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Control of Substrate Conformation by Hydrogen Bonding in a Retaining β-Endoglycosidase

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Bacterial β-glycosidases are hydrolytic enzymes that depolymerize polysaccharides such as β-cellulose, β-glucans and β-xylans from different sources, offering diverse biomedical and industrial uses. It has been shown that a conformational change of the substrate, from a relaxed $^4C_1$ conformation to a distorted $^1S_3/^{1,4}B$ conformation of the reactive sugar, is necessary for catalysis. However, the molecular determinants that stabilize the substrate's distortion are poorly understood. Here we use quantum mechanics/molecular mechanics (QM/MM)-based molecular dynamics methods to assess the impact of the interaction between the reactive sugar, i.e. the one at subsite −*1*, and the catalytic nucleophile (a glutamate) on substrate conformation. We show that the hydrogen bond involving the C2 exocyclic group and the nucleophile controls substrate conformation: its presence preserves sugar distortion, whereas its absence (e.g. in an enzyme mutant) knocks it out. We also show that 2-deoxy-2-fluoro derivatives, widely used to trap the reaction intermediates by X-ray crystallography, reproduce the conformation of the hydrolysable substrate at the experimental conditions. These results highlight the importance of the 2-OH···nucleophile interaction in substrate recognition and catalysis in endoglycosidases and can inform mutational campaigns aimed to search for more efficient enzymes.

Introduction

Glycoside hydrolases or glycosidases (GHs) are the enzymes responsible for the cleavage of glycosidic bonds in carbohydrates and glycoconjugates. These enzymes play a major role in health and disease and have a myriad of industrial applications, such as in food, detergent, oil, gas, and biotechnology industries.[1] The molecular mechanism of GHs has been of interest to chemists since the early 50's. Most GHs share a common mechanism in which two essential residues, either aspartic acid or glutamic acid, participate in the chemical reaction.[2] The reaction can take place with retention or inversion of the anomeric stereochemistry. Retaining GHs often follow a double displacement mechanism, in which a covalent glycosyl-enzyme intermediate (GEI) forms (Figure 1A). The GEI intermediate is subsequently hydrolysed by reacting with a water molecule, to reach the product state with the same anomeric configuration as the initial compound. The transition state (TS) of the reaction is known to have strong oxocarbenium ion-like character, as demonstrated by quantum mechanics calculations, mass spectrometry and kinetic measurements of the isotope effect.[3]

A number of structural analyses of GHs in complex with their carbohydrate substrates (Michaelis complexes, MCs) have shown that the substrate distorts upon binding to the enzyme. In particular, the reactive sugar (i.e., the one bearing the C–O bond to be hydrolysed by the enzyme), is distorted away from the ground-state $^4C_1$ conformation.[4] This change from a relaxed to a distorted -and typically higher energy- conformation is believed to help catalysis. Sugar distortion orients the glycosidic bond axially and elongates it, bringing the substrate to a conformation that resembles that of the transition state of the reaction, thus pre-activating the enzyme·substrate complex for catalysis[5,6] (Figure 1A). The specific distorted conformation that a substrate adopts depends on the particular active site architecture[4a,b]. In addition, computer simulations have shown that the conformation adopted by the substrate in the enzyme active site is among the ones that exhibit certain "suitable" structural, energetic and electronic properties (e.g. long and axial C1-O bond, low energy and high anomeric charge) of the free substrate, i.e., in absence of the enzyme. In this respect, GHs have evolved to use intrinsic properties of their sugar substrates for a most efficient catalysis.[6–7,8,9] Knowing the

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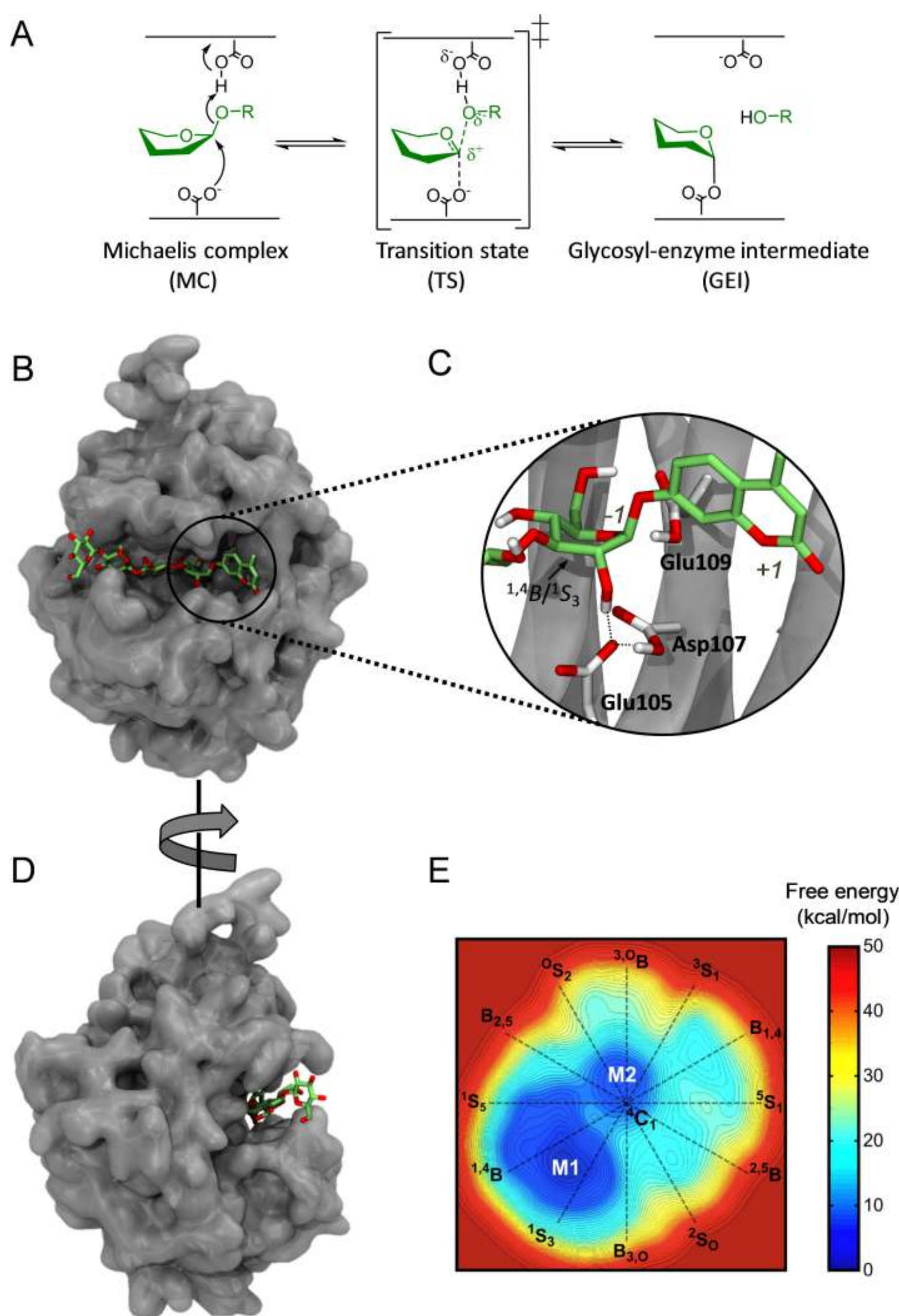

Figure 1. (A) First step of the reaction catalysed by retaining β-endoglucanases, in which a glycosyl-enzyme intermediate (GEI) forms. (B–D) Michaelis complex of 1,3-1,4-β-endoglucanase in complex with the $(Glc)_4$-MU substrate and close view of the active site, showing the distortion of the reactive sugar and the 2-OH···nucleophile interaction. (E) Conformational free energy landscape of the -1 sugar in the active site of 1,3-1,4-β-endoglucanase, computed by QM/MM metadynamics.[10] The most stable conformation corresponds to $^{1}S_{3}/^{1,4}B$ (distorted conformation, M1), whereas the $^{4}C_{1}$ conformation (relaxed chair, M2) is higher in energy by 6 kcal/mol. Copyright 2011 American Chemical Society (panel E).

substrate distortion in the MC is crucial to decipher the enzyme mechanism, which in turn can help the design of specific inhibitors.

The conformation of the substrate in GH·substrate complexes (hereafter substrate conformation) can be elucidated by X-ray crystallography, using strategies to slow or knock-out the enzymatic reaction, such as mutating the catalytic residues, working at non-optimal pHs or using sugar-based inhibitors. Of particular interest is the fluoro-substitution strategy, pioneered by Withers et al.,[11] in which the hydroxyl group at position 2 of the reactive sugar (2-OH) is substituted by a fluorine atom. These substrate derivatives destabilize the electron-deficient transition state,[12] allowing analysis of MC complex-like species by X-ray crystallography.[13] In some retaining GHs, the combination of a 2-deoxy-2F derivative with a good leaving group that occupies the $+1$ subsite (e.g. 2,4-dinitrophenyl) leads to suicide

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inhibitors that can overcome the nucleophilic displacement (Figure 1A), enabling trapping of the glycosyl-enzyme reaction intermediate (GEI).[13–14] The same substrate analogue can be used to trap either the MC or the GEI by working at different pH conditions, as shown for a family GH5 cellulase.[13] At low pH, the catalytic nucleophile is protonated, thus the MC is obtained. At neutral pH, the nucleophilic displacement can take place, leading to the GEI.

From a chemical point of view, substitution of the hydroxyl group by a fluorine atom slows down the reaction by two complementary effects: the inductive effect of the F atom and, most importantly, the absence of the hydrogen bond between the 2-OH and the catalytic nucleophile.[15] Previous studies suggested that there is a relation between the 2-OH··nucleophile hydrogen bond and the conformation of the reactive sugar. In particular, the hydrogen bond might be important to compensate for the energy destabilization to reach a distorted (but catalytically competent) substrate.[16] However, a systematic study of the effect of the 2-OH··nucleophile hydrogen bond in substrate conformation is lacking.

Here we investigate the interplay between intermolecular interactions through the 2-OH group and the conformation of the substrate in 1,3-1,4-β-endoglucanase, a prototypical endo-β-GH that hydrolyses linear β-glucans containing β-1,3 and β-1,4 linkages such as cereal β-glucans and lichenan. 1,3-1,4-β-endoglucanase belongs to family GH16 and displays strict cleavage specificity for β-1,4 glycosidic bonds on 3-*O*-substituted glucosyl residues.[17] Bacterial 1,3-1,4-β-glucanases are important biotechnological aids in the brewing and animal feedstuff industries, thus it is important to dissect their mechanisms of action to provide hints for enzyme engineering. Previous studies showed that the β-glucose at the -1 subsite (hereafter the -1 sugar) is distorted in a $^{1}S_{3}/^{1,4}B$ conformation (Figure 1).[5] To investigate the impact of the 2-OH···nucleophile interaction (2-OH···Glu105 in *B. licheniformis* 1,3-1,4-β-endoglucanase[17]) in substrate conformation, we consider either changes on the C2 exocyclic substituent (OH vs. F) or the residue at position 105 (Glu, Asp, Gln, Ala and Ile, i.e., taking into account residues of different polarity and size). For each system (Table 1), we determine the substrate conformation by means of quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations. Our results show that the hydrogen bond interaction between the substituent at C2 and an active site residue is essential to keep the substrate in a distorted conformation similar to the one observed for the WT enzyme and we identify the factors that contribute to this crucial interaction. We also show that 2-deoxy-2-fluoro inhibitors (substitution of 2-OH by F) are *bona fide* mimics of the MC, in the conditions in which the X-ray experiments are typically conducted.

Experimental Section

Initial structures

The structure of the Michaelis complex (MC) of 1,3-1,4-β-endoglucanase with a 4-methylumbelliferyl tetrasaccharide, $(Glc)_4$-MU, was taken from previous QM/MM MD simulations of the WT enzyme.[5] Ten models were chosen to assess the influence of charge, protonation state, polarity and size, of the residues involved in the C2···nucleophile interaction (Table 1). On one hand, the C2 substituent was taken as either a hydroxyl (as in the natural substrate) or a fluorine atom (as in 2-deoxy-2-F-inhibitors). On the other, the nucleophile residue (Glu105) was mutated in silico to Asp, Gln, Ala or Ile. Neutral pH conditions were considered, unless specified, whereas low pH conditions were modelled by manually protonating the nucleophile Glu105 and/or the neighbouring residue Asp107 to its acid form. The acid/base residue (Glu109) was considered as protonated in all cases, as this is the protonation state of this residue at both neutral and low pH conditions.[18]

QM/MM MD simulations

All simulations were performed using the CPMD program[19] coupled with the QM/MM interface developed by Laio, Vandevondele and Röthlisberger,[20] in which the QM region is described by Car-Parrinello molecular dynamics (MD).[21] The starting structure for the simulations was taken as the enzyme complex with $(Glc)_4$-MU, equilibrated by molecular dynamics (MD) and QM/MM MD.[5] The QM region included the side chain of residue 105 (Glu in WT), Glu109 and Asp107, as well as the sugar units at subsites -1 to $+1$

Table 1. Models considered in the QM/MM MD simulations of 1,3-1,4-β-endoglucanase.

Model	Residue 105 (nucleophile)	Residue 107 (assistant)	Reactive sugar	Conformation
1 (WT)	Glu105 (−)	Asp107 (H)	Glc	$^{1,4}B/^{4}E$
2	Glu105 (H)	Asp107 (H)	2-deoxy-2-F-Glc	$^{1,4}B/^{1}S_{3}$
3	Glu105 (−)	Asp107 (H)	2-deoxy-2-F-Glc	$^{4}C_{1}$
4	Gln105	Asp107 (H)	Glc	$^{1,4}B$
5	Gln105	Asp107 (−)	Glc	$^{1,4}B/^{1}S_{3}$
6	Asp105 (−)	Asp107 (H)[a]	Glc	$^{1,4}B$
7	Ala105	Asp107 (H)	Glc	$^{1,4}B/^{1}S_{5}$
8	Ile105	Asp107 (H)	Glc	$^{4}C_{1}$
9	Ala105	Asp107 (−)	Glc	$^{1}S_{5}$
10	Ile105	Asp107 (−)	Glc	$^{1,4}B/^{1}S_{5}$

[a] The system evolves towards the alternative protonation states, i.e. Asp105 (H)···Asp107 (−), during the simulation.

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(i.e. a glucose unit and the MU aglycon). Monovalent pseudopotentials were used to saturate the QM region.[22] The size of the QM systems ranged from 63 to 72 atoms, depending on the system investigated. The rest of the substrate (sugar units at subsites −4 to −2), the protein, the water solvent and chloride counterions were included in the MM region. The NN/MIX/ESP regions cutoff for the electrostatic interactions between the QM and the MM parts of the system[23] were set to 10/12/14 a.u. respectively. In all cases, the QM region was enclosed in a supercell large enough to ensure a minimum distance of 7 a.u. from any atom to its closest boundary. Troullier-Martins ab initio pseudopotentials[24] were used to describe the core electrons and the Perdew–Burke–Ernzerhoff generalized gradient-corrected approximation (PBE)[25] was considered, in consistency with previous work.[5,26] The atoms of the MM region were described with the Cornell et al. force field[27] (protein), TIP3P (water),[28] GLYCAM04 (carbohydrate)[29] and GAFF (MU).[30] The time-step and electron mass chosen for all the simulations were taken as 5 a.u. and 850 a.u., respectively. The simulations were performed at constant temperature by coupling the system to a Nosé-Hoover thermostat[31] at 300 K. All the systems were initialized using the following protocol. First, an optimization of the starting structure was performed by means of molecular dynamics with annealing of the ionic velocities until the highest nuclear gradient was of $5\cdot10^{-4}$ H/a.u. Afterwards, the system was heated up to 300 K and the MD was extended until a stable structure was reached. The total simulation time ranged from 6.70 ps to 15 ps depending on the system studied. The first two picoseconds of each simulation were discarded as equilibration time. All the images were done using VMD.[32] Home-made Python3 scripts and the matplotlib library[33] were used to analyse the sugar conformation for each system and the cpptraj tool of Amber[34] was used to obtain representative structures of the most populated clusters along the simulations. The data that support the findings of this study is openly available in Zenodo at http://doi.org/10.5281/zenodo.8385519.[36]

Results

The reference system: 1,3-1,4-β-glucanase in complex with $(Glc)_4$-MU

The complex of the WT enzyme with the hydrolysable substrate $(Glc)_4$-MU (MU = 4-methylumbelliferyl) was taken as reference for forthcoming analyses. Our QM/MM MD simulations showed that the sugar hydroxyl group at C2 (2-OH) forms a persistent hydrogen bond with the nucleophile Glu105 (1.8 Å in average; Figure 2A). At the same time, the proton of Asp107 forms a low energy barrier hydrogen bond with Glu105. The reactive glucose (−*1* sugar) adopts a conformation around $^{1,4}B$, oscillating between $^{1}S_3$, $^{1,4}B$ and ^{4}E. This is consistent with the global minimum of its conformational free energy landscape (Figure 1E). The distortion of the −*1* sugar is most likely stabilized by the hydrogen bond interaction between the catalytic nucleophile Glu105 and the 2-OH. Previous work showed that this interaction is also kept during catalysis, even though the 2-OH changes hydrogen bond partner from one Glu105 oxygen atom to the other one to enable the nucleophilic attack.[10]

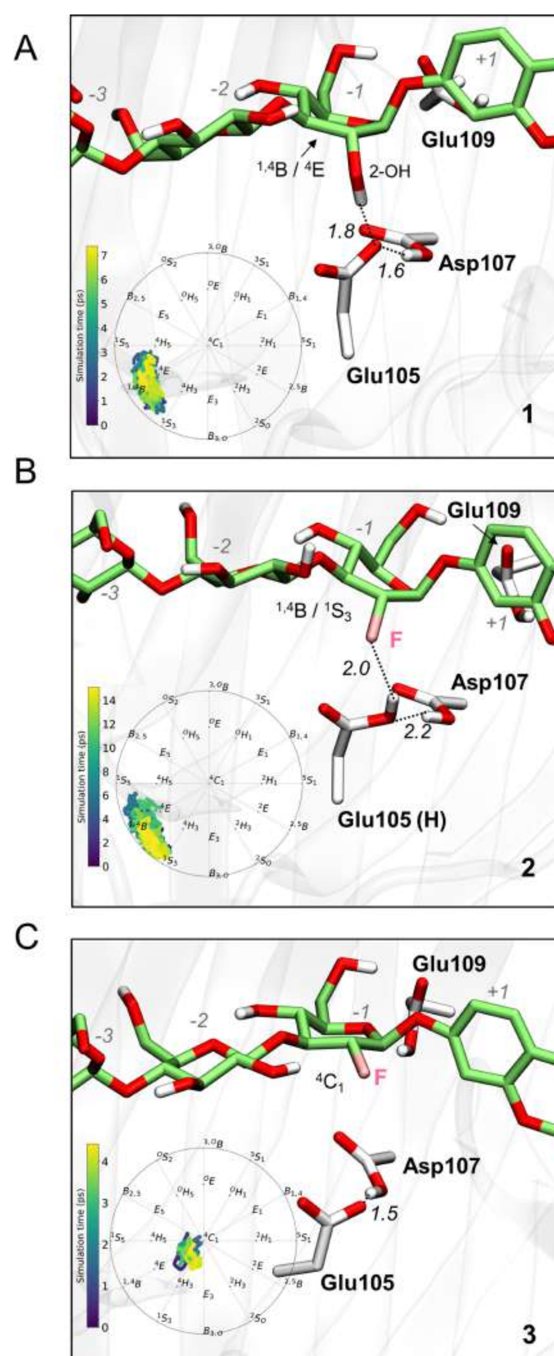

Figure 2. Representative structure of the most populated clusters of the QM/MM MD simulations of the complex between the wild-type enzyme and (A) the $(Glc)_4$-MU substrate; (B) The wild-type enzyme in complex with the corresponding 2-deoxy-2F-substrate at low pH conditions; (C) The wild-type enzyme in complex with the 2-deoxy-2F-substrate at neutral pH. The evolution of the substrate conformation along the simulation is shown in a Stoddard representation (from purple colour at time zero to yellow colour at the end of the simulation). Hydrogen bond interactions are shown as dotted black lines. Carbon atoms of the substrate and protein residues are coloured in green and grey, respectively.

Substitution of the 2-OH by a fluorine atom

Substitution of the 2-OH by a fluorine atom is the basis of the successful 2-deoxy-2-fluoro strategy that is commonly adopted

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in X-ray crystallography to characterize both the MC and the GEI of retaining β-GHs, working either at low or neutral pH, respectively. Both cases were considered in the simulations.

At low pH, the protonated nucleophilic residue Glu105 forms a hydrogen bond with the exocyclic 2-F substituent of the -1 sugar (i.e. 2-F···H-OOC$_{Glu105}$),[13] which adopts a $^{1,4}B/^{1}S_{3}$ conformation (Figure 2B). This is a similar scenario as the one obtained in the complex between the natural substrate (2-OH) and the wild-type enzyme at neutral pH, when Glu105 is deprotonated (Figure 2A). Therefore, the 2-F···H-OOC$_{Glu105}$ interaction observed in the enzyme-inhibitor complex at low pH has the same effect on substrate conformation as the 2-OH···$^{-}$OOC$_{Glu105}$ interaction present in the natural substrate at neutral pH.

The conformation of the substrate changed significantly when the simulation was performed at neutral pH (deprotonated Glu105). Here, the -1 sugar evolved rapidly to a relaxed chair ($^{4}C_{1}$) (Figure 2C). This can be attributed to the lack of interaction between the Glu105 (negatively charged) and the fluorine atom at position C2, which is no longer attractive but repulsive, pushing the sugar away from the nucleophile residue (the average distance between the fluorine atom and the carboxylate group of Glu105 acid is of 5.2 Å). These results highlight the significance of the hydrogen bond between the C2 substituent and the enzyme nucleophile: its presence preserves the distorted conformation, but its absence cancels it out.

Substitution of the Glu105 nucleophile by a polar residue (Gln)

Replacement of the nucleophile Glu105 by a neutral residue such as Gln is expected to weaken the hydrogen bond interaction through the 2-OH that stabilize the substrate distortion in the wild-type enzyme. Therefore, it is not clear to which extend the Glu105Gln mutation can keep the conformation of the substrate.

Replacement of Glu105 by a neutral residue probably affects the pKa of its neighbouring Asp107 residue (Figure 1C), since the two residues form a strong hydrogen bond in the WT enzyme. Therefore, we considered the possibility that Asp107 could be either protonated (low pH) or deprotonated (neutral pH). When Asp107 is protonated (Figure 3A), Gln105 adopts an orientation in which its carbonyl oxygen forms two hydrogen bonds: one with Asp107 and another one with the 2-OH. The distance of the 2-OH···O=C$_{Gln105}$ hydrogen bond (2.0 Å) is longer than the same interaction in the wild-type enzyme (1.8 Å), indicating a weaker interaction due to the decrease of the charge of residue 105. Nevertheless, the interaction is strong enough to maintain the distorted conformation of the -1 sugar that was observed in the wild-type enzyme ($^{1,4}B/^{1}S_{3}$). Therefore, the Glu105Gln mutant at low pH mimics the structure of the active site in the wild-type enzyme (Figure 2A).

When Asp107 is deprotonated (Figure 3B), Gln105 adopts a different orientation than in the previous case and interacts with Asp107 via one of the amide hydrogen atoms, whereas the other one forms a hydrogen bond with the 2-OH of the -1 sugar. The hydrogen bond distance (1.9 Å in average) is slightly longer than the one found in the WT enzyme (1.8 Å), indicating a weaker interaction due to the decrease in the net charge of residue 105. Nevertheless, this is enough to stabilize the same $^{1,4}B/^{1}S_{3}$ distorted conformation of the -1 sugar that is observed in the wild-type enzyme. Therefore, substitution of Glu105 by a neutral polar residue of similar size, such as Gln, at neutral pH, does not affect substrate conformation.

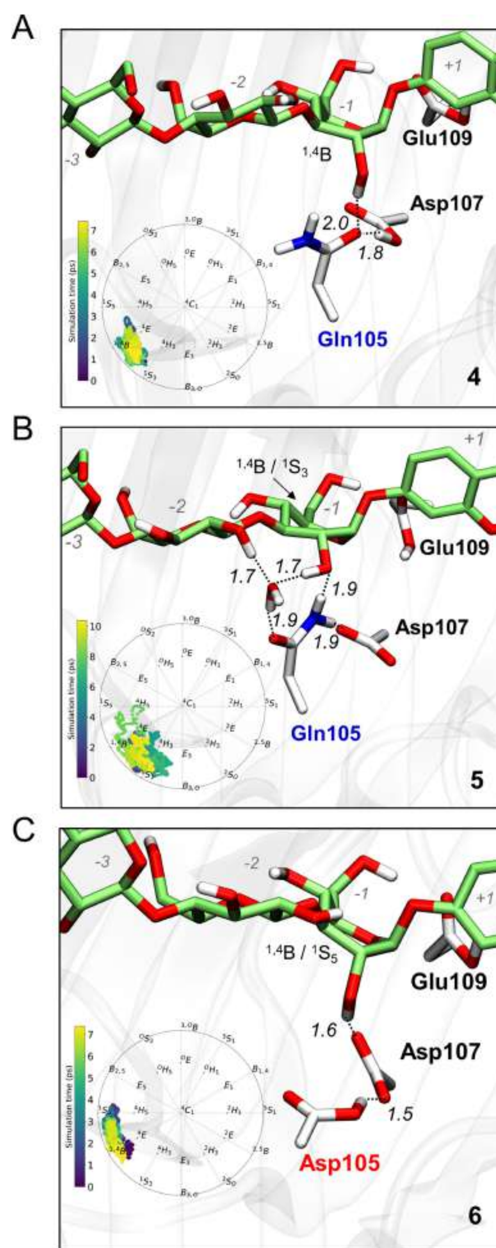

Figure 3. Representative structure of the most populated clusters of the QM/MM MD simulations of (A) the Glu105Gln enzyme mutant in complex with (Glc)$_4$-MU at low pH; (B) Glu105Gln at neutral pH); (C) Glu105Asp at neutral pH.

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Substitution of the Glu105 nucleophile by a smaller and charged residue (Asp105)

Replacement of Glu105 by an aspartate (Figure 3C) introduces additional space in the active site, which prevents the -1 sugar from interacting with the nucleophile while maintaining the charge of the active site. In this case, the simulations were performed with protonated Asp107 and deprotonated Asp105, therefore considering only neutral pH conditions.

The results of the QM/MM MD simulations showed that the 2-OH substituent is far from Asp105 and thus does not interact with it. However, the 2-OH is able to form an alternative hydrogen bond with Asp107 (2-OH···$^{-}$OOC$_{Asp107}$ = 1.6 Å), which ends being negatively charged due to proton transfer between the two aspartates. The tight H-bond between the neighbouring Asp107 and the -1 sugar stabilizes a distorted $^{1,4}B/^{1}S_5$ conformation that is similar to the one observed for the wild-type enzyme ($^{1,4}B/^{1}S_3$).

Substitution of the Glu105 nucleophile by non-polar residues (Ala and Ile)

Replacement of Glu105 with non-polar residues, either a bulky one (isoleucine) or small one (alanine), should in principle preclude the formation of a hydrogen bond via 2-OH. As in the Glu105Gln model, we considered Asp107 as both protonated and deprotonated.

For the protonated Asp107 scenario, replacement of Glu105 for alanine (Figure 4A) gave similar results as the (previously discussed) mutation to aspartate, also a small residue. In both cases, the active site can rearrange so that Asp107 forms a hydrogen bond with the 2-OH. This preserves the distorted conformation of the -1 sugar, albeit the conformation shifts slightly from $^{1,4}B/^{1}S_3$ to $^{1,4}B/^{1}S_5$. In contrast, Asp107 is not able to restore the hydrogen bond in the case of the Glu105Ile mutant (Figure 4B), which results in a rapid evolution of the -1 sugar towards a relaxed $^{4}C_1$ conformation.

Considering the case in which Asp107 is deprotonated (Figures 4C and 4D), the simulations of the Glu105Ala mutant showed that the substrate keeps a distorted $^{1,4}B/^{1}S_5$ conformation, due to the formation of a 2-OH···Asp107 interaction. The same situation is observed for the isoleucine mutant (Figure 4D).

Overall, we conclude that the presence of a hydrogen bond that locks the C2-exocyclic group of the -1 sugar in an axial orientation, towards the α face of the sugar, is necessary to maintain the distorted substrate conformation observed in the wild-type enzyme. The primary source of this hydrogen bond is the nucleophile Glu105, but a similar hydrogen bond interaction can be restored by additional elements of the active site when the nucleophile is replaced by other amino acids.

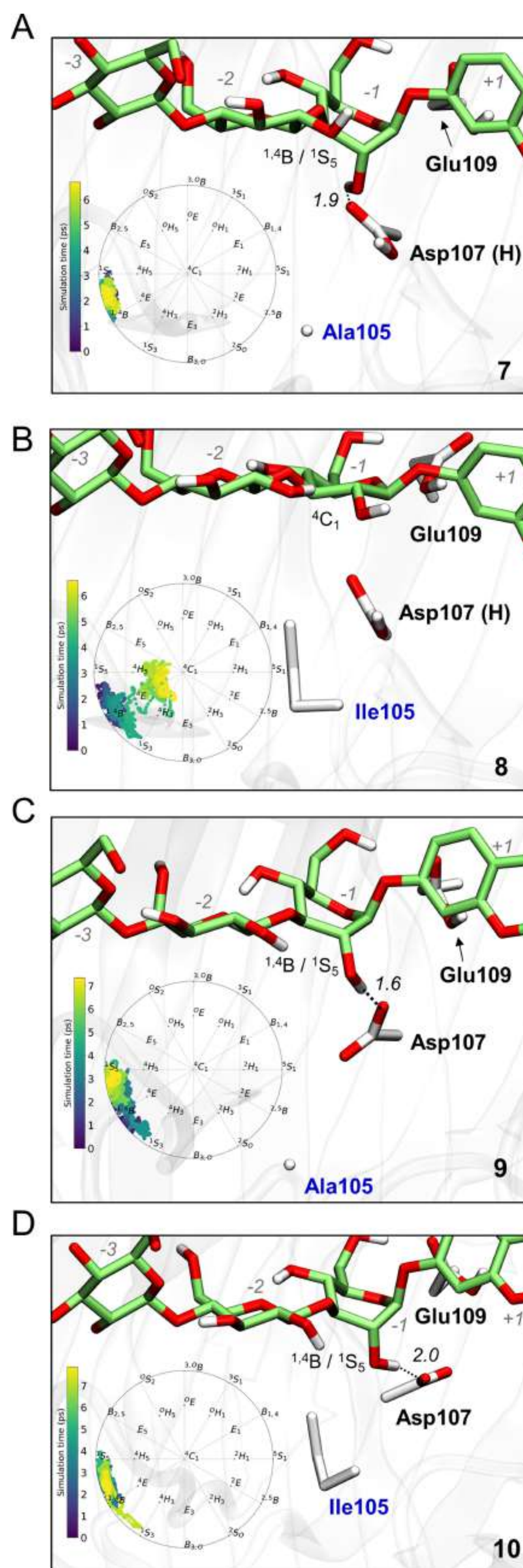

Figure 4. Representative structures of the most populated clusters of the QM/MM MD simulations of (A) the Glu105Ala enzyme mutant in complex with (Glc)$_4$-MU at low pH; (B) Glu105Ile mutant, at low pH; (C) Glu105Ala mutant at neutral pH; (D) Glu105Ile mutant, at neutral pH.

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Discussion

Substrate distortion is a well stablished mechanism of substrate preactivation in glycosidases.[6,26] Previous studies in retaining β-glycosidases[5,10, 15–16, 35] highlighted the strong interaction between the catalytic nucleophile and the 2-OH substituent of the sugar at subsite -1. Our study on 1,3–1,4-β-glucanase shows that small changes in either of the two hydrogen bond partners strongly affect substrate conformation.

Substitution of the 2-OH group by a fluorine atom does not affect sugar distortion and active site configuration ($^{1,4}B/^{1}S_3$) when low pH conditions are considered (i.e. Glu105 is protonated). Therefore, the commonly used crystallographic strategy of using 2-deoxy-2-fluoro inhibitors should also reproduce the substrate conformation of the enzyme complex with the natural substrate. This scenario varies when neutral pH conditions are considered (i.e. Glu105 is deprotonated). In this case the substrate loses the distortion and evolves towards a relaxed $^{4}C_1$ conformation (Figure 5).

As mentioned in the introduction, experiments using the 2-deoxy-2-fluoro inhibitor at neutral pH conditions allows capturing (in a long period of time) the reaction GEI, even though the reaction is difficult due to the destabilizing inductive effect of the fluorine atom substitution. Our results suggest that, in addition, the inhibitor adopts a non-distorted $^{4}C_1$ conformation at pH $\geq$ 7.5, which contributes further to the slow turn-over of the reaction. It is likely that the hydrolysis reaction of the 2-deoxy-2-fluoro at neutral pH follows a circular $^{4}C_1 \rightarrow [^{4}H_3] \rightarrow {}^{4}C_1$ conformational itinerary.

Our QM/MM MD simulations on several in silico mutations of the Glu105 nucleophile to residues of different size and polarity (Glu, Asp, Ala, Gln and Ile) show that the substrate keeps the $^{1,4}B/^{1}S_3$ distortion of the WT enzyme only when the new residue can stablish a hydrogen bond interaction with the substituent at C2 (e.g. the Glu105Gln mutant). In the case of smaller residues at position 105 (e.g. Asp or Ala, as in models 6 and 7 in Table 1), the active site exhibits significant plasticity, enabling an alternative 2-OH···Asp107 interaction that changes substrate conformation towards $^{1,4}B/^{1}S_5$, even if Asp107 is protonated. Such plasticity is reduced for mutants involving bulkier substituents (e.g. Glu105Ile). In this case, the substrate can only keep the distorted conformation when Asp107 is deprotonated (model 10), as this ensures the formation of a 2-OH···Asp107 hydrogen bond.

Overall, the simulations show that the conformation of the sugar at subsite -1 is very sensitive to changes in the interaction between the C2 exocyclic substituent and the enzyme. However, the active site shows a high degree of plasticity, so that the loss of the 2-OH···nucleophile hydrogen bond can easily be compensated by a suitable movement of the substrate towards a nearby protein residue to create a new hydrogen bond interaction. The presence of the neighbouring Asp107 in GH16 1,3-1,4-β-glucanase, which is an important factor in enabling this plasticity, is common to many retaining GH families that exhibit additional polar residues forming the often called catalytic triad in retaining GHs. In these cases, other residues that show hydrogen bond interactions with the catalytic nucleophile in the WT enzymes may perform similar roles. Some examples include an Asp residue in GH7 endoglucanase from *Fusarium oxysporum* (PDB 3OVW), or GH12 endo-1,4-b-glucanase from *Streptomyces lividans* (PDB 1NLR), a His in GH10 β-1,4-glycanase Cex from *Cellulomonas fimi* (PDB 1EXP), Tyr residues in GH1 β-glucosidase from *Bacillus polymxa* (PDB 1BGG), GH3 β-glucan glucohydrolase from *Hordeum vulgare* (1IEQ) and GH17 1,3-1,4-β-glucanase from *Hordeum vulgare* (1AQ0), just to name few examples. These observations can facilitate the interpretation of structures of Michaelis complexes of β-GHs, such as those obtained by X-ray crystallography in conjunction with active-site mutants and C2 exocyclic group modifications of the ligands. In addition, our results can be useful for the engineering of retaining β-GHs for biotechnological applications.

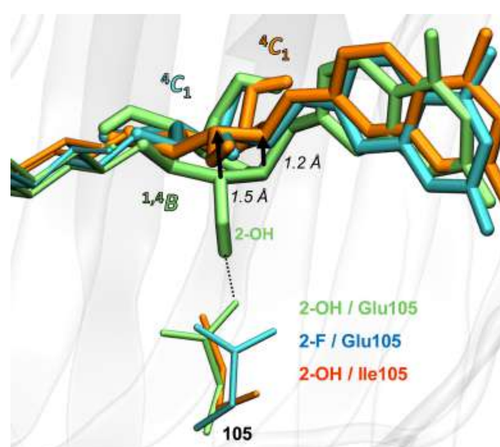

Figure 5. Superposition of representative structures of the wild-type enzyme in complex with the substrate, in green, the wild-type enzyme in complex with the 2-fluoro derivative substrate at neutral pH, in blue; and the Glu105Ile mutant at low pH. The crucial 2-OH···Glu105 interaction in the wild-type model is shown as a dotted black line and the upwards movement of the -1 sugar with respect to the WT is shown with black arrows.

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Conflict of Interests

The authors declare no conflict of interest.

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Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.8385519, reference number [36].

Keywords: ab initio molecular dynamics · carbohydrate-active enzymes · carbohydrate conformations · enzyme catalysis · glycosidases

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