QM/MM Insight on Enzymatic Reactions of Glycosyltransferases

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Abstract: Glycosyltransferases comprise a group of enzymes that catalyze the transfer of glycosyl residues from donors containing nucleoside phosphates to other molecules. The molecular details of the catalytic mechanism involving these enzymes are not well understood. Hybrid QM/MM methods have become important in providing new insights into the atomic details of enzymatic reactions. The QM/MM calculations of GnT-I and β4GalT-1 show that inverting glycosyltransferases utilize an S_N2 type mechanism, with one amino acid functioning as a base catalyst. In addition, the computed transition state structures provide a rational basis for the design of transition state analog inhibitors.

Keywords: Glycosyltransferases, catalytic mechanisms, QM(DFT)/MM methods, transition state structures.

1. INTRODUCTION

Interest in carbohydrates has rapidly increased in recent years, with a growing recognition of their essential role in many biological processes. The remarkable structural complexity of glycans found in biological systems, which greatly exceeds that of DNA or proteins, reflects the vital functions that oligosaccharides and glycoconjugates play in living cells and organisms; from simple structural or reserve substances to high information signals in cell biology. A vast repertoire of glycan structures requires the existence of diverse enzymes involved in the formation and breakdown of glycosidic linkages. The enzymes involved in selective biosynthesis of glycan structures are glycoside hydrolases and glycosyltransferases. While great progress has been made in our knowledge of the mechanism used by glycoside hydrolases to catalyze hydrolysis of the glycosidic bond [1], an understanding of the catalytic mechanism of glycosyltransferases involved in glycoside bond formation has remained elusive [2-6].

Glycosyltransferases comprise a group of enzymes that transfer glycosyl residues from a nucleoside phosphate to other molecules [7-10]. The result of this reaction, catalyzed by these enzymes, is the formation of a new glycosidic linkage. The structural and functional diversity of the products of the glycosyl transfer implies the existence of distinct and specific glycosyltransferases. Glycosyltransferases (GT's, a general nomenclature for glycosyltransferases is EC 2.4.x.y) display low sequence homology [11,12], and they have recently been classified into ninety-two families, GT-1 to GT-92 [13]. Structural similarities between glycosyltransferases were recently reviewed [4,14-17]. In contrast to glycoside hydrolases, available X-ray structures of glycosyltransferases revealed that all solved structures, even without a detectable sequence similarity, adopt only two general folds called GT-A and GT-B superfamilies [17-20]. The number of superfamilies was extended by a prediction of GT-C fold [21] and the first structure with this fold [22] was recently solved.

2. THE CATALYTIC MECHANISM OF GLYCOSYLTRANSFERASES

The catalytic reaction of glycosyltransferases can be considered as a nucleophilic displacement of the nucleoside diphosphate e.g. uridine 5'-pyrophosphate (UDP) functional group at the anomeric carbon C1 of the transferred saccharide residue of a donor by a hydroxyl group of a specific acceptor. The rate constants k_{cat} for glycosyltransferases are sparse and are in the range [23-27] between 50 and 0.1 s^{-1}. Using the standard thermodynamic equation, the estimated free energy barriers are between 15 and 19 kcal/mol. The glycosylation reaction can mechanistically proceed with either inversion or retention of stereochemistry at the anomeric carbon C1 of the donor saccharide. Glycosyltransferases are, therefore, classified as either retaining or inverting depending on the stereochemical outcome of the reaction (Fig. **1**). While the GT-A and GT-B superfamilies have different folds, different active sites, and likely different mechanisms, it is surprising that the GT-A and GT-B folds do not control the stereochemical outcome of the transfer reaction since some retaining glycosyltransferases have folds related to the inverting glycosyltransferases [12].

Despite considerable progress, many aspects of the functions and catalytic mechanisms of glycosyltransferases are still unknown. Therefore, molecular modeling methods can be useful in gaining a deeper understanding of mechanistic strategy employed by glycosyltransferases. These methods have been established as a valuable tool for describing catalytic reactions on an atomic level, and thus providing insights that are not available through experimental means. Quantum mechanical methods (QM) are required for a description of the bond forming and bond breaking processes during the catalytic reaction. However, QM methods are restricted to systems of up to a few hundred atoms. The computational demands of QM methods, in terms of memory and central processor unit (CPU) usage, are impractical for large systems and can essentially only be used on small models of the enzymatic reaction. Alhough QM investigations using truncated models have provided useful information into the microscopic characteristics of the catalytic mechanism of glycosyltransferases [28-31] it is clear that for a more realistic description of their reaction mechanism it would be necessary to include the whole enzyme environment into the computations. The size and complexity of enzymes requires methods capable of treating up to several thousand atoms. However, most of the efficient, force-field based molecular mechanics (MM) methods that are commonly used to describe structures of large systems are not very useful when describing a chemical reaction. The logical approach is to combine both methods and apply a QM treatment for the reaction region and an MM method for the rest of the enzymatic environment. Such approaches are commonly called hybrid QM/MM methods and have recently become a valuable tool for modelling enzymatic reactivity. These methods combine the accuracy of a QM description with the low cost of MM. Herein, we will focus entirely on the application of QM/MM methods to further elucidate the catalytic mechanisms of glycosyltransferases. We will begin with a brief introduction into the basic characteristics of the QM/MM approach. The subsequent section will discuss two examples illustrating the major insights gained from applying QM/MM methods to the catalytic mechanisms of glycosyltranseferases. We will conclude with a summary and outlook discussion.

3. QM/MM METHOD

The combined quantum mechanics-molecular mechanics methodology was first outlined by Warshel and Levitt [32]. However, a

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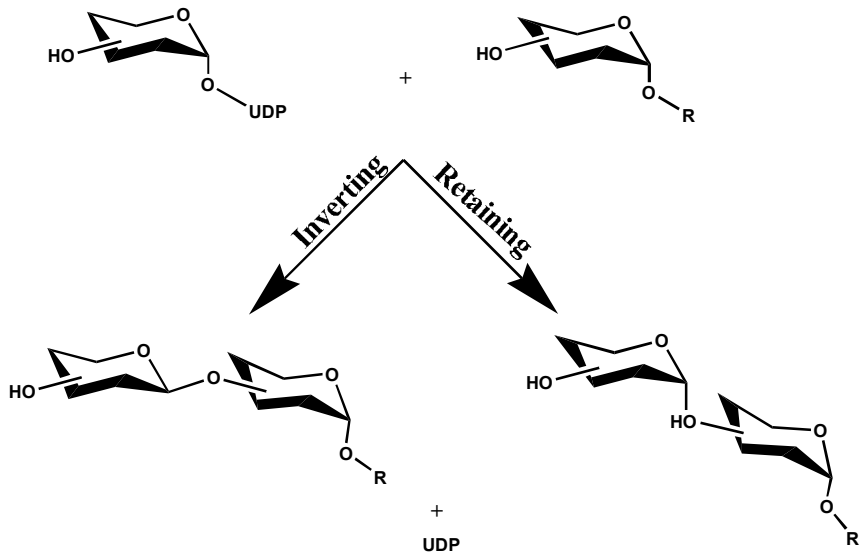

Fig. (1). Schematic representation of overall reactions catalyzed by glycosyltranferases leading to either inversion or retention of configuration at the anomeric carbon of the donor.

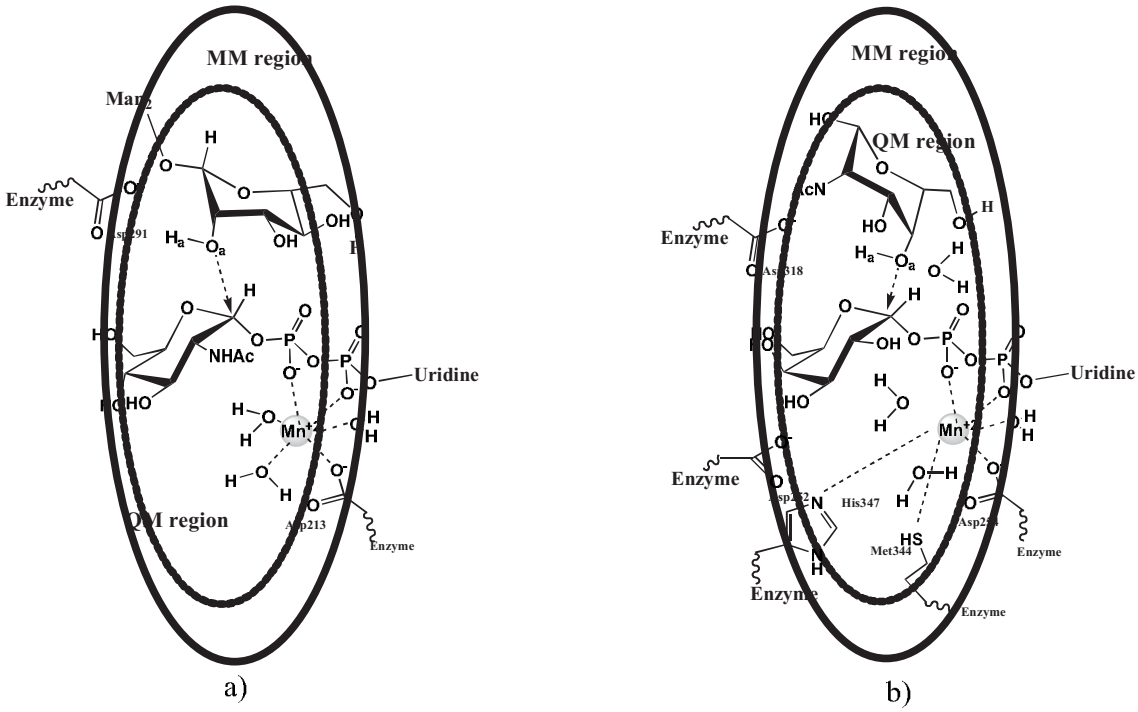

Fig. (2). Schematic representation of partitioning of the entire complex into QM and MM regions for (**a**) GnT-I and (**b**) β4Gal-T1.

comprehensive overview of QM/MM approaches is beyond the scope of this mini-review. The development and applications of QM/MM methodology were recently discussed in a number of review papers [33-37]. Therefore, herein we briefly describe only basic principles and readers should refer to those reviews for further details. The general idea of the QM/MM method is illustrated in Fig. (**2**) on two inverting glycosyltransferases. The entire system (composed of the enzyme, substrates, metal cofactor, and water molecules) is partitioned into QM and MM regions, respectively. The QM region is usually centered on the active site where a chemical reaction proceeds and includes structural moieties involved in the catalytic reactions e.g. crucial parts of the substrates, metal cofactor, catalytic amino acid(s), amino acids directly interacting with substrates, relevant water molecules, and amino acids in the vicinity. The MM region consists of the remaining enzyme and, if necessary, also an aqueous environment.

The total internal energy of the QM/MM system can be formally written as

$$E_{QM/MM} = E_{QM} + E_{MM} + E_{QM\text{-}MM} \quad (1)$$

The first two terms on the right-hand side are the QM energy of the QM region and the MM energy of the MM region. The third term is the interaction energy between these two regions. Various schemes were developed to calculate the total energy ($E_{QM/MM}$) of the QM/MM system, e.g. subtractive and additive [37]. The

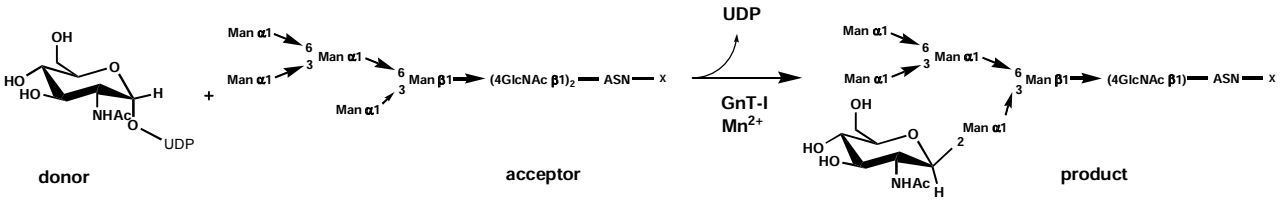

Fig. (3). Schematic diagram of the enzymatic reaction catalyzed by GnT-I.

QM/MM algorithm can use almost any combination of QM and MM methods, and the choice of the QM method depends on the goal of the application. Formerly, semi-empirical methods were usually employed for QM calculations, though currently more and more applications use density functional theory (DFT) methods. For the MM portion there are several force fields available for biomolecular systems allowing parameterization for proteins, nucleic acids and carbohydrates.

The key issue with QM/MM methods concerns the procedural treatment of the boundary region, especially when the boundary often goes through one or more covalent bonds. Depending on the type of QM/MM scheme, the boundary region may contain additional atoms (link atoms) that cap the QM system and are not part of the entire system, or it may consist of atoms with special features that are calculated by use of both the QM and MM methods. Another approach uses localized orbitals (frozen orbitals) between the QM and MM regions that are determined by calculations on small molecules. Various approaches have been developed to treat the interactions (coupling) between the QM and MM regions. The energy term for the coupling consists of both bonded (bond stretching, bond bending, and torsion terms) and non-bonded (van der Waals and electrostatic) interactions. The key component of the coupling is the electrostatic interactions between the QM charge density and the atomic charges in the MM region. Depending on their treatment, QM/MM methods can be divided into three basic models: mechanical embedding, electrostatic embedding, and polarized embedding. The groups differ, essentially, by the extent of mutual polarization of two regions. In the mechanical embedding model, electrostatic interactions between the QM and MM regions are treated at the MM level, with the same charge model applied to the QM region. In this model, the QM charges are not polarized by the electrostatic environment. This might serve to create a problem, especially when charge distribution of the region varies considerably, e.g. during a reaction. In the electrostatic or electric embedding model, the QM calculations are performed in the presence of MM charges by incorporating the MM charges as one-electron terms in QM Hamiltonian. In this way, the QM charges can follow changes in the MM region and are polarized by it, which overall provides a more accurate description than mechanical embedding. However, since the electrostatic interactions between the QM and MM regions in this model are treated at the QM level, the computational demands are considerably increased. A natural next step is to use a flexible MM charge model that is polarized by the QM electric field. However, until now there were no polarizable force fields developed for carbohydrates and applications of such a model to the enzymatic reactions could not be carried out.

There are several programs accessible for calculations using the QM/MM methods that were developed using three basic strategies: 1) QM code was extended by adding MM functionalities (examples of this approach are Gaussian [38], ADF [39], MOZYME [40], GAMESS [41], and QSite [42]; 2) adding the QM module to the MM traditional package, examples include AMBER [43] and CHARMM [44]; 3) coupling of QM and MM modules using the central interfacing module. ChemShel [45] is an example of this type of architecture. Each of these approaches has its merits and disadvantages. The third approach is the most flexible, yet requires the transfer of large amounts of data between both modules, which may decrease its efficiency. On the other hand, the first two are based on QM or MM packages and allow for the use of many features from the original programs that were already quite well refined. For example, MM programs are developed to take care of very large systems and provide tools for simulation and analysis, whereas QM codes provide efficient algorithms used to locate stationary points on potential energy surfaces (PES).

4. QM/MM STUDIES ON GLYCOSYLTRANSFERASES

An investigation of the catalytic mechanism of glycosyltransferases using a QM/MM method is not a trivial task. The crucial part is the generation of the model system prior to the actual calculations. Given that the glycosyltransferases complexes having both a donor and acceptor (or their analogues) bound to the enzyme are not solved, the first step requires building a model system containing all reacting molecules. The available crystal structure of glycosyltransferase complexes usually have only one substrate (donor or acceptor) bound in the active site, and therefore, the missing substrate has to be inserted into the active site. The docking procedures are used for this purpose. The result of docking is usually a cluster of structures, and choosing the most probable one is guided by experimental data. The next step is to divide the entire system into the QM and MM parts and define the reaction coordinate(s).

In calculating the reaction mechanism, the choice of reaction coordinate(s) is very important. An improper choice of reaction coordinate can bias the calculation and yield slower convergence. The QM/MM calculated PES of the entire system provides a clear picture of possible catalytic mechanisms, as well as quantitative (at a given level) energetic information regarding the reactants, Michaelis complex (ES), transition state(s) (TS), and products. The minimum energy path on PES represents the reaction path that connects ES with products via TS. The reaction path also provides a structural basis for detailed analysis of specific interactions involved in the catalytic mechanism, with the benefit of the QM/MM method allowing for the investigation of the role of the enzyme microenvironment. Also, TS plays a special role; its structure can be used in the design of new transition state analogue inhibitors. Next, we will briefly discuss two examples of applying QM/MM methods for the examination of enzymatic catalysis by two inverting glycosyltransferases.

4.1. *N*-acetylglucosaminyltransferase-I

The enzyme *N*-acetylglucosaminyltransferase I (UDP-*N*-acetyl-D-glucosamine:α-3-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase I, GnT-I, EC 2.4.1.101) catalyzes the transfer of a GlcNAc residue (2-acetamido-2-deoxy-α-D-glucopyranose) from the nucleotide-sugar donor UDP-GlcNAc [uridine 5'-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate)] to the acceptor, which is the C2-hydroxyl group of a mannose residue in the trimannosyl core of the $Man_5GlcNAc_2$-Asn-X oligosaccharide [46] (Fig. **3**). The transfer reaction of the GlcNAc residue occurs in the Golgi apparatus and is the first step in the biosynthesis of hybrid and complex *N*-linked glycans [8]. The crystal structure of the catalytic domain of rabbit GnT-I was determined [47] in complex with UDP-GlcNAc/Mn^{2+}, and more recently in complex with donor substrate analogs [48]. The fold of GnT-I belongs to the GT-A superfamily

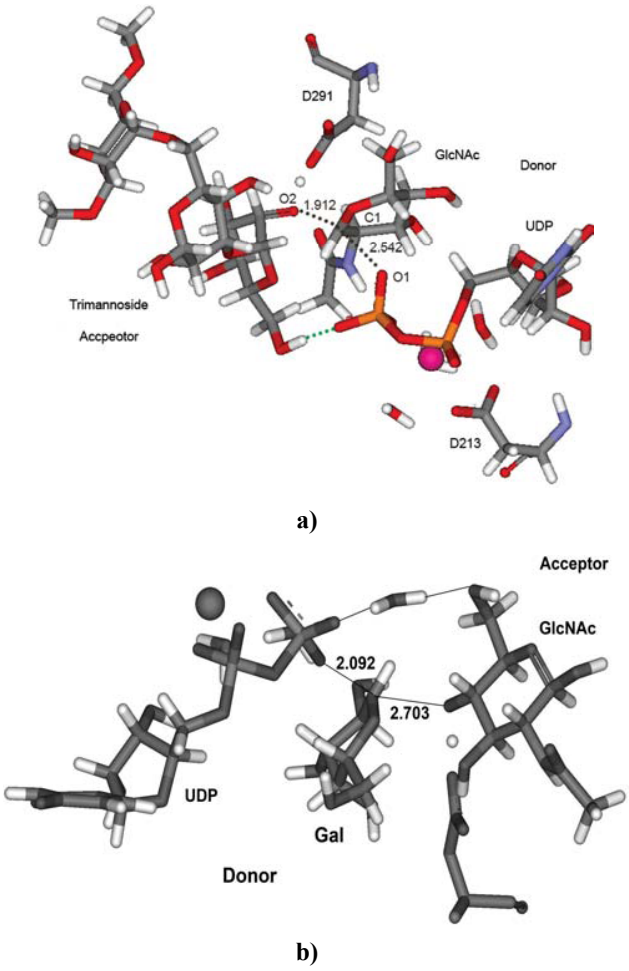

Fig. (4). A geometrical representation of the transition state model obtained by applying the MM(DFT)/MM method for (**a**) GnT-I and (**b**) β4Gal-T1. Green dot lines show the stabilizing hydrogen bonds.

and experiments have shown that GnT-I follows an ordered kinetic mechanism [46], where the enzyme first binds both the metal cofactor and UDP-GlcNAc and subsequently the $Man_5GlcNAc_2$-Asn-X oligosaccharide acceptor. The oligosaccharide product, $GlcNAcMan_5GlcNAc_2$-Asn-X, is then released, followed by UDP.

The catalytic mechanism of GnT-I has become one of the first systems for computational investigations of the glycosyltransferase mechanism. Early, QM studies, using simple models of glycosyltransferases and methods from HF/6-31G* to B3LYP/6-31++G** [28,29], found that the catalytic mechanism requires a single catalytic base for the reaction [28] and suggested an S_N2 type of mechanism. In QM(DFT)/MM calculations [49], the missing acceptor was inserted into the X-ray crystallographic structure of GnT-I in complex with UDP-GlcNAc using the docking procedure implemented in the Glide program from Schrödinger Inc [50]. The trimannosyl core Manα1-3(3,6-OMe-Manα1-6)Manβ of the $Man_5GlcNAc_2$-Asn-X oligosaccharide, that represents the minimal acceptor binding determinant [51], was used as the acceptor. DFT with BP functional and TZP basis sets, and using the ADF package [52], was applied to the reaction site model (Fig. **2a**) that includes the DP-GlcNAc portion of the sugar-donor molecule; the Manα1-3 mannose residue of the trisaccharide-acceptor, aspartate D291, the divalent metal cofactor Mn^{2+} fully coordinated by three water molecules, and aspartate D213 (altogether 88 atoms). The AMBER95 all-atom force field [53] was applied to the remaining part of the substrates as well as the enzyme, altogether 5633 atoms. The reaction path was calculated along a predefined coordinate $r_{C1\text{-}O2}$ describing the nucleophilic attack of the acceptor oxygen (O_a2) on the anomeric carbon C1 of the donor. The obtained activation barrier of 19 kcal/mol correlated well with available experimental data and supports a concerted S_N2-type mechanism. These conclusions were further supported by analyzing in detail the structural and electronic characteristics of TS, as well as the interactions of substrates with specific residues along the reaction path. The overall effect of the protein environment on reaction energetics was found to be quite important by decreasing the overall reaction barrier by 9 kcal/mol. The analysis of results showed that protein environment prefers the TS and PC structures over the ES structure and two specific interactions responsible for the TS stabilization were identified. The role of the enzyme appears to consist mainly in activating the acceptor by deprotonation of the nucleophile O_a2 and by hydrogen bond interactions between the hydrogen atom H_b6 and β-phosphate oxygen. A strong, low-barrier hydrogen bond between the O_a2 hydroxyl group and the carboxylate oxygen of the catalytic base D291 facilitates nucleophilic attack, and the HO6 - β-phosphate oxygen interaction stabilizes the building of a negative charge on the β-pyrophosphate. It was found that removal of the C6 hydroxyl group decreases enzyme activity and that an O6 methylated acceptor is tolerated as a substrate but has no catalytic activity, which supports the importance of the above interactions.

The structure of the active site in the TS model is displayed in Fig. (**4a**). The TS features nearly simultaneous nucleophilic addition and dissociation steps, as evidenced by a C1-O_a2 distance of 1.912 Å and a C1-O1 bond elongated to 2.542 Å. As expected, the

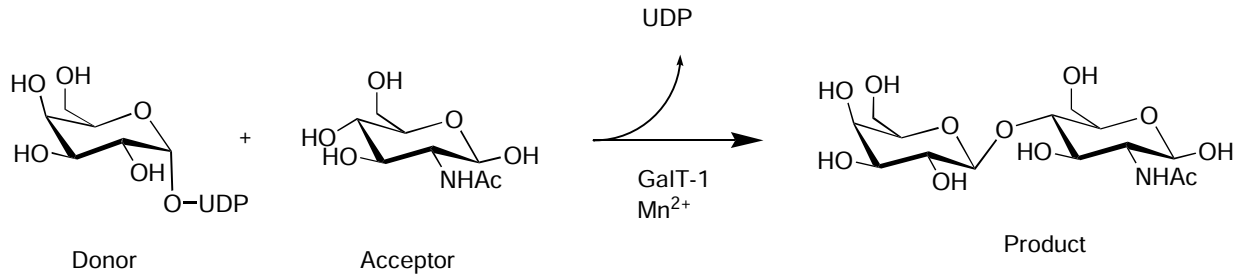

Fig. (5). Schematic diagram of the enzymatic reaction catalyzed by β4Gal-T1.

major structural changes were observed for the glucopyranose ring of GlcNAc that adopts the $^4H_3$ conformation in the TS model. The almost planar arrangement around anomeric carbon facilitates coincident interactions of C1 with the leaving group and attacking nucleophile. The partial double-bond character of the C1-O5 bond, characterized by this bond length of 1.333 Å, is a result of delocalization of the O5 lone pairs to C1. This delocalization stabilizes the partial positive charge on the anomeric carbon C1. In TS, the HO6 - β-phosphate oxygen distance is 1.66 Å, suggesting a quite strong stabilizing interaction. Cleavage of the C1-O1 glycosidic bond is accompanied by the 17° rotation of the β-phosphate oxygen, with the remaining part of UDP not showing any other relevant changes. The TS structure was supported by a normal-mode analysis of the QM part of the model and, together with the calculated a-deuterium kinetics effect (KIE), supported an S_N2-type mechanism.

4.2. β−1,4-galactosyltransferase-1

Galactosyltransfrerases constitute a family of enzymes that use the same donor, UDP-Gal [uridine 5'-(2-acetamido-2-deoxy-α-D-galactopyranosyl pyrophosphate)], to transfer α-D-galactopyranose (Gal) to different acceptors. This enzyme, in the absence of α-lactalbumin, catalyzes the transfer of the Gal residue from UDP-Gal to the O_a4 oxygen of *N*-acetylglucosamine (GlcNAc) in the presence of a Mn^{2+} metal ion (Fig. **5**). The structure of the inverting β−1,4-galactosyltransferase-1 (β4Gal-T1; EC 2.4.1.38) has been extensively investigated [54-60]. Experimental data support a direct displacement S_N2-type mechanism, with Asp318 serving as the catalytic base [60]. The crystal structures of the catalytic domain of β4Gal-T1 have been solved both in *apo* form and with bound substrates [54,56] and were used recently to generate the starting complex for a QM(DFT)/MM study [62]. The structure of the enzyme complex of the catalytic domain of recombinant bovine Gal-T1 with its native substrates was generated through the alignment of two crystal structures of GalT-1 in complex with UDP-Gal and GlcNAc, respectively. The QM subsystem, consisting of 253 atoms (Fig. **2b**), was formed by the acceptor and donor substrates, the metal cofactor, and the side chains of 11 amino acids involved in the enzymatic reaction, binding the substrates, or bearing a charge near the reaction center. This system was treated using either the DFT/B/DZP or DFT/BP/TZ2P method [53]. The MM region was composed of the remaining enzyme atoms and was described using the AMBER95 all-atom force field [53]. Prior to potential energy surface calculations, a geometry optimization of the whole system was performed to refine the location of both substrates.

The reaction mechanism was followed using the distance $r_{C1\text{-}O4}$ between the anomeric carbon of the donor and the nucleophilic oxygen O_a4 of the acceptor as the predefined reaction coordinate. The results of the QM/MM study support a concerted S_N2-type mechanism. Such a structure is characteristic for the early transition state [2]. The TS structure (Fig. **4b**) is characterized by synchronous bond breaking of the C1-O1 (2.092 Å) bond and formation of the C1-O_a4 bond (2.703 Å), accompanied by the abstraction of the H_a proton from the nucleophile O_a4 oxygen by the catalytic base D318, by a distorted ring shape with almost planar arrangement around the anomeric carbon and by a rotation of the diphosphate group. Since a similar movement was found for GnT-I, it was suggested that this might be the distinguishing feature for the S_N2 mechanism of inverting glycosyltransferases. The calculated barrier of 15 kcal/mol is consistent with the experimentally observed barriers. The QM(DFT)/MM calculations revealed how the β4Gal-T1 environment influences the catalytic reaction by stabilizing the transition state structure. The low-barrier hydrogen bond between O_a4 and O(D318) facilitates nucleophilic attack on C1. Similarly, as in the case of GnT-I, the primary hydroxyl group (H-O6) of the acceptor was found to interact with the β-phosphate oxygen stabilizing the developing charges on diphosphate. Though this interaction is mediated by water molecules in β4Gal-T1, one can speculate whether or not this kind of stabilization is a general characteristic of the catalytic mechanism of inverting glycosyltransferases.

4.3. Transition State Structure

Important outcomes of the QM/MM calculations represent the structures of transition states. The QM/MM investigations of the inverting glycosyltransferases of the GT-A superfamily revealed [49,61] that the donor in transition state of the ring conformation resembles a half-chair conformation with oxo-carbenium character at the anomeric carbon; the distance C1-O1 is longer than the normal C-O bond length; a new β-glycosidic bond is being created with a bond length larger than the normal bond length; both forming and breaking bonds are oriented almost perpendicularly with respect to the plane defined by C2-C1-O5 atoms. Of course, the "entire" transition state for a glycosyltransferase also consists of an acceptor oligosaccharide that is linked to the donor. These structural features provide a blueprint for the design of transition state analog inhibitors. Recently, a new scaffold was suggested [62] based on this information, and progress in its synthesis was also reported [63].

5. SUMMARY AND OUTLOOK

QM(DFT)/MM methods are well suited for investigations aimed at understanding the origin of the catalytic power of glycosyltransferases. While existing approaches provide a reasonably good description of these systems, there is an apparent need for improvement, especially in higher accuracy of the DFT functional for calculating interactions between carbohydrates and enzymes. This is very important since the TS structure cannot be directly determined by experimental studies.

QM(DFT)/MM results support a concerted S_N2-like displacement mechanism that involves one amino acid functioning as a base catalyst for the inverting glycosyltransferases with a GT-A fold. The calculations also furnish details on the structure of transition state models that can be used as a guide for the design of transition state analogue inhibitors for glycosyltransferases with a large therapeutic potential.

Until now, only so-called reaction-path techniques were used for this purpose. However, we have to keep in mind that for enzymatic reactions a stationary point on PES is only one of many such

states, simply because there are many degrees of freedom. Therefore, a future challenge is to elucidate the role of coupling between the enzyme dynamic and the reaction process. This is an important issue especially for glycosyltransferases, where the mobility of a loop is part of the catalytic cycle.

It is also apparent that more structural, enzymological, and computational studies remain to be done in order to fully understand the catalytic machinery of glycosyltransferases. Particularly, the mechanism of retaining glycosyltransferases remains less clear, as well as the strategy used by glycosyltransferases possessing a GT-B fold in the catalytic mechanism.

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