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Crystallization and preliminary structural studies of lactose-specific enzyme IIA from *Lactococcus lactis*

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Abstract

Lactose-specific enzyme IIA of the phosphoenol:pyruvate-dependent sugar phosphotransferase system from *Lactococcus lactis* has been crystallized in phosphate buffer. The crystals belong to space group $P4_12_12$ or its enantiomorph $P4_32_12$ with unit-cell axes $a = b = 90.9$ and $c = 82.4$ Å. The packing parameter (Matthews parameter) V_m of 2.48 Å^3 Da^{-1} is consistent with one trimer per asymmetric unit and non-crystallographic threefold symmetry has been confirmed by calculating a self-rotation function. The crystals diffract X-rays to at least 2.3 Å resolution, are stable in an X-ray beam and are therefore appropriate for structure determination. Native data to 2.3 Å resolution have been collected using a MAR image-plate system at a synchrotron source. One isomorphous heavy-atom derivative has been identified and the presence of an isomorphous signal in the data has been confirmed by Patterson methods.

1. Introduction

The phosphoenolpyruvate:sugar transferase system (PTS) found in bacteria is responsible for binding, transmembrane transport and phosphorylation of numerous sugar substrates in prokaryotes. The system is also involved in several regulatory events including catabolite repression, catabolite inhibition and chemotaxis (for a review, see Postma, Lengeler & Jacobson, 1993).

The PTS system consists of two non-specific, energy-coupling components, enzyme I and a heat-stable phosphocarrier protein (HPr), as well as several sugar-specific multidomain permeases known as enzymes II. The hydrophilic intracellular domains of enzyme II, EIIA and EIIB, are phosphorylated in the phosphorylation cascade, while domain EIIC acts as a sugar channel in the bacterial membrane. The phosphoryl group from phosphoenolpyruvate, the only source of energy for sugar translocation, is transferred through intracellular components of the system. In the final stage of the transfer it phosphorylates the sugar.

$$\text{phosphoenolpyruvate} \xrightarrow{P} \text{EI} \xrightarrow{P} \text{HPr} \xrightarrow{P} \text{EIIA} \xrightarrow{P} \text{EIIB} \xrightarrow{P} \text{sugar}.$$

Based on differences in amino-acid sequences and on limited structural information, enzymes IIA have been classified into four families. The best studied representatives of these families are the (1) glucose-, (2) mannose-, (3) mannitol- and (4) lactose-specific proteins, respectively. Detailed X-ray structures are available for glucose-specific enzymes IIA and a model of the interaction between $EIIA^{glu}$ and HPr has been proposed (Herzberg, 1992). The mannose- and mannitol-specific families of enzyme IIA have been studied using NMR methods, which revealed different secondary structures and folding topologies for those enzymes. Lactose and cellobiose-specific enzymes IIA, which are homologous to each other, are the members of the only family for which no structural data has been reported so far, although crystals have been described for IIA^{lac} from *Staphylococcus aureus* (Celikel *et al.*, 1991). For a review of structural information about proteins from the PTS system, see Herzberg & Klevit (1994).

Enzyme IIA from the lactose permease of *Lactococcus lactis* is a small, 11.5 kDa protein expressed from the *lac* gene as a monomer of 105 residues. Primary sequence comparison with other proteins of known structure and the application of reverse-folding algorithms suggest that the fold of the lactose family of enzyme IIA is not homologous with any of the other known structures. Structure prediction algorithms indicate that in contrast to other families of enzymes IIA, $EIIA^{lac}$ is highly helical.

In addition, antibody binding experiments imply that the protein might undergo major conformational changes upon phosphorylation (Deutscher, Beyreuther, Sobek, Stuber & Hengstenberg, 1982). To confirm the validity of these models in structural terms we have started an X-ray crystallographic study of enzyme IIA^{lac}. Here, we report the crystallization and preliminary characterization of the crystals.

2. Materials and methods

The *L. lactis* $EIIA^{lac}$ was purified from an overproducing *Escherichia coli* strain as described de Vos, Boerrigter, Rooyen, Reiche & Hengstenberg (1990). After purification, the protein was stored as an ammonium sulfate precipitate at 277 K.

For crystallization trials, the ammonium sulfate precipitate was disssolved in 0.15 *M* Na/K phosphate buffer, pH 6.4. The protein solution was then desalted using a Nap10 column (Pharmacia Biotech, Uppsala, Sweden) and brought to a protein concentration of 10 mg ml^{-1} using Centricon3 concentrators (Amicon, Beverly, Massachusetts, USA).

Crystals of enzyme IIA^{lac} were obtained by the sitting-drop vapor-diffusion technique (McPherson, 1985) at room temperature using only a small increase in the phosphate concentration to achieve crystallization. The protein solution (2 µl) was mixed with 2 µl of 0.15 *M* Na/K phosphate buffer, pH 6.4, and equilibrated against 0.40 *M* Na/K phosphate buffer at the same pH. The largest crystals (0.25 × 0.25 × 0.25 mm) appeared in *ca* a week. They resembled hexagonal prisms (Fig. 1).

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Table 1. *Data-collection statistics*

Cell parameters	
Space group	$P4_12_12/P4_32_12$
Cell dimensions (Å)	$a = b = 90.9$
	$c = 82.4$
Solvent (%)	48
V_m (Å^3 Da^{-1})	2.48

Data collection	Native	Derivative
Resolution (Å)	2.3	2.3
No. of unique reflections	15009	15417
Multiplicity	6.1	4.8
$\langle I\rangle/\sigma(I)$	18.2/4.9*	14.9/5.0*
Completeness	97.7%/98.9%*	99.8/99.9%*
$R_{\rm merge(I)}$†	8.6/36.3*	10.9/34.0*
$R_{\rm deriv(I)}$‡	N/A	15.0/21.7

*In highest resolution range (2.4–2.3 Å). †$R_{\rm merge(I)} = (\sum|I - \langle I\rangle|/\sum I) \times 100$, where I is the observed intensity, and $\langle I\rangle$ is the average intensity obtained from multiple observations of symmetry-related reflections. ‡$R_{\rm deriv(I)} = (\sum I_D - I_N|/\sum I_N) \times 100$, where I_N and I_D are the observed reflection intensities for the native and heavy-atom substituted protein, respectively, calculated using unique reflections.

Native X-ray diffraction data were collected from a single crystal on the X31 beamline of the EMBL outstation at the DESY synchrotron source in Hamburg, Germany, using the MAR image-plate detector. The experimental design of this beamline has been described by Wilson (1989). Crystals were mounted in thin-walled glass capillaries. The crystal-to-detector distance was set to 200 mm. Exposure time was linked to flux and at an average ring current of 99 mA, a typical exposure was 3 min for a 1° oscillation. Data were indexed, integrated and reduced on a Silicon Graphics workstation using the program package *DENZO* (Otwinowski, 1993; Minor, 1993). Data-reduction statistics are shown in Table 1.

An isomorphous heavy-atom derivative was obtained by soaking native crystals for 7 d in 0.15 *M* Na/K phosphate buffer, pH 6.4, containing 20 m*M* trimethyl lead acetate. The crystals were stable and diffraction data were collected from one crystal with the same parameters as for the native crystal. A wavelength of $\lambda = 0.94$ Å was used to increase the anomalous signal from the lead derivative.

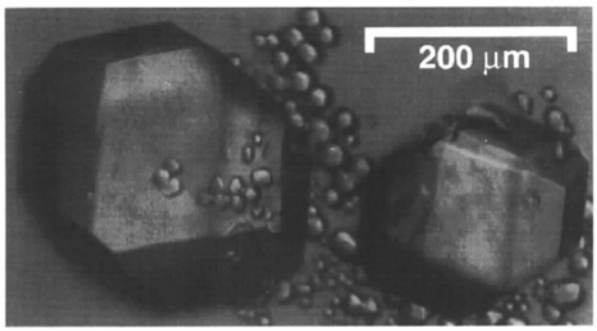

Fig. 1. Crystals of lactose-specific enzyme IIA from *L. lactis*. Growth occurred at 0.15 *M* Na/K phosphate buffer, pH 6.4, equilibrated against 0.40 *M* Na/K phosphate buffer, pH 6.4, at 298 K.

3. Results and discussion

Native crystals of enzyme IIA were stable in the synchrotron X-ray beam for several hours and data to 2.3 Å resolution were collected. Analysis of the data by the program package *DENZO* (Otwinowski, 1993; Minor, 1993) indicated that the crystals of *L. lactis* enzyme IIA belong to the tetragonal space group $P4_12_12$ or $P4_32_12$ (distortion index 0.19 *versus* 8.83% for primitive cubic) with unit-cell parameters $a = b = 90.9$ and $c = 82.4$ Å.

Based on the cell parameters and molecular weight of the protein, the Matthews parameter V_m was calculated to be 2.48 Å^3 Da^{-1}, assuming three monomers per asymmetric unit. The predicted solvent content would thus be 48%, well within the range usually observed for globular proteins (Matthews, 1968).

It has been reported previously that EIIA$^{\rm lac}$ forms a functional trimer in solution (de Vos, Boerrigter, Rooyen, Reiche & Hengstenberg, 1990). A self-rotation function computed on the native data in the 3.5–8 Å resolution range shows a characteristic threefold non-crystallographic symmetry peak at 6σ above the map average located at $\varphi = 55$, $\psi = 45$ and $\kappa = 120°$ (Fig. 3). This suggests that the functional trimeric complex of the protein is also present in the crystal. The noncrystallographic symmetry should prove helpful for phase determination, through the application of averaging methods.

Although we had performed an extensive search for suitable heavy-atom derivatives, including low concentrations of trimethyl lead acetate, all experiments had failed. When diffraction data, obtained with Cu $K\alpha$ radiation from a rotating-anode source, were analyzed they either did not show any differences at all or the resulting difference-Patterson map was not interpretable. Alternative approaches included cross-

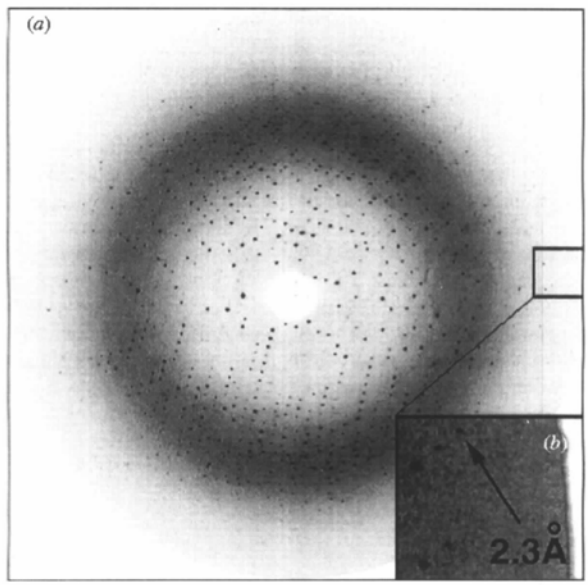

Fig. 2. (*a*) A typical 1° oscillation image for a crystal of lactose-specific enzyme IIA from *L. lactis*. The image was recorded on an 18 cm diameter MAR image plate on beamline X31 of the EMBL outstation of DESY, Hamburg, Germany. With a crystal-to-film distance of 200 mm, $\lambda = 0.94$ Å and a ring current of 99 mA, the typical exposure time was 3 min. (*b*) Enlargement of the edge of the diffraction image showing reflections at high resolution (up to 2.3 Å).

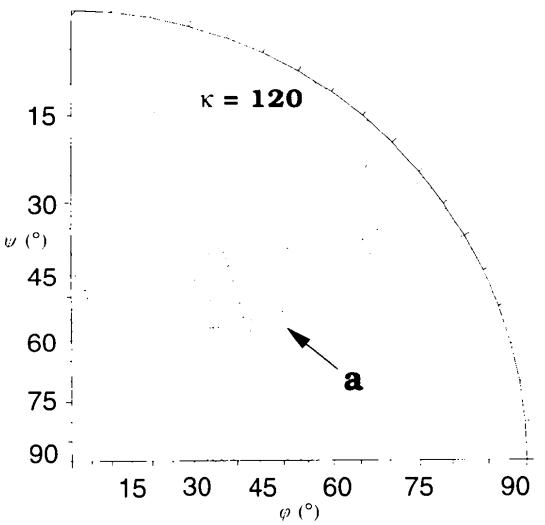

Fig. 3. $\kappa = 120°$ section of the self-rotation function calculated on the native data set. The best results were obtained using data in the 8–3.5 Å resolution range and a Patterson function radius of 15 Å. The map was contoured at 0.5σ levels starting at 3σ above the map average. The non-crystallographic peak (*a*) corresponding to 6σ above the map average is clearly seen and its height is 29% of the origin.

linking with glutaraldehyde in order to stabilize crystals which were cracking during soaking or the introduction of sulfhydryl groups (Mowbray & Petsko, 1983). Both experiments did not meet with success.

A high concentration (20 m*M*) of trimethyl lead acetate soaked for a longer time (7 d), however, gave promising results. The isomorphous difference-Patterson map is very clean. Fig. 4 shows a Harker section with eight symmetry-related peaks (14σ above map average), indicating a single metal binding site per asymmetric unit. Combining the isomorphous and anomalous signals from this lead derivative and applying methods such as solvent flattening and symmetry averaging should make it possible to solve the structure of $EIIA^{lac}$ without the need for any additional derivatives.

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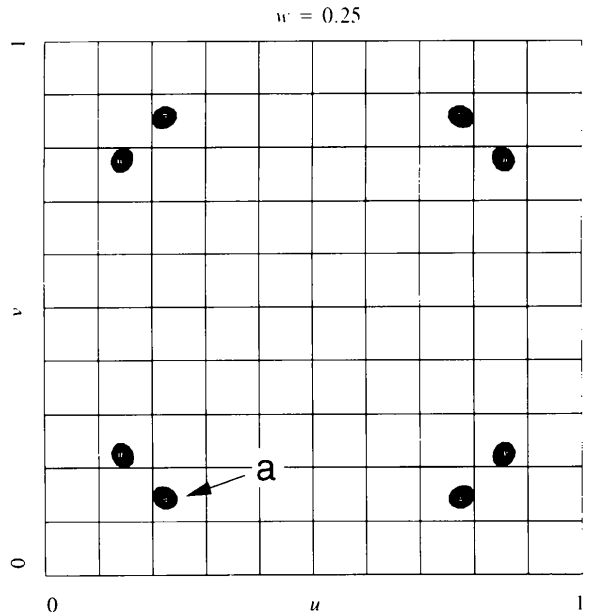

Fig. 4. $uv\frac{1}{4}$ section of the isomorphous Patterson difference map produced with *PHASES* (Furey, 1991). Contours are drawn at levels of 1σ starting at 3σ. The peak *a* which is observed at $u = 0.223$, $v = 0.145$ and $w = 0.249$ has a height of 14σ, which represents 8.3% of the height of the origin peak. All other peaks are related to peak *a* by crystallographic symmetry and correspond to a single binding site of trimethyl lead acetate.

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