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Crystallization and preliminary X-ray data of amine oxidase from bovine serum

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A copper-containing amine oxidase extracted from bovine serum (BSAO) and purified to homogeneity has been deglycosylated and crystallized. The crystals obtained belong to space group $P2_12_12_1$, with unit-cell parameters a = 77.68, b = 131.19, c = 134.00 Å, and diffract to at least 2.4 Å resolution. BSAO is the first mammalian amine oxidase to be crystallized.

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1. Introduction

The structural characterization of copper-containing amine oxidases [amine-oxygen oxidoreductases (deaminating) (copper/TPQ-containing); EC 1.4.3.6] is of broad importance in order to understand the structure–function relationships of these enzymes, which play a variety of fundamental functions in the metabolism of cellular and extracellular amines. Copper-containing amine oxidases are a class of ubiquitous enzymes that catalyze the two-electron oxidation of primary amines to the corresponding aldehydes, with reduction of molecular oxygen to hydrogen peroxide (Knowles & Dooley, 1994). These enzymes are homodimers, with a subunit size in the range 70–95 kDa. They are glycosylated, with their carbohydrate content depending on the enzyme source. Human plasma amine oxidase was found to be linked to pathologies such as diabetes (Yuen, 1987; Gronvall-Nordquist *et al.*, 2001), congestive heart failure (Boomsma *et al.*, 1997, 2000), atherosclerosis and other vascular endothelial damage (Boomsma *et al.*, 1997; Magyar *et al.*, 2001). Amine oxidases have been the subject of intense kinetic and spectroscopic investigations over many years (Klinman & Mu, 1994) and the nature of the covalently bound cofactor (2,4,5-trihydroxyphenylalanine quinone; TPQ), as well as the reaction mechanism, are well established (Padiglia *et al.*, 2001). Conversely, despite the primary structures being well defined for about ten copper amine oxidases (Tipping & McPherson, 1995), crystal structures of these quinoenzymes are only available for the amine oxidases from *Escherichia coli* (Parsons *et al.*, 1995), pea seedling (Kumar *et al.*, 1996), *Arthrobacter globiformis* (Wilce *et al.*, 1997) and *Hansenula polymorpha* (Li *et al.*, 1998). To date, the three-dimensional structure of the enzyme from higher animals and, in particular, from mammalian organisms has not been determined. A crystalline form of 'highly purified' bovine and porcine plasma enzyme was reported in an old paper (Yamada & Yasunobu, 1962), but no crystal structure has yet been solved. One reason for the difficulty in crystallization is probably the presence of the glycoside portion. Moreover, the deglycosylated enzymes have poor stability.

One of the most studied enzymes in this family is bovine amine oxidase (BSAO), the carbohydrate content of which accounts for about 4.3% of its weight (Watanabe & Yasunobu, 1970). In this paper, we report the production and preliminary characterization of crystals of deglycosylated BSAO, which currently represents the best model for the human enzyme.

2. Experimental and results

2.1. Purification and deglycosilation

Bovine serum amine oxidase was purified according to the procedure reported by Vianello *et al.* (1992). The specific activity of the preparation was 0.36 U mg^{-1} (Tabor *et al.*, 1954), where one enzyme unit (U) corresponds to the transformation of 1 µmol of benzylamine per minute. The concentration of the purified enzyme was determined according to the method of Bradford (1976), assuming a molecular mass of 180 kDa.

Deglycosylation of the purified enzyme was carried out using a 'glycoprotein deglycosylation kit' from Calbiochem containing the following deglycosidases: *N*-glycosidase F from *Chryseobacterium meningosepticum* (activity 5000 U ml^{-1}), endo-α-*N*-acetylgalactosaminidase from *Streptococcus pneumoniae* (activity 1.25 U ml^{-1}), α-2–3,6,8,9-neuroaminidase from *Arthrobacter ureafaciens* (activity 5.3 U ml^{-1}) and β-1,4-galactosidase from *S. pneumoniae* (activity 1.66 U ml^{-1}). The deglycosylation was carried out in 50 m*M* sodium phosphate pH 7.0, incubating the samples at 310 K for 4 d, under the conditions (1 µg BSAO per microlitre of deglycosidase) suggested by Calbiochem.

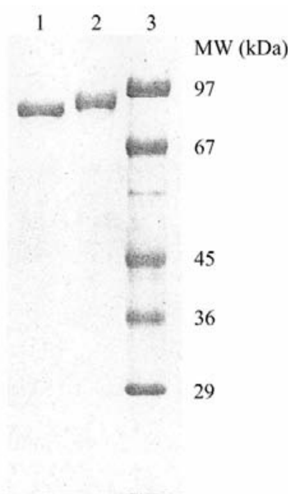

Figure 1
SDS–polyacrylamide gel electrophoretic pattern of deglycosylated BSAO. The gel, with 9% acrylamide concentration, was prepared according to Laemmli (1970). Lane 1, deglycosylated BSAO, 2 µg; lane 2, native BSAO, 2 µg; lane 3, molecular-weight markers, 2 µg.

The purity of the native BSAO and the degree of deglycosylation were assessed electrophoretically.

Denaturing SDS–PAGE was carried out according to the method of Laemmli (1970). The following proteins were used as molecular-weight markers: phosphorylase b (97 kDa) bovine serum albumin (66 kDa), ovoalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa) and carbonic anhydrase (29 kDa).

2.2. Crystallization

Single crystals of BSAO were obtained at 295 K using the vapour-diffusion method with either hanging or sitting drops. Tests were performed with Crystal Screen 2 and PEG–Ion Screen from Hampton Research (Laguna Niguel, CA, USA) and Structure Screens 1 and 3D from Molecular Dimensions Ltd (Soham, UK). In a successful experiment, a 4 µl drop was prepared by mixing 2 µl of protein solution containing 2.0–2.3 mg ml^{-1} BSAO in 10 m*M* HEPES, 10 m*M* NaCl pH 7.3 with an equivalent amount of the precipitant reservoir solution [PEG–Ion Screen solution No. 41; 0.2 *M* KH_2PO_4, 20%(*w*/*v*) PEG 3350 pH 4.7]. Small plate-shaped crystals of maximum dimensions 0.1 × 0.1 × 0.02 mm were obtained in a few days.

2.3. Data collection

Native diffraction data were collected at the diffraction beamline of the Elettra synchrotron (Trieste, Italy) at 100 K using a single crystal. Before measurement, the crystal was soaked for few seconds in a cryoprotectant solution prepared by mixing the precipitant solution with 10%(*v*/*v*) glycerol. During measurement, the ring was operated at 1.99 GeV, with a current ranging from 250 to 190 mA. A single wavelength of 1.2 Å was selected through the (111) face of a silicon double-crystal monochromator. Diffraction data were collected with a CCD

Figure 2
Sequence alignment of bovine serum AO with enzymes from other sources whose crystal structure is known. BSAO, amine oxidase from bovine serum; 1A2VHp, amine oxidase from *H. polymorpha*; 1AVKAg, amine oxidase from *A. globiformis*; 1KSIPs, amine oxidase from pea seedlings.

Table 1
X-ray data-collection and processing statistics.

Values in parentheses refer to the highest resolution shell, 2.50–2.37 Å.

Wavelength (Å)	1.2
Detector	MAR CCD
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 77.68, b = 131.19, c = 134.00
Mosaicity (°)	0.5
Resolution range (Å)	25–2.37 (2.50–2.37)
No. of unique reflections, $I \geq 0$	47368 (4684)
Completeness (%)	93.7 (77.3)
Redundancy	3.5 (3.4)
$\langle I/\sigma(I)\rangle$ of measured data	10.1 (2.1)
$R_{sym}(I)$†	0.106 (0.390)

† $R_{sym}(I) = \sum_{hkl}\sum_i |I_{hkl,i} - \langle I_{hkl}\rangle| / \sum_{hkl}\sum_i |I_{hkl,i}|$, where $\langle I_{hkl}\rangle$ is the mean intensity of the multiple $I_{hkl,i}$ observations of symmetry-related reflections.

detector (MAR Research) positioned at a distance of 180 mm from the sample, with oscillations of 0.7°. Data processing and scaling was performed with *MOSFLM* (Leslie, 1991) and *SCALA* (Collaborative Computational Project, Number 4, 1994). Processing statistics are summarized in Table 1.

3. Results and discussion

3.1. Protein preparation and deglycosylation

All the BSAO preparations used for crystallization, both of the native and of the deglycosylated form, were pure to homogeneity (>95%), displaying a single band under denaturing conditions. According to SDS–PAGE, the deglycosylated enzyme is characterized by a reduction of the molecular mass of about 4% (from ∼93 to ∼89 kDa; Fig. 1), apparently corresponding to the carbohydrate content reported by Watanabe & Yasunobu (1970). Furthermore, the deglycosylation of BSAO does not affect its kinetic characteristics; the deglycosylated enzyme shows a specific activity equal to that of the native enzyme within experimental error.

3.2. Crystallization results

Crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 77.68, b = 131.19, c = 134.00 Å. Assuming the presence of one dimer in the asymmetric unit, the V_M crystal packing parameter is 2.06 $Å^3\,Da^{-1}$, corresponding to a solvent content in the unit cell of 39% (Matthews, 1968). The crystal used diffracted to a maximum resolution of 2.4 Å, as reported in Table 1.

At present, the three-dimensional structures of four different amine oxidases (from *E. coli*, pea seedling, *A. globiformis* and *H. polymorpha*) are known. Their sequence alignment is reported in Fig. 2; it shows that a relatively high homology can only be detected in the central part of the protein, between amino-acid residues 290 and 700 approximately. A partial model of the dimer comprising only those amino acids was built with the ExPASy modelling server (http://www.expasy.org). Using this partial model, a preliminary molecular-replacement solution was obtained using the program *AMoRe* (Navaza, 1994). Refinement and the model building of the remaining part of the model is in progress in our laboratory.

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