Postnatal changes in sialylation of glycoproteins in rat liver

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Glycoproteins containing *N*-linked oligosaccharides were prepared from plasma and liver microsomes of rats aged 0–5 weeks, and galactose and sialic acid content were determined. The sialic acid/galactose ratios in plasma membrane *N*-glycans remained at about 1 throughout the postnatal period, suggesting that most of the galactose residues are sialylated. In the same way, it was suggested that most of the galactose residues of microsomal *N*-glycans were sialylated at 0, 4 and 5 weeks of age, but that the degree of sialylation was lower at the other ages, with a minimum at 2 weeks. When the activities of sialyltransferase and galactosyltransferase in liver Golgi membranes were determined, age-dependent changes were found, not only in the specific activities of the enzymes, but also in the Golgi membrane content per g of liver. The activity of galactosyltransferase per g of liver increased immediately after birth, whereas that of sialyltransferase remained at a low level for 2 weeks and then increased to a constant level at 4 weeks. It is probable that this delayed increase in the activity of sialyltransferase results in the decreased sialylation of microsomal *N*-glycans at 1, 2 and 3 weeks. Sialyltransferase was solubilized from the liver microsomes of rats aged 2, 3 and 4 weeks and characterized. Phosphocellulose column chromatography separated the activity into two subfractions, designated transferase I and transferase II in the order of elution. The increase in total sialyltransferase activity during this period was caused mainly by an increase in transferase I. Rechromatography of each transferase from 3-week-old rats after neuraminidase treatment showed that transferase I but not transferase II contained sialic acid residue(s) and that desialylated transferase I was eluted in a similar way as transferase II. Although the apparent K_m value for CMP-*N*-acetylneuraminic acid and the heat stability of transferase I were different from those of transferase II, the difference was abolished by treating transferase I with neuraminidase, suggesting that transferase II may be a desialylated form of transferase I. These changes in the sialylation of membrane glycoproteins, including sialyltransferase, may be related to the control of liver growth during postnatal development.

INTRODUCTION

Sialic acid residues occupying terminal positions in *N*-linked oligosaccharides of glycoproteins have been shown to play important roles in a variety of biological functions [1], including the anti-recognition effect, i.e. protection of serum glycoproteins from clearance through the hepatic asialoglycoprotein receptor [2]. Increases in the sialic acid content of cell surface glycoproteins are often accompanied by malignant transformation [3]. A postnatal decrease in the sialic acid content of microsomal and plasma membrane glycoproteins has been shown in rabbit skeletal muscle [4] and in the microvilli of rat small intestine [5]. Thus the alteration in sialic acid content in membrane glycoproteins appears to be related to cellular growth and development.

On labelling liver slices with [^3H]mannose, we have previously found that the glycopeptides from livers of 2-week-old rats were less sialylated than those from livers of neonatal and 5-week-old rats, but that glycopeptides in the secretions from the liver slices were not changed [6]. In the present paper we have studied the changes in the sialylation of glycoproteins and the enzymic basis for these changes. Our results show consistent age-dependent changes in the activities of liver sialyltransferase and galactosyltransferase and in the sialylation of microsomal glycoproteins, including sialyltransferase itself.

MATERIALS AND METHODS

Chemicals

UDP-galactose, CMP-*N*-acetylneuraminic acid, human transferrin, ovomucoid (trypsin inhibitor, Type III-O), asialofetuin (Type I), β -galactose dehydrogenase (EC 1.1.1.48) from

Pseudomonas fluorescens and BSA (fraction V) were purchased from Sigma, St. Louis, MO, U.S.A. Neuraminidase (EC 3.2.1.18) from *Arthrobacter ureafaciens* was from Nacalaitesque, Kyoto, Japan. UDP-[U- ^{14}C]galactose (297 mCi/mmol) and CMP-*N*-acetyl[U- ^{14}C]neuraminic acid (351 mCi/mmol) were purchased from Amersham International, Amersham, Bucks., U.K., and were diluted with UDP-galactose and CMP-*N*-acetylneuraminic acid to give specific radioactivities of 1.0 and 1.5 mCi/mmol respectively. Asialotransferrin was prepared from human transferrin by mild acid hydrolysis, as described by Spiro [7]. Phosphocellulose was purchased from Whatman, Maidstone, Kent, U.K., and Sephadex G-25 columns (PD-10) were from Pharmacia, Uppsala, Sweden.

Animals

Pregnant Wistar rats (Nihon Rat Co., Urawa, Japan) were fed *ad libitum* with laboratory chow in a room at a constant temperature (23.5 °C) with 12 h each of light (06:30–18:30 h) and darkness. Neonatal rats within 15 h post-partum, rats aged 1 and 2 weeks taken from the mother regardless of sex, and male Wistar rats aged 3, 4 and 5 weeks from different litters (Nihon Rat Co.) were killed by decapitation between 09:00 and 10:30 h.

Determination of galactose and sialic acid linked to *N*-glycans in plasma and liver microsomes

Plasma and liver microsomes were prepared from rats as described previously [8]. The sample (about 10 mg of protein) was mixed with 10 vol. of ice-cold 10% (w/v) trichloroacetic acid, kept on ice for 30 min and centrifuged at 1000 *g* for 10 min at 4 °C. The precipitate was washed with 2 × 4 ml of ice-cold 10% trichloroacetic acid, and then successively with 4 ml each of

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ethanol, ethanol/diethyl ether (1:1, v/v) and ether. From the resulting precipitate, an alkali-stable and trichloroacetic acid-precipitable fraction of glycoproteins containing *N*-linked oligosaccharides was prepared [9]. Sialic acid was released by mild acid hydrolysis and determined as described previously [10]. The residue obtained after removing sialic acid was heated in a sealed tube at 100 °C for 6 h with 5 ml of 0.5 M-H₂SO₄ and then cooled to room temperature. After centrifugation at 1000 *g* for 10 min, the supernatant was neutralized with 5.0 M-NaOH, and galactose content was determined by the galactose dehydrogenase method [11].

Preparation of Golgi membranes

Golgi membranes were prepared at 4 °C from liver microsomes essentially as described by Rothman & Fries [12]. The microsomes were suspended with 3 strokes of a Teflon-glass homogenizer (500 rev./min) in 0.25 M-sucrose/TMK buffer (50 mM-Tris/HCl, pH 7.4, 5 mM-MgCl₂ and 25 mM-KCl) to give a protein concentration which is twice that of the postmitochondrial supernatant. After adding 2.0 M-sucrose in TMK buffer to the suspension to adjust the sucrose concentration to 1.25 M, a 3.5 ml sample was overlaid in a Hitachi 40T ultracentrifuge tube successively with 2.8 ml each of 1.05 M-, 1.0 M- and 0.25 M-sucrose in TMK buffer. After centrifugation at 150 000 *g* for 90 min, the band of Golgi membranes at the 0.25 M/1.0 M-sucrose interface was collected and suspended in 0.25 M-sucrose in TMK buffer. The suspension was centrifuged at 180 000 *g* for 45 min, and the pellet was suspended again in 0.25 M-sucrose in TMK buffer to give a protein concentration of about 5 mg/ml.

Assays of enzymes

The activities of glucose-6-phosphatase (EC 3.1.3.9) and 5'-nucleotidase (EC 3.1.3.5) were assayed by the methods of Aronson & Touster [13] and Heppel & Hilmoie [14] respectively.

Galactosyltransferase activity was assayed essentially by the method of Franc *et al.* [15]. The standard assay mixture (50 µl) contained 0.1 M-Mes/NaOH, pH 6.3, 20 mM-MnCl₂, 0.5 % (w/v) Triton X-100, 1 mM-UDP-[¹⁴C]galactose and the membrane fraction (about 10 µg of protein). Incubation was at 37 °C for 30 min in the presence or the absence of 2 mg of ovomucoid. The reaction was stopped by adding 1 ml of ice-cold 1 % (w/v) phosphotungstic acid in 0.5 M-HCl; BSA (2 mg) was added to the mixture as a carrier protein. The tube was kept in an ice bath for 30 min and then centrifuged at 1000 *g* for 10 min at 4 °C. The pellet was washed with 2 × 1 ml of ice-cold 1 % (w/v) phosphotungstic acid in 0.5 M-HCl. The radioactivity in the pellet was measured as described previously [16]. The difference between the radioactivity recovered in the pellet in the presence of ovomucoid and that in its absence was used for the calculation of the enzyme activity. The enzyme activity was linear with incubation time up to 45 min. One unit of enzyme activity was defined as the amount which catalysed the transfer of 1 nmol of galactose/h.

Sialyltransferase activity was assayed as described by Schachter *et al.* [17]. The standard assay mixture (50 µl) contained 0.1 M-Mes/NaOH, pH 6.3, 4 mM-CMP-*N*-acetyl[¹⁴C]neuraminic acid, 0.2 mg of BSA (pretreated at 65 °C for 15 min), 0.5 % (w/v) Triton X-100 and the membrane fraction (about 10 µg of protein). Incubation was at 37 °C for 30 min in the presence or absence of 2 mg of asialotransferrin or asialofetuin. Thereafter, the reaction mixture was processed in the same way as in the galactosyltransferase assay. The difference between the radioactivity recovered in the pellet in the presence of asialoglycoprotein and that in its absence was used for the calculation of the enzyme activity. The enzyme activities toward asialotransferrin and

asialofetuin were linear with time up to 60 min and 30 min respectively. One unit of enzyme activity was defined as the amount which catalysed the transfer of 1 nmol of sialic acid/h.

Solubilization and phosphocellulose column chromatography of sialyltransferase

Sialyltransferase was solubilized from liver microsomes as described by Miyagi & Tsuiki [18]. All of the procedures were done at 4 °C. Microsomes obtained as a pellet (150 mg of protein) were suspended with 3 strokes of a Teflon-glass homogenizer (500 rev./min) in 10 ml of 10 mM-potassium phosphate buffer, pH 6.8, containing 25 mM-KCl, 1 mM-EDTA, 0.25 M-sucrose and 2 % (w/v) Triton X-100, and were stirred for 30 min. After centrifugation at 105 000 *g* for 60 min, about 80 % of the activity was recovered in the supernatant. Ammonium sulphate was gradually added to the supernatant, with stirring, to 50 % saturation. Then the mixture was allowed to stand for 30 min and centrifuged at 15 000 *g* for 20 min. The precipitate and the floating pellicle were washed with 2 × 10 ml of ammonium sulphate (50 % satn.) in 50 mM-potassium phosphate buffer, pH 6.8, containing 1 mM-EDTA (referred to as 'standard buffer'). The resulting pellet was dissolved in 3 ml of standard buffer. Ammonium sulphate was removed by passing the enzyme solution through a Sephadex G-25 column (PD-10) equilibrated with standard buffer. The excluded fraction containing 8–10 mg of protein was applied to a phosphocellulose column (1.0 cm × 4.5 cm) equilibrated with standard buffer. The column was washed with 20 ml of standard buffer and eluted with a linear gradient of 0–0.3 M-NaCl in 50 ml of standard buffer. The flow rate was 20 ml/h and 2 ml fractions were collected.

Preparation of two subfractions of sialyltransferase

Sialyltransferase was solubilized from liver microsomes of 3-week-old rats, precipitated with ammonium sulphate and dissolved again in standard buffer on a 2-fold scaled up version of the procedure described above. After removing ammonium sulphate by gel filtration, the enzyme fraction was divided into four aliquots and each was separately subjected to phosphocellulose column chromatography as described above. Fractions 4–10 and fractions 12–19 (as shown in Fig. 4b) were respectively pooled and concentrated at 4 °C to 2 ml by ultrafiltration on an Amicon ultrafiltration unit using a PM-10 filter (Amicon Corp., Danvers, MA, U.S.A.). NaCl was removed by passing each enzyme concentrate through a PD-10 column equilibrated with standard buffer. The excluded fractions obtained from fractions 4–10 and from fractions 12–19 of phosphocellulose chromatography were designated transferase I and transferase II respectively, and were used for analyses.

Determination of proteins

Protein in insoluble fractions were determined by the method of Lowry *et al.* [19], with BSA as a standard. Protein in soluble fractions was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA, U.S.A.).

Neuraminidase digestion of sialyltransferase

A portion of enzyme solution (1.2 mg of protein) was adjusted to pH 5.5 with 0.2 M-KH₂PO₄ and incubated at 37 °C for 40 min with 0.4 unit of neuraminidase in a final volume of 2 ml. The mixture was chilled on ice and was adjusted to pH 6.8 with ice-cold 0.2 M-K₂HPO₄, mixed with 10 mM-EDTA and diluted with ice-cold distilled water to give final concentrations of phosphate and EDTA of 50 mM and 1 mM respectively.

Table 1. Contents of galactose and sialic acid linked to *N*-glycans in plasma and liver microsomes

Glycoproteins containing *N*-linked oligosaccharides were obtained from plasma and liver microsomes of 3–12 rats at different ages as indicated, and galactose and sialic acid were measured as described in the Materials and methods section. The contents of galactose and sialic acid are shown as nmol/mg of protein in both plasma and liver microsomes. Each value is the average of the values obtained from three separate experiments ($\pm$ S.E.M.).

Source	Content (nmol/mg)		Ratio <i>a/b</i>
	Sialic acid (<i>a</i>)	Galactose (<i>b</i>)	
Plasma			
Neonatal	13.8±1.14	11.9±3.38	1.16
1-week-old	15.1±0.43	14.9±1.42	1.01
2-week-old	14.2±1.15	16.3±1.14	0.87
3-week-old	19.3±0.74	17.0±1.77	1.13
4-week-old	21.3±0.12	20.0±2.10	1.07
5-week-old	21.9±0.70	25.0±3.55	0.88
Liver microsomes			
Neonatal	0.85±0.34	0.68±0.22	1.25
1-week-old	0.80±0.16	1.59±0.15	0.50
2-week-old	0.78±0.13	4.50±0.30	0.17
3-week-old	2.05±0.19	4.18±0.80	0.49
4-week-old	2.36±0.24	2.90±0.18	0.81
5-week-old	2.27±0.15	2.35±0.50	0.97

RESULTS

Postnatal changes in contents of galactose and sialic acid linked to *N*-glycans in plasma and liver microsomes

In order to evaluate the degree of sialylation of *N*-glycans in the liver, we measured the contents of galactose and sialic acid linked to *N*-glycans in plasma and liver microsomes. As shown in Table 1, the contents of both sialic acid and galactose linked to *N*-glycans in plasma increased after birth with similar patterns. The sialic acid/galactose ratio remained at around 1 after birth, suggesting that most of the galactose residues of plasma *N*-glycans are sialylated throughout the postnatal period. The content of galactose linked to *N*-glycans in liver microsomes

Fig. 1. Postnatal changes in Golgi membrane content in rat liver

Golgi membranes were prepared from 3–12 rats aged 0–5 weeks as described in the Materials and methods section. The recoveries in Golgi membranes of the total liver activities of galactosyltransferase, 5'-nucleotidase and glucose-6-phosphatase were 70, 1.8 and 0.6 % respectively. The specific activity of galactosyltransferase in Golgi membranes was about 60-fold that in the total homogenate. These values did not change with the age of the rats. Thus the Golgi membranes obtained are almost free from plasma membrane and endoplasmic reticulum contamination. Values represent means $\pm$ S.E.M. of three separate experiments.

increased to a maximum at 2 weeks postnatally, and then decreased. On the other hand, the sialic acid content remained at a low level for 2 weeks and then increased up to a constant level at 4 weeks. The sialic acid/galactose ratio in liver microsomes was about 1 at birth, decreased to a minimum at 2 weeks and returned to about 1 at 4 weeks.

As polysialylated galactose residues have never been found in *N*-linked oligosaccharides of glycoproteins [20], a sialic acid/galactose ratio of less than 1 suggests the presence of non-sialylated galactose residues and/or polylactosamine sequences. However, the latter has never been shown in rat liver glycoproteins. Furthermore, we did not detect any high-molecular-

Fig. 2. Postnatal changes in the specific activity (a) and activity relative to liver weight (b) of galactosyltransferase

The assays were done using liver Golgi membranes from 3–12 rats aged 0–5 weeks as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of three separate experiments. The activity per g wet wt. of liver was calculated using the values shown in Figs. 1 and 2(a).

Fig. 3. Postnatal changes in the specific activity (a) and activity relative to liver weight (b) of sialyltransferase toward asialotransferrin

The assays were done using liver Golgi membranes from 3–12 rats aged 0–5 weeks as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of three separate experiments. The activity per g wet weight of liver was calculated using the values shown in Figs. 1 and 3(a).

mass glycopeptides, that may contain a poly-lactosamine sequence, in rat liver throughout the postnatal period [6]. Therefore it is suggested that most of the galactose residues in microsomal *N*-glycans were sialylated at birth and at 4 and 5 weeks, and that the degree of sialylation was lower at the other ages, with a minimum at 2 weeks. These age-dependent changes in the sialylation of *N*-glycans in liver microsomes, but not plasma, are consistent with and confirm our previous findings [6].

Postnatal changes in the activities of galactosyltransferase and sialyltransferase

For the determination of the activities of galactosyltransferase and sialyltransferase, the Golgi membranes from livers of rats at different ages were used, since it is well established that these enzymes are located mainly in the Golgi apparatus [20]. The content of Golgi membranes per g wet weight of liver showed an age-dependent change (Fig. 1), whereas total protein per g wet weight of liver did not (results not shown). These results agree well with the electron microscopic observation that the volume of Golgi apparatus in rat hepatocytes doubled between 3 and 5 weeks post-partum [21]. It has been reported that an increase in Golgi membranes after injection of turpentine resulted in a 2-fold enhancement of the activities of the glycosyltransferases per g wet weight of liver, without changing their specific activities [22]. Therefore, in view of the age-dependent change in the content of Golgi membranes, the activities of galactosyltransferase and sialyltransferase were compared in terms of activity/g of liver as well as specific activity.

As shown in Fig. 2(a), the specific activity of galactosyltransferase began to increase immediately after birth. After reaching a maximum at 2 weeks, it decreased and levelled off at 4 weeks. The galactosyltransferase activity/g wet weight of liver increased until 2 weeks, like the specific activity. However, it did not decline thereafter but increased gradually (Fig. 2b) due to the increased content of Golgi membranes (Fig. 1).

The specific activity of sialyltransferase toward asialotransferrin, which contains two *N*-linked oligosaccharides but lacks *O*-linked oligosaccharides [23,24], remained constant for 2 weeks post-partum, and then increased to a constant level at 4 weeks (Fig. 3a). The enzyme activity/g wet weight of liver showed a similar pattern, except that the increase after 2 weeks was very large (Fig. 3b) due to the increases in both the specific activity

(Fig. 3a) and the content of Golgi membranes (Fig. 1). Sialyltransferase activity/g of liver did not increase before 2 weeks post-partum, whereas galactosyltransferase activity/g of liver increased immediately after birth. This delay in the increase in sialyltransferase activity may result in the decreased sialylation of microsomal *N*-glycans at 1, 2 and 3 weeks. Therefore we examined whether the properties of sialyltransferase change depending on the age of the rats or not.

Separation of solubilized sialyltransferase from liver microsomes of 2-, 3- and 4-week-old rats

Sialyltransferase which acts on asialofetuin in the membrane fraction of adult rat livers consists of immunologically indistinguishable sialylated and non-sialylated forms which can be separated by phosphocellulose column chromatography [18]. Therefore we examined possible changes in the composition of rat liver sialyltransferase during the postnatal period. Since the total activity of sialyltransferase in liver microsomes towards asialotransferrin and asialofetuin was similar to that in the Golgi membranes, the microsomes were used as the enzyme source. In this series of experiments, the enzyme activity was assayed using asialofetuin as an acceptor, as previously described [18]. Though fetuin contains three *N*-linked and three *O*-linked oligosaccharides, approx. 75% of galactose residues are bound to the *N*-linked oligosaccharides [25–27]. Indeed, the activity towards asialofetuin was consistently about 10 times that towards asialotransferrin at every age (results not shown).

Phosphocellulose column chromatography of sialyltransferase solubilized from microsomes separated the activity into two subfractions, designated transferase I and transferase II in order of elution (Fig. 4). Both transferase I and transferase II appeared to consist of at least two components. As is evident in Fig. 4(b), transferase I contained a minor component which was eluted as a shoulder at lower ionic strength. A component of transferase I which eluted with 0.1 M-NaCl became predominant with increasing age. A compartment of transferase II which was eluted with NaCl at a higher concentration than 0.2 M decreased with age and was barely detectable at 4 weeks, whereas that eluted at lower ionic strength did not change markedly. Sialyltransferase activity recovered in the two subfractions (transferase I and transferase II) are shown in Table 2. The activity recovered as transferase I at 4 weeks was 18 times that at 2 weeks, whereas the

Fig. 4. Phosphocellulose column chromatography of solubilized sialyltransferase from liver microsomes of 2-week-old (a), 3-week-old (b) and 4-week-old (c) rats

Sialyltransferase was solubilized from the liver microsomes of 3–10 rats aged 2–4 weeks and chromatographed on a phosphocellulose column as described in the Materials and methods section. After elution with a linear gradient of NaCl (---), the activity of sialyltransferase (●) was determined with asialofetuin as an acceptor using 30 μ l of each fraction. Recovery of the activity from the column was about 90% in all cases.

Table 2. Age-dependent changes in the composition of sialyltransferase

Sialyltransferase was solubilized from liver microsomes of rats at the ages indicated and separated into two subfractions, designated as transferase I and transferase II, by phosphocellulose column chromatography as described in the Materials and methods section. Enzyme activity was determined using asialofetuin as an acceptor. The activity recovered in each subfraction was calculated from the values in Fig. 4 and the contents of microsomal protein per g of liver. The values in parentheses are relative to the 2-week-old value as 1.00.

Age of rats (weeks)	Activity (units/g wet wt. of liver)		
	Transferase I	Transferase II	I/(I + II)
2	47.1 (1.00)	214.5 (1.00)	0.18
3	241.7 (5.13)	241.7 (1.13)	0.50
4	830.2 (17.63)	373.0 (1.74)	0.69

activity recovered as transferase II was only 2-fold greater. Transferase I activity was only 18% of the total at 2 weeks, but was 69% at 4 weeks. These results suggest that transferase I is responsible for the regulation of sialyltransferase activity during development.

Properties of two sialyltransferases separated by phosphocellulose column chromatography

Transferase I and transferase II prepared from liver microsomes of 3-week-old rats were further characterized. A half

Fig. 5. Chromatography on a phosphocellulose column of sialyltransferase subfractions before and after neuraminidase treatment

Transferase I (a) and transferase II (b) were prepared from livers of 3-week-old rats and portions were digested with neuraminidase as described in the Materials and methods section. After phosphocellulose column chromatography, the activity of sialyltransferase toward asialofetuin was determined using 30 μ l of each fraction. ●, Before neuraminidase treatment; ○, after neuraminidase treatment.

Fig. 6. Heat stability of transferase I, transferase II and desialylated transferase I

Transferase I and transferase II were obtained from liver microsomes of 3-week-old rats as described in the Materials and methods section. A portion of transferase I was digested with neuraminidase and used as desialylated transferase I. Transferase I (●, 0.68 mg of protein/ml), transferase II (○, 0.81 mg of protein/ml) and desialylated transferase I (△, 0.36 mg of protein/ml) were incubated at 50 °C in 300 μ l of standard buffer. At the indicated time points, 5, 5 and 10 μ l of the incubation mixtures containing transferase I, transferase II and desialylated transferase I respectively were taken for assay of enzyme activity toward asialofetuin. Initial specific activities of transferase I, transferase II and desialylated transferase I were 580, 490 and 550 units/mg of protein respectively.

portion of each transferase was rechromatographed on a phosphocellulose column before and after neuraminidase treatment. As shown in Fig. 5(a), the sample of transferase I contained another activity which was eluted at about the same position as transferase II, indicating a conversion of transferase I by some unknown mechanism. However, the majority of transferase I activity was eluted at about the same position as transferase II after neuraminidase treatment. On the other hand, neuraminidase treatment did not affect the elution profile of transferase II (Fig. 5b). These results indicate the presence of sialic acid residue(s)

in transferase I but not in transferase II. Apparent K_m values for CMP-*N*-acetylneuraminic acid of transferase II and desialylated transferase I were the same (125 μM), whereas transferase I showed a lower K_m value (42 μM). After incubation at 50 °C for 60 min at pH 6.8, transferase I lost 43 % of its original activity, while desialylated transferase I and transferase II did not lose their activities at all (Fig. 6). It seems that transferase I became heat-stable after the removal of sialic acid residues. Overall, desialylated transferase I showed similar properties to transferase II, suggesting that transferase II is a desialylated form of transferase I, as indicated by Miyagi & Tsuiki [18].

DISCUSSION

We studied the postnatal changes in the sialylation of *N*-glycans by determining the contents of sialic acid and galactose linked to *N*-glycans in plasma and liver microsomes (Table 1). Whereas most of the galactose residues of plasma *N*-glycans were sialylated for the 5 weeks after birth, those of microsomal *N*-glycans were not all sialylated at 1, 2 and 3 weeks postnatally, with the lowest degree of sialylation at 2 weeks. These results are consistent with our previous findings, obtained by labelling liver slices with [^3H]mannose [6]. The DNA content [28], [^3H]thymidine-labelling index [29] and mitotic index [30] of rat liver in the postnatal period indicate that the decrease in postnatal proliferation begins at about 2 weeks. In addition, decreased binding of insulin [31] and epidermal growth factor [32] in liver plasma membranes of 2-week-old rats has been reported. Since many receptors for hormones and growth factors are glycoproteins, it is possible that the change in the *N*-glycans of membrane glycoproteins may influence the growth of hepatic cells by affecting the function of these receptors.

In most cases, the changes in sialylation of glycoproteins have been ascribed to a change in the activity of sialyltransferase. However, in our case, sialylation of microsomal *N*-glycans decreased from 1 to 3 weeks after birth, with a minimum at 2 weeks (Table 1), without any decrease in sialyltransferase activity (Fig. 3). The activities of galactosyltransferase (Fig. 2) and of enzymes in the dolichol pathway [9] and in the early stage of oligosaccharide processing [6] began to increase immediately after birth and had reached their respective maxima by 2 weeks. It is most likely that the synthesis of *N*-glycans, which act as the substrate for sialyltransferase, increases just after birth and levels off at 2 weeks, whereas sialyltransferase activity does not increase until 2 weeks after birth and is not high enough to sialylate all of the substrate.

Sialylation of sialyltransferase itself also changed during postnatal development and may be related to the regulation of its activity. Miyagi & Tsuiki [18] have speculated that non-sialylated sialyltransferase may be inactive *in vivo*, comparing its K_m value for CMP-*N*-acetylneuraminic acid (165 μM) with the pool size of CMP-*N*-acetylneuraminic acid (41 μM) in hepatocytes [33]. They have also speculated that the conversion of the non-sialylated form to sialylated form may enhance sialyltransferase activity in cells. These considerations may hold true in our case. However, it is also possible that the age-dependent changes in the sialylation of sialyltransferase are caused by the change in neuraminidase activity. In regenerating rat liver, sialyltransferase activity is increased [34–36] and non-sialylated sialyltransferase is relatively decreased [37]. The situation in developing rat liver is similar, and may be a characteristic of normally growing liver.

The glycosidic linkage formed by the catalysis of sialyltransferase was not determined in this study. The existence of four different sialyltransferases towards glycoproteins has now been reported in adult rat liver. One formed a NeuAca2 $\rightarrow$ 6Gal sequence on β -galactose residues of *N*-linked oligosaccharides,

and two other enzymes formed a NeuAca2 $\rightarrow$ 3Gal sequence on β -galactose residues of *N*-linked or *O*-linked oligosaccharides respectively [38–40]. Finally, GlcNAc α 2 $\rightarrow$ 6-sialyltransferase participates in the formation of the disialylated sequence NeuAca2 $\rightarrow$ 3Gal β $\rightarrow$ 3(NeuAca2 $\rightarrow$ 6)GlcNAc-, which terminates *N*-linked oligosaccharides [41]. However, the activity of β -galactoside α 2 $\rightarrow$ 6-sialyltransferase is predominant and the activities of the other three enzymes are negligibly low in adult rat liver [38–43]. Indeed, Miyagi & Tsuiki [18] found that two forms of sialyltransferase toward asialofetuin are both β -galactoside α 2 $\rightarrow$ 6-sialyltransferases in adult rat liver. Therefore transferase I and transferase II described in the present paper are likely to correspond to this enzyme. However, it is possible that a minor component of transferase I that was eluted from the phosphocellulose column at lower ionic strength, and a minor component of transferase II that was eluted with NaCl at a higher ionic strength (Fig. 4), are other sialyltransferases. Our preliminary data shown that about 20 % of sialic acid transferred to asialofetuin was attached to *O*-linked oligosaccharides in liver microsomes of 3-week-old rats. Moreover, the activity of α 2 $\rightarrow$ 3-sialyltransferase toward lactose is reported to be higher than that of α 2 $\rightarrow$ 6-sialyltransferase in the rat liver during postnatal development [44]. Therefore it would be of interest to determine the types of sialyltransferases present in developing rat liver.

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