Catalytic mechanism of the inverting N-acetylg glucosaminyltransferase I:
DFT quantum mechanical model of the reaction pathway and
determination of the transition state structure

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The complex N-glycan structures on glycoproteins play important roles in cell adhesion and recognition events in metazoan organisms. A critical step in the biosynthetic pathway leading from high mannose to these complex structures includes the transfer of N-acetylg glucosamine (GlcNAc) to a mannose residue by the inverting N-acetylg glucosaminyltransferase I (GnT-I). The catalytic mechanism of this enzymatic reaction is explored herein using DFT quantum chemical methods. The computational model used to follow the reaction is based on the X-ray crystallographic structure of GnT-I and contains 127 atoms that represent fragments of residues critical for the substrate binding and catalysis. The mechanism of the catalytic reaction was monitored by means of a 2D potential energy map calculated as a function of predefined reaction coordinates at the B3LYP/6-31G** level. This potential energy surface revealed one transition state associated with a reaction pathway following a concerted mechanism. The reaction barrier was estimated, and the structure of the transition state was characterized at the B3LYP/6-311++G**//B3LYP/6-31G** level.

Key words: catalytic mechanism N-acetylg glucosaminyltransferase I/quantum chemical calculations/reaction pathway/transition state

Introduction

Glycosyltransferases (GTs) are found in most living organisms, and they are involved in the biosynthesis of glycans, which play important roles in many biological events (Varki, 1993). These enzymes attach a sugar molecule to a specific acceptor, thus creating a glycosidic linkage (Schachter, 1991). Many aspects of the functions and catalytic mechanisms of GTs are, however, still unknown, and high-level quantum mechanical calculations can be used to gain some insight into many characteristics of the enzymatic reaction catalyzed by these enzymes.

In a previous article, we used ab initio computational methods to investigate the catalytic mechanism of inverting N-acetylg glucosaminyltransferases (GnTs) (Tvaroska et al., 2000). We then examined different possible reaction pathways and determined the structure of all transition states and intermediates in the catalytic reaction of inverting GnTs. These results suggested that a stepwise catalytic mechanism involving a single catalytic base is energetically preferred over mechanisms using both a catalytic base and a catalytic acid for the reaction. These findings are supported by all currently available X-ray crystal structures of inverting GTs (Charnock and Davies, 1999; Unligil and Rini, 2000; Unligil et al., 2000; Mulichak et al., 2001; Gastinel et al., 1999; Ramakrishnan and Qasba, 2001; Ramakrishnan et al., 2001; Ha et al., 2000; Pedersen et al., 2000; Tarbouriech et al., 2001). A comparison of the structures of inverting GTs belonging to the GT-2 SpsA family (Tarbouriech et al., 2001), also identified as the GT-A superfamily according to the carbohydrate-active enzymes nomenclature (Coutinho and Henriussat, 1999), has indeed shown the existence of a conserved catalytic and recognition machinery. The common catalytic elements identified are made up of three carboxylate (aspartate or glutamate) residues, one carboxylate involved in the stabilization of the uracil, one in the metal chelation, and, last, one interacting with the hydroxyl groups on the ribose moiety and also forming part of the DxD motif. Additionally, one of the most significant findings in that study (Tarbouriech et al., 2001) was the identification of a fourth carboxylate residue, a conserved Brønsted base, functioning as the catalytic base.

When the structure of the first inverting GlcNAc-T, namely, the UDP-N-acetylg glucosaminic:α-1,3-β-mannosidase β-1,2-GnT I, (Unligil et al., 2000) (EC 2.4.1.101) became available (Pdb codes 1F08, 1F09, and 1FOA), the opportunity arose to use a structural model based on the geometry of an actual active site to investigate the catalytic mechanism of this class of enzymes. GnT-I 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 Man3GlcNAc2-Asn-X oligosaccharide (Scheme 1). The attachment of the GlcNAc sugar is the first step in the biosynthesis of the hybrid and complex N-glycans (Schachter, 1991). This transfer reaction of the GlcNAc residue occurs in the Golgi apparatus and can be regarded as a nucleophilic displacement of the UDP functional group at the anomeric carbon C1 of the GlcNAc residue of UDP-GlcNAc by the hydroxyl group at C2 of the oligosaccharide acceptor, which leads to an inversion of the anomeric configuration (Nishikawa et al., 1988). In this

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Results

Experimental data on the mechanism of GnT-I indicate an ordered sequential mechanism (Nishikawa et al., 1988) in which the enzyme first binds to both the metal cofactor and UDP-GlcNAc and subsequently to the Man5GlcNAc2-Asn-X oligosaccharide acceptor. The oligosaccharide product, GlcNAcMan5GlcNAc2-Asn-X, is then released, followed by UDP. The crystal structure of GnT-I was solved in the presence of UDP-GlcNAc/Mn2⁺ (Unligil et al., 2000). Although binding of the substrates occurs in a sequential manner, the computational investigation of the catalytic reaction requires simultaneous presence of the three components (donor, acceptor, and metal cofactor) in the active site.

To generate a structural model to follow the reaction mechanism, it was first necessary to determine the location where the acceptor binds. For that, the Man5GlcNAc2 oligosaccharide acceptor was docked into the X-ray crystallographic structure of GnT-I complexed with UDP-GlcNAc. The mannohexopyranose derivative shown in Scheme 2 was then placed at the corresponding location of the mannohexopyranose residue of the docked oligosaccharide. It is worth mentioning that this position is in very good accordance with the crystallographic position of the acceptor substrate analog determined experimentally for glucuronyltransferase I (Pedersen et al., 2000), also belonging to the GT-2 SpSA superfamily. The structural model represented in Scheme 2 was used to follow the enzymatic reaction of GnT-I. This reaction model is shown in the active site of GnT-I in Figure 1a. The reaction model contains the entire donor molecule, the fully coordinated metal cofactor, the catalytic base (D291), part of the acceptor (mannoxyranose derivative), as well as some selected enzyme side chains (D144, D212, D213) interacting with the donor substrate (Figure 1b). This model was used to calculate the potential energy surfaces (PESs) given in Figures 2 and 3.

Our starting model corresponds to the structure of the reactants when the whole system was relaxed by energy minimization at the B3LYP/6-31G** level. During all calculations, the relative position of the α-carbons representing the backbone of the GnT-I structure was constrained to their crystallographic positions. However, the remaining parts of the amino acid residues (representing the side chains) were allowed to move. Movements of the oxygen atoms of the water molecules were also restricted. There was no need to restrain the nucleotide conformation because the interactions between UDP and the adjacent amino acid side chains from the enzyme resulted in a sufficient restriction of the movement of the nucleotide. Clearly inclusion of four carboxylates and the hydration shell of the metal in the reaction model prevented the motion of the uridine moiety seen in our previous work (Tvaroska et al., 2000). The minimized geometry used for the calculations was nearly identical to the crystallographic structure (Unligil et al., 2000); only very subtle differences were observed. More detailed structural information on each stationary point is given in Table I, and their relative energies (E) determined at various levels, are listed in Table II. Figure 4 represents the reaction pathway found for the transfer of GlcNAc by GnT-I and the geometrical changes observed for the different stationary points, reactants (R), transition state (TS), and product (P), detected in Figure 2 and calculated at the B3LYP/6-311++G**/B3LYP/6-31G** level. A geometrical representation of the transition state TS calculated at the B3LYP/6-31G** level is given in Figure 5.

PESs

Figure 2 shows the PES calculated in terms of the distance between the anomeric carbon C1 and the Oa oxygen atom of the attacking mannose residue (C1-Oa) and the distance between the Ha proton and the Oa oxygen atom of the acceptor molecule (Ha-Oa) at the B3LYP/6-31G** level. On this contour diagram, the horizontal axis represents the glycosidic C1-Oa bond formation, and the vertical axis represents the proton transfer to the catalytic base. Reactants and products are located in potential wells at the
The calculated PES is clearly asymmetrical, which indicates that the proton Ha is located nearer one of the oxygen atoms. The cross-sections along the vertical axis describe the energetics for the transfer of the Ha proton at different stages of the nucleophilic attack, characterized by the C1-Oa distance. Examination of these cross-sections reveals that the proton exists in a single potential well that shifts its position during the course of the catalytic reaction. The diagonal across the PES, going from the lower-left-hand corner to the upper-right-hand corner, represents the development of the charge at the nucleophile oxygen atom Oa. The structure in the lower-left-hand corner refers to the product with the protonated glycosidic oxygen and a formal $1^+$ charge at the oxygen Oa. The structure in the upper-right-hand corner corresponds to the acceptor with a deprotonated oxygen atom Oa and a formal $1^-$ charge. Both of these structures correspond to high-energy structures.

**Table 1.** *Ab initio* calculated geometric parameters of the points

<table>
<thead>
<tr>
<th>Conformer</th>
<th>C1-Oa</th>
<th>Ha-Oa</th>
<th>Ha-OB</th>
<th>C1-O1</th>
<th>C1-O5</th>
<th>C1-O5-C5</th>
<th>C1-O5-C5-C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>3.000</td>
<td>1.100</td>
<td>1.428</td>
<td>1.440</td>
<td>1.405</td>
<td>117.7</td>
<td>53.2</td>
</tr>
<tr>
<td>TS</td>
<td>1.881</td>
<td>1.233</td>
<td>1.182</td>
<td>2.380</td>
<td>1.330</td>
<td>121.5</td>
<td>36.7</td>
</tr>
<tr>
<td>P</td>
<td>1.397</td>
<td>2.651</td>
<td>1.013</td>
<td>3.286</td>
<td>1.449</td>
<td>111.2</td>
<td>67.3</td>
</tr>
</tbody>
</table>

**Fig. 1.** Representation of the reaction model used to describe the reaction of GnT-I. (a) Reaction model placed in the active site of the X-ray crystallography structure of Rabbit GnT-I (Unigil et al., 2000). (b) Reaction model used.

**Fig. 2.** PES calculated at the B3LYP/6-31G** level using $r_{C1-Oa}$ and $r_{Ha-Oa}$ distances as reaction coordinates.

**Fig. 3.** PES calculated at the B3LYP/6-31G** level as a function of $r_{C1-O1}$ and $r_{C1-Oa}$ distances.
The PES clearly shows the presence of a single transition state (TS) in the central region of the map on the diagonal going from reactants to products. The presence of only one transition state and maxima at two corners of the map indicates a concerted mechanism, in which the formation of the C1-Oa bond and the proton transfer from the acceptor to the catalytic base occur simultaneously. This differs from our previous findings on the general mechanism of inverting GTs (Tvaroska et al., 2000). In the mechanism described by our previous calculations (Tvaroska et al., 2000), the nucleophilic attack would occur as a first step, followed by a proton transfer, in agreement with a stepwise mechanism.

The only reaction coordinates we have considered are the two distances describing the nucleophilic attack on the anomeric carbon of the donor and the proton transfer to the catalytic base. As observed in our previous study (Tvaroska et al., 2000), the current results clearly show that the C1-O1 distance varies in a continuous manner with the rC1-Oa distance. As the nucleophilic attack on the anomeric carbon proceeds, the breakdown of the C1-O1 linkage progresses. In this study, the C1-O1 bond has not been considered as a reaction coordinate of the catalytic mechanism of GTs because we believe that changes in the C1-O1 bond length are only a consequence of the nucleophilic attack at C1. However, we have investigated this hypothesis further by calculating the B3LYP/6-31G** PES as a function of the distance between C1 and the Oa oxygen atom of the attacking mannose residue (rC1-Oa) and the distance between the anomeric carbon C1 and the glycosidic oxygen O1 (rC1-O1) (Figure 3).

This energy contour map clearly confirms the existence of a correlation between these two distances. The path connecting reactants and products follows the diagonal of the 2D map. The structures in the lower-left-hand and upper-right-hand corners represent the pentacoordinated anomeric carbon and the carbocation, respectively. Both of these structures are unstable, and the structure with the pentacoordinated anomeric carbon corresponds, as expected, to the highest energy structure. The stationary point detected on the map is located at $r_{C1-Oa} = 1.9$ Å and $r_{C1-O1} = 2.5$ Å. These distances also coincide with the values of these two distances determined in the transition state structure (TS) observed in Figure 2. This suggests a strong correlation between the $r_{C1-Oa}$ and $r_{C1-O1}$ distances; our calculations clearly show how the equilibrium $r_{C1-O1}$ distance elongates with the shortening of $r_{C1-Oa}$. On the other hand, we have not observed the reverse effect of $r_{C1-O1}$ on $r_{C1-Oa}$. These results support the nucleophilic attack as the driving force for this reaction. It is quite difficult to conceive how the elongation of the $r_{C1-O1}$ bond could induce a significant change in the $r_{C1-Oa}$ distance, unless small variations in the $r_{C1-O1}$ bond could also induce conformational changes in the enzyme and bring the substrates closer to each other. However, this situation seems very unlikely.

**Geometries**

All geometries discussed were calculated at the B3LYP/6-31G** level. Analysis of the geometrical changes occurring along the reaction pathway clearly showed that, during the nucleophilic attack of the acceptor on the anomeric

### Table II. Comparison of the ab initio relative energies (kcal/mol) calculated by various methods for the points

<table>
<thead>
<tr>
<th>Geometry</th>
<th>B3LYP/6-31G‖</th>
<th>B3LYP/6-31G‖</th>
<th>B3LYP/6-31G‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>0.00a</td>
<td>0.00b</td>
<td>0.00c</td>
</tr>
<tr>
<td>TS</td>
<td>44.83</td>
<td>43.85</td>
<td>42.18</td>
</tr>
<tr>
<td>P</td>
<td>30.42</td>
<td>30.11</td>
<td>29.10</td>
</tr>
</tbody>
</table>

$a E = -2,954,618.04$ kcal/mol.

$b E = -2,954,769.52$ kcal/mol.

$c E = -2,955,410.32$ kcal/mol.
carbon c1 of the GlcNAc, the C1-O1 linkage is being cleaved while the C1-Oa bond is formed (Figure 4). Values given in Table I show the formation of the C1-Oa bond, going from as far apart as 3.0 Å in R to 1.881 Å in TS and 1.397 Å in P. At the same time, the elongation of the C1-O1 bond is occurring, going from 1.440 Å in R to 2.380 Å in TS and finally 3.286 Å in P. The geometry of the reactants is characterized by values of 1.440 Å and 1.405 Å for the C1-O1 and C1-O5 bond lengths, respectively.

As the reaction proceeds, a conformational rearrangement of the glucopyranose ring occurs, and variations in these two geometrical variables reflect the changes in the ring conformation. The geometry of the GlcNAc residue in P is characterized by C1-O1 and C1-O5 bonds of 3.286 Å and 1.449 Å, respectively. The C1-O5 bond in TS is shorter by about 5% (~0.075 Å) compared to the reactants (1.405 Å), which is consistent with the charge delocalization occurring in the six-atom ring during the formation of the oxocarbenium ion. In TS, the anomeric proton is located in the plane defined by O5, C1, and C2 atoms, which alleviates the interactions of the leaving and attacking groups at the anomeric carbon. The conformations adopted by the GlcNAc ring go from the 4C1 chair in R through a 4H3 half-chair in TS, and then back to a 4C1 chair conformation after the glycosyl transfer reaction has occurred. At that final stage, the C1-O5 bond is back to a standard value of 1.449 Å. Complexes of glycosidases with a substrate or a product, in which a sugar ring is substantially deformed, have been experimentally observed (Davies et al., 1998; Strynadka and James, 1991; Sulzenbacher et al., 1996). Ring distortions induced by these enzymes in ground states have been assumed to be crucial for their reaction mechanism.

As the acceptor substrate binds in the active site, the catalytic reaction begins and the GlcNAc residue becomes distorted while moving away from the nucleotide moiety. These subtle conformational changes lead to the formation of specific interactions between the GlcNAc residue and the enzyme, which results in the stabilization of the TS during the rate-limiting step. Although mechanisms are distinct (concerted versus stepwise), a substantial resemblance can be observed in the geometry of the transition state described in this study and the so-called late transition states described in our earlier general study of inverting GTs (Tvaroska et al., 2000). In that particular study, late transition states were characterized by short C1-Oa bonds around 1.5 Å and elongated C1-O1 bonds of about 3.0 Å. In the present case, the geometry of TS is characterized by a C1-Oa bond almost formed at about 1.9 Å and a C1-O1 bond elongated to about 2.4 Å which is in fair agreement with the earlier described geometry of the late transition states.

**Reaction barrier**

In the calculations, the geometries for the PES were optimized through energy minimization at the B3LYP/6-31G** level. To provide a more rigorous basis for the comparison of energies along the reaction pathway, we have calculated the energy of the stationary points R, TS, and P using DFT at the B3LYP/6-31++G** and B3LYP/6-311++G** levels, respectively. These values are listed in Table II. From this table, it is clear that the calculated reaction barrier decreases as the basis set is enlarged. However, even at the best level of theory, the calculated barrier along the reaction pathway R (0.0 kcal/mol) → TS (42.2) → P (29.1) is considerably higher than the range of experimentally determined barriers of 15–25 kcal/mol for GTs (Seto et al., 1999).

**Discussion**

In this study, the catalytic mechanism of the GlcNAc transfer by the inverting GT was investigated using DFT quantum chemical methods and the reaction model based on the available crystal structure of GnT-I. The modeled catalytic reaction represented by the PES, shown in Figure 2, clearly proceeds along the horizontal axis representing the nucleophilic attack of the mannose oxygen Oa on the anomeric carbon C1 of the GlcNAc while the proton Ha is still located in the energy well corresponding to this hydroxyl group. However, when the rC1-Oa distance approaches 2 Å, the preferred location of the proton moves to the energy well located at the oxygen OB of the catalytic base. The energetic surface in the direction along the rHa-Oa coordinate is very steep, up to the location of the transition state at rHa-Oa ~ 1.4 Å. This position indicates the point at which the transfer of the proton Ha from Oa to the catalytic base oxygen OB becomes energetically favorable. Indeed, optimization of the structure of the system with rC1-Oa < 2.1 Å always led to proton located at OB. A possible explanation of this behavior could reside in the Oa-OB distance in this reaction model. Indeed, the Oa-OB distance is about 2.4 Å in this model, whereas it was fixed at 2.8 Å in the model used in our previous article (Tvaroska et al., 2000).

As has been previously reported (Lu and Voth, 1998), we believe that a decrease in the O-O distance is responsible for a significant decrease of the energy barrier for the proton transfer between both oxygen atoms. Concerted mechanisms are more likely to be observed in reactions with no significant barrier for one of the reaction steps. In this particular case, the decrease in the proton transfer barrier because of the enzyme flexibility is reflected by a change in the type of mechanism adopted in comparison to our previous study. These results also show how small changes in the structure of the enzyme can influence the energetics of the catalytic mechanism. The basicness of the attacking hydroxyl group decreases with the amount of bonding to the anomeric carbon. For C1-Oa distances lower than 2.1 Å, the OBH group is more basic than Oa˚H (Tvaroska et al., 2000). In our earlier article, we anticipated this type of effect on the shape of the PES due to a decrease in the proton transfer barrier.

Several factors may affect the calculated PES and the resulting reaction barrier. First, the difference observed between the substrate-free and the substrate-bound enzyme structures of GnT-I (Unligil et al., 2000) may have significant consequences for the energetics of the catalytic reaction. It was reported that in the native structure no electron density could be observed for the 13 residues forming a loop adjacent to the donor binding site. However, in the complex with UDP-GlcNAc, the loop is well structured and partially covers the UDP-GlcNAc moiety (Unligil et al., 2000). The catalytic mechanism of GnT-I corresponds to an ordered
The enzyme first binds the donor UDP-GlcNAc complexed with Mn\(^{2+}\) and then the Man\(_6\)GlcNAc\(_2\) acceptor; the oligosaccharide GlcNAc-Man\(_5\)GlcNAc\(_2\) product is subsequently released, followed by UDP. In view of this mechanism, it has been suggested that the binding of UDP-GlcNAc triggers certain conformational changes of the enzyme, which results in the creation of the acceptor binding site where the catalytic reaction can then take place. Such movements of the different domains, as observed for GnT-I, appear to be frequent in enzymes (Vrielink et al., 1994; Morera et al., 2001), which might indicate that such induced-fit interactions with the nucleotide donor can be inherently important for the catalytic mechanism of GTs. Energetic consequences of this movement on the stability of the enzyme–substrate complex, represented in our reaction model by R, were not accounted for because the model contains none of the 13 loop residues.

A comparison of the structures of the substrate-free and the substrate-bound enzyme reveals intensified hydrogen bonding and electrostatic interactions that engage the pyrophosphate group and the metal cofactor (Ungil et al., 2000). As a result, the loop becomes constrained during the assembly of the active site. This structuring also buries ~600 Å\(^2\) of the protein surface next to the donor binding site. The rough proportionality between the buried surface and the hydrophobic Gibbs free energy, 25 cal/Å\(^2\) (Chothia, 1974), suggests that the loop structuring might liberate approximately ~15 kcal/mol. On the other site, the complex of GnT-I with UDP-GlcNAc is relatively weak (K\(_{\text{on}}\) ~ 0.04 mM, ΔG ~ ~6 kcal/mol) (Nishikawa et al., 1988). This suggests that the structure of the enzyme complexed with UDP-GlcNAc is destabilized in comparison to the native state, which might be responsible for the decrease in the binding affinity of UDP-GlcNAc. Using these values, we estimated the conformational free energy for the loop structuring to be around 8–10 kcal/mol and supposed that this represents the energetic cost to change the GnT-I conformation to a less favorable form. This value is comparable to the values estimated for ATP binding to tyrophosphyl-tRNA synthetase (Retailleau et al., 2003). Noteworthy in both cases, is the fact that the phosphate moieties are involved in induced-fit interactions. We suggest that the structure of the GnT-I complex with UDP-GlcNAc characterizes a structure somewhere between the ground state and the transition state on the reaction pathway. This structure may therefore represent the so-called pre-TS complex and contribute to the rate acceleration by destabilization of the ground state. Experimental evidence for this suggestion could be provided by crystal structures of GnT-I complexes with the acceptor and product. Unfortunately, the states relevant for each step of this mechanism have not yet been solved. If we use the value of 8–10 kcal/mol estimated for the ground state destabilization and correct the calculated reaction barrier, we reach a reaction barrier of about 30–35 kcal/mol.

Of course, other factors that may have significant effects on the reaction barrier are the approximations that have been used in the present calculations. Due to computational limitations, the reaction model used does not contain all the residues present in the active site. Interactions between the amino acids left out in this model and the substrates were not accounted for, which might influence the calculated overall structure of R, TS, or P and therefore lower the reaction barrier in the real enzyme active site. We have attempted to estimate qualitatively those interactions that could stabilize TS over R by placing the three stationary points (R, TS, and P) in the active site, bearing in mind that this is a very simplified approach to describing the missing interactions. Generated complexes of the crystal structure of GnT-I with R, TS, and P clearly show that the calculated structures of the stationary points are easily accommodated in the active site without any steric constraints. As expected, and because the UDP part of the molecule is not subjected to any large movement, the main changes in the interactions occur in the region surrounding the GlcNAc ring. In lieu of the so-called DxD motif identified in many GT families, a ~21\(^{\text{1EDD}}\) motif is present in GnT-I. One of the residues (E211) constituting this motif makes direct hydrogen bonds with the GlcNAc O3 and O4 hydroxyls. These interactions are already present in R, and as the reaction proceeds, interactions between the carboxylic group of E211 and these hydroxyls become tighter as the distances O3...Os1(E211) and O4...Os2(E211) decrease. Moreover, location of the α-carbons of four amino acids representing the enzyme active site in our model, were held fixed to ensure that these amino acids would not move to form any stabilizing interactions with the substrates. In the real enzyme active site, these amino acids would be free to move as the enzyme adjusts its structure to the substrate/reactants movement. To consider these effects would require the inclusion of the surrounding protein in the calculations using a hybrid quantum mechanics/molecular mechanics (QM/MM) method. However, these types of calculations would necessitate the inclusion of the complete donor and acceptor molecules, which means over 200 atoms would be in the QM region, making the processor time requirements to investigate the enzymatic reaction prohibitive.

During the reaction process, the electrostatic interactions with the surrounding residues and solvent are also expected to change. The most charged species involved in the reaction is the transition state; one could expect this structure to be more stabilized by interactions with the solvent as compared to the less charged reactants. To probe this effect we have treated the electrostatic environment as a dielectric continuum using a procedure implemented in Jaguar (Schrödinger, 1998). Treated this way, reaction barriers are decreased by 7 kcal/mol and 15 kcal/mol in distinct environments, such as cyclohexane (ε = 2) and water (ε = 78), respectively.

The present results shed some light on the catalytic mechanism of the GlcNAc transfer by the inverting GnT-I and suggest a concerted reaction mechanism with a calculated reaction barrier of 42.2 kcal/mol at the B3LYP6-311++G**/ B3LYP6-31G** level of theory. After the corrections discussed earlier regarding the cost of adopting a less favorable conformation of the enzyme and solvent effect, the reaction barrier could be more realistically estimated around 20–25 kcal/mol. This result is then in fair agreement with experimental values (Seto et al., 1999).

The structure of TS is characterized by a C1-O1 bond elongated to about 2.4 Å and a C1-Oa bond almost formed.
at about 1.9 Å, which resembles the geometry of the late transition states. These results, together with the analysis of the GnT-I structure, led us to suggest that the rate acceleration of the reaction catalyzed by GnT-I might be due to the destabilization of the ground state by induced-fit interactions and the stabilization of the transition state. However, it is clear that more structural, enzymological, and computational work remains to be done to take into account the effects discussed earlier and fully understand the catalytic machinery of GTs. As part of this very challenging goal, we have very recently followed a similar approach using also DFT quantum mechanical methods to investigate the enzymatic mechanism of retaining GTs (André et al., 2003).

Materials and methods

**Model**

The X-ray crystallography structure of GnT-I complexed with UDP-GlcNAc and solved at 1.8 Å (Pdb code: 1FOA) (Unlilig et al., 2000) was used as a guide to design the reaction site model that allowed us to analyze computationally the mechanism of the GlcNAc transfer by GnT-I. We included in this reaction site model all relevant parts of the donor and acceptor substrates, the metal cofactor, and key amino acids involved either in the enzymatic reaction or in the binding of the substrates. The reaction site model defined accordingly (Scheme 2) contains the complete sugar-donor molecule, UDP-GlcNAc; a manno.pyranoside derivative representing the oligosaccharide acceptor; the divalent metal cofactor modeled by Mg²⁺ and fully coordinated by three water molecules and aspartate D212 as found in the X-ray structure (Unlilig et al., 2000) (Figure 1a–b); a portion of aspartate D291 presumed to be the catalytic base; and the essential fragments of aspartate D212 and aspartate D144 interacting with the uridine part of the donor. This reaction site model is consistent with the findings, mentioned earlier, that there exists a conserved catalytic and recognition machinery made up of four carboxylate residues common to all inverting GTs belonging to the GT-1 SpsA family (Tarbouriech et al., 2001) to which GnT-I also belongs. All together, this model contains 127 atoms (Scheme 2). The location of the acceptor model (mannopyranoside derivative) was derived from docking Man₃GlcNAc₂ (the oligosaccharide acceptor) into GnT-I using the AutoDock suite of programs (Goodsell et al., 1996).

**PES**

The reaction that GnT-I catalyzes is the formation of one new glycosidic linkage, between the nucleophile and the donor, cleavage of the donor glycosidic linkage, and removal of a proton from the nucleophile. This reaction mechanism was monitored by means of a 2D PES, as represented in Figure 2. The procedure followed was as previously described for inverting GTs (Tvaroska et al., 2000). Two distances were used as reaction coordinates (Scheme 2): the rC₁-Oa distance between the anomeric carbon C₁ and the oxygen Oa of the acceptor hydroxyl group and the distance rHₓ-Oa between the proton Ha of the sugar-acceptor and the oxygen Oa of the acceptor molecule. These geometrical variables reflect the nucleophilic attack of the sugar acceptor on the anomeric C₁ and the proton transfer process from the sugar acceptor to the catalytic base, respectively. Although we do not consider the rC₁-O₁ distance between the anomeric carbon C₁ and the glycosidic oxygen O₁ to be a reaction coordinate of the GlcNAc transfer reaction, we have still calculated the potential energy surface, represented in Figure 3, as a function of the rC₁-Oa and the rC₁-O₁ distances. This PES helps to shed some light on the relationship between the formation and cleavage of the C₁-O₁ and C₁-Oa glycosidic bonds.

The energy of the model calculated as a function of the two distances gives the two PESs displayed in Figures 2 and 3. Each calculated point on the PES corresponds to the optimized structure and arrangement of the model for the given pair of values of rHₓ-Oa, rC₁-Oa and rC₁-O₁, and rC₁-O₁ distances, respectively. These distances were varied by 0.2 Å increments, within a 0.9–1.9 Å range for rHₓ-Oa, within a 3.0–1.3 Å range for rC₁-Oa, and within a 1.35–3.5 Å range for rC₁-O₁. During the optimization, all geometrical parameters were optimized, with the exception of those defining the location of the α-carbon of the amino acids. As a result, each point on the respective PES is represented by fixed values of the rHₓ-Oa, rC₁-Oa and rC₁-O₁, and rC₁-O₁ distances, and all points have all their geometrical variables relaxed to their most stable values. The calculations were carried out using the Jaguar program (Schrödinger, 1998). The optimization of the geometry was performed using the B3LYP density functional method with the 6-31G* basis set (1347 basis functions). The geometries of all stationary points on the PES were then fully optimized with no constraints on the rHₓ-Oa, rC₁-Oa and rC₁-Oa, and rC₁-O₁ distances. To better characterize the individual reaction paths, the location and structure of the transition state were calculated using the three points nearest to the particular barrier on the PES using the QST-guided search of the Jaguar software (Schrödinger, 1998). Ultimately, selected geometries determined at the B3LYP/6-31G** level were used to estimate the effect of the basis set by calculating their single point energy with the B3LYP/6-31+G** and B3LYP/6-311++G** basis sets (1684/1963 basic functions), respectively.

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**Abbreviations**

DFT, density functional theory; GnT, N-acetylglucosaminyltransferase; GT, glycosyltransferase; P, product; PES, potential energy surface; QM/MM, quantum mechanics/molecular mechanics; R, reactants; TS, transition state

**References**

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