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Crystallization and preliminary X-ray analysis of recombinant human acid β-glucocerebrosidase, a treatment for Gaucher's disease

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Acid β-glucocerebrosidase (*N*-acylsphingosyl-1-*O*-β-D-glucoside: glucohydrolase) is a lysosomal glycoprotein that catalyzes the hydrolysis of the glycolipid glucocerebroside to glucose and ceramide. Inadequate levels of this enzyme underly the pathophysiology of Gaucher's disease. Cerezyme (Genzyme Corporation, Cambridge, MA, USA) is a partially deglycosylated form of recombinant human acid β-glucocerebrosidase that is used in the treatment of Gaucher patients. Although acid β-glucocerebrosidase belongs to a large family of glycosidases, relatively little is known regarding its structural biology. Here, the crystallization and the initial diffraction analysis of Cerezyme are reported. The crystals are *C*-centered orthorhombic, with unit-cell parameters a = 285.0, b = 110.2, c = 91.7 Å. A 99.9% complete data set has been collected to 2.75 Å with an $R_{\rm sym}$ of 8.8%.

1. Introduction

Gaucher's disease is an autosomal recessive disorder characterized by decreased levels of the enzyme acid β-glucocerebrosidase. It is the most common single-enzyme lysosomal storage disorder and affects 20 000–30 000 patients worldwide (Zhao & Grabowski, 2002). A deficiency of acid β-glucocerebrosidase, which catalyzes the hydrolysis of the glycolipid glucocerebroside to glucose and ceramide, results in the accumulation of glucocerebroside within the lysosomes of the monocyte/macrophage system. Lipid-engorged cells with eccentric nuclei, known as Gaucher cells, displace healthy normal cells in the liver, spleen and bone marrow and are associated with hepatosplenomegaly, organ dysfunction and skeletal deterioration (Charrow *et al.*, 1998; Elstein *et al.*, 1997).

Over 20 clinically relevant genetic mutations have been mapped to the gene for acid β-glucocerebrosidase on chromosome 1 (Whitfield *et al.*, 2002). Biochemical, genetic and homology studies with other glycosyl hydrolases suggest a conserved active site in which Glu340 serves as the critical nucleophile. The glycosyl-enzyme intermediate is formed and hydrolyzed *via* an oxocarbenium ion-like transition state in a double-displacement reaction (Miao *et al.*, 1994; Durand *et al.*, 1997). A variety of active-site inhibitors and substrate analogues are available.

Cerezyme is a modified recombinant human acid β-glucocerebrosidase that is used clinically to treat Gaucher patients. The drug differs from the enzyme purified from placenta (alglucerase/Ceredase) in that it contains a histidine in place of an arginine at amino-acid residue 495, a Man3GlcNac2Fuc in place of an oligomannose at amino-acid residue 19 and a Man3GlcNac2Fuc in place of a Man3GlcNac2 at amino-acid residue 146. During manufacturing, the two enzymes undergo sequential removal of terminal sialic acid, galactose and *N*-acetyl-glucosamine sugars, exposing additional mannose sugars and improving macrophage uptake. Ceredase and Cerezyme are pharmacologically equivalent (Friedman *et al.*, 1999).

Enzyme-replacement therapy is effective in reducing the hepatic, hematopoietic and skeletal effects of Gaucher's disease (Barton *et al.*, 1991; Grabowski *et al.*, 1998; Niederau *et al.*, 1998; Hayes *et al.*, 1998). Unfortunately, enzyme replacement is expensive, requires intravenous administration and must continue throughout the patient's lifetime (Russell & Clarke, 1999). Clinical efficacy of Cerezyme/ Ceredase is variable, with neuronopathic patients (Gaucher Types 2 and 3) seldom showing benefit (Erikson, 2001; Tylki-Szymanska & Czartoryska, 1999).

2. Materials and methods

Samples of Ceredase and Cerezyme were obtained as gifts from the Genzyme Corporation. Crystallization trials were undertaken using the hanging-drop method of vapor diffusion with a sparse-matrix strategy (McPherson, 1999). Data-collection quality Cerezyme crystals were obtained by mixing 2.0 µl of enzyme (25 mg ml^{-1}) with 2.0 µl of reservoir solution in the hanging drop. The

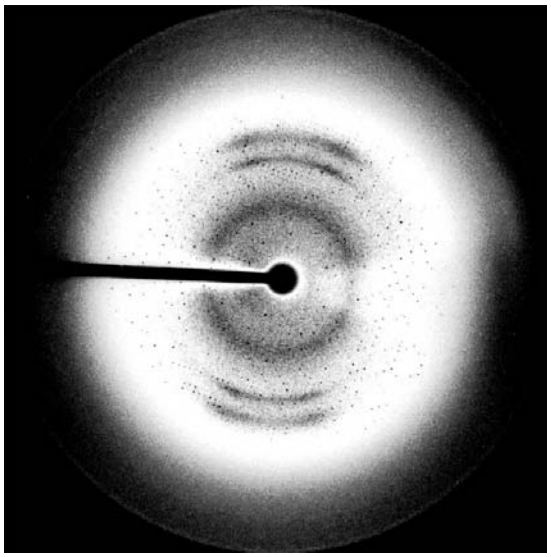

Figure 1
Imaging-plate data from a cryocooled Cerezyme crystal. The arrow indicates a diffraction spot at 2.5 Å.

Table 1
Data-processing statistics for Cerezyme.

Values in parentheses refer to parameters for the highest resolution data shell.

No. of images	180
No. of crystals	1
Space group	$C222_1$
Unit-cell parameters (Å)	a = 285.0, b = 110.2, c = 91.7
Mosaicity (°)	0.62
Resolution range (Å)	30–2.75 (2.85–2.75)
No. measured reflections	141842
No. unique reflections	38217
R_{sym}† (%)	8.8 (29.8)
Completeness (%)	99.9 (100.0)
$\langle I/\sigma(I)\rangle$	10.3 (3.7)
Data with $I > 3\sigma$ (%)	72.5 (46.1)

† $R_{sym} = \sum[(I - \langle I\rangle)^2]/\sum(I^2)$, where I is the observed intensity and $\langle I\rangle$ is the average intensity from multiple observations of symmetry-related reflections.

reservoir contained 100 m*M* cacodylate pH 6.0 and 1.6 *M* ammonium sulfate. The crystals appeared in 2–3 d and grew to final dimensions of 0.1 × 0.05 × 0.03 mm after 10–14 d. Crystals of Ceredase suitable for data collection were not obtained despite multiple trials.

Crystals of Cerezyme diffracted in the laboratory to 3.0 Å. A complete data set was subsequently collected at beamline 7-1 of the Stanford Synchrotron Radiation Laboratory (SSRL) using a MAR 345 mm imaging-plate detector. Cerezyme crystals were briefly exposed to 2,3-butanediol before undergoing flash-cooling in the 100 K gas stream on the SSRL beamline. The crystal-to-detector distance was set at 300 mm with a beam wavelength of 1.08 Å. The data-collection strategy was optimized with the computer program *MOSFLM* (Leslie, 1992). A total of 180 images were collected and processed using the *HKL* program package (Otwinowski & Minor, 1997).

3. Results

The trapezoidal Cerezyme crystals were determined to be *C*-centered orthorhombic. The asymmetric unit contains two enzyme molecules based on a calculated Matthews coefficient of 2.9 Å^3 Da^{-1}, corresponding to 57% solvent content (Matthews, 1968). Although the crystals nominally diffracted to 2.2 Å (Fig. 1), integration statistics steeply declined beyond 2.8 Å. The R_{sym} at 2.75 Å was 8.8%, with the data 99.9% complete. Based on systematic absences, the space group was determined to be $C222_1$. The results of the data collection are summarized in Table 1.

In order to rule out the inadvertent crystallization of carriers added to the Cerezyme preparation during manufacturing, crystals of Cerezyme were washed to remove mother liquor and dissolved in electrophoresis buffer. SDS–PAGE gels revealed a single band migrating at approximately 62 kDa corresponding to the known molecular weight of Cerezyme (data not shown).

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