Differential Recognition of the Type I and II H Antigen Acceptors by the Human ABO(H) Blood Group A and B Glycosyltransferases*

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The human ABO(H) blood group A and B antigens are generated by the homologous glycosyltransferases A (GTA) and B (GTB), which add the monosaccharides GalNac and Gal, respectively, to the cell-surface H antigens. In the first comprehensive structural study of the recognition by a glycosyltransferase of a panel of substrates corresponding to acceptor fragments, 14 high resolution crystal structures of GTA and GTB have been determined in the presence of oligosaccharides corresponding to different segments of the type I (α-L-Fucp-1→2)-β-D-Galp-1→3)-β-D-GlcNAc-OR, where R is a glycoprotein or glycolipid in natural acceptors) and type II (α-L-Fucp-1→2)-β-D-Galp-1→4)-β-D-GlcNAc-OR) H antigen trisaccharides. GTA and GTB differ in only four “critical” amino acid residues (Arg/Gly-176, Gly/Ser-235, Leu/Met-266, and Gly/Ala-268). As these enzymes both utilize the H antigen acceptors, the four critical residues had been thought to be involved strictly in donor recognition; however, we now report that acceptor binding and subsequent transfer are significantly influenced by two of these residues: Gly/Ser-235 and Leu/Met-266. Furthermore, these structures show that acceptor recognition is dominated by the central Gal residue despite the fact that the L-Fuc residue is required for efficient catalysis and give direct insight into the design of model inhibitors for GTA and GTB.

Many pathogenic bacteria display a repertoire of glycoconjugates that are comprised of monosaccharides and synthesized by corresponding glycosyltransferases (GTs) not found in their mammalian hosts, and a number of disease-associated GTs are coming under increasing scrutiny as targets for a new class of therapeutics to treat bacterial and fungal infections as well as disorders such as diabetes. However, there is little sequence homology among the hundreds of known GTs. There are currently 79 different families of GTs; but to date, only 20 contain structurally characterized examples. Despite the sequence diversity, common themes have emerged from GT study. For example, all GTs can be classified as either “inverting” or “retaining” depending on whether the anomeric stereochemistry of the donor sugar is changed or conserved during addition.

The human ABO(H) blood group A and B antigens are synthesized by the specific GTs A (GTA) and B (GTB), which transfer GalNac from UDP-GalNAc and Gal from UDP-Gal, respectively, to the H antigen disaccharide α-L-Fucp-1→2)-β-D-Galp-1→3)-β-D-GlcNAc-OR (where R is a glycoprotein or glycolipid). Individuals with the gene for GTA have blood group A; those with the gene for GTB have blood group B; those with genes for both enzymes or a cis-acting form of GTA or GTB have blood type AB; and those with a mutated inactive form of enzyme have blood group O.

GTA and GTB are prototypic retaining GTs and are model probes of this class of enzyme. They are the two most homologous, naturally occurring GTs known to specifically transfer distinct naturally occurring donors and differ only in 4 of 354 amino acid residues: Arg/Gly-176, Gly/Ser-235, Lee/Met-266, and Gly/Ala-268. Given that these enzymes share the same acceptor, it had been postulated that the four “critical” amino acid differences would impact only donor recognition. However, studies using GTA/GTB chimeric enzymes showed that donor recognition is significantly influenced only by Lee/Met-266 and Gly/Ala-268 (6). The remaining two critical residues (Arg/Gly-176 and Gly/Ser-235) were found to have little effect on donor specificity. This pattern was understood upon the determination of the structures of GTA and GTB in the presence and absence of UDP and the H antigen disaccharide α-L-Fucp-1→2)-β-D-Galp(OCH₃)₂CH₃ (7), which showed that only Lee/Met-266 and Gly/Ala-268 are positioned to affect donor recognition. Interestingly, Gly/Ser-235 was observed to affect the conformation of the aliphatic tail on the synthetic H antigen substituted at the position at which the disaccharide is normally displayed on cell-surface glycoconjugates, indicating that this residue can influence acceptor recognition despite the acceptor substrate being common to GTA and GTB.

In nature, there exist at least four H antigens on glycolipids and glycoproteins that are recognized by GTA and GTB (8). The most common are the type I H antigen (α-L-Fucp-1→2)-β-D-Galp-1→3)-β-D-GlcNAc-OR) and the type II H antigen (α-L-Fucp-1→2)-β-D-Galp-1→4)-β-D-GlcNAc-OR) (see Fig. 1). Less common are the type III H antigen (α-L-Fucp-1→2)-β-D-Galp-1→3)-α-D-GalNAc-OR) and the type IV H antigen (α-L-Fucp-1→2)-β-D-Galp-1→3)-β-D-GalNAc-OR) (9). GTA and GTB can form the terminal A and B antigens, respectively, using any of the H antigen-type acceptors and some fragments of the H
antigens. Omitting the (1→4)-β-D-GlcNAc or (1→3)-β-D-GlcNAc residue from the type I or II H antigen trisaccharide (Fig. 1), respectively, creates the so-called “H antigen disaccharide,” which is an excellent acceptor for both GTA and GTB. However, removing the α-L-Fuc residue (to form N-acetyllactosamine (LacNAc) in the case of the type II H antigen: β-D-Galp-(1→4)-β-D-GlcNAc) results in a weak acceptor for GTA, indicating that the fucose residue is essential for efficient catalysis (10).

The importance of the fucose residue was surprising in light of the structures of GTA and GTB determined in the presence of the H antigen: β-D-Galp-(1→2)-β-D-GlcNAc-OR (11, 12). The analogs HA-octyl and HI-octyl were carried out with 10-fold less enzyme and half of the incubation times of 90 and 60 min, respectively. Kinetic measurements were carried out at 37 °C in a total volume of 12 μl containing 1 mM acceptor, 1 mM UDP-Gal or UDP-GalNAc donor, and enzyme in 50 mM MOPS (pH 7.0) with 1 mg/ml bovine serum albumin and 20 mM MnCl₂. For relative rate measurements, 520 ng of GTA and 440 ng of GTB were used with incubation times ranging from 30 to 120 min.

Enzyme Isolation and Characterization—Soluble catalytic domains of GTA and GTB (amino acids 63–354) were expressed in Escherichia coli and purified by successive ion-exchange (SP-Sepharose fast flow) and affinity (UDP-hexanolamine) chromatography as described previously (17, 18). The relative rates of transfer were determined using a radiochemical assay with the hydrophobic acceptors α-L-Fucp-(1→2)-β-D-Galp-O(CH₂)₇CH₃ (referred to as HA-octyl), Gal-gr, β-D-Galp-(1→4)-β-D-Glcp-O(CH₂)₇CH₃ (referred to as Lac-gr), β-D-Galp-(1→4)-β-D-GlcNAcp-O(CH₂)₇CH₃ (referred to as LacNAc-gr), Hi-octyl, and HiI-gr. Assays were carried out at 37 °C in a total volume of 12 μl containing 1 mM acceptor, 1 mM UDP-Gal or UDP-GalNAc donor, and enzyme in 50 mM MOPS (pH 7.0) with 1 mg/ml bovine serum albumin and 20 mM MnCl₂. For relative rate measurements, 520 ng of GTA and 440 ng of GTB were used with incubation times ranging from 30 to 120 min. Kinetics for Hi-octyl and HiI-gr were determined by varying the acceptor concentrations with the donor concentration fixed at 5 mM; the assays contained 52 ng of GTA or 44 ng of GTB at incubation times of 90 and 60 min, respectively. Kinetic measurements for Hi-octyl were carried out with 10-fold less enzyme and half of the reaction times. The initial rate conditions were linear with no more than 10% of the substrate consumed. The kinetic parameters kcat and Km were obtained by nonlinear regression analysis of the Michaelis-Menten equation with the GraphPad PRISM Version 3.0 program.

Preparative Reactions—To ensure that products were formed with the lactoside acceptor, a small-scale reaction containing Lac-gr (5.7 mg, 11 μmol), UDP-Gal donor (12.6 mg, 19.6 μmol), GTB (1.8 μunits), and alkaline phosphatase (2 units) in 1 ml of 50 mM MOPS (pH 7.0) containing 0.2 mg/ml bovine serum albumin and 2 mM MnCl₂ was incubated at ambient temperature with gentle rotation for 6 days. The product was isolated by application of the mixture to a Sep-Pak reverse-phase cartridge. The cartridge was washed with 100 ml of water to remove the donor, enzyme, and buffer, and the product was eluted with 10 ml of high pressure liquid chromatography-grade methanol. The solvent was evaporated to yield a residue, which was redissolved in water (2 ml) and passed through a Millipore Millex filter (0.22 μm). Lyophilization furnished a product that was shown to contain α1–3-linked Gal by 1H NMR analysis.

To confirm transfer to LacNAc, a mixture of LacNAc-gr (4 mg, 7.2 μmol), UDP-Gal donor (7.1 mg, 11 μmol), GTB (3.6 units), and alkaline phosphatase (2.8 units) in 0.8 ml of 50 mM MOPS (pH 7.0) containing 0.2 mg/ml bovine serum albumin and 2 mM MnCl₂ was incubated at ambient temperature with gentle rotation for 6 days. The product was...
isolated and characterized as described above and shown to contain α1–3-linked Gal by 1H NMR analysis.

A mixture of LacNAc-gr (2.8 mg, 5 μmol), UDP-GalNAc donor (3.3 mg, 5 μmol), GTA (4.5 units), and alkali phosphatase (0.6 units) in 0.5 ml of 50 mM MOPS (pH 7.0) containing 0.2 mg/ml bovine serum albumin and 2 mM MnCl2 was incubated at ambient temperature with gentle rotation for 20 days. The product was isolated and characterized as described above and shown to contain α1–3-linked GalNAc by 1H NMR analysis.

RESULTS

The details of data collection and refinement results for the enzyme complexes are provided in Table 1 for GTA and Table 2 for GTB. GTA and GTB were both crystallized in the presence of seven acceptor fragments: Gal, Gal-gr, Lac, LacNAc, βMeLac, HI-octyl, and HII-gr. The 14 possible complex structures are GTA-Gal, GTA-Gal-gr, GTA-Lac, GTA-LacNAc, GTA-βMeLac, GTA-HI-octyl, GTA-HII-gr, GTB-Gal, GTB-Gal-gr, GTB-Lac, GTB-βMeLac, GTB-LacNAc, GTB-HI-octyl, and GTB-HII-gr. Diffraction data were collected to a maximum resolution of 1.85–1.49 Å, with final Rfree ranging from 0.197 to 0.211 and Rfree ranging from 0.220 to 0.236. All structures show excellent electron density along the entire length of the polypeptide chain with the exception of the disordered loop (α-helices ~175–195) and the C-terminal amino acid residues, both of which are also disordered in the native GTA/GTB structures (7). The electron density observed surrounding the acceptor fragments is shown in Fig. 2. Of the 14 possible complex structures, electron density corresponding to the acceptor fragments was seen in every complex except GTA-Lac and GTA-βMeLac. Like the H antigen disaccharide (7), the alkyl tails on synthetic Gal-gr were observed ordered in the active site (see Fig. 5c); however, the corresponding tails on the type I and II H antigen trisaccharides were not observed to have an ordered conformation in either GTA or GTB. The Gal, Lac, and LacNAc acceptor analog fragments were purchased commercially and do not possess alkyl tails; consequently, each of these substrates contains reducing sugar residues that can exist in solution in either the α- or β-anomer.

Gal Monosaccharide—The Gal monosaccharide was observed to bind in the active sites of both GTA and GTB in the β-anomeric form,
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![Diagram of structures](image)

FIGURE 2. Electron density (magenta) corresponding to acceptor analogs bound in GTA (left) and GTB (right) showing the Gal monosaccharide (a), the Lac disaccharide (b), the LacNAc disaccharide (c), and the type I (d) and type II (e) H antigen trisaccharides. Not shown are Gal-gr and β-MeLac. Atoms in the acceptor analogs are colored red for oxygen, white for carbon, and blue for nitrogen. In each case, the acceptor molecule exhibited lower occupancy while bound to GTA. There was no observed electron density corresponding to the alkyl tail on the synthetic type I and II H antigens, and they have been omitted from the figure for clarity.

which corresponds to the anomeric conformation of the corresponding Gal residue of the H antigens. However, electron density maps and refined isotropic temperature factors indicate a significantly lower occupancy of the Gal residue in GTA, although the substrate is visible (Fig. 2a). With one exception, the contacts between the Gal residue and the enzymes were as observed previously for the H antigen disaccharide (7), where the O-4 hydroxyl group forms hydrogen bonds with the side chains of His-233 and Glu-303; the O-6 hydroxyl group forms a hydro-

gen bond with the side chain of Thr-245; and the monosaccharide interacts with the hydrophobic face of Trp-300 (Fig. 3).

The exception to the pattern of interactions reported for the H antigen disaccharide (7) is a water molecule that bridges the O-3 and O-4 hydroxyl groups of Gal and the side chain of Glu-303. This bridging water molecule was observed in the GTB-Gal complex, but not in the GTA-Gal complex due to the change of the critical residue 266 from methionine to leucine (Fig. 3).

Gal-gr (β-D-Galp-O(CH₂)₈CO₂CH₃)—Well defined electron density for the Gal residue with the 8-methoxycarbonyloctyl aglycone was observed in GTA-Gal-gr and GTB-Gal-gr (data not shown). The Gal contacts correspond to those reported for the native H antigen disaccharide (see Fig. 5c). The aliphatic tail (required for product purification) is curled into the active site normally occupied by the i-Fuc residue, but does not contribute any hydrogen bonds.

Lac Disaccharide (β-D-Galp-(1→4)-d-Glcp)—Well defined electron density for the α-anomer of Lac was observed only in GTB-Lac (Fig. 2b). The Gal contacts for Lac correspond to those reported for the native H antigen disaccharide. The Glc residue displays only a single hydrogen bond to the enzyme, between the O-1’ hydroxyl group and the side chain of the critical residue Ser-235 (Fig. 4a). Very weak electron density was observed in the acceptor recognition site of the GTA-Lac complex (Fig. 2b).

LacNAc Disaccharide (β-D-Galp-(1→4)-d-GlcNAcp)—As with the co-crystallizations with Lac, well defined electron density for LacNAc was observed only in the GTB-LacNAc complex (Fig. 2c). The contacts with the Gal residue in the GTB-LacNAc complex (Fig. 4d) were as observed in the GTB-Lac, GTB-Gal, and H antigen disaccharide complexes (7). The GlcNAc residue in the LacNAc complex is in a somewhat different conformation compared with the corresponding residue in the Lac complex, with the result that the single hydrogen bond from the critical residue Ser-235 is with the O-3’ hydroxyl group. The electron density for the LacNAc residue in the GTA-LacNAc complex was fragmented, but more clearly visible than that for GTA-Lac (Fig. 2c). The bridging water molecule observed in all GTB complexes was again not observed in GTA-LacNAc (Fig. 4c).

β-MeLac Disaccharide (β-D-Galp-(1→4)-β-D-Glcp- OCH₃)—Like the co-crystallizations with Lac and LacNAc, well defined electron density for β-MeLac was observed only in the GTB-β-MeLac complex (data not shown). The contacts for β-MeLac in the GTB-β-MeLac complex (Fig. 4b) were as observed in the GTB-Lac and GTB-LacNAc complexes.

HII-octyl Trisaccharide (α-1-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GlcNAcp-OR)—Electron density corresponding to the type II H antigen trisaccharide acceptor was observed in both GTA and GTB (Fig. 2d). The Gal contacts with the enzyme in both GTA and GTB were as described above, with both enzymes showing the water molecule bridging the O-3’ and O-4’ hydroxyl groups of Gal and the side chain of Glu-303 (Fig. 5a). Some disorder is present for both the Fuc and GlcNAc residues in both GTA-HII-octyl and GTB-HII-octyl, but this disorder is more pronounced in the GTA-HII complex. As with the H antigen disaccharide, the Fucose residue displays only a single hydrogen bond to Asp-326.

HI-gr Trisaccharide (α-1-Fucp-(1→2)-β-D-Galp-(1→4)-β-D-GlcNAcp-OR)—Electron density corresponding to the type I H antigen trisaccharide acceptor was observed in both GTA and GTB (Fig. 2e). The Gal contacts with the enzyme in both GTA and GTB were as described above, including the bridging water molecule (Fig. 5b). Unambiguous electron density is present for both the Fuc and Gal residues in GTA-HII-gr; however, the GlcNAc residue is disordered. GTB-HI-gr displays unambiguous density for all three of the carbohy-
drate residues and a number of water molecules that bridge the acceptor and protein.  

Kinetics—The relative rates of transfer for GTA and GTB with each of the acceptor fragments relative to the H antigen disaccharide are shown in Table 3. The kinetic constants determined with the respective donors (5 mM) for both GTA and GTB are shown in Table 4. The relative rates of transfer to non-fucosylated acceptors agree with those in the early report by Schwyzer and Hill (10) with GTA from porcine submaxillary glands; they noted low rates of transfer to galactose and other galactoside acceptors. Removal of fucose dramatically reduces the rate of transfer. To ensure that the low rates of transfer observed in the radiochemical analysis correspond to product formation, we determined that α1–3-linked products were formed by transfer to LacNAc-gr (both GTA and GTB) and Lac-gr (GTB).

DISCUSSION

Two Critical Residues Dramatically Alter the Stability of the Acceptor Recognition Site—In the 12 complex structures in which substrate is observed, the overwhelming bulk of interactions is with the Gal residue; however, the most striking feature is the systematic difference with
which GTA and GTB bind each of these substrates. Although the enzymes share the H antigens as a common acceptor, the structures presented in this work show that two of the four critical amino acid residue differences between GTA and GTB (originally thought to affect only donor preference) significantly influence the stability of the acceptor in its recognition site.

Despite the crystals being grown under the same conditions and with the same substrate concentrations, each of the antigen fragments shows higher occupancy and order in GTB than in GTA (Fig. 2). This can be correlated with interactions involving the two critical residues Gly/Ser-235 and Leu/Met-266. The structures can be grouped according to the critical residue with which they have significant contact, where the Gal...
TABLE 3
Relative rates of transfer at substrate concentrations of 1 mM for the acceptor and respective donors as a percent of the HA-octyl disaccharide (α-L-Fucp-(1→2)-β–D-Galp-(1→3)β–D-Glcp)
All values have an estimated uncertainty of 11%.

| Acceptor fragment | GTA | GTB |
|-------------------|-----|-----|
| Gal-gr            | 2.0 | 0.52|
| Lac-gr            | 0.48| 0.21|
| LacNAc-gr         | 0.30| 0.16|
| HA-octyl          | 100 | 100 |
| HI-octyl          | 41  | 69  |
| HII-gr            | 79  | 90  |

TABLE 4
Kinetic constants determined at 5 mM nucleotide sugar donor

|          | GTA | GTB |
|----------|-----|-----|
|          | \(k_{on}\) | \(k_{cat}\) | \(k_{on}\) | \(k_{cat}\) |
| HA-octyl | 87 \(\pm 6\) | 7.6 \(\pm 0.3\) | 22 \(\pm 3\) | 3.7 \(\pm 0.1\) |
| HI-octyl | 1300 \(\pm 140\) | 1.3 \(\pm 0.1\) | 1600 \(\pm 180\) | 0.5 \(\pm 0.1\) |
| HII-gr   | 490 \(\pm 50\) | 1.8 \(\pm 0.1\) | 184 \(\pm 23\) | 1.2 \(\pm 0.05\) |

monosaccharides contact only Leu/Met-266 (Fig. 3); the Lac and LacNAc disaccharides interact with Leu/Met-266 and Gly/Ser-235 (Fig. 4); and HI-octyl and HII-gr interact with Leu/Met-266, Gly/Ser-235, and the residues involved in I-Fuc recognition (Fig. 5).

The differential impact of residue 266 on the ability of GTA and GTB to bind the antigen fragments is evident in the complexes of Gal, Lac, and LacNAc (Figs. 3 and 4), where the bulkier side chain of Met-266 in GTA positions a water molecule to bridge between Gal and the side chain of Glu-303. This bridging water molecule is shifted in GTB, where the smaller side chain of Leu-266 allows it to be displaced from Glu-303 to form interactions with two other water molecules (Figs. 3 and 4c).

Similarly, the Lac and LacNAc structures are influenced by Gly/Ser-235, which in GTB is able to form a hydrogen bond with the Glc and GlcNAc residues, respectively, but can make no such bond in GTA. Together, these two interactions result in an almost complete lack of electron density for Lac and BMeLac in GTA.

The question of the position of the bridging water molecule in GTA versus GTB does not arise when examining the structure of antigen fragments containing the I-Fuc residue (Fig. 5), as this water molecule is displaced by the fucose residue into its bridging position in both enzymes. This is also seen in the Gal-gr structures, where the aliphatic tail is curled into the position normally occupied by the fucose residue (Fig. 5a).

**GTAs and GTBs Show a Lack of Complementarity to the Third Acceptor Residue**—The Lac and LacNAc substrates used in the crystallization experiments contain a reducing Glc and GlcNAc residue, respectively, that can maturate between the α- and β-anomers. The electron density of these bound substrates shows clearly that the α-anomer is selected by GTB in both Glc (in Lac) and GlcNAc (in LacNAc), even though the GlcNAc residue of the natural acceptors is substituted in the β-anomeric form. This selection of the sterically disfavored α-anomer of Lac in GTB is due to a direct hydrogen bond between the anomeric hydroxyl and O-γ of Ser-235. Surprisingly, GTB also binds the α-anomer of LacNAc without forming the corresponding hydrogen bond with the anomeric hydroxyl group.

Crystalization with BMeLac, which corresponds to the natural antigen with the glycosidic linkage in the β-anomeric conformation, revealed no interaction between the Glc residue and Ser-235 in GTB. Like Lac, BMeLac was not observed bound to GTA. In fact, the Glc/GlcNAc residues of the Lac, LacNAc, and BMeLac substrates bound to GTB display distinct conformations. The difference with which GTA and GTB interact with the Glc/GlcNAc residue demonstrates the lack of specificity of the enzymes toward this part of the antigen.

This forgiving fit between the enzyme and the Glc/GlcNAc residue in Lac/LacNAc/BMeLac is necessary given the range of natural H antigen acceptor structures that are recognized by GTA and GTB. This is seen particularly in the structures of the two major H antigens in complex with GTA and GTB (Fig. 5, a and b). The different glycosidic linkages with the GlcNAc residue (β1–3 in the type I H antigen and β1–4 in the type II H antigen) (Fig. 1) (19) result in significantly different conformations for this residue, requiring different sets of interactions with Gly/Ser-235. As with the other complexes, there is incomplete electron density corresponding to the HI-octyl and HII-gr antigens in GTA, whereas the corresponding electron density in GTB is unambiguous. This can again be traced to the critical residue Gly/Ser-235. For GTB, HI-octyl, Ser-235 hydrogen bonds to the O-6 hydroxyl group of the GlcNAc residue. For GTB-HII-gr, the side chain of Ser-235 stabilizes a network of water molecules that bridge to the HII-gr antigen GlcNAc residue. There is no hydrogen bond or water network present about Gly/Ser-235 in the GTA complexes, resulting in poorly defined electron density for the entire HI-octyl and HII-gr antigens in GTA, particularly the GlcNAc residue.

**GTAs and GTBs Display Different Transfer Kinetics toward the Same Acceptor Fragments**—Given the expected low transfer rates for the antigen fragments lacking the I-Fuc residue, the determination of complete kinetic parameters for these substrates was not possible. Only the relative rates of transfer for the synthetic acceptor analogs are presented in Table 3. The rates are normalized to those observed for GTA and GTB to the H antigen disaccharide acceptor HA-octyl and their respective donors. Overall, the results agree with the general conclusion of Schwyzer and Hill (10) that the I-Fuc residue is required for efficient catalysis. However, significant detail has emerged regarding the ability of the enzymes to discriminate between the type I and II H antigen trisaccharides. Both antigen trisaccharides displayed decreased activity compared with the disaccharide, but the greatest decrease was for HI-octyl, which showed relative rates of 41 and 69% for GTA and GTB, respectively. HII-gr displayed transfer rates of 79 and 90%.

The antigens lacking the I-Fuc residue (Lac-gr, LacNAc-gr, and Gal-gr) all showed markedly reduced rates of transfer compared with the H antigen disaccharide. The highest observed rate of transfer in this group (of 2%) was to Gal-gr in GTA, which was unexpected, as the Gal residue is among the least specific acceptor analogs possible. To help explain this observation, we determined the structures of GTA and GTB in the presence of Gal-gr. Although the Gal residue occupies the same position in the active site (Fig. 5c), the aliphatic tail curls into the space normally occupied by I-Fuc, especially in GTA. This occupation of the catalytically required I-Fuc site for Gal-gr bound to GTA may explain the higher observed rate of transfer.

The kinetic constants determined for transfer of the respective donors to the H antigen disaccharide and trisaccharides (which contain the I-Fuc residue) are presented in Table 4. The \(K_m\) for the H antigen acceptor is somewhat lower for GTB than for GTA (22 versus 87 \(\mu\)M), which might be attributed to the Leu/Met-266 interaction (7); however, the \(K_m\) for type I and II H antigen acceptors dramatically increased for both enzymes. HI-octyl was the most affected, with \(K_m\) values of 1300 and 1600 \(\mu\)M in GTA and GTB, respectively. Detailed two-substrate kinetics with HI-octyl and HII-gr were not possible because insufficient material was available for such studies given the high \(K_m\) values observed. The discrepancies between the relative rates of transfer and the kinetic data are consistent with higher HI-octyl and HII-gr α-values.
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for GTA compared with GTB, where $\alpha$-values reflect the effect that the binding of one substrate has on the other (6, 18).

Caution must always be exercised in drawing any direct correlations between a resting crystal structure and kinetic data; however, it must be noted that the elevated $K_m$ values for HI-octyl versus HI-gr are consistent with the decreased contacts due to the higher observed disorder of fucose with GTA and GTB (Fig. 2d). Similarly, the observed decrease in the type I over type II H antigen relative activity for both GTA and GTB is consistent with the observed electron density because the Fuc residue has been shown to be required for efficient catalysis (10). Finally, the systematically lower occupancies of the H antigen fragments and analogs bound to GTA also suggest that the A antigen products might be less tightly bound to the enzyme than the corresponding B antigen products, which explains the generally higher turnover rates observed for GTA versus GTB. The differential ability of GTA and GTB to utilize the type I and II H antigen acceptors raises the possibility that these preferences may also be reflected in vivo; however, there are no comprehensive studies to date on the relative distribution of type I and II H antigens between the ABO(H) A and B blood groups.

Dominance of the Gal Residue and Generation of Prototypic Inhibitors—The central Gal residue in each of the observed H antigen fragments in complex with GTA or GTB forms the majority of interactions observed between the enzyme and acceptor substrate, which is the same hydrogen bonding pattern as the original H antigen disaccharide structure (7). This is surprising, as the 1-Fuc residue has been known for some time to be essential for efficient catalysis (10). However, it provides key insight into experiments to generate prototypic inhibitors of these enzymes. Initial designs of inhibitors of GTA and GTB were made in the absence of any structural information concerning GTs and consisted of chemical modification of hydroxyl groups on the central galactose residue of HA-octyl (12, 13). These studies have provided some of the few GT-inhibitor complexes available (11). In the present study, both enzymes have been shown to form an overwhelming number of interactions with the Gal residue such that its location in the active site is reproduced regardless of the other substituted saccharides. Similarly, both enzymes have been shown to only loosely recognize the GalNAc residues of the antigen trisaccharides, and so future prototypic inhibitor studies will be focused on modifying the essential fucose residue.

Conclusions—The critical residues Leu/Met-266 and Gly/Ser-235 in GTA and GTB were previously believed to be involved strictly in donor recognition, but we have demonstrated that they have a significant differential impact on the binding of acceptor substrates. Although Leu-266 in GTA and Met-266 in GTB affect only the central galactose residue common to all acceptor fragments used, Gly/Ser-235 clearly causes the enzymes to discriminate among the glycoconjugate structures that display the common H antigen disaccharide acceptor, leading to the prediction that this discrimination will be reflected in vivo.

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