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Review Article

Insights into the structural biology of Gaucher disease

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ABSTRACT

Gaucher disease, the most common lysosomal storage disorder, is caused by mutations in the gene encoding the acid-β-glucosidase lysosomal hydrolase enzyme that cleaves glucocerebroside into glucose and ceramide. Reduced enzyme activity and impaired structural stability arise due to > 300 known disease-causing mutations. Several of these mutations have also been associated with an increased risk of Parkinson disease (PD). Since the discovery of the acid-β-glucosidase X-ray structure, there have been major advances in our understanding of the structural properties of the protein. Analysis of specific residues has provided insight into their functional and structural importance and provided insight into the pathogenesis of Gaucher disease and the contribution to PD. Disease-causing mutations are positioned throughout the acid-β-glucosidase structure, with many located far from the active site and thus retaining some enzymatic activity however, thus far no clear relationship between mutation location and disease severity has been established. Here, we review the crystal structure of acid-β-glucosidase, while highlighting important structural aspects of the protein in detail. This review discusses the structural stability of acid-β-glucosidase, which can be altered by pH and glycosylation, and explores the relationship between known Gaucher disease and PD mutations, structural stability and disease severity.

1. Introduction

Acid-β-glucosidase (GCase) (IUBMB enzyme nomenclature number EC 3.2.1.45) is a lysosomal enzyme involved in cleaving the glycolipid glucocerebrosidase (also known as glucosylceramide or GlcCer) into glucose and ceramide (Brady et al., 1965). Mutations in the glucocerebrosidase (GBA) gene encoding this enzyme cause Gaucher disease (GD), an autosomal recessive lysosomal storage disorder (LSD). This results in the lysosomal accumulation of GlcCer in the macrophages of the reticuloendothelial system as a consequence of decreased GCase activity or stability. Clinically, GD is associated with enlarged organs, hepatomegaly, splenomegaly and in a minority of cases a neurological deficit (Grabowski, 2008). That said there is evidence of a range of neurological involvement across the entire spectrum of Gaucher disease (Beavan et al., 2015). The disease can be classified into three clinical subtypes Type 1, Type 2 and Type 3 based upon the severity of neurological features. Type 1, the most common variant, is non-neuronopathic and can be asymptomatic with onset at any age. On the other hand, both Type 2 and Type 3 are neuronopathic. Type 2 GD has severe neurological involvement, with disease onset occurring in the first few months of life and the disorder rapidly progressing until death during infancy. Type 3 is a chronic neuronopathic disease, with patients surviving infancy but having neurological features for the rest of their lives (Sidransky, 2012). Although GD occurs in the general population with a frequency of approximately 1 in 50,000, prevalence is much greater among the Ashkenazi Jewish population with the incidence around 1 in 800 (Charrow et al., 2000; Horowitz et al., 1998).

Over 300 mutations have been identified in GD patients including nonsense mutations, deletions and insertions and complex alleles. The most prevalent are missense mutations (Beutler et al., 2005; Hruska et al., 2008). Two important disease-causing missense mutations are N370S and L444P, which account for the majority of mutations found in the Ashkenazi Jewish population (Sidransky and Lopez, 2012). The N370S mutation causes a mild Type 1 GD in patients, whereas the L444P mutation results in a severe neurological disorder, such as Type 2 and 3. Over the past 15 years, it has been reported that GD may increase the risk for PD, the most common neurodegenerative movement disorder, with many GD patients developing parkinsonian symptoms (Aharon-Peretz et al., 2004; Beavan et al., 2015; Bembi et al., 2003; McNeill et al., 2012; Tayebi et al., 2003).

Normally, native GCase is synthesised on endoplasmic reticulum (ER)-bound ribosomes and translocated to the ER for correct folding before being transported to the lysosome. When the protein is mutated, however, it is identified as misfolded and retained in the ER where it is eliminated by the ubiquitin proteasomal system (UPS), leading to a reduction in enzyme concentration at the lysosome (Schapira and Gegg, 2013). Interestingly, when analysed *in vitro* several mutated GCase variants, including N370S and L444P, produce stable proteins with

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residual activity (Alfonso et al., 2004; Liou et al., 2006). This observation has led to the development of small molecular chaperones, which are designed to bind the misfolded protein to increase trafficking and reduce degradation and thus have the potential to offer a therapeutic benefit to GD patients (Jung et al., 2016).

Until the past decade or so, the three-dimensional structure of GCase was poorly understood. Therefore, exploration of the relationships between mutations, structure, disease manifestation and enzymatic activity were difficult. The first review of the structure of GCase was published in 2008 (Kacher et al., 2008), with the first X-ray structure of GCase elucidated only in 2003 (Dvir et al., 2003). This review focuses on the main structural aspects of the GCase enzyme and the contribution of current literature to our knowledge of the protein structure and potential relationships with common GD and PD mutations.

2. Structure of acid-β-glucosidase

2.1. Key structural features

The mature GCase polypeptide is a glycoprotein consisting of 497 residues (Grabowski et al., 1990) with a molecular weight between 59 and 69 kDa depending on post-translational modification of the protein (Bergmann and Grabowski, 1989). GCase comprises of three discontinuous domains (Fig. 1). Domain I (residues 1–27 and 383–414) consists of an antiparallel β-sheet flanked by a loop. Within this domain exists two disulphide bridges (residues 4–16 and 18–23) which may aid proper folding of the protein (Dvir et al., 2003; Moharram et al., 2006). Domain II (residues 30–75 and 431–497) is an 8-stranded β-barrel, which forms an independent domain to resemble an immunoglobulin (Ig) fold (Lieberman et al., 2009). The third domain, domain III (residues 76–381 and 416–430) is the catalytic domain and comprises of a $(\beta/\alpha)_8$ triosephosphate isomerase (TIM) barrel. Domain III harbours the active site in addition to having three free cysteines (residues 126, 248 and 342) (Dvir et al., 2003; Moharram et al., 2006). It is thought that these free cysteines and the cysteines involved in disulphide bond formation in domain I are essential for preservation of an active enzyme, allowing the breakdown of GlcCer into glucose and ceramide and preventing substrate accumulation. In particular, Cys 342 is thought to exert important stabilisation effects due to its close proximity to the active site (Fig. 2a) (Liou et al., 2006).

2.2. Active site

The active site is a catalytic dyad, consisting of two important residues Glu 340 and Glu 235 (Fig. 2). Site-directed mutagenesis has revealed Glu 235 is the acid/base catalyst (Fabrega et al., 2002), with electrospray tandem mass spectrometry identifying Glu 340 as the nucleophile (Miao et al., 1994). Both residues are located near the c-terminus of β-strands 4 and 7 in domain III, existing on opposite sides of the glycosidic bonds to be hydrolysed, approximately 5 Å away from each other. In addition to these two catalytic residues, several residues that line the active site region are thought to be important although not directly involved in catalysis (Lieberman, 2011), potentially playing a role in stabilising the substrate or modulating protonation sites. These include Arg 120, Asp 127, Phe 128, Trp 179, Asn 234, Tyr 244, Phe 246, Tyr 313, Cys 342, Ser 345, Trp 381, Asn 396, Phe 397 and Val 398 (Fig. 2b & 2c). Of these residues, seven are aromatic and line the active site pocket (Phe 128, Trp 179, Tyr 244, Phe 246, Tyr 313, Trp 381, Phe 397) with a potential role in recognising the GlcCer substrate (Chi et al., 1999).

Located at the entrance to the active site are several hydrophobic residues that may facilitate protein interaction with the lysosomal membrane, helping transport the protein to the area where activity takes place. These residues may also aid the interaction between GCase and the activator protein, Saposin C (Wilkening et al., 1998). Saposin C is thought to associate with the GCase and activate the hydrolysis of GlcCer (Ho and O'Brien, 1971; Tamargo et al., 2012; Vaccaro et al., 2010). A deficiency in Saposin C levels through mutations leads to a GD phenotype, most commonly the neurological variants (Vaccaro et al., 2010). This occurs despite normal GCase protein activity, suggesting if structural alterations occurred in mutant GCase to ameliorate it's interaction with Saposin C there may be a significant reduction in GlcCer hydrolysis and the development of GD. Although the hydrophobic residues at the entrance to the active site may play some role in GCase binding of Saposin C, several GCase specific residues have been thought

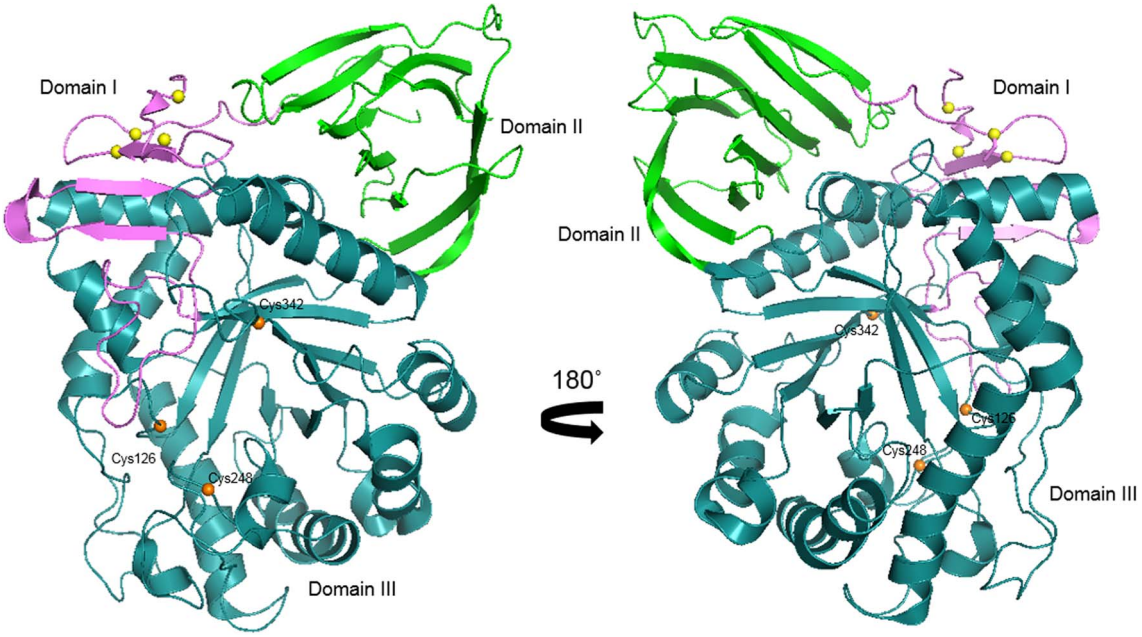

Fig. 1. The X-ray structure of acid-β-glucosidase (PDB code 3GXD) created using PYMOL (http://www.pymol.org). Domain I is shown in pink and houses the two disulphide bridges, with the sulphur atoms depicted by yellow spheres. Domain II is shown in green and is an Ig-like domain. Domain III, the catalytic domain, is shown in teal and is a TIM barrel structure. This domain contains the three free cysteines, of which are labelled by orange spheres.