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A Model of Photoprobe Docking with β1,4-Galactosyltransferase Identifies a Possible Carboxylate Involved in Glycosylation Steps

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Abstract—A molecular docking study has been performed on the interaction of β1,4-galactosyltransferase with an acceptor site photoprobe. This is based on an acceptor site peptide fragment which was recently identified by the use of a photoprobe. The present model strongly suggests that the carboxylate group of Asp318 could be involved in the activation of the acceptor sugar 4-OH for the efficient galactosyltransfer. The result also exemplified that the combination of photoaffinity labeling with crystallography is a powerful method for the detailed structural analysis of ligand–protein complex.

Glycosylation reactions are of fundamental importance to both prokaryotes and eukaryotes. The biosynthesis of oligosaccharides is dependent on a series of highly specific enzymes, glycosyltransferases, which elongate saccharide chains sequentially by transferring glycosyl groups to acceptor sugars.[1] Recent successes in the cloning of glycosyltransferases have illustrated characteristic aspects of their molecular structure.[2] Most of these enzymes are located in the Golgi apparatus and share a common topology of type II membrane proteins, consisting of a short N-terminal cytoplasmic tail, a transmembrane segment, and an extended stem region which is followed by the C-terminal catalytic domain. Despite these topological similarities, few regions of homology have been found among the different glycosyltransferase families or even within one family catalyzing different reactions. Recent systematic sequence analysis has allowed the extraction of several conserved motifs for each glycosyltransferase family.[3]

Galactosyltransferases (GalTs) are one of the most widely studied families among these enzymes. GalTs comprise enzymes that transfer galactose from UDP-α-D-galactose (UDP-Gal) to various acceptor substrates.[4] A motif DxD has been found to be conserved in almost all GalTs and also in many other glycosyltransferases. β1,4-Galactosyltransferase (β4GalT, EC 2.4.1.38) transfers galactose from UDP-Gal to the acceptor sugar, *N*-acetyl-glucosamine (GlcNAc), forming a β-(1→4) linkage with inversion at the anomeric configuration of the galactosyl linkage.[5] The crystal structure of recombinant β4GalT1 in a UDP-Gal bound complex at 2.4 Å resolution has recently been solved.[6] The analysis of the catalytic domain structure confirmed the involvement of the DxD motif in the donor UDP-Gal binding. Several amino acids were also suggested to be located in the acceptor binding site.

Recently, we reported a rapid and efficient method, photoaffinity biotinylation, for identifying photolabeled sites within proteins. Using a photoreactive biotinyl GlcNAc analogue as a nonradioisotopic probe, the approach yielded, for the first time, information on the acceptor site peptides in this enzyme.[7] Here we show a docking model involving the photochemically identified peptide, which suggests a mechanism for the acceleration of galactose transfer.

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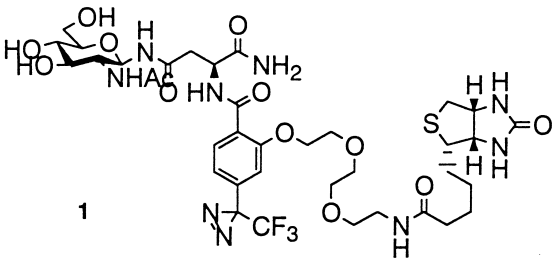

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The crystal structure of β4GalT1 contained the UDP moiety of UDP-Gal but lacked the galactose moiety.[6] Thus, two low-energy conformations of the galactose moiety generated in the active site were examined for the complex formation with the photoprobe **1**. The photoprobe **1** docked into the active site of UDP-Gal-bound β4GalT1 structure was properly located in the proximity to the Oδ atom of Asp318 for galactosyl-transferation in only one of the two ternary complex structures.[8] In the plausible ternary complex structure, the 4-*O* atom of galactose formed hydrogen bonds with the 2-*N* atom of the GlcNAc and the main-chain carbonyl oxygen of the Asn residue in the photoprobe. These interactions may contribute to the specific recognition of the donor and acceptor at the binding site and may also restrain the direction of the photoactive diazirine moiety toward the photolabled peptide, Tyr197-Lys230 (Fig. 1).

In the most stable complex structure, the three-membered photoreactive diazirine ring is located close to the Met225 residue on the photolabeled sequence (Fig. 2). Since the labeled peptide was relatively long and the yields of PTH amino acids were decreased in the last half of the fragment, the previous result of sequencing was not enough for pinpointing the labeled residue.[7b] The PTH amino acid yield of Met225 is, however, considerably lower than that of unlabeled control peptide.[7b,10] The result of molecular docking is consistent with this observation of photoaffinity labeling.

From the mechanistic viewpoint of enzymatic glycosylation, an amino acid is expected to act as an active-site base that abstracts a proton from the 4-hydroxyl group of GlcNAc.[2] The 4-hydroxyl group then increases in nucleophilicity so that it can accomplish an S_N2 attack on the C1 carbon of UDP-Gal from the backside of α-diphosphate bond (Fig. 3). The overall reaction results in the formation of the desired 1→4 linkage by an inversion of the glycosidic bond from α to β.

The crystal structure of β4GalT1 has identified a well conserved negative cluster, EDDD. The cluster is located at the bottom surface of catalytic domain and has been suggested to be involved in acceptor binding.[6] Our model has revealed that the carboxyl residue of Asp318 on the EDDD segment can be located within 2.5 Å distance of the 4-hydroxyl group of GlcNAc (Fig. 4). The other vicinal carboxyl residue of Glu317 is beyond the distance required for the formation of a strong hydrogen bond (6.5 Å).

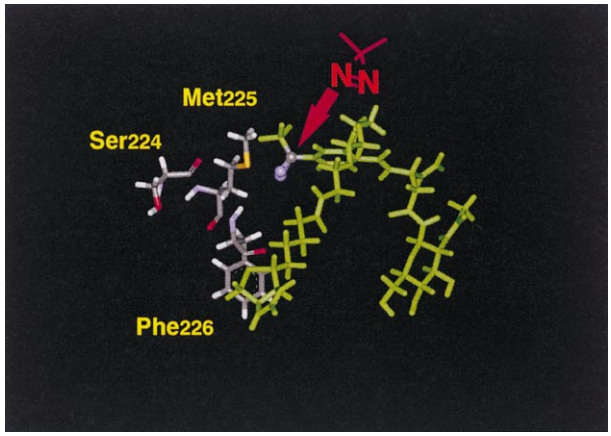

Figure 2. Amino acid residues near to the photoreactive diazirine moiety (arrow) on the probe structure of **1** (green).

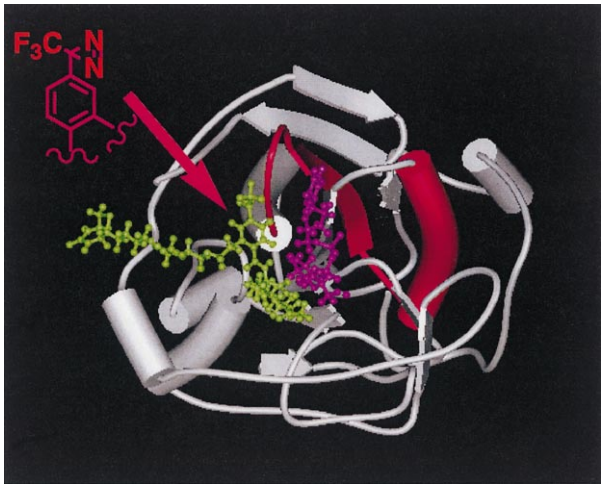

Figure 1. A docking model of β4GalT1. The photolabeled peptide, Y197-K230, is colored in red. The photoprobe (green) and UDP-Gal (pink) are shown as a ball-and-stick drawing.

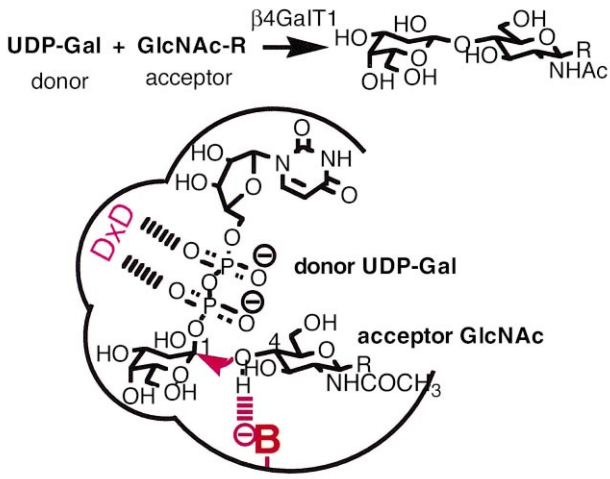

Figure 3. A schematic representation for the possible β-(1→4)-galactosyl bond formation.

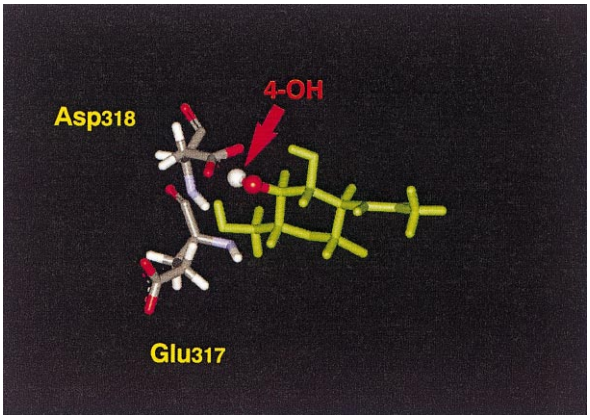

Figure 4. Graphical representation of GlcNAc residue (green) showing the location of Glu317 and Asp318. The 4-hydroxyl group of GlcNAc is shown as a ball-and-stick drawing.

In conclusion, the present model strongly supports the previous assumptions concerning the galactosyltransfer mechanism of β4GalT1 in which the carboxylate group of Asp318 is the likely candidate for the abstraction of 4-OH proton from the acceptor sugar. The result also exemplifies that the method of photoaffinity biotinylation is complementary to the crystallography. The combination of both methods would be a powerful approach for the detailed structural analysis of ligand–protein complex.

Acknowledgements

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8. The UDP-Gal structure was generated at the active site of the crystal structure and then a low-energy conformation search by use of a molecular dynamics/energy-minimization procedure at 300 K for 100 ps afforded two plausible conformations of the galactose portion. Typical dihedral angles for P–O–C1–C2 were 100° and 170°. The photoprobe molecule was docked into the active site manually, where the 4-hydroxyl group of the GlcNAc moiety (4-OH-GlcNAc) was placed at an appropriate position for S_N2 replacement at C-1 of the galactose moiety (C-1-Gal). The complex model was initially energy-minimized to remove severe steric interactions between the photoprobe and the protein under a distance restraint (3.4–3.6 Å between 4-OH-GlcNAc and C-1-Gal). Then, more relaxed complex structures were searched under the distance constraint with the molecular dynamics/energy-minimization procedure by use of DISCOVER 3 (Molecular Simulations Inc., San Diego, USA) as described previously.[9]
9. Ishiguro, M. *J. Am. Chem. Soc.* **2000**, *122*, 444.
10. The PTH amino acid yields for the labeled peptide-I and that for the control peptide (in parentheses) are: Ser224 3.6 pmol (3.2 pmol), Met225 1.6 pmol (5.7 pmol), and Phe226 5.3 pmol (5.3 pmol). The data were adopted from Fig. 6 in ref 7b.