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Crystallization and preliminary X-ray analysis of the *Saccharomycopsis fibuligera* glucoamylase expressed from the *GLU*1 gene in *Escherichia coli*

ADRIANA SOLOVICOVÁ, JURAJ GAŠPERÍK, JOZEF ŠEVČÍK AND EVA HOSTINOVÁ* *at Institute of Molecular Biology, Slovak Academy of Sciences, Dúbravská cesta 21, 842 51 Bratislava, Slovakia. E-mail: umbihost@savba.savba.sk*

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Abstract

The active non-glycosylated glucoamylase, overexpressed from the *Saccharomycopsis fibuligera GLU*1 gene in *Escherichia coli* BL21(DE3), has been purified from the solubilized inclusion bodies and then renatured *in vitro*. Crystals of the recombinant glucoamylase were obtained by vapour diffusion using PEG as precipitant. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell dimensions of $a = 58.1$, $b = 87.8$ and $c = 99.9$ Å, and diffract to 1.7 Å resolution. This is the first report of the crystallization of the full-length glucoamylase corresponding to the mature enzyme.

1. Introduction

Glucoamylase (1,4-α-D-glucan glucohydrolase, E.C. 3.2.1.3) is an exohydrolase that catalyses the release of β-D-glucose from the non-reducing ends of starch and maltooligosaccharides. The enzyme has potentially important industrial applications. The primary industrial use of glucoamylase is in conversion of starch to glucose syrups.

Glucoamylases are produced mainly by molds and yeasts (Saha & Zeikus, 1989). The dimorphous yeast *Saccharomycopsis fibuligera* belongs to a family of good producers of glucoamylases (De Mot, 1990). The enzyme has, besides its commercial application, interesting molecular properties, namely a capability to renew its catalytic activity after exposure to adverse conditions such as heat denaturation (Gašperík & Hostinová, 1993). The glucoamylase of the native producer is a glycoprotein which exists in multiple forms due to differences in extent of glycosylation (Gašperík, Kováč & Mináriková, 1990). Two wild-type genes *GLU*1 (Itoh, Ohtsuki, Yamashita & Fukui, 1987) and *GLA*1 (Hostinová, Balanová & Gašperík, 1991) were isolated from different genomic libraries encoding the mature proteins consisting of 492 amino-acid residues. The glucoamylases Glu1 and Gla1 differ in seven amino-acid residues.

The three-dimensional structure of a catalytically active fragment of glucoamylase I from *Aspergillus awamori* var. X100, a representative of the mold-*O*-glycosidically modified glucoamylase, has been solved recently by Aleshin *et al.* at 2.2 Å resolution (Aleshin, Golubev, Firsov & Honzatko, 1992; Aleshin, Hoffman, Firsov & Honzatko, 1994). The fragment of 471 amino-acid residues has 32% sequence identity to the mature glucoamylase Glu1 from *S. fibuligera*.

The aim of the present work is to further contribute to the understanding of the structure–function relationships of this industrially important group of enzymes. Here we report crystallization and preliminary X-ray diffraction studies of the non-glycosylated form of *S. fibuligera* glucoamylase expressed from the *GLU*1 gene in *Escherichia coli* (EcGlu1).

2. Materials and methods

The truncated *GLU*1 gene encoding the mature enzyme was overexpressed in *E. coli* BL21(DE3) carrying the PCR fragment cloned into the pET-3d vector. Plasmid pSf Glu1 (Yamashita, Itoh & Fukui, 1985) was used as a template for PCR amplification. The nucleotide sequence of the construct was verified by DNA sequencing using a T^7 Sequencing Kit (Pharmacia Biotech.). Conditions for PCR amplification, bacterial expression, purification and enzyme assay of the recombinant glucoamylase have been described previously (Solovicová, Gašperík & Hostinová, 1996).

Crystallization conditions were screened using the hanging-drop vapour-diffusion technique at room temperature (McPherson, 1982). The drops were prepared by mixing equal volumes of protein and reservoir solutions. The best crystals were obtained with reservoir solution containing 30–32%(*w*/*v*) PEG 8 K and 100 m*M* acetate buffer at pH = 4.7–5.5, and a protein solution of 20 mg ml^{-1} in water.

X-ray data were collected at room temperature from a single crystal with a MAR Research imaging-plate scanner on the X-31 beamline at the Doris storage ring, DESY, Hamburg, Germany. The X-ray wavelength used was 0.93 Å. The data were processed with *DENZO* (Otwinowski, 1993).

3. Results and discussion

The specific activity of the recombinant non-glycosylated glucoamylase EcGlu1 was 80 ± 5 U mg^{-1} of the lyophilized enzyme. This value is lower than that of the glycosylated counterpart ScGlu1 (100 U mg^{-1}) isolated from *S. cerevisiae* (Gašperík & Hostinová, 1993).

Crystals of EcGlu1 suitable for crystallographic analysis appeared after 4–6 weeks and continued to grow for the next four weeks up to a maximum size of $0.3 \times 0.4 \times 0.15$ mm (Fig. 1). The crystals are orthorhombic in $P2_12_12_1$ space group. Unit-cell dimensions are $a = 58.1$, $b = 87.8$ and $c = 99.9$ Å. Two sets of data with resolution 4.0 and 1.7 Å were measured with an oscillation range 3.0–0.7°, respectively. For both sets 95° was covered. There were 56654 unique reflections and the completeness of data in the range 30.0–1.7 Å is 99.4%. The merging R factor ($R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$) is 4.2%.

The crystals prepared from the glucoamylase EcGla1 (the *GLA*1 gene expressed in *E. coli*), grown under identical conditions, were not suitable for high-resolution data collection. We attribute the different qualities of the EcGlu1 and EcGla1 crystals to their amino-acid differences (Table 1). The revised sequencing of the *GLA*1 gene revealed in its putative mature gene product seven instead of eight amino-acid differences (Hostinová, Balanová & Gašperík, 1991) relative

Table 1. *Differences in the amino-acid composition of the mature glucoamylases derived from the GLU1 and GLA1 genes*

Position of amino-acid residue	*GLU*1	*GLA*1
27	Glu	Asp
46	Asn	Asp
166	Glu	Lys
377	Ala	Val
410	Ala	Gly
461	Tyr	Asn
467	Gly	Ser

to the *GLU*1 gene product. Our preliminary studies indicate that some of the four amino-acid alterations in the C-terminal region of the protein influence its enzyme activity and substrate specificity (Gašperík & Hostinová, 1993). The results of studying the two enzymes obtained so far as well as the crystals of EcGlu1 suitable for X-ray diffraction analysis provide the basis for using protein engineering to modify the properties of *S. fibuligera* glucoamylases. The possibility of producing large quantities of recombinant, non-glycosylated and glycosylated *GLU*1 and *GLA*1 gene products as well as their mutants should provide valuable material for further studies.

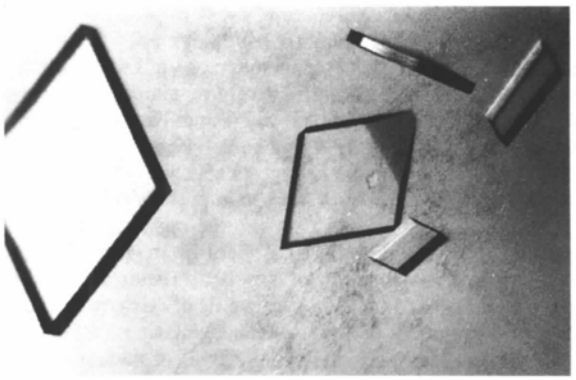

Fig. 1. Crystals of the recombinant non-glycosylated glucoamylase expressed from the *GLU*1 gene.

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