Structure-function Relationship of UDP-GalNAc: Polypeptide N-Acetylgalactosaminyltransferase 1 (GalNAc-T1)

——Function of Conserved Aromatic Residues in the Gal/GalNAc-T Motif of GalNAc-T1——

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Abstract

UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferases (GalNAc-transferases) catalyze an initial step of mucin-type carbohydrate biosynthesis by transferring GalNAc to a hydroxylamino acid on acceptor proteins. It is reported that the GalNAc-transferases catalyze two distinguishable reactions; one is the initial reaction that transfers GalNAc to unglycosylated acceptors, and the other is the follow-up reaction that glycosylates partially glycosylated acceptors. Here, I report the structure-function relationship of the Gal/GalNAc-T motif, which occurs in both the GalNAc-transferases and the β4-galactosyltransferases. I investigated the role of the six conserved aromatic residues in this motif in GalNAc-T1, one of the most ubiquitous isozymes, and evaluated the role of each aromatic residue, using site-directed mutagenesis, as follows. 1) Tyr302 and Phe325 might not be important for reaction. 2) Phe303 and Tyr309 might be modestly involved in the interactions with both the sugar-donor and sugar-acceptor substrates. 3) Trp328 and Trp316 are important sites for enzymatic activity, since the mutations at either site resulted in complete inactivation of the enzyme. I then investigated the role of Trp328 and Trp316 in more detail by generating additional mutants and analyzing the resultant mutants kinetically. Their role was deduced as follows. 1) Trp328 is an essential residue for the activity, since all the mutants generated were inactive and Trp328 is conserved in all the isozymes cloned to date. 2) An aromatic residue is required for the activity at the position of 316. 3) Trp316 is an important binding site with acceptors, primarily involved in the initial glycosylation of acceptor polypeptides. This study first pinpointed the location of the amino acid residue associated with the initial activity of the GalNAc-transferases.

Keywords: mucin-type O-glycosylation, GalNAc-transferase, Gal/GalNAc-T motif, structure-function relationship, site-directed mutagenesis

Contents

1. Introduction

Mucin-type O-glycosylation of proteins is one of the most important post-translational modifications in the cells [1,2]. Its biosynthetic initial step is catalyzed by a large family of UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferases (EC 2.4.1.41) (GalNAc-transferases) by transferring GalNAc from UDP-GalNAc to Ser/Thr residues of proteins [1–3]. To date, 14 members of the mammalian GalNAc-transferase family have been identified [4–21]. They all have common structural features; the presence of an N-terminal cytoplasmic tail, followed by a transmembrane region, a stem region, a putative catalytic domain, and a C-terminal lectin domain that consists of the three tandem $(QXW)_3$ repeats (Fig. 1A). The catalytic domain contains two subdomains; the GT1 motif and the Gal/GalNAc-T motif [22].

It is reported that the reaction catalyzed by the GalNAc-transferases consists of two mechanisms that proceed in biochemically distinct manners; one is the initial glycosylation, and the other the follow-up glycosylation [23–26]. The former is the reaction that transfers GalNAc to unglycosylated acceptors. The latter reaction is involved in the glycosylation of partially glycosylated acceptors and is dependent on the recognition of GalNAc residues on the acceptors by the lectin domain of the enzymes. As a consequence, the follow-up activity is inhibited by free GalNAc, while the initial activity is not. Some isozymes, such as GalNAc-T4 and -T7, are reported to predominantly catalyze the follow-up reaction [8,12]. Recently, I demonstrated that GalNAc-T1, which is one of the most ubiquitous isozymes and had been regarded as having the initial glycosylation activity, catalyzes both the initial and the follow-up reactions [26,27]. I also showed that the lectin domain of GalNAc-T1 is functional, and identified aspartic acid in the α repeat of the $(QXW)_3$ repeats to be predominantly involved in the follow-up reaction. By contrast, no structural information on the initial activity is available so far.

In this report, I investigated the structure-function relationship of the Gal/GalNAc-T motif of GalNAc-T1. Although it is reported that the Gal/GalNAc-T motif, a sequence similarly found in β4-galactosyltransferases, contains the essential carboxylates for the GalNAc-T1 activity [22], the functional role of this motif still remains to be elucidated. I investigated the role of the conserved aromatic amino acid residues in the Gal/GalNAc-T motif using site-directed mutagenesis and kinetic analysis of the mutants. I found that some of the aromatic residues in this domain are involved in binding with both the sugar donor and the acceptor substrates. Among them, invariant Trp328 was found to be essential for the enzyme activity of GalNAc-

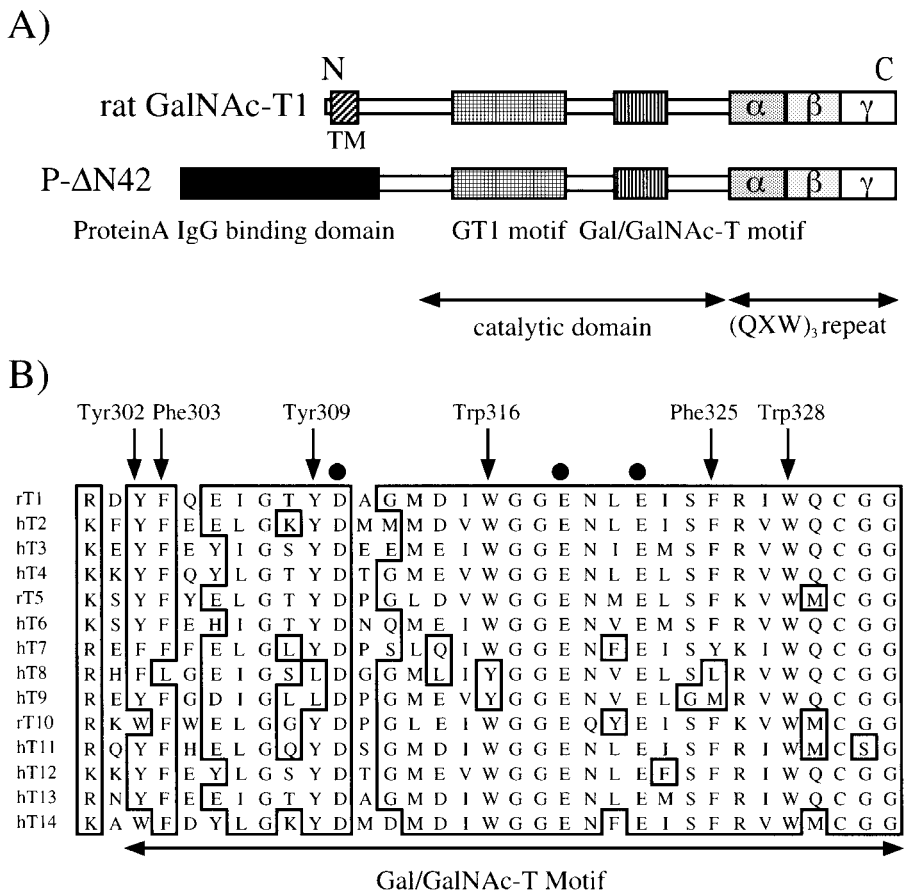

Fig. 1 Schematic representation of GalNAc-T1 and P-ΔN42, and sequence comparison of the Gal/GalNAc-T motif among isozymes
A) Schematic representation of GalNAc-T1 and P-ΔN42. B) Amino acid alignment of the Gal/GalNAc-T motif. Identical amino acid residues are boxed. Closed circles indicate essential acidic residues for the activity of GalNAc-T1 [22].

T1. I also report the structural element required for the initial activity for the first time. Trp316 in the Gal/GalNAc-T motif was an important residue related to the initial activity.

2. Materials and Methods

2.1 Site-directed mutagenesis of soluble rat recombinant GalNAc-T1

Rat GalNAc-T1 cDNA was obtained as outlined by Hagen *et al.*[28]. The plasmid, *pInsProAΔN*42, containing cDNA for soluble, tagged, rat GalNAc-T1 was prepared by deleting the coding sequence for the cytoplasmic tail and the transmembrane domain from full-length GalNAc-T1 cDNA, and by fusing cDNA for an insulin signal sequence and a Protein A-IgG binding domain to the resulting 5'-end of the truncated GalNAc-T1 cDNA

as described previously [29]. Site-directed mutagenesis on *pInsProAΔN*42 was performed using primers listed below [29]. Nucleotides expressed in boldfaces are altered nucleotides to introduce mutations to GalNAc-T1. Y302L, ATTTCCTGAAA**GAG**ATCTCT; F303L, ATTTCCTG**AAG**GTAATCTCT; Y309L, TTCCAGCATC**AGC**TGTTCCA; W316Y, TTCTCCTCC**ATA**AATATCCA; W316F, TTCTCCTCC**GAA**AATATCCA; W316L, TTCTCCTCC**GAG**AATATCCA; W316A, TTCTCCTCC**CGC**AATATCCA; W316H, TTCTCCTCC**GTG**AATATCCA; F325L, CTGCCAAATCCT**AAG**GGAAA; W328L, CACACTG**CAG**AATCCTAAAG; W328A, CACACTG**CGC**AATCCTAAAG; W328Y, CTG**ATA**AATCCTAAAGGAAA; W328F, CTG**GAA**AATCCTAAAGGAAA. The nucleotide sequence of the mutant clones was verified by DNA sequencing using ALFexpress II (Amersham Pharmacia Biotech).

2.2 Expression of P-ΔN42 and mutant P-ΔN42 in COS7 cells

The fusion protein (P-ΔN42), containing a Protein A-IgG binding domain at the N-terminus, and mutant P-ΔN42 were expressed in COS7 cells and purified from the conditioned medium as described previously [29]. SDS-PAGE and Western blotting of the recombinant molecules were also carried out as described [29]. The protein bands on the immunoblots were scanned and quantitated with a Luminoimage Analyzer LAS-1000 PLUS (Fujifilm). The activities of P-ΔN42 and mutant P-ΔN42 were determined as described below and the activity levels were corrected for the enzyme protein concentration in the medium.

2.3 GalNAc-transferase assay

GalNAc-transferase assays using apomucin and peptide acceptors as acceptors were carried out as described in [29] and [26], respectively. Kinetic analysis of P-ΔN42 and its mutants were carried out as described previously [29].

3. Results

3.1 Mutagenesis of the conserved aromatic residues in the Gal/GalNAc-T motif

Amino acid sequence alignment of the Gal/GalNAc-T motif among the GalNAc-transferases (Fig. 1B) shows that this motif contains six highly conserved aromatic amino acid residues among the isozymes (Tyr302, Phe303, Tyr309, Trp316, Phe325, and Trp328). Among them, Trp328 is the only residue that is strictly conserved within all the GalNAc-transferases cloned to date. The other aromatic amino acids, although highly conserved, are replaced in some of the isozymes. However, the substitution of these residues is conservative in most cases; *i.e.*, they are replaced by another aromatic residue, such as phenylalanine, tryptophan, and tyrosine, suggesting the functional importance of aromatic residues at these sites.

In order to evaluate the role of the conserved aromatic residues in the Gal/GalNAc-T motif, I carried out site-directed mutational analysis on these residues. I used the recombinant rat GalNAc-T1 for this experiment. The recombinant enzyme was constructed by deleting the cytoplasmic tail and the transmembrane region from the rat isozyme and by fusing an in-

sulin signal sequence and a protein A-IgG binding domain to the resulting N-terminus of the truncated GalNAc-T1 (Fig. 1A). The recombinant GalNAc-T1 thus prepared was expressed in COS7 cells and the secreted fusion protein (P-ΔN42), which lacks N-terminal 42 amino acid residues of the enzyme, was purified from the culture medium on IgG-Sepharose as described under Materials and Methods. As reported previously [29], P-ΔN42 is fully active, having almost identical K_m values for the sugar donor and the acceptor substrates as compared with the full-length GalNAc-T1.

I first prepared the single point mutants of P-ΔN42, in which the respective aromatic residue was replaced by a leucine residue (Fig. 2). Leucine does not contain an aromatic ring, but is as hydrophobic as the aromatic amino acids. It is, therefore, expected that the substitution of leucine for an aromatic residue may minimize the local conformational change in the enzyme molecules due to the altered hydrophobicity and make it possible to evaluate the functional role of the replaced aromatic residues. P-ΔN42 and its single-point mutants were expressed in COS7 cells and the secreted fusion proteins were recovered from culture medium. The amount of secreted fusion protein was evaluated by Western blotting, as described under Materials and Methods. For the assay for the activity, apomucin was used as an acceptor. Apomucin is an efficient substrate of GalNAc-T1 and is characterized by the presence of multiple acceptor sites on the polypeptide [30–35]. The enzymatic activity was corrected for recombinant protein expression and expressed as the relative activity to that of P-ΔN42.

Fig. 2 shows that the protein expression level of all the six leucine mutants was roughly

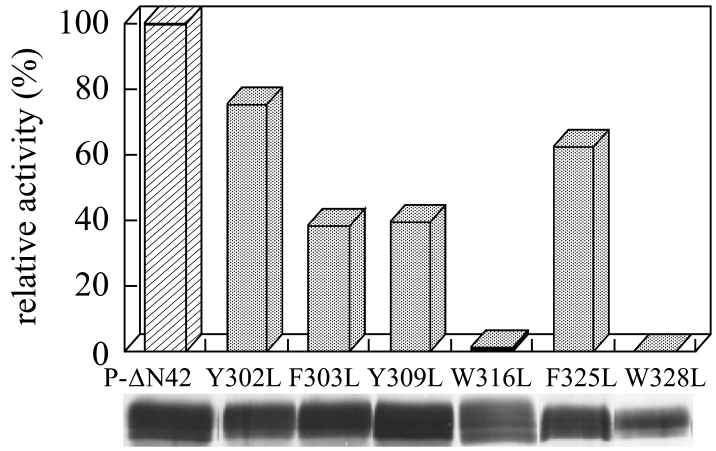

Fig. 2 Expression and enzymatic activity of GalNAc-T1 mutant enzymes
P-ΔN42 and mutant enzymes were expressed in COS7 cells and the secreted recombinant proteins in the medium were recovered from the medium. The mutant enzymes were purified on IgG-Sepharose as described under Materials and Methods. The amount of the secreted protein was determined by Western blotting followed by the densitometric scanning of the blot (lower panel). The enzymatic activity secreted in the medium was determined using apomucin as acceptor and corrected for the amount of the mutant in the medium and expressed as the relative activity to that of the wild type, P-ΔN42. The solid bars show the percent enzyme activity relative to that of P-ΔN42 (hatched bar).

comparable to that of the P-ΔN42. On the other hand, the relative activity of these mutants was significantly reduced. Especially, the activity of the two mutants (W316L and W328L) was decreased almost to the background level, suggesting the functional importance of these residues. By contrast, the other four mutants (Y302L, F303L, Y309L, and F325L) exhibited more moderately reduced activity at the levels of 40–80% of the parent enzyme.

3.2 Kinetic analysis of the mutant enzymes

In order to evaluate the catalytic roles of the aromatic residues, I investigated kinetic properties of four leucine mutant enzymes that retained activity to allow the kinetic analysis (Table 1). First, the K_m values were determined for the donor substrate, UDP-GalNAc. Of the mutants examined, the affinity of F303L and Y309L was most severely affected with the approximately 3-fold increase in K_m values. On the other hand, no significant alteration was observed for the mutants, Y302L and F325L, which retained the highest activities with apomucin among the mutants (Fig. 2). Affinity of the mutants for the sugar acceptor, apomucin, was also investigated (Table 1). The affinity of F303L for apomucin was decreased as well, with approximately 3-fold increase in the K_m value. No significant or only modest change in K_m values was observed for the other mutants. The kinetic study of the mutants indicates that some of the aromatic amino acids in the Gal/GalNAc-T motif are involved in the binding with both UDP-GalNAc and apomucin.

3.3 Mutagenesis of Trp316 and Trp328 in the Gal/GalNAc-T motif

Among the six leucine mutants, the activity of W316L and W328L were most severely affected (Fig. 2). To examine the importance of the aromatic residues at these two positions, I prepared several additional mutants (Figs. 3A and B). As to the position 316, four mutants (W316Y, W316F, W316A, and W316H) were generated. Of these, W316Y and W316F, in which tryptophan was replaced by another aromatic amino acid, tyrosine and phenylalanine, respectively, exhibited significantly higher activity than W316L, with approximately 40% and 20% of the activity of the wild type, respectively. The preference of tryptophan and tyrosine to phenylalanine may suggest the requirement of an indole group or an aromatic ring with a polar

Table 1 Kinetic analysis of the leucine mutants

	UDP-GalNAc		apomucin	
	K_m (mM)	-fold	K_m (mg/ml)	-fold
P-ΔN42	5.1 ± 0.8	1.0	4.7 ± 0.1	1.0
Y302L	5.2 ± 0.1	1.0	5.0 ± 0.2	1.0
F303L	14.2 ± 1.1	2.8	13.6 ± 1.9	2.8
Y309L	16.0 ± 0.3	3.2	7.1 ± 0.4	1.5
F325L	6.2 ± 0.6	1.2	6.7 ± 1.7	1.4

K_m for UDP-GalNAc and apomucin was determined as described under Materials and Methods. Values are the average of three distinct reactions.

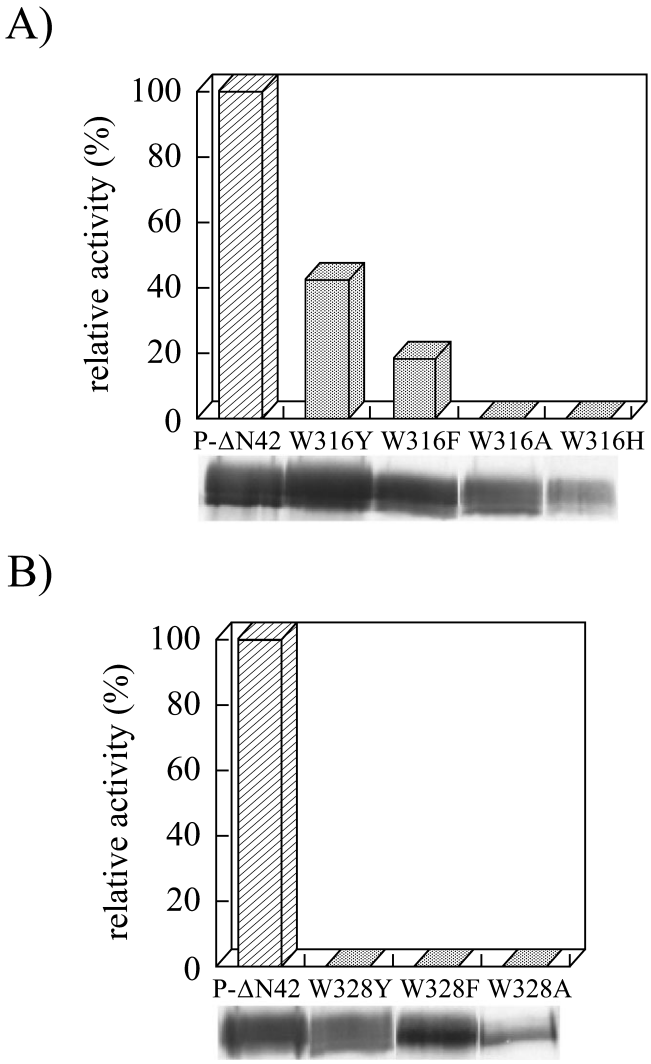

Fig. 3 Expression and enzymatic activity of mutant enzymes at Trp316 and Trp328
Expression and enzyme assay of P-ΔN42 and the mutant proteins were carried out as described in the legends to Fig. 2. The solid bars show the percent enzyme activity relative to that of P-ΔN42 (hatched bar). A) Mutants at Trp316 and B) mutants at Trp328.

functional group at this site. W316H contains a polar imidazole group, instead of an aromatic group at this site. This mutant, however, was completely inactive. W316A had no activity, as would be expected by the loss of the aromatic ring and by the low hydrophobicity of alanine. Consequently, an aromatic amino acid is considered essential at the position 316 for the activity and moreover, the aromatic residue with a polar functional group would be more favorable.

I, then, investigated the role of Trp328. Similarly, I generated three additional mutants,

W328Y, W328F, and W328A. Contrary to the position 316, these three mutants were completely inactive (Fig. 3B). This indicates that the tryptophan residue is essential at the position 328 for the GalNAc-T1 activity. It is noteworthy that Trp328 is strictly conserved within all known isozymes, suggesting that it serves the important function common to the family.

3.4 Kinetic analysis of the Trp316 mutant enzymes

To investigate the functional role of Trp316 in catalysis, I carried out the kinetic analysis of W316Y and W316F (Table 2). The two mutants exhibited modestly decreased affinity for UDP-GalNAc. On the other hand, their affinity for apomucin was more severely affected. Moreover, the reduced affinity for apomucin of these mutants was correlated well with the reduced activity (Fig. 3A and Table 2). W316F, which had lower activity than W316Y, exhibited lower affinity for apomucin with 9.1-fold increase in K_m value. This indicates that Trp316 is the major site interacting with the acceptors. A polar group would be necessary for the efficient binding with acceptors, as demonstrated by the low affinity of W316F.

3.5 Trp316 is involved in the initial glycosylation of an acceptor polypeptide

Since apomucin contains multiple glycosylation sites, the altered K_m values for apomucin include the overall decrease in the affinity related to both the initial and the follow-up activity (Fig. 3A and Table 2). In the previous report, I demonstrated that the inclusion of a high concentration of free GalNAc in the reaction interferes with binding of the enzymes to partially glycosylated acceptors, and selectively inhibits the successive follow-up glycosylation [25,26]. Hence, the enzymatic activity remaining in the presence of a high concentration of GalNAc represents the initial O-glycosylation activity of the mutants. I investigated the effects of GalNAc on the glycosylation of apomucin by W316Y and W316F. If the mutation at 316 primarily affects the initial glycosylation of apomucin, the inhibition by GalNAc should be more prominent in the mutants. This was, indeed, the case with the W316F and W316Y (Fig. 4). In addition, W316F, which had lower activity with apomucin than W316Y, was more severely inhibited by free GalNAc. These data indicate that the reduced glycosylation of apomucin by W316F and

Table 2 Kinetic analysis of the Trp316 mutants

	UDP-GalNAc		apomucin	
	K_m (mM)	-fold	K_m (mg/ml)	-fold
P-ΔN42	5.1 ± 0.8	1.0	4.7 ± 0.1	1.0
W316Y	9.0 ± 0.1	1.8	17.4 ± 3.6	3.6
W316F	9.1 ± 0.7	1.8	43.9 ± 3.8	9.1

K_m for UDP-GalNAc and apomucin was determined as described under Materials and Methods. Values are the average of three distinct reactions.

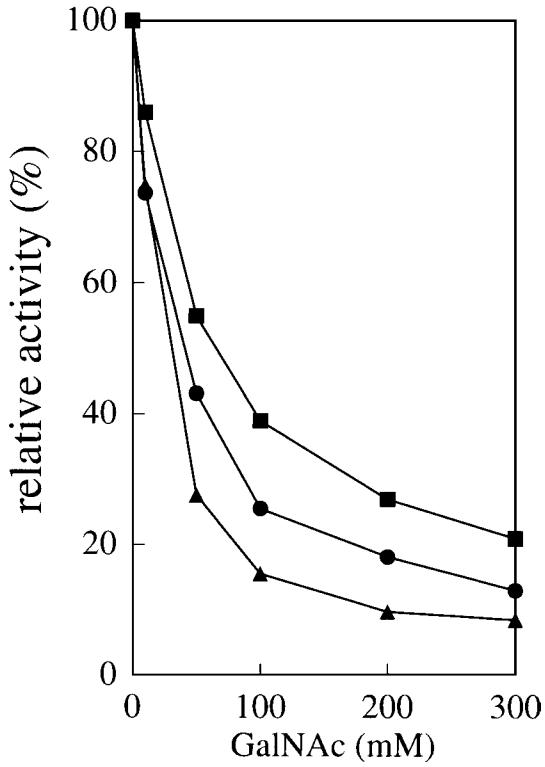

Fig. 4 Inhibition of P-ΔN42 and the Trp316 mutants with free GalNAc
Recombinant enzymes, expressed in COS7 cells and purified from the conditioned medium on IgG-Sepharose, were assayed for the activity using apomucin as acceptor in the presence of increasing concentrations of GalNAc. ■, P-ΔN42; ●, W316Y; ▲, W316F.

W316Y can be primarily ascribed to the decreased initial glycosylation.

3.6 Involvement of Trp316 in the binding of acceptor peptides with a single acceptor site

I determined the activity of W316Y and W316F to glycosylate peptides with a single acceptor site, in order to evaluate the effect of the mutation on the initial glycosylation of acceptors. In this experiment, I used two synthetic peptides, PPDAA**T**AAPL and GVVP**T**VVPG, which are efficient acceptors of GalNAc-T1 [3,26]. As I expected, the two mutants had drastically decreased activity with these peptides (Fig. 5), while they retained significant activity with apomucin (Fig. 3A). This also supports the idea that Trp316 is the major site involved in the initial glycosylation.

The kinetic analysis of W316Y and W316F was also carried out using these two peptides. Table 3 shows that the decrease in the activity of W316Y and W316F was primarily ascribed to the reduced affinity of the mutants for the acceptors. Considering the moderately reduced affinity for UDP-GalNAc of the mutants (Table 2), the increase in K_m for both peptides, for the most part, accounts for the low activity of the mutants. These results indicate that Trp316

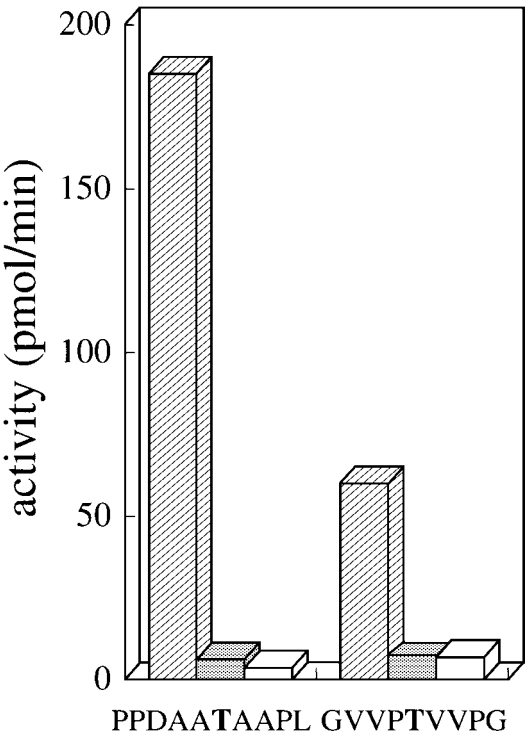

Fig. 5 Enzyme activity of P-ΔN42 and the Trp316 mutants with peptides with a single acceptor site
Hatched, solid, and white bars indicate the enzyme activity of P-ΔN42, W316Y, and W316F, respectively.

in GalNAc-T1 serves as an important binding site for the initial glycosylation of the acceptor polypeptides.

4. Discussion

In this study, I demonstrated that the Gal/GalNAc-T motif participates in the binding with both the sugar donor and acceptor substrates based on the combination of site-directed mutagenesis and kinetic analysis of the mutant enzymes. I also identified some of the conserved aromatic residues to be involved in the enzyme function. The effects of the mutations were dependent on the positions of the mutated aromatic residues (Fig. 2 and Table 1). The mutations at Tyr302 and Phe325 gave only a minor reduction (20–30%) in the activity. Kinetic analysis also showed that these sites are basically unrelated to binding with the substrates. On the other hand, the mutations at Phe303 and Tyr309 caused moderately reduced activity with reduced affinities for both UDP-GalNAc and apomucin. This suggests that these residues may weakly interact with both the sugar donor and the acceptor substrates. These residues are highly conserved, although conservatively substituted by other hydrophobic amino acids in some isozymes (Fig. 1). They may not be crucial for the basic catalytic function of GalNAc-transferases, but rather im-

portant in defining the catalytic properties of some of isozymes. Contrary to these residues, all the mutations at Trp328 (W328L, W328A, W328Y, and W328F) resulted in complete inactivation of the enzyme (Figs. 2 and 3). This, together with the fact that Trp328 is strictly conserved within all known isozymes, suggests that this residue may serve the basic enzyme function common to the family (Fig. 1). As to Trp316, the mutational effects depend on the type of substituted amino acids. When leucine, alanine, or histidine was introduced, enzymatic activity was lost. On the other hand, substitution with another aromatic residue, such as tyrosine and phenylalanine, gave a reduced, but significant activity (W316Y and W316F) (Figs. 2 and 3). All these results, together with the increased sensitivity of the mutant of the inhibition by free GalNAc, indicate that Trp316 is a biding site with acceptor peptide to perform the initial glycosylation.

It seems likely that the GalNAc-transferases have a repertoire of amino acid residues (subsites) that can interact with the acceptor substrates [3,36,37]. Supposedly, there would be numerous combinations of the interactions between substrates and the substrate-binding sites of the GalNAc-transferases, which might determine the binding affinity for the acceptors. As a consequence, the enzyme would have broad rather than defined substrate specificity as reported, and the variation in the subsites could provide each isozyme with distinct kinetic properties [1,3,36,38,39]. This report demonstrated that Trp316 is one of the important subsites in GalNAc-T1. This is supported by the fact that the mutation at 316 in GalNAc-T1 selectively impaired their initial activity (Figs. 4 and 5, Table 3). In accordance with this, our preliminary experiments using a panel of undecapeptides indicate that the activity of the mutants was, on the whole, decreased to the level as low as a few percentages of that of the parent enzyme (personal communications). It should be noted that Trp316 is conserved among the GalNAc-transferases except GalNAc-T8 and GalNAc-T9 (Fig. 1). These two isozymes contain a tyrosine residue in place of Typ316. Accordingly, they are expected to have a lower or restricted initial activity than the other isozymes. Together with their restricted expression in the tissue [13,14], they may catalyze tissue-specific glycosylation.

Clarified three-dimensional structure of the β4-galactosyltransferases demonstrates that Trp314 in β4-galactosyltransferases, which corresponds to Trp316 in GalNAc-T1, interacts with UDP-Gal [40–45]. Interestingly, Trp314 is involved in the conformational change of the Gal/GalNAc-T motif, which occurs following the binding of UDP-Gal to the β4-

Table 3 Affinity of the Trp316 mutants for peptides

	PPDAA**T**AAPL		GVVP**T**VVPG	
	K_m (mM)	-fold	K_m (mM)	-fold
P-ΔN42	0.35 ± 0.05	1.0	1.74 ± 0.3	1.0
W316Y	13.5 ± 0.7	38.5	16.0 ± 0.3	9.2
W316F	15.6 ± 0.6	44.6	17.6 ± 0.5	10.1

K_m for peptides with a single acceptor site was determined as described under Materials and Methods. Values are the average of three distinct reactions.

galactosyltransferases. This conformational change leads to alteration of the β4Gal-transferases from the inactive to the active state, making the binding of the sugar acceptors as well as α-lactoalbumin to the enzyme possible [40–45]. It may also be possible that Trp316 in GalNAc-T1 plays a similar role in catalysis. Our kinetic data suggest that Trp316 can bind with both UDP-GalNAc and the acceptor substrates (Tables 2 and 3). Moreover, GalNAc-T1 apparently catalyzes the ordered reaction; the binding of UDP-GalNAc precedes the binding with the acceptor substrates [3,26]. Thus, the binding of UDP-GalNAc to the binding sites in the Gal/GalNAc-T motif may cause the conformational change in GalNAc-T1 and make the following interactions with the acceptors possible.

Here, I reported the role of the aromatic residues in the Gal/GalNAc-T motif in the binding with both UDP-GalNAc and the acceptor peptides for the first time. Through our present and previous studies on the structure-function relationship of GalNAc-T1, I have demonstrated that the characteristic domain structures of the GalNAc-transferases contribute to the common function of this family. Still, basic characteristics of the GalNAc-transferases remain to be elucidated, such as substrate specificity, and the detail mechanism of the initial and follow-up activities. More in-depth analysis of each motif, using biochemical and molecular biological approaches, together with crystallographic study of each enzyme, would be necessary for understanding catalytic mechanism of the GalNAc-transferases.

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Footnotes

Abbrevations: EDTA, ethylenediaminetetraacetic acid; GalNAc, N-acetylgalactosamine; GalNAc-transferase, UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase; GT1, glycosyltransferase 1; PCR, polymerase chain reaction; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

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UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)の構造活性相関

——Gal/GalNAc-Tモチーフ中の保存された芳香族アミノ酸残基の機能解析——

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要　旨

UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase (GalNAc-T) は，糖タンパク質のムチン型糖鎖生合成開始反応を触媒する酵素である．この酵素は，糖鎖を持たないペプチド上に GalNAc を転移する initial 反応と，部分的に糖付加されたアクセプター上に GalNAc を転移する follow-up 反応という生化学的に機構の異なる 2 つの反応を触媒することが知られている．筆者は，GalNAc-T の中でも最も幅広い組織分布を示す GalNAc-T1 をモデル酵素として，Gal/GalNAc-T モチーフの機能を調べた．このモチーフはβ4-galactosyltransferase にも見いだされる配列であり，糖転移酵素に重要な機能を付与する配列であると考えられている．筆者は，Gal/GalNAc-T モチーフ内で高度に保存されている 6 つの芳香族アミノ酸残基を部位特異的に改変した変異体を作製し，それらの性質を解析することで次に示す結果を得た．1) Tyr302 と Phe325 は酵素活性に関与しない．2) Phe303 と Tyr309 は糖ドナーとアクセプターの両方の結合に関与する．3) Trp328 と Trp316 は酵素活性に不可欠なアミノ酸残基である．Trp328 と Trp316 については，さらに多くの種類の変異体を作製してより詳細な解析を行い，以下の知見を得た．1) すべてのアイソザイム間で保存されている Trp328 は GalNAc-T1 の酵素活性に不可欠である．このトリプトファン残基を他のアミノ酸に改変すると酵素は完全に失活した．2) 316 番目の位置には芳香族アミノ酸残基が必要である．3) Trp316 は基質タンパク質との結合，特に initial 反応に重要な役割を果たす．本研究を通じて，筆者は GalNAc-T の initial 反応に関係するアミノ酸残基を同定することに初めて成功した．

キーワード：ムチン型 O-グリコシレーション，GalNAc 転移酵素，Gal/GalNAc-T モチーフ，構造活性相関，部位特異的変異