Understanding functional dynamics and conformational stability of beta-glycosidases

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ENGLISH SUMMARY

β-glycosidases play fundamental roles in maintaining the cellular homeostasis in living organisms. In the last decades, this widespread enzyme family has found multiple industrial applications. Given the importance of these enzymes, characterization of the biophysical properties of β-glycosidases is a necessity for deepening our understanding of their structure/function relationship. **Chapter 1** is a general introduction describing the biophysical and structural characteristics of β-glycosidases that belong to the GH5, GH11 and GH30 families, as well as the commonly used inhibitors that modulate their activities.

In humans, ganglioside catabolism comprises consecutive hydrolysis of the oligosaccharide moieties by different exo β-glycosidases. Deficiency in ganglioside processing enzymes leads to a range of metabolic disorders collectively classified as glycosphingolipidoses. In bacteria, ganglioside metabolism follows a different path, in which the entire oligosaccharide chain is removed from the ceramide moiety in a single step. One of the first enzymes identified to be able to perform this reaction is endoglycoceramidase II (EGCII) from *Rhodococcus* sp. In theory, this enzyme could be applied to treat human glycosphingolipidoses, and insight into factors that influence EGCII conformational stability is an important prerequisite for its application as a therapeutic agent. **Chapter 2** describes the characterization of EGCII using activity-based probes (ABPs) initially developed against the human β-glucocerebrosidase (GBA.) The protein is heat labile and its stability is markedly enhanced by formation of covalent complexes with ABPs based on cyclophellitol linked to hydrophobic substituents. This is evidenced by an increased melting temperature, resistance against tryptic digestion, changes in ^{15}N-^{1}H NMR spectra of the enzyme, and changes in the degree of exposed hydrophobic surface area, as determined by 8-anilino-1-naphthalenesulfonic acid fluorescence.

Deficiency of GBA function leads to the accumulation of its glucocerebroside substrate in the lysosomal compartment of the cell, thus causing a syndrome called Gaucher disease. The application of small chemical compounds that rescue the activity of GBA mutants in Gaucher patients is under intense investigation. This new therapeutic approach represents a promising avenue for treatment of Gaucher patients with symptoms that also include neural manifestation of the disease. However, the effects of those compounds on the protein conformation in solution remain unclear. Therefore, **Chapter 3** describes the utilization of a variety of active site binders to dissect their stabilization mechanisms on the GBA fold. Amphiphilic ABPs, which are supposed to simultaneously occupy the glycon and aglycon pockets of GBA binding site, exert superior stabilization effects presumably by promoting a further structural compactness of GBA through hydrophobic interactions.

Despite the differences in folds and in substrate specificities between the β-glycosidases, these enzymes share a common catalytic mechanism, in which two catalytic residues act in a synchronized manner on the glycosidic link, causing its hydrolysis. The chemistry that governs this mechanism became widely accepted, which somehow limited the study of the contribution of the conformational changes of the enzyme and the dynamics in the catalytic reaction. In **chapter 4** this topic is addressed by following the dynamics of *Bacillus circulans* xylanase during its catalytic pathway using standard and advanced NMR spectroscopy techniques. In its resting state, the protein shows minor millisecond time scale dynamics. The Michaelis complex formation seems to follow an induced fit mechanism and the exchange between the two bound forms of the enzyme is revealed to be in the slow exchange regime. It is hypothesised that this step with a high activation barrier is a change in the protein conformation that is necessary for substrate positioning and distortion, which could represent a rate limiting step of the reaction. In the covalent intermediate state of the reaction, the protein preserves its free state dynamics behaviour although it assumes a different conformational state. A drastic enhancement of the millisecond motion of the protein in the last step of the catalytic reaction (product dissociation) suggests a role of such dynamics in assisting the product dissociation from the enzyme binding site. The study provided evidence for the involvement of β-glycosidases motions in their paradigmatic hydrolysis mechanism.

Chapter 5 discusses and suggests future perspectives for the insights generated in this thesis.