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Crystallization and preliminary characterization of human recombinant *N*-acetylgalactosamine-4-sulfatase.

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Abstract

Crystals of human recombinant *N*-acetylgalactosamine-4-sulfatase have been grown using vapour diffusion. The protein contains approximately 13%(*w*/*w*) carbohydrate. The crystals belong to the tetragonal space group $P4_12_12$ or its enantiomorph $P4_32_12$ with $a = b = 108.0$ and $c = 145.5$ Å. The crystals diffract to 2.7 Å resolution.

Introduction

The sulfated glycosaminoglycans (mucopolysaccharides) are degraded in mammalian cells to produce inorganic sulfate and monosaccharides. These reactions occur in the lysosome following partial degradation in endosomes. This process is initiated and completed once the substrate comes in contact with a family of hydrolytic enzymes. One of these enzymes responsible for intralysosomal degradation of dermatan sulfate and chondroitin 4-sulfate is *N*-acetylgalactosamine-4-sulfate sulfatase (*N*-acetylgalactosamine-4-sulfatase, 4-sulfatase, arylsulfatase B, E.C. 3.1.6.12) (O'Brien, Cantz & Spranger, 1974; Matalon, Arbogast & Dorfman, 1974).

Deficiencies in these enzymes in humans are the cause of a family of lysosomal storage diseases termed mucopolysaccharidoses (MPS) (Hopwood & Morris, 1990). In particular a lack of 4-sulfatase results in Maroteaux–Lamy syndrome (mucopolysaccharidosis type VI). Patients with this disorder store and excrete abnormal amounts of dermatan sulfate and chondroitin 4-sulfate. Symptoms include skeletal deformities, corneal clouding, growth retardation and hepatosplenomegaly (Hopwood & Morris, 1990). However, neurological development is generally normal even in severe cases. The accessibility of all sites of pathology to the enzyme from circulation, renders this syndrome a possible candidate for enzyme-replacement therapy (Anson *et al.*, 1992).

Human 4-sulfatase has been expressed in several different forms (Gibson, Saccone, Brooks, Clements & Hopwood., 1987; Taylor, Gibson, Brooks & Hopwood, 1990; Peters *et al.*, 1990; Anson *et al.*, 1992). The precursor form is a single polypeptide lacking the 40-residue N-terminal signal peptide. This form has a molecular weight of 67 kDa of which about 9 kDa is associated with glycosylation and there are up to four *N*-linked oligosaccharide chains (Peters *et al.*, 1990). This form when proteolytically cleaved yields a mature form, comprising three disulfide-linked polypeptides, 43, 8 and 7 kDa (Kobayashi *et al.*, 1992) which still retain the four potential oligosaccharide attachment sites. We have purified both the precursor (67 kDa) and mature forms (47, 8 and 7 kDa) expressed in Chinese hamster ovary (CHO)-4S2 cells (Anson *et al.*, 1992).

Experimental and results

The crystals of the precursor form were grown by vapour diffusion using the hanging-drop technique (McPherson, 1982). Broad screening was carried out using the incomplete factorial method (Carter & Carter, 1979), using the list of conditions given by Jancarik & Kim (1991). Initially, crystals were grown from a 4 µl drop containing a mixture of precursor and mature forms of the protein in a concentration of 5 mg ml^{-1} in the presence of 0.025 *M* KH_2PO_4 and 10% PEG 8K. The reservoir contained 0.05 *M* KH_2PO_4 and 20% PEG 8K. The pH of the unbuffered solution was 5.0 at 277 K. Obelisk-shaped crystals appeared after 12 h and grew up to 0.4 × 0.2 × 0.2 mm in 10 d. Crystals of the same size and form were subsequently grown using the same conditions from a protein solution containing only the precursor form. Optimal conditions for crystal growth have been found using 30 µl sitting-drop wells (microbridges) with a reservoir solution of 0.2 *M* KH_2PO_4 and 14% PEG 8K. The crystals appear in 4 or 5 d and grow to maximum size in 10 d.

Preliminary characterization of the crystals was made using precession photographs recorded using Cu $K\alpha$ radiation from a Rigaku RU200 rotating-anode generator. Intensity data have been recorded using the Weissenberg camera of Professor N. Sakabe on beamline 6A2 at the Photon Factory, Tsukuba, Japan with 1 Å radiation and using an R-AXIS image-plate system on an RU-200 rotating-anode generator in Sydney with Cu $K\alpha$. The symmetry and systematic absences indicate a tetragonal space group $P4_12_12$ or its enantiomorph $P4_32_12$ with $a = b = 108.0$ and $c = 145.5$ Å. The Matthews coefficient calculated assuming one 67 kDa molecule in the asymmetric unit is 3.2 Å^3 Da^{-1}. This lies in the range of values found from a survey of protein structures (Matthews, 1977).

The crystals are very sensitive to removal from the mother liquor. Within seconds of removal, the crystals develop small cracks and a faint yellow colour. The chemical nature of this degradation is unknown. The protein is stable in solution for periods of weeks to months at 277 K. It has been necessary to use techniques that ensure the crystals are never exposed to the atmosphere during mounting. That is, the crystals must be mounted in a small drop of mother liquor. We are currently attempting flash freezing of the crystals to obviate this problem.

Native data to a resolution of 2.7 Å have been recorded using a Weissenberg camera on beamline 6A2 at the Photon Factory, Tsukuba, Japan (Sakabe, 1991). The data were processed with progam *WEIS* (Higashi, 1989; Fields, Guss,

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Lawrence & Nakagawa, 1992) and then scaled and merged with *PROTEIN* (Steigemann, 1974). The overall merging R factor for data with $I \geq \sigma(I)$ was 0.081. In the highest resolution shell, 2.76 Å $\geq d \geq$ 2.70 Å, 31% of the possible data were observed $[I \geq \sigma(I)]$ and for all data to 2.70 Å resolution, 78% of the data were observed. Putative heavy-atom data are currently being recorded on a Rigaku R-AXIS IIc imaging-plate detector in Sydney.

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