

# Sugar Conformations that Enhance Cleavage of Glycosidic Bonds in Carbohydrate-Active Enzymes

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**Abstract-** Carbohydrate-active enzymes select particular conformations of one sugar of their carbohydrate substrates to enhance catalysis. The evolution of this conformation during catalysis, known as the catalytic itinerary, is of fundamental interest in glycobiology. Here we calculate the free energy landscape of  $\beta$ -xylose to predict the possible catalytic itineraries of  $\beta$ -xylan enzymes. Our results discard one of the three catalytic itineraries proposed on the basis of X-ray structures of mutant enzymes. Additional classical and quantum mechanics/molecular mechanics (QM/MM) calculations indicate that complexes obtained from modified enzymes do not necessarily represent the structures that take place in the reaction coordinate.

## I. INTRODUCTION

The covalent bond that joins a simple carbohydrate molecule (also known as sugar) to another group, which may or may not be another carbohydrate, is known in chemistry as *glycosidic bond*. These bonds are extremely stable in solution and display half-lives of several million years. However, in the presence of enzymes such as glycoside hydrolases (GHs), the half-life of glycosidic bonds can decay up to the millisecond range [1]. How do these enzymes enhance the breakage of glycosidic bonds is a topic that has been extensively studied since 1894 Emil Fisher's 'lock and key' model [2].

It is nowadays accepted that, for beta oligosaccharides (carbohydrate molecules bound by beta glycosidic bonds), one of the sugar molecules changes shape upon binding to the GH enzyme [3], facilitating catalysis by placing the leaving group of the reaction in an axial position. In particular, a key sugar molecule deforms from its ground state *chair* conformation in solution to a new conformation (typically a *boat*, *skew-boat* or *half-chair*) that is less populated in the same conditions (Fig. 1). Knowing the precise conformation of the sugar molecule in the active site of the enzyme and how this shape changes during catalysis (the catalytic itinerary) is important not only to understand how these enzymes work, but also to design new drugs for GHs involved in human health and disease [4], as well as engineering GHs for their use in food, detergent, oil, gas and biotechnological industries. In particular,  $\beta$ -xylanases are GH enzymes responsible for the hydrolysis of glycosidic bonds in  $\beta$ -xylans, a group of hemicelluloses of high biotechnological interest that are found in plant cell walls. An intriguing aspect of these enzymes is that, unlike other GHs, their  $\beta$ -xylan carbohydrate substrates are assumed to adopt more than one conformation in the enzyme active site, thus leading to several conformational itineraries during catalysis (Fig. 2 B). Based on crystallographic structures, some  $\beta$ -xylanases have even been predicted to adopt conformations that disobey previously established rules for enzymatic glycosidic bond cleavage [5].

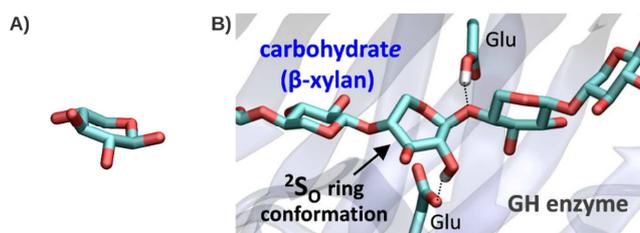


Fig. 1. A)  $\beta$ -xylose sugar in the most populated conformation in solution: *chair*  ${}^4C_1$ . B)  $\beta$ -xylose oligosaccharide bound to a family 11 GH enzyme. The key sugar, which is located between the two glutamic catalytic residues, displays a  ${}^2S_0$  skew-boat conformation. Almost all hydrogens have been omitted for the sake of clarity.

In recent years, our group has contributed to the understanding of how GHs tune carbohydrate conformations to enhance catalysis [6,8]. Specifically, we demonstrated that the computed conformational free energy landscape (FEL) of isolated monosaccharides (e.g. glucose and mannose) correlate with the observed X-ray structures of enzyme-ligand complexes [7]. In other words, one can predict the conformation and electronic properties that a given carbohydrate will exhibit in the active sites of GHs from gas phase calculations [8,9]. Here we investigate all possible conformations of a  $\beta$ -xylose sugar molecule using state-of-the-art computational techniques based on molecular dynamics (MD). In particular, we compute the conformational FEL of  $\beta$ -xylose by *ab initio* metadynamics and use it to predict the most likely catalytic itineraries for  $\beta$ -xylanases. Moreover, we employ classical and quantum mechanics/molecular mechanics (QM/MM) MD simulations to check whether common enzyme modifications used in experiments can affect the observed xylose conformation, leading to incorrect assignments of catalytic itineraries.

## II. METHODS

Metadynamics [10] is a molecular dynamics based technique that enhances phase space exploration by the addition of repulsive potentials on a set of collective variables (functions of atomic positions) that enclose the slowest modes that are relevant to the process of interest.

We used metadynamics on the phase space of  $\beta$ -xylose described by Density Functional Theory, using  $\theta$  and  $\Phi$  Cremer and Pople puckering coordinates [11] as collective variables (Fig. 2 A). All *ab initio* and hybrid QM/MM molecular dynamics simulations were carried out within the Car-Parrinello approach, as implemented in the CPMD 3.15.1

