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Crystallization and preliminary X-ray studies of recombinant amylosucrase from *Neisseria polysaccharea*

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Recombinant amylosucrase from *Neisseria polysaccharea* was crystallized by the vapour-diffusion procedure in the presence of polyethylene glycol 6000. The crystals belong to the orthorhombic space group $P2_12_12$, with unit-cell parameters a = 95.7, b = 117.2, c = 62.1 Å, and diffract to 1.6 Å resolution. A *p*-chloromercuribenzene sulfonate (pcmbs) derivative has been identified and a selenomethionine-substituted protein has been produced and crystallized.

1. Introduction

Amylosucrase is a hexosyltransferase (E.C. 2.4.1.4) found in *N. polysaccharea* isolated from the throats of healthy children in Europe and Africa. With sucrose as the unique energy source, it catalyses the synthesis of an amylose-like polysaccharide composed of only α-(1→4)-glucosidic linkages. Sequencing of the gene and the expression of a fully active enzyme in *Escherichia coli* has been achieved (Potocki de Montalk *et al.*, 1999), making the production of large quantities of protein and mutational studies possible. Recombinant amylosucrase derived from a glutathione S-transferase fusion protein consists of a single polypeptide chain with 626 amino-acid residues, including six cysteines and 15 methionines, and a calculated molecular weight of 71 750 Da. Based on amino-acid sequence similarities, the enzyme has been placed in the retaining glycoside hydrolase (GH) family 13 (Henrissat, 1991; Davies & Henrissat, 1995). This family mainly contains polysaccharide degrading enzymes such as α-amylase; however, it also has members that have glycosyltransferase activity, such as cyclomaltodextrin glucanotransferase. The three-dimensional structure of amylosucrase is unknown, although alignments and structure prediction suggest that it contains a $(\beta/\alpha)_8$ barrel (Potocki de Montalk *et al.*, 1999). Structural information from related enzymes is quite limited. Of 207 subclasses in the hexosyltransferase group (E.C. 2.4.1.*x*), only four have a model structure; among the members of GH13 that have glycosyltransferase activity, only the structure of cyclomaltodextrin glucanotransferase (E.C. 2.4.1.19) is known. The structure determination of amylosucrase will make a large number of substrate/inhibitor–enzyme complex studies and mutational studies possible. This will hopefully contribute to a detailed understanding of the reaction mechanism of this class of enzymes.

2. Materials and methods

2.1. Overexpression and purification of amylosucrase

Fully active recombinant amylosucrase from *N. polysaccharea* was expressed in *E. coli* and was purified and characterized as described by Potocki De Montalk *et al.* (1999). Selenomethionine-substituted amylosucrase was produced by standard procedures (Doublié, 1997) and was characterized by SDS–PAGE and activity was monitored using the native amylosucrase assay (Potocki de Montalk *et al.*, 1999). The substitution level was checked by X-ray fluorescence spectroscopy with S (from Cys) as internal standard and was found to be at least 65%.

2.2. Circular dichroism spectroscopy

Circular dichroism (CD) spectra were measured in the 180–260 nm region on a Jasco J-710 spectropolarimeter. The measurement was performed at 298 K under N_2 and the protein buffer was purged with N_2 10 min before use. A quartz cuvette (0.01 cm path length) was employed and the protein concentration was 0.5 mg ml^{-1}. Eight scans from 260 to 178 nm (0.5 nm resolution, 4 s response time and 1.0 nm bandwidth) were collected. Background correction was performed and the final spectrum was deconvoluted using the *CDNN* program (Bohm *et al.*, 1992).

2.3. Crystallization and data collection

Crystallization conditions were screened according to the sparse-matrix method (Jancarik & Kim, 1991) using commercially available buffers (Hampton Research, Laguna

Hills, CA, USA) and the hanging-drop vapour-diffusion technique (McPherson, 1992). Polyethylene glycol (PEG) 6000 was found to be a good crystallization medium and several parameters were refined.

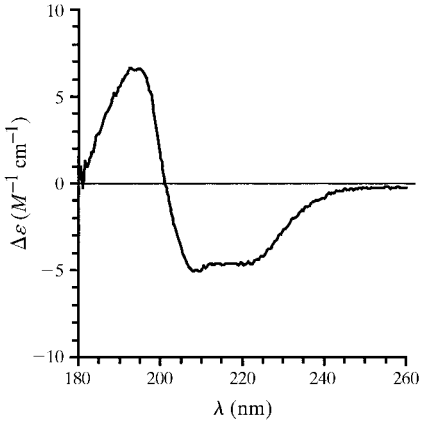

Figure 1
CD spectrum of amylosucrase. Solution, 7 µ*M* in 150 m*M* NaCl, 50 m*M* Tris–HCl pH 7.0, 1 m*M* EDTA and 1 m*M* α-dithiothreitol. Temperature, 298 K. Δε is given as the mean amino-acid residue value.

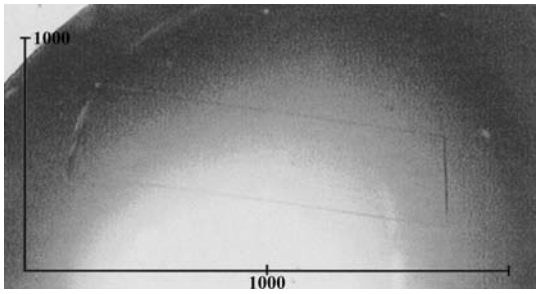

Figure 2
Orthorhombic crystals of recombinant *N. polysaccharea* amylosucrase. The scale is in micrometres.

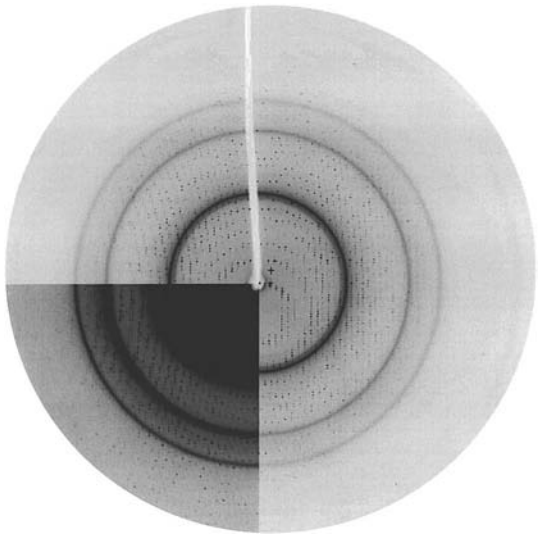

Figure 3
Diffraction pattern from the 1.6 Å native amylosucrase data set with two shading levels. Exposure time was 30 s with 1.0° oscillations, an 85 mm crystal-to-detector distance and a 130 mm image plate. The edge of the plate corresponds to 1.6 Å resolution.

Hanging drops were prepared by mixing 2.5 µl of protein solution (4–6 mg ml^{-1}, 150 m*M* NaCl, 50 m*M* Tris–HCl pH 7.0, 1 m*M* EDTA and 1 m*M* α-dithiothreitol) with 2.5 µl reservoir solution [30%(*w*/*v*) PEG 6000 and 100 m*M* HEPES pH 7.0] and were equilibrated against 500 µl reservoir solution at 277 K. Colourless crystals of amylosucrase appeared after 10 d incubation and grew to maximum dimensions of 1.5 × 0.6 × 0.04 mm. The solution from which the crystals formed was used directly in the following cryo-experiments. Crystals were flash-cooled in liquid nitrogen and a native 1.6 Å data set with 69 218 unique reflections was collected at 110 K at beamline ID 14-3 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The overall completeness was 72.7% and $R_{\rm merge}$ was 0.092.

In order to produce heavy-atom derivatives, α-dithiothreitol was removed by dialysis. Hanging drops containing crystals were transferred to dialysis buttons and were dialyzed extensively against a solution containing 30%(*w*/*v*) PEG 6000, 100 m*M* HEPES pH 7.0 and 150 m*M* NaCl. Heavy-atom compounds [$HgCl_2$, K_2PtCl_4, *p*-chloromercuribenzene and $Lu_2(SO_4)_3$] were added to fresh dialysis solution and allowed to react for 24 h. Selenomethionine-substituted amylosucrase was crystallized under conditions similar to the native protein; a slightly lower protein concentration and micro-seeding with native crystals was used. Diffraction data on a *p*-chloromercuribenzene (pcmbs) derivative of native amylosucrase were collected at beamline 711, MAX-LAB, Lund, Sweden. A 2.5 Å data set with 24 230 unique reflections was collected using a MAR345 image-plate system with a crystal-to-detector distance of 350 mm and an oscillation range of 1.0°. 180° of data was collected in order to permit complete coverage of the anomalous data. The overall completeness was 95.6% and $R_{\rm merge}$ was 0.092.

All data were processed using the *HKL* suite (Otwinowski & Minor, 1997) and the *CCP*4 program package (Collaborative Computational Project, Number 4, 1994). Maximum-likelihood heavy-atom parameter refinement of Hg sites was performed with the *SHARP* software (de la Fortelle & Bricogne, 1997).

3. Results and discussion

Recombinant amylosucrase was used in the crystallization experiments. Deconvolution of the CD spectrum (Fig. 1) showed that the protein is folded and supports the predicted $(\beta/\alpha)_8$ barrel structure with a high content of α-helix and β-sheet (44% and 26%, respectively). Plate-like crystals of amylosucrase formed using 30–35% PEG 6000 as crystallization medium. Many unsuccessful experiments have been carried out to attempt to enhance growth in the third dimension and to limit nucleation. However, in a small percentage of identical experiments large crystals formed (Fig. 2).

From the symmetry of the intensity-weighted reciprocal lattice and systematically absent reflections, the space group of the orthorhombic crystals was determined to be $P2_12_12$, with unit-cell parameters a = 95.7, b = 117.2, c = 62.1 Å. Using the Matthews formula (Matthews, 1968), one molecule in the asymmetric unit and a water content of 49.7% were predicted. Fig. 3 shows a 1° oscillation image of a native crystal. Data

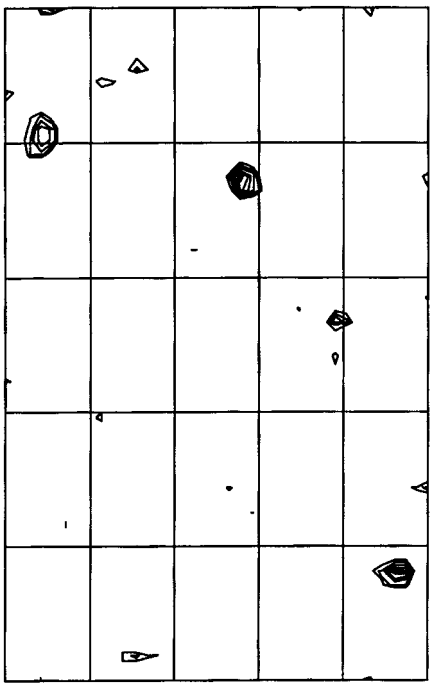

Figure 4
Anomalous Patterson map for the pcmbs derivative of amylosucrase. The origin is in the upper left corner, with *u* down and *w* to the right.

collections on a large number of heavy-atom soaked crystals were undertaken. These crystals were always severely non-isomorphous to the native crystals. However, from data collected on a pcmbs-soaked crystal it was possible to locate three mercury sites in the anomalous Patterson (Fig. 4) and to refine these sites (isomorphous and anomalous phasing powers after refinement were 0.86 and 1.6, respectively, to 2.0 Å resolution). It therefore seems highly likely that the structure can be solved using the multi-wavelength anomalous dispersion (MAD) method either on the pcmbs derivative or selenomethionine-substituted amylosucrase.

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References

Bohm, G., Muhr, R. & Jaenicke, R. (1992). *Protein Eng.* **5**, 191–195.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Davies, G. & Henrissat, B. (1995). *Structure*, **3**, 853–859.
Doublié, S. (1997). *Methods Enzymol.* **276**, 523–530.
Fortelle, E. de la & Bricogne, G. (1997). *Methods Enzymol.* **276**, 472–494.
Henrissat, B. (1991). *Biochem. J.* **280**, 309–316.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
McPherson, A. (1992). *J. Cryst. Growth*, **112**, 161–167.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Potocki De Montalk, G., Remaud-Simeon, M., Willemot, R. M., Planchot, V. & Monsan, P. (1999). *J. Bacteriol.* **181**, 375–381.