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Crystallization and preliminary X-ray characterization of G_{M2}-activator protein

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Abstract

G_{M2} activator protein is a glycolipid transfer protein cofactor necessary for the hydrolytic degradation of G_{M2} ganglioside by β-hexosaminidase A. Its absence in human tissue results in symptoms of type AB Tay–Sachs disease. The protein prepared by recombinant techniques using an *E. coli* expression system, has been crystallized by the vapor-diffusion technique. The crystals are well ordered and belong to the orthorhombic space group $P2_12_12_1$. The unit-cell dimensions are $a = 42.40$, $b = 39.82$, $c = 113.61$ Å. One molecule is contained in the asymmetric unit.

1. Introduction

G_{M2} activator protein is a monomeric protein of 18.6 kDa which acts as an essential cofactor in the enzymatic hydrolysis of G_{M2} [GalNAc-β1,4-(NeuNAc-α2,3)Gal-β1,4-Glc-ceramide] (GalNAc = *N*-acetyl-D-galactosamine, NeuNAc = *N*-acetyl-D-neuraminic acid) by β-hexosaminidase A (HexA) (review by Fuerst & Sandhoff, 1992). Evidence for its presence in cell extracts was first reported by Li, Mazzotta, Wan, Orth & Li (1973). Deficiency of the enzyme or the G_{M2}-activator protein causes accumulation of G_{M2} resulting in symptoms of Tay–Sachs disease or G_{M2} gangliosidosis (Sandhoff, 1977). The protein is well characterized genetically and with respect to its biochemical properties (Wu *et al.*, 1994; Fuerst & Sandhoff, 1992). The natural protein has a higher molecular weight (21.5–23.5 kDa), as it contains a high-mannose Asn-linked carbohydrate moiety. The amino-acid composition exhibits an abundance of acidic amino acids accounting for the low isoelectric point (4.8) of the protein. The eight cysteine residues, likely to be present in disulfide linkages, are thought to contribute to the stability of the protein at high temperatures (333 K).

A wide range of studies have suggested that G_{M2}-activator functions through direct binding to the exposed sugar chain of the membrane embedded ganglioside G_{M2}, presenting it subsequently in some fashion to HexA for hydrolysis. Either it extracts the ganglioside completely or it binds to it and induces a conformational change, perhaps by disrupting hydrogen-bond interactions within the tetrasaccharide (Koerner, Koerner, Prestegard, Demou & Yu, 1983; Levery, 1991) to allow hydrolysis by HexA. It is believed that HexA is unable to attack the carbohydrate moiety directly because of the fact that this tetrasaccharide is not sufficiently extended from the membrane surface. In addition, free access to the tetrasaccharide may be masked by tight packing of phospholipid head groups. The binding interactions are thought to be of a hydrophobic nature (Conzelmann & Sandhoff, 1979; Neuenhofer & Sandhoff, 1985). K_m values for the binding of G_{M2} are in the micromolar range (1.9 μM). Evidence suggests that both the terminal GalNAc and NeuNAc are involved in the activation process (Wu *et al.*, 1994), as chemical modification studies of these sugars have resulted in reduced hydrolysis efficiencies and their removal abolishes binding of G_{M2}-activator.

2. Methods

The protein used for crystallization trials was prepared by recombinant techniques. The cDNA encoding only the 162 amino acids of the mature protein (Wu *et al.*, 1994) was generated by polymerase chain reaction (PCR) from the 1093-base pair cDNA clone which was originally isolated from human placental λgt11 cDNA libraries (Nagarajan, Chen, Li, Li & Lockyer, 1992). The PCR product was used to construct a plasmid, pT513, using the pT7-7 expression vector. pT513 was transformed into *E. coli* BL21 (DE3) and the production of the activator protein was induced by isopropyl thio-β-galactoside. The recombinant G_{M2} activator protein was solubilized from the inclusion bodies of *E. coli* and refolded as described by Marston *et al.* (1984). The insoluble proteins were first extracted under denaturing conditions (4 *M* guanidine HCl). Subsequently a solution containing 4 *M* urea, 1 m*M* EDTA, 1 m*M* glycine and 100 m*M* glutathione (pH 8.0) was added and the thick suspension was centrifuged at 17 000*g*. The supernatant was added dropwise to 10 vols of rapidly stirring 20 m*M* Tris–HCl buffer (pH 9.0), and the pH was adjusted to a final range of 6.0–6.3 by dropwise addition of 3 *N* HCl. After storage of this suspension at 277 K overnight, it was again centrifuged and the supernatant, which contained the refolded protein, was concentrated and further purified by Sephadex G-75 column chromatography at pH 7.0 (25 m*M* phosphate buffer). The purified recombinant activator protein had the same specific activity as that of the native activator protein in the range of $1.1–1.9 \times 10^7$ units (mg protein)$^{-1}$ (Wu *et al.*, 1994).

Crystallization trials were carried out with the Hampton crystallization kit (Crystal Screen I) using both the sitting and hanging-drop techniques. Crystallization solutions contained equal volumes of protein at 10 mg ml^{-1} and reservoir buffer. The buffer consisted of 15–30% PEG 4000 buffered in the pH range of 4.5–5.5 with 0.1 *M* sodium acetate and 0.2 *M* ammonium acetate at pH 7.5 with 0.1 *M* sodium Hepes buffer. A preliminary intensity data set was taken on a small crystal (dimensions $0.27 \times 0.20 \times 0.17$ mm) in order to assess crystal quality and stability in the X-ray beam. This crystal was grown by the sitting-drop method using a 2 ml reservoir (30% PEG 4000 in 0.1 *M* sodium acetate/0.2 *M* ammonium acetate) and an initial drop volume of 14 µl, which contained 8.2 mg ml^{-1} protein, 4.2% PEG 4000 and 0.14 *M* ammonium acetate/71 m*M* sodium acetate. The crystal was mounted in mother liquor in a sealed glass capillary tube. Data to 2.5 Å resolution were measured on an R-AXIS IIC imaging-plate system with a Rigaku rotating-anode X-ray source. The X-ray intensity data were processed and scaled with the *R-AXIS* software package of the Molecular Data Corporation.

3. Results and discussion

Well ordered prism-shaped crystals appeared after a period of 4–6 weeks of equilibration (see Fig. 1) and continued to grow for several additional weeks before reaching maximum size (about 0.05–0.3 mm). The protein precipitated initially under these conditions. Crystals started forming only slowly within the precipitate. These crystals belong to space group $P2_12_12_1$ with unit-cell dimensions $a = 42.40$, $b = 39.82$, $c = 113.61$ Å. A V_m value of 2.572 Å^3 Da^{-1} is consistent with the presence of one molecule per asymmetric unit. This value is in the range of values tabulated by Matthews (1968). The solvent content is estimated to be 52%.

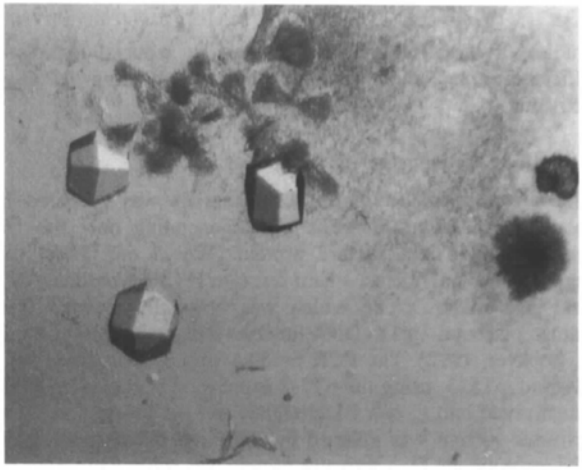

Fig. 1. Photomicrograph of orthorhombic crystals of the G_{M2} activator protein. The crystal size corresponds to approximately $0.2 \times 0.25 \times 0.15$ mm.

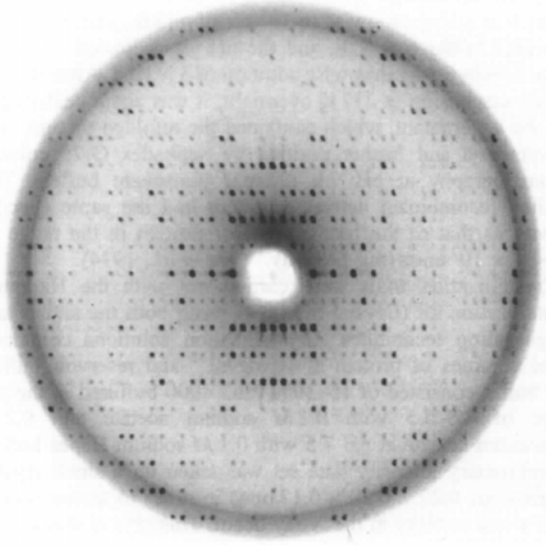

Fig. 2. 10° Precession photograph of the *h0l* zone of a G_{M2} activator crystal after exposure for data collection on the R-AXIS image-plate detector. The outer edge of the photograph corresponds to a Bragg spacings of 4.6 Å.

Table 1. *Diffraction-data statistics*

Resolution (Å)	No. of reflections	Completeness (%)	$\langle F^2/\sigma \rangle$	R_{merge} (%)
15.0	46	90.2	12.4	5.2
10.0	91	93.7	15.2	4.6
7.5	166	93.8	14.2	5.6
5.0	626	94.1	11.0	7.6
3.5	1584	90.0	7.2	9.5
3.0	1235	81.3	3.7	15.0
2.75	832	66.9	2.2	19.0
2.5	973	54.6	1.7	21.3
Overall	5552	78.1	6.5	9.6

As listed in Table 1, the merged 2.4 Å intensity data scaled with an overall R factor of 9.6%. Data-collection statistics indicated a clear fall-off in intensity beyond 3 Å resolution (see Table 1). This is likely to be a result of the small size of the crystal diffracting only weakly at high resolution. The unit-cell parameters of this crystal refined to values of $a = 42.30$, $b = 39.74$ and $c = 113.80$ Å with standard deviations of 0.080, 0.087 and 0.136, respectively. The crystal remained fairly stable during data collection. As illustrated in Fig. 2, the diffraction pattern continued to be well ordered after additional exposure to high-intensity X-rays for 24 h. Since there is no knowledge of a possible structural relationship of this protein with other known protein structures, the structure will be solved by the method of isomorphous replacement.

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