crystal form II was fragile and tended to stick to the cover slip. Therefore, form I crystals were preferred for derivative searches.

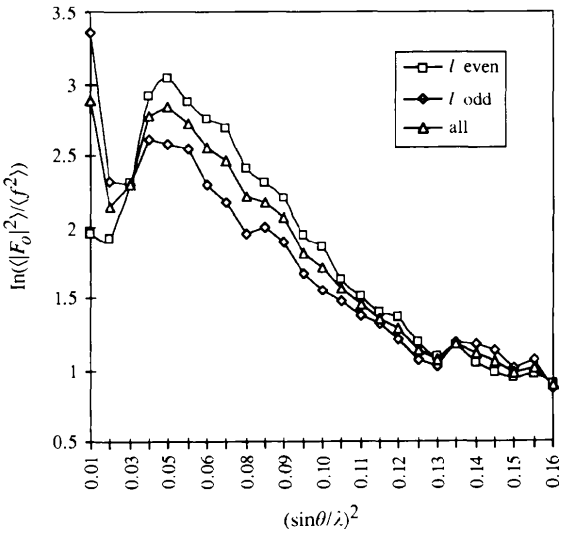

Fig. 2. Wilson plot for crystal form I*a* with intensity data to 2.5 Å resolution. The intensity data are classified into three groups in which the indexes of the reflections are $l = 2n$, $l = 2n + 1$ and all l. Particularly at low resolution, the average of the amplitudes squared, $\langle F_o^2 \rangle$, are noticeably weaker for those reflections with l odd. This fact implies that two independent molecules are related by pseudotranslational symmetry of about $\frac{1}{2}$ of the unit-cell along the c axis.

X-ray diffraction data were collected on a San Diego MultiWire (SDMW) X-ray detector (Hamlin, 1985). The intensities were integrated with software provided by SDMW (Howard *et al.*, 1985) and scaled with *CCP*4 (Collaborative Computational Project, Number 4, 1994). The X-ray source was Cu $K\alpha$ from a Rigaku RU200 rotating anode operated at 40 kV and 100 mA with a graphite monochromator and 0.3 mm collimator. Crystal form I diffracted to a resolution of 2.5 Å at room temperature and was of an orthorhombic space group with unit-cell dimensions a = 87.7, b = 120.5 and c = 76.7 Å. The data were 90% complete to 2.5 Å resolution and had an R_{sym} = 4.0%. Assuming a dimer per asymmetric unit, a V_m value of 2.40 Å^3 Da^{-1} was obtained (Matthews, 1968), corresponding to a solvent content of 49%. Analysis of the diffraction data showed the following systematic absences: $h = 2n + 1$ for $h00$; $k = 2n + 1$ for $0k0$ and $l = 2n + 1$ for $00l$, satisfying the condition for space group $P2_12_12_1$. In addition, all of the reflections in the $h0l$ zone with $l = 2n + 1$ were unobserved or very weak. Further analysis of the X-ray diffraction data showed that all reflections with $l = 2n + 1$ were weaker on average than reflections with $l = 2n$ (Fig. 2). These observations suggest that pseudo-translational symmetry exists along the c axis. Native Patterson maps calculated with

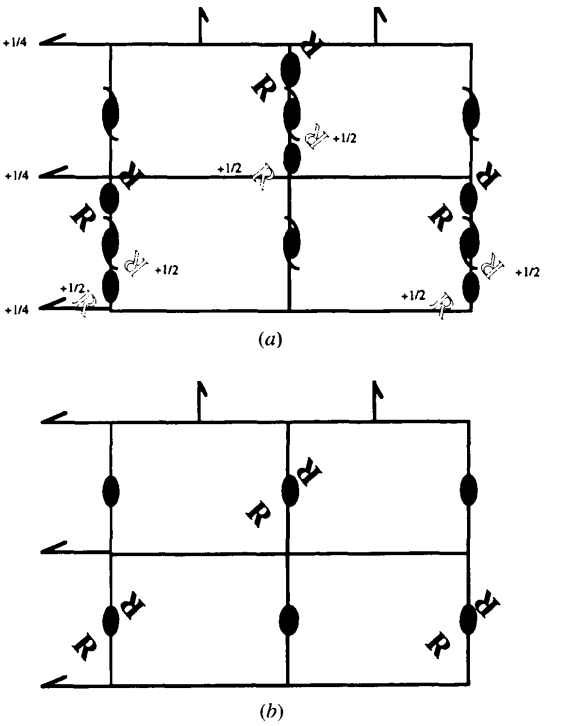

Fig. 3. Packing diagrams to illustrate the phase change that occurs for form I crystals of thiaminase I. This view is down the c axis with the b axis horizontal. (*a*) At room temperature ($P2_12_12_1$ cell, a = 87.7, b = 120.5, c = 76.7 Å), the non-crystallographic twofold axis is parallel to the $P2_1$ axis along the c axis direction. The separation between non-crystallographic twofold and $P2_1$ screw axis is only 1.4 Å. (*b*) At low temperature ($P2_12_12$ cell, a = 85.5, b = 117.5, c = 36.6 Å), the c axis is half the length of the room-temperature crystal.

various resolution limits showed a strong peak (half the height of the origin) at $(u, v, w) = (0.03, 0.00, 0.50)$. This arises from a combination of $P2_12_12_1$ symmetry and a non-crystallographic twofold axis that happens to be parallel to the c axis. Thus, there is a translational relation between the monomer of one dimer and the monomer of a second, crystallographically related dimer (Fig. 3).

Upon freezing form I crystals of thiaminase I for data collection at the Cornell High Energy Synchrotron Source (CHESS), the crystallographic symmetry appears to change due to a displasive transformation. These X-ray diffraction data were collected with a CCD detector (Walter *et al.*, 1995), were processed with the program *HKL* (Otwinowski & Minor, 1998), were 89% complete to 2.0 Å resolution and had an R_{sym} of 6.0%. At the lower temperature, the unit-cell remained orthorhombic, but the unit-cell dimensions became $a = 85.5$, $b = 117.5$ and $c = 36.6$ Å. Assuming a monomer per asymmetric unit, a V_m value of 2.18 Å^3 Da^{-1} was obtained, corresponding to a solvent content of 44%. Compared with the a and b axes from room-temperature crystal form I, the respective a and b axes at lower temperature were 2.5% smaller, and the c axis of the low-temperature crystal was half the length of the c axis of the room-temperature crystal. Other examples of a phase change in protein crystals show the opposite phenomenon, where a cell axis is doubled upon cooling the crystal (Love & Stroud, 1986; Ealick *et al.*, 1984). It appears that the phase change involved cell shrinkage and the superposition of the non-crystallographic twofold axis onto the $P2_1$ axis along c, resulting in a space group change (Fig. 3). Phase transitions of this type are not uncommon for inorganic materials, in which the high- and low-temperature forms are related by disordering or displacive transitions (Buerger, 1960). The room-temperature crystal form I and the low-temperature crystal form I will be referred to as form I*a* and form I*b*, respectively.

The third crystal form that was characterized (form II crystals) diffracted to 2.7 Å resolution at room temperature. The space group was $P2_12_12_1$ with cell edges of $a = 114.4$, $b = 123.1$ and $c = 92.5$ Å. A complete native data set was collected with a SDMW detector, as for the form I*a* crystals. Assuming three molecules per asymmetric unit, a V_m value of 2.58 Å^3 Da^{-1} was obtained, corresponding to a solvent content of 52%. These crystals were unstable and difficult to handle. Therefore, the search for heavy-atom derivatives will be limited to form I*a* crystals initially.

In addition to the atomic details for the mechanism of thiamin degradation, the structure determination of the three crystal forms will provide insight into protein packing interactions and the phase change between the two temperatures. Some potential derivatives of crystal form I*a* have been found for a structure solution by multiple isomorphous replacement. The structure solution of thiaminase-I form I*b* crystals and form II crystals will be solved by molecular replacement.

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References

Abe, M., Ito, S., Kimoto, M., Hayashi, R. & Nishimune, T. (1987). *Biochem. Biophys. Acta*, **909**, 213–221.
Buerger, M. J. (1960). *Crystal Structure Analysis*, p. 56. New York: John Wiley.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Costello, C. A., Kelleher, N. L., Abe, M., McLafferty, F. W. & Begley, T. P. (1996). *J. Biol. Chem.* **271**, 3445–3452.
Cudney, R., Patel, S., Weisgraber, K., Newhouse, Y. & McPherson, A. (1994). *Acta Cryst.* D**50**, 414–423.
Douthit, H. A. & Airth, R. L. (1966). *Arch. Biochem. Biophys.* **113**, 331–337.
Duffy, X., Morris, X. & Neilson, X. (1981). *Am. J. Clin. Nutr.* **34**, 1584–1592.
Ealick, S. E., Cook, W. J., Fontecilla-Camps, J. C., Suddath, F. L., Bugg, C. E. & Watt, D. D. (1984). *J. Biol. Chem.* **259**, 12081–12083.
Earl, J. W. & McLeary, B. V. (1994). *Nature (London)*, **368**, 683–684.
Evans, C. (1975). *Vitam. Horm.* **33**, 467–504.
Fujita, A. (1954). *Adv. Enzymol.* **15**, 389.
Fujita, A. (1972). *J. Vitaminol.* **18**, 67–72.
Hamlin, R. (1985). *Methods Enzymol.* **114**, 416–452.
Hayashi, X. (1957). *Nutr. Rev.* **15**, 65–67.
Howard, A. J., Nielsen, C. & Xuong, N. H. (1985). *Methods Enzymol.* **114**, 452–472.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kelleher, N. L., Costello, C. A., Begley, T. P. & McLafferty, F. W. (1995). *J. Am. Soc. Mass Spectrom.* **6**, 981–984.
Lienhard, G. (1970). *Biochemistry*, **9**, 3011–3020.
Love, R. A. & Stroud, R. M. (1986). *Protein Eng.* **1**, 37–46.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Murata, K. (1982). *Ann. NY Acad. Sci.* **378**, 146–156.
Otwinowski, Z. & Minor, W. (1998). *Methods Enzymol.* **276**. In the press.
Pearson, W. R. & Lipman, D. J. (1988). *Proc. Natl. Acad. Sci. USA*, **85**(8), 2444–2448.
Petersen, J. F. W., Kadziola, A. & Larsen, S. (1994). *FEBS Lett.* **339**, 291–296.
Puzach, S., Gorbach, Z. & Ostrovskii, Y. (1984). *Biochemistry (USSR)*, **49**, 1010–1016.
Tate, M. W., Eikenberry, E. F., Barna, S. L., Wall, M. E., Lowrance, J. L. & Gruner, S. M. (1995). *J. Appl. Cryst.* **28**, 196–205.
Walter, R. L., Thiel, D. J., Barna, S. L., Tate, M. W., Wall, M. E., Eikenberry, E. F., Gruner, S. M. & Ealick, S. E. (1995). *Structure*, **3**, 835–844.