Knockout of mouse β1,4-galactosyltransferase-1 gene results in a dramatic shift of outer chain moieties of N-glycans from type 2 to type 1 chains in hepatic membrane and plasma glycoproteins

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INTRODUCTION

The outer chains of complex-type N-glycans consist of two backbone structures called type 1 chain (Galβ1→3GlcNAc) and type 2 chain (Galβ1→4GlcNAc). These structures are also found in O-glycans and glycolipids. Modifications of the two types of outer chains by several glycosyltransferases leads to the synthesis of a variety of terminal carbohydrate structures, such as Lewis a, Lewis b or sialyl Lewis antigens on type 1 chains and Lewis x, Lewis y, sialyl Lewis x or polyglactosamine structures on type 2 chains. These modified structures have been suggested to have physiologically and pathologically important roles in cell-cell interactions. For example, sialyl and/or sulphated Lewis x serve as ligands for selectins and mediate leucocyte-endothelial cell interactions [1–3]. These ligands, as well as sialyl Lewis, play important roles in interaction of tumour cells with endothelial cells during the process of metastasis [4].

Recently, two groups [5,6] have independently generated mice deficient in β1,4-galactosyltransferase (β4Gal-T), which is involved in the synthesis of type 2 chain. These mice show several abnormal phenotypes; semi-lethality after birth, growth retardation, enhanced epithelial cell proliferation of skin and small intestine, abnormal differentiation of intestinal villi, pituitary insufficiency, etc. Examination of glycoproteins by lectin staining and of the enzyme activity, indicated that residual β4Gal-T activity and its products were considerable in brain tissue and were found at lower levels in other tissues of β4Gal-T-knockout mice [5–7]. Structural analysis of erythrocyte membrane glycoproteins also indicated that β1,4-galactosylation of core 2 O-glycans is severely impaired, but considerable amounts of outer chains of N-glycans are galactosylated in homozygous β4Gal-T knockout mice (β4Gal-T−/−) [8], suggesting that other galactosyltransferases which efficiently β1,4-galactosylate N-glycans are working in erythroid cells.

Gene cloning studies have clearly shown that human β4Gal-T consists of multiple members [9–13]. In mice, β4Gal-T1[14,15], β4Gal-T2[16], β4Gal-T5[16] and a novel β4Gal-T[17] have been cloned. Multiple members of a β3Gal-T gene family have also been cloned in humans [18–21] and mice [22]. Thus galactosylation seems to be regulated in a complicated manner in vivo. It is likely that deficiency of β4Gal-T results in a variety of alterations in terminal glycosylation depending on tissues or cell types. To examine such a possibility, it is important not only to understand apparently normal phenotypes and the pathology of the β4Gal-T knockout mice, but also to obtain valuable information about how terminal glycosylation is regulated in vivo. To approach this issue by analysis of glycans produced in vivo, in the present study, N-glycans of plasma and hepatic membrane glycoproteins from wild-type (β4Gal-T+/+) and β4Gal-T−/− mice have been compared.

Key words: galactosylation, sialylation, N-glycans.

Abbreviations used: β4Gal-T, β1,4-galactosyltransferase; β4Gal-T+/+, heterozygous β4Gal-T1 knockout mice; β4Gal-T−/−, homozygous knockout β4Gal-T1 mice; β4Gal-T+/+, wild-type β4Gal-T1 mice; β3Gal-T, β1,3-galactosyltransferase; MALDI–TOF–MS, matrix-assisted laser-desorption ionization–time-of-flight MS; 2-AB, 2-aminobenzamide; HPAEC, high-pH anion-exchange chromatography; SSA, Sambucus sieboldiana agglutinin.

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**EXPERIMENTAL**

**Materials**

2-Aminobenzamide (2-AB), dimethylamine–borane complex, trifluoroacetic acid, and neuraminidase from *Arthrobacter ureafaciens* were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Anhydrous hydrzone was obtained from Tokyo Kasei Inc. (Tokyo, Japan); Nanodex microconcentrators (molecular-mass cut-off 10 kDa) were from Pall Filtron Co. (Northborough, MA, U.S.A.), Sep-Pak C18 cartridges were from Waters Co. (Milford, MA, U.S.A.) and the Micro BCA Protein Assay reagent kit was from Pierce (Rockford, IL, U.S.A.). Jack bean β-N-acetylhexosaminidase, β-galactosidase from *Streptococcus 6646K*, and *Sambucus sieboldiana* agglutinin (SSA)-agarase were purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Neuraminidase from *Salmonella typhimurium* LT2 and recombinant β,1,3-galactosidase of *Xanthomonas manihotis* were obtained from New England BioLabs Inc. (Beverly, MA, U.S.A.); β-galactosidase from *Diplococcus pneumoniae* was from Boehringer-Mannheim (Yamanouchi, Tokyo, Japan). AG-3 × 4A anion-exchange resin and AG-50W-X8 cation-exchange resin were purchased from Bio-Rad (Hercules, CA, U.S.A.).

**Preparation of hepatic plasma membrane from wild-type and knockout mice**

β4-Gal-T1 heterozygous (β(4Gal-T1+/−)), β4Gal-T1−/− and β4Gal-T1+/+ mice were generated as described previously [5]. Hepatic plasma membranes were prepared as follows. All procedures were carried out on ice using chilled buffer solutions, and centrifugation was performed at 4 °C. Livers from each of the mouse strains (9-week-old mice) were removed and washed with PBS, chopped in PBS, using glass slides, and allowed to stand for 5 min. After removal of the precipitate, the tissue was centrifuged at 400 g for 10 min. The resulting pellet was suspended in haemolysis buffer (0.15 M NH₄Cl/1 mM KHCO₃/0.1 mM EDTA, adjusted to pH 7.4 with 1 M HCl) and allowed to stand for 5 min. The precipitate was washed twice with PBS, finally resuspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.4) and centrifuged at 150 g for 5 min. The supernatants were then centrifuged at 25000 g for 30 min. The precipitate was washed twice by centrifugation with TE buffer and once with 10 mM ammonium acetate, and the pellet was freeze-dried. The membrane fraction thus prepared was delipidated by extraction with chloroform/methanol [2:1 (v/v) and 1:1 (v/v)] as described previously [8].

**Preparation of 2-AB-labelled N-glycans**

The hepatic membrane fraction and aliquots of plasma were dried under reduced pressure, and subjected to hydrazinolysis followed by N-acetylation as described previously [23]. The desalted N-glycans were labelled with 2-AB, as described previously [24]. The reaction mixture was applied to a small AG-50 (H⁺ form) column and eluted with distilled water. The eluate was subjected to FPLC on a fast-desalting HR 10/10 column (Pharmacia Biotech Japan, Tokyo, Japan) using 50 mM pyridine/acetate buffer (pH 5.4), and the oligosaccharide fractions were recovered and freeze-dried.

**Fractionation of 2-AB-labelled N-glycans by HiTrap Q-FPLC and high-pH anion-exchange chromatography (HPAE)**

HiTrap Q-FPLC (Pharmacia Biotech Japan) was performed using an isocratic elution from 0–6 min with 1 mM ammonium acetate buffer, pH 7.0, and a gradient elution from 6–57 min with 1–120 mM ammonium acetate buffer, pH 7.0. HPAEC was performed using a Bio-LC system (Dionex Co., Sunnyvale, CA, U.S.A.) equipped with a CarboPac PA-1 column (4 mm × 250 mm), a pulsed amperometric detector, an ASRS-2 anion micromembrane suppressor (Dionex Co.) and a RF-10AXL fluorescent monitor (Shimadzu Co., Kyoto, Japan) as described previously [25].

**Immobilized lectin column chromatography**

The sample was applied to an SSA–agarose column (1 ml) equilibrated with 50 mM Tris/HCl, pH 7.4. The unbound fraction was eluted with 5 ml of the same buffer, and the bound fraction was eluted with 5 ml of 50 mM Tris/HCl (pH 7.4) containing 0.1 M lactose. The recovered fractions were concentrated and desalted by FPLC on a fast-desalting HR 10/10 column.

**Glycosidase digestion**

Glycans were incubated at 37 °C overnight in 50 μl of one of the following enzyme solutions: neuraminidase from *Sal. typhimurium* LT2 (10 units in 0.05 M sodium citrate buffer, pH 6.0), neuraminidase from *A. ureafaciens* (100 m-units in 0.05 M citrate/phosphate buffer, pH 5.0), *D. pneumoniae* β-galactosidase (10 m-units in 0.05 M citrate/phosphate buffer, pH 6.0), β-galactosidase from *Str. 6646K* (5 m-units in 0.05 M citrate/phosphate buffer, pH 6.0), or β,1,3-galactosidase from *X. manihotis* (10 m-units in 0.05 M citrate/phosphate buffer, pH 4.5). After incubation, the digest was applied to a Nanodex microconcentrator to remove the enzyme, and desalted by passage through a C18 cartridge.

**Matrix-assisted laser-desorption ionization–time-of-flight MS (MALDI–TOF-MS)**

MALDI-TOF-MS was carried out using a Voyager-DE Pro Bio spectrometer (PerSeptive Biosystems) in the linear mode. Samples were prepared by mixing 0.5 μl each of an aqueous solution of glycan and a solution of 2,5-dihydroxybenzoic acid in 50% acetonitrile (10 mg/ml).

**RESULTS**

**N-Glycans of membrane glycoproteins of liver are differently galactosylated in β4Gal-T1-deficient and wild-type mice**

N-Glycans released by hydrazinolysis from hepatic membrane glycoproteins of β4Gal-T1−/− and β4Gal-T1+/+ 9-week-old mice were labelled with 2-AB. The labelled glycans were separated by anion-exchange chromatography using a HiTrap Q column. Acidic oligosaccharides, which amounted to 50%, (β4Gal-T1−/−) and 30% (β4Gal-T1+/+) of the total respectively, were almost all converted into neutral ones by desialylation. The desialylated glycans and their products, obtained by digestion with β-galactosidase from *D. pneumoniae*, which cleaves the Galβ1→4GlcNAc linkage but not the Galβ1→3GlcNAc linkage [26], and with β-galactosidase from *Str. 6646K*, which cleaves both Galβ1→3 and 4GlcNAc linkages [27], were analysed by HPAEC (Figure 1). Elution profiles of digests from β4Gal-T1−/− mice (Figures 1B and 1C) were different from that of the non-digested sample (Figure 1A). A slight difference in the elution profiles was observed between the products obtained by digestion with the two distinct galactosidases. On the other hand, the desialylated glycans from β4Gal-T1−/− mice (Figure 1D) were only slightly susceptible to β1,4-linkage-specific galactosidase (Figure 1E),...
glycans from specificity (Figure 1F). These results indicate that hepatic N-
shown in Figure 2A, the 2-AB-labelled glycans from
but cleavage with β-galactosidase from Str. 6646K had a broad
Sialylation of N-glycans of plasma glycoproteins from β4Gal-T1-deficient mice
Since plasma glycoproteins are synthesized largely by and
acido-glycans: N/A1/A2/A3/A4 = 43:11:25:8:13. Notably, the positions of acidic glycans were different in the samples from β4Gal-T1+/− and β4Gal-T1−/− mice. All acidic fractions of the three samples were neutralized by digestion with neuraminidase from A. ureafaciens (results not shown). The results indicate that sialylated N-glycans with distinct structures are expressed on plasma glycoproteins of β4Gal-T1−/− mice, as is the case with hepatic membrane glycoproteins.

Plasma glycoproteins from β4Gal-T1-deficient mice express a different set of N-glycans
The total glycans were desialylated and then analysed by HPAEC. As shown in Figure 3, the samples from β4Gal-T1+/+ (Figure 3A) and β4Gal-T1−/− mice (Figure 3B) had quite different elution profiles. On the other hand, glycans from β4Gal-T1−/− mice had a similar profile to that of β4Gal-T1−/− mice (results not shown). The elution profiles of other preparations from β4Gal-T1+/− mice were, without exception, similar to those shown in Figure 3B (results not shown). Thus detailed comparative analysis of glycans from β4Gal-T1+/+ and β4Gal-T1−/− mice was performed as described below.

To investigate the detailed features of altered glycosylation, analysis by MALDI–TOF-MS was first performed with desialylated preparations of the total glycans. As shown in Figure 4, the samples from β4Gal-T1+/+ (Figure 4A) and β4Gal-T1−/− (Figure 4B) mice had different spectra. The mass spectra of the major fractions separated by HPAEC [peaks a–d in Figure 3(A) and e–k in Figure 3(B)] were also measured, and the results are...
Figure 3 Analysis of neutral and asialo N-glycans of plasma glycoproteins

A mixture of neutral and asialo N-glycans were analysed by HPAEC. Arrows and numbers indicate elution times of reduced glucose oligomers (internal standards) and their glucose units respectively. (A) β4Gal-T1+/+ mice, (B) β4Gal-T1−/− mice. Fractions a–k were collected at the elution times shown by horizontal bars beneath the peaks.

summarized in Table 1. The samples from β4Gal-T1+/+ mice yielded signals at m/z 1785 (peaks a and c) and m/z 2150 (peaks b–d), which correspond to fully galactosylated bi- and tri-antennary glycans respectively. Samples from β4Gal-T1−/− mice yielded signals at m/z 1460 (Figure 3B, peak f), m/z 1826 (peak i) and m/z 1988 (peak k), corresponding to non-, mono- and di-galactosylated triantennary glycans respectively. It should be noted that there was no correlation between the elution positions of fractions shown in Figure 3 and their molecular masses derived by MS. A typical example is that peaks a, c, h and j all have the same molecular masses, corresponding to biantennary glycan, but their elution positions are different (Figure 3). Thus it is suggested that these are isomers, possibly containing different galactosyl linkages.

Galactose residues are predominantly β1,4-linked to GlcNAc of N-glycans from wild-type mice, but β1,3-linked to GlcNAc of those from knockout mice

Galactosyl linkages of glycans from the β4Gal-T1+/+ mice (Figure 3A) and those from the knockout mice (Figure 3B) were analysed by HPAEC before and after β-galactosidase digestion, similarly to those shown in Figure 1. In Figure 3, peaks a and b were susceptible to D. pneumoniae β-galactosidase, which was specific for Galβ1→4 GlcNAc linkages, and the products were eluted at positions of degalactosylated bi- and 2,6-branched triantennary glycans respectively. Peaks c (7.8 glucose units) and d (8.7 glucose units), shown in Figure 3, were susceptible to D. pneumoniae β-galactosidase, and the products were eluted at similar times to peaks g (7.4 glucose units) and i (7.9 glucose units) respectively. Under the chromatographic condition used, one residue of β1,4-linked galactose corresponds to approx. 0.4 glucose units [25]. Therefore this result suggests that one residue of galactose was removed from peak c and two from peak d. These products required further digestion with β-galactosidase from Str. 6646K, with a broad specificity, or recombinant β1,3-galactosidase [28] to be converted completely into degalactosylated bi- and tri-antennary glycans. As summarized in Table 1, these results suggest that peaks a and b (77%) contain only type 2 chains, and peaks c and d (23%) contain both type 1 (Galβ1→3GlcNAc) and type 2 chains. By contrast, peaks g–k, with the exception of peak h, were resistant to D. pneumoniae β-galactosidase but were completely degalactosylated by β-galactosidase from Str. 6646K or β1,3-galactosidase. Peak h was digested similarly to peak c. Thus the results indicate that most of galactosylated glycans from the knockout mice contain only type 1 chains and that the minor peak h contains both type 1 and 2 chains (Table 1).

Figure 4 Mass spectra of neutral and asialo N-glycans of plasma glycoproteins

A mixture of neutral and asialo N-glycans was subjected to MALDI–TOF-MS analysis. (A) β4Gal-T1+/+ mice, (B) β4Gal-T1−/− mice. Molecular masses are indicated above the relevant peaks.
The predominant expression of type 1 chain in the acidic glycans of plasma glycoproteins from wild-type and knockout mice are differently sialylated.

Table 1 Mass spectrometric data of neutral and desialylated oligosaccharides

<table>
<thead>
<tr>
<th>Peak</th>
<th>Mass found (as M+Na)^+</th>
<th>Calculated [M+Na]^+</th>
<th>Composition</th>
<th>Gal → GlcNAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (68)</td>
<td>1784.5</td>
<td>1784.7</td>
<td>5 4</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>b (9)</td>
<td>2149.7</td>
<td>2150.0</td>
<td>6 5</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>c (17)</td>
<td>1784.8</td>
<td>1784.7</td>
<td>5 4</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>d (6)</td>
<td>2149.5</td>
<td>2150.0</td>
<td>6 5</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>e (25)</td>
<td>1460.4</td>
<td>1460.4</td>
<td>3 4</td>
<td>GlcNAc,R</td>
</tr>
<tr>
<td>f (8)</td>
<td>1664.2</td>
<td>1663.6</td>
<td>3 5</td>
<td>GlcNAc,R</td>
</tr>
<tr>
<td>g (31)</td>
<td>1622.6</td>
<td>1622.6</td>
<td>4 4</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>h (6)</td>
<td>1784.9</td>
<td>1784.7</td>
<td>5 4</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>i (7)</td>
<td>1826.3</td>
<td>1825.8</td>
<td>4 5</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>j (18)</td>
<td>1785.1</td>
<td>1784.7</td>
<td>5 4</td>
<td>Gal,GlcNAc,R</td>
</tr>
<tr>
<td>k (5)</td>
<td>1988.3</td>
<td>1987.9</td>
<td>5 5</td>
<td>Gal,GlcNAc,R</td>
</tr>
</tbody>
</table>

* A trace of signal at m/z 1826.1 was detected.

Table 2 Mass spectrometric data of acidic oligosaccharides

The 2-AB-labelled acidic oligosaccharides from fractions A1–A4, shown in Figure 2 were analysed by MALDI–TOF-MS in negative ion mode. Signals detected as [M-H]^- ions are shown. The average molecular masses were calculated from the probable carbohydrate composition plus 2-AB. Structures are proposed on the basis of the mass and composition of known glycans; -R indicates the trimannosyl core, Man3,GlcNAc-GlcNAc-2-AB. Presence (+) or absence (−) of type 1 (Galβ1→3GlcNAc) and type 2 chains (Galβ1→4 GlcNAc) was based on susceptibility to D. pneumoniae galactosidase (see the text). Abbreviations: H, hexose (galactose or mannose); N, N-acetylgalactosamine (N-acetylgalactosamine).

(a) βGal-T1+/−

<table>
<thead>
<tr>
<th>Peak</th>
<th>Mass found ([M−H]−)</th>
<th>[M−H]− calculated</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1890.6*</td>
<td>1889.9</td>
<td>4 4 1</td>
</tr>
<tr>
<td>A2</td>
<td>2343.3</td>
<td>2343.2</td>
<td>5 4 2</td>
</tr>
<tr>
<td>A3</td>
<td>3000.3</td>
<td>2999.8</td>
<td>6 5 3</td>
</tr>
<tr>
<td>A4</td>
<td>2634.0</td>
<td>2634.5</td>
<td>5 4 3</td>
</tr>
</tbody>
</table>

* A trace of signal was detected.

(b) βGal-T1−−

<table>
<thead>
<tr>
<th>Peak</th>
<th>Mass found ([M−H]−)</th>
<th>[M−H]− calculated</th>
<th>Composition</th>
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</thead>
<tbody>
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<td>A1</td>
<td>1889.4</td>
<td>1889.9</td>
<td>4 4 1</td>
</tr>
<tr>
<td>A2</td>
<td>2180.9</td>
<td>2181.1</td>
<td>4 4 2</td>
</tr>
<tr>
<td>A3</td>
<td>2384.6</td>
<td>2384.3</td>
<td>4 5 3</td>
</tr>
<tr>
<td>A4</td>
<td>2837.9</td>
<td>2837.7</td>
<td>5 5 3</td>
</tr>
</tbody>
</table>

N-Glycans of plasma glycoproteins from wild-type and knockout mice are differently sialylated

The predominant expression of type 1 chain in the acidic glycans from β4Gal-T1−−/− mice suggests the occurrence of an over-sialylated type 1 chain, Neu5Acα2→3Galβ1→3 (Neu5Acα2→6) GlcNAc. Thus we analysed acidic glycans by MALDI–TOF-MS in the negative-ion mode. As shown in Table 2, signals corresponding to monosialyl biantennary (m/z 2053), disialyl biantennary (m/z 2343) and trisialyl triantennary (m/z 3000) glycans were detected in fractions A1, A2 and A3 of the β4Gal-T1−−/− mouse glycans respectively (see Figure 2). Signals that corresponded to trisialyl biantennary (m/z 2634) and tetrasialyl triantennary (m/z 3000) glycans were also detected in fractions A3 and A4. Glycans from β4Gal-T1−−/− mice yielded several unique signals that corresponded to biantennary glycans containing three and four N-acetylenameric acid residues (m/z 2634 and 2925), monogalactosyl biantennary glycans containing two N-acetylenameric acid residues (m/z 2181), monogalactosyl triantennary glycans containing two N-acetyl-
neuraminic acid residues (m/z 2385), and digalactosyl triantennary glycan containing three and four N-acetyleneuraminic acid residues (m/z 2838 and 3129). Thus most of the knockout mouse glycans contained more sialic acid residues than galactose residues. These results support the predicted occurrence of disialylated type 1 chains in the glycans from $\beta 4$Gal-T1−/− mice.

To analyse the sialyl linkages of glycans, each of the total acidic oligosaccharide fractions from $\beta 4$Gal-T1−/− and $\beta 4$Gal-T1+/+ mice was subjected to affinity chromatography using a SSA–agarose column, which has been shown to bind sialylated glycans with the Neu5Ac2→6Gal(GalNAc) group but not those with the Neu5Ac2→3Gal group [29]. A striking contrast in the binding properties of the fractions was observed in that 85% of the acidic glycans from $\beta 4$Gal-T1−/− mice bound to the column, whereas 95% of those from $\beta 4$Gal-T1+/+ mice did not bind. Under the conditions used, authentic monosialyl biantennary glycans containing Neu5Ac2→6Gal1→4GlcNAc bound to the column; therefore, it is suggested that the major N-glycans from $\beta 4$Gal-T1−/− express the Neu5Ac2→6Gal group, but those from $\beta 4$Gal-T1+/+ mice do not. The major unbound fraction from $\beta 4$Gal-T1−/− mice consisted of four acidic fractions A1–A4 (Figure 5A). By digestion with α2,3-neuraminidase from Sal. typhimurium [30], one residue of sialic acid was removed from each of A1 and A2, and two residues were removed from each of fractions A3 and A4 (Figures 5B–5E). These results indicate that the major acidic glycans from $\beta 4$Gal-T1−/− mice contain one or two Neu5Ac2→3Gal group. On the other hand, the major SSA-bound fraction of $\beta 4$Gal-T1+/+ mice consisted of four acidic fractions (A1/A2/A3/A4 = 9.66:22:3), and its product, obtained by digestion with α2,3-neuraminidase, was separated into two acidic fractions A1 and A2 in a ratio of 36:64 by HiTrap Q-FPLC (results not shown). Thus acidic glycans from $\beta 4$Gal-T1+/+ mice contain one or two α2,6-linked sialic acid residues and some of them contain extra α2,3-linked sialic acid(s).
→ 3GlcNAc-R, has been shown to occur in the Golgi apparatus [35] and microsomes [36] of rat liver. Based on the known acceptor specificities of these sialytransferases, it has been proposed that the galactose residue of the type 1 chain is first \(\alpha_2,3\)-sialylated and then the GlcNAc residue is \(\alpha_2,6\)-sialylated, resulting in the formation of oversialylated type 1 chain. Therefore, it is suggested that a similar mechanism for sialylation of N-glycans is present in the liver of \(\beta_4\)Gal-T1\(^{-/-}\) mice (Scheme 1).

Previous studies have shown that reduced \(\beta_1,4\)-galactosylation occurs in many tissues [5,6], but that of brain glycoproteins does not change significantly in \(\beta_4\)Gal-T1\(^{-/-}\) mouse [7]. N-glycans of erythrocyte glycoproteins from \(\beta_4\)Gal-T1\(^{-/-}\) mice are considerably galactosylated in \(\beta_1,4\)-linkage, whereas galactosylation of core 2-O-glycans is severely impaired [8]. In the present study, we show that deficiency of the \(\beta_4\)Gal-T1 gene causes a dramatic shift from \(\beta_1,4\)- to \(\beta_1,3\)-galactosylation of N-glycans of hepatic membrane and plasma glycoproteins, which leads to altered sialylation. These variable effects of \(\beta_1\)-T1 deficiency on terminal glycosylation of glycans must be considered in relation to the tissue distribution of glycosyltransferases. It has been shown, so far, that \(\beta_4\)Gal-T1 [37], other \(\beta_4\)Gal-Ts (\(-T2, -T3\) and \(-T5\)) [16] and sialyltransferases [38] are differently expressed in various mouse tissues. Transcripts of three members of a mouse \(\beta_3\)Gal-T family cloned recently are mainly detected in brain tissue and at low levels in other tissues [22]. Thus each tissue seems to express a distinct set of glycosyltransferases responsible for the synthesis of terminal structures of glycans. This might account for the tissue-dependent change in glycosylation by knockout of the \(\beta_4\)Gal-T1 gene. Considering that both backbone structures, Gal\(\beta_1\) → 3GlcNAc and Gal\(\beta_1\) → 4GlcNAc, are widely found in N- and O-glycans, the altered galactosylation, with or without changes in sialylation, is also likely to take place in other tissues of \(\beta_4\)Gal-T1\(^{-/-}\) mice. Further analysis of glycosylation in various tissues of Gal-T1\(^{-/-}\) mice at structural and enzymic levels will unveil the mechanism which may regulate the synthesis and modification of the two types of backbone structure in a tissue-dependent manner. Such an approach will also aid the understanding, on a molecular basis, of abnormal and apparently normal phenotypes found in the knockout mice [5,6]. Because of the expression of distinct sialyl linkages in \(\beta_4\)Gal-T1\(^{-/-}\), these mice may serve as valid tools for the analysis of sialic acid recognition phenomena, which mediate interactions between some pathogens and host cells or between certain types of cells [39].

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