Postnatal changes in *N*-linked oligosaccharides of glycoproteins in rat liver

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[³H]Mannose-labelled glycopeptides in the slices of livers from neonatal and 1-, 2-, 3- and 5-week-old rats were characterized by column chromatographies on Sephadex G-50 and concanavalin A-Sepharose and by endo- β -*N*-acetylglucosaminidase H digestion. The proportion of complex-type glycopeptides was increased with time until 2 weeks *post partum* and then returned to the neonatal level. This was mainly due to the increased proportion of concanavalin A-bound (biantennary) species. These changes were accompanied by consistent changes in the activities of processing enzymes in liver microsomal fraction, especially of *N*-acetylglucosaminyltransferase I. Complex-type glycopeptides from neonatal and 2- and 5-week-old rat livers were further characterized by column chromatographies on Bio-Gel P-6 and DE 52 DEAE-cellulose in combination with neuraminidase digestion. No significant difference was found between concanavalin A-bound species from neonatal liver and those from liver 5 weeks *post partum*, most of which were sialylated. Concanavalin A-bound species 2 weeks *post partum* were comparatively smaller in size and less sialylated. On the other hand, there was no significant difference among concanavalin A-unbound species from the three different sources, most of which were sialylated. Since glycoproteins from regenerating rat liver also contain a higher proportion of complex-type oligosaccharides, as previously reported, such changes in *N*-linked oligosaccharides of glycoproteins may be related to control of the growth of liver cells.

INTRODUCTION

N-Linked oligosaccharides of glycoproteins are implicated in cellular interaction, which is important in growth, development, and maintenance and regeneration of tissues, and in the intracellular and intercellular localization of glycoproteins as such (Olden *et al.*, 1982, 1985; Sharon, 1984). We have previously reported that glycoproteins from regenerating rat liver contain a higher proportion of complex-type oligosaccharides (Kato & Akamatsu, 1984) and biantennary species with a core fucose residue (Kato & Akamatsu, 1985) than do controls, and also that the proportion changes in a time-dependent manner after partial hepatectomy. Our results present a contrast with those indicating that growing cells are rich in high-*M_r* fucosyl glycopeptides (Buck *et al.*, 1971; Muramatsu *et al.*, 1973; Ceccarini, 1975) and in high-mannose-type glycopeptides (Muramatsu *et al.*, 1976; Ceccarini & Atkinson, 1977; Rupar & Cook, 1982). Therefore it is likely that such growth-dependent changes in *N*-linked oligosaccharides of glycoproteins as in regenerating liver should be specific to liver cells under normal control of growth.

The DNA concentration of rat liver is doubled during the first 5 days *post partum* and thereafter falls towards the adult level within 25 days (Oliver *et al.*, 1962). In addition, the decrease in [³H]thymidine-labelling index (Stöcker *et al.*, 1972) and mitotic index (Houbrechts & Barbason, 1972) begins at about 2 weeks *post partum*. Thus developing rat liver as well as regenerating rat liver is considered to be a good model system for the study of the glycopeptides in growing liver cells. In the present

investigation we studied the properties of glycopeptides in developing rat liver to obtain more information about the changes in *N*-linked oligosaccharides of glycoproteins during the growth of liver cells, and the results are discussed together with our previous findings in regenerating rat liver.

MATERIALS AND METHODS

Chemicals

Endo- β -*N*-acetylglucosaminidase H (EC 3.2.1.96) from *Streptomyces griseus* was purchased from Seikagaku Kogyo, Tokyo, Japan; neuraminidase (EC 3.2.1.18) from *Arthrobacter ureafaciens* was from Nakarai Chemicals, Kyoto, Japan; Pronase P was from Kaken Kagaku, Tokyo, Japan. D-[2-³H]Mannose (13.2 Ci/mmol), D-[1-¹⁴C]glucosamine hydrochloride (55.5 mCi/mmol), UDP-D-[U-¹⁴C]glucose (295 mCi/mmol), GDP-D-[U-¹⁴C]mannose (203 mCi/mmol) and UDP-*N*-acetyl-D-[U-¹⁴C]glucosamine (229 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Con A-Sepharose was purchased from Pharmacia, Uppsala, Sweden; DE 52 DEAE-cellulose was from Whatman, Maidstone, Kent, U.K. Ovalbumin (grade V) and swainsonine were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Other chemicals were of the best grade available commercially.

Animals

Pregnant Wistar rats (Nihon Rat, Urawa, Japan) were fed *ad libitum* with laboratory chow in a room at a

Abbreviations used: Con A, concanavalin A; [¹⁴C]Glc- α -glucosidase activity, α -glucosidase activity towards [¹⁴C]glucose-labelled oligosaccharide; PNP- α -glucosidase activity, α -glucosidase activity towards *p*-nitrophenyl α -glucoside; [¹⁴C]Man- α -mannosidase activity, α -mannosidase activity towards [¹⁴C]mannose-labelled oligosaccharide; PNP- α -mannosidase activity, α -mannosidase activity towards *p*-nitrophenyl α -mannoside.

constant temperature (23.5 °C) with 12 h each of light (6:30–18:30 h) and darkness. Neonatal rats (within 15 h *post partum*) and rats aged 1 and 2 weeks taken from the mother regardless of the sex, and male Wistar rats aged 3 and 5 weeks (Nihon Rat) from different litters, were killed by decapitation between 9:00 h and 10:30 h.

Preparation of liver slices and incubation procedure

Liver slices were prepared and incubated at 37 °C for 4 h with 5 μ Ci of [^{3} H]mannose as described previously (Kato & Akamatsu, 1984).

Preparation of glycopeptides

Tissue and medium glucopeptides were prepared as described previously (Kato & Akamatsu, 1984). The Pronase digest was centrifuged at 1000 *g* for 10 min and the supernatant was used for analyses. The radioactivity in the precipitate was negligibly low as compared with that in the supernatant.

Chromatography

The glycopeptides were desalted by passage through a Sephadex G-25 (medium grade) column (1.2 cm $\times$ 48 cm), and used for analyses. Sephadex G-50 column chromatography was done as described previously (Kato & Akamatsu, 1985). The glycopeptide fractions were combined and used for further analyses. A Bio-Gel P-6 (–400 mesh) column (1 cm $\times$ 57 cm) was equilibrated with 0.1 M-ammonium acetate containing 0.02 % NaN_3 . Flow rate was adjusted to 4 ml/h by pumping, and 1 ml fractions were collected.

A Con A-Sepharose column (0.6 cm $\times$ 3.6 cm) was equilibrated with 10 mM-Tris/HCl buffer, pH 8.0, containing 0.15 M-NaCl, 1 mM- CaCl_2 , 1 mM- MgCl_2 and 0.02 % NaN_3 . The glycopeptides (derived from 250 mg of liver slices) dissolved in 1 ml of the same buffer were applied to the column and eluted stepwise with 5 ml of the buffer, 12 ml each of 10 mM- and 200 mM-methyl α -D-glucopyranoside in the buffer and 6 ml of 500 mM-methyl α -D-glucopyranoside in the buffer successively. Flow rate was about 6 ml/h, and 1 ml fractions were collected.

A DE 52 DEAE-cellulose column (0.6 cm $\times$ 3.6 cm) was equilibrated with 10 mM-pyridine/acetate buffer, pH 6.5. The glycopeptides (one-quarter portion of each Con A fraction) dissolved in 2 ml of the same buffer were applied to the column and eluted stepwise with 8 ml of the buffer and 10 ml each of 20 mM- and 100 mM-NaCl in the buffer successively. Flow rate was about 8 ml/h, and 2 ml fractions were collected.

Descending paper chromatography was done on Toyo no. 50 paper (Tokyo, Japan) in the following solvents: A, butan-1-ol/ethanol/water (13:8:4, by vol.); B, ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by vol.); C, butan-1-ol/pyridine/water (6:4:3, by vol.).

Enzymic digestions

Digestions with Pronase and endo- β -N-acetylglucosaminidase H were done as described previously (Kato & Akamatsu, 1984). Neuraminidase digestion was done as described previously (Kato & Akamatsu, 1985).

Mild acid hydrolysis

Lipid intermediates were hydrolysed as described previously (Oda-Tamai *et al.*, 1985).

Preparation of rat liver microsomal fraction

Rat liver microsomal fraction was prepared as described previously (Oda-Tamai *et al.*, 1985). The fraction also contains Golgi apparatus (Eriksson & Glaumann, 1983).

Preparation of [^{14} C]oligosaccharides for glycosidase assays

[^{14} C]Glucose-labelled oligosaccharide was prepared as described previously (Oda-Tamai *et al.*, 1985).

[^{14} C]Mannose-labelled oligosaccharide–lipid was extracted with chloroform/methanol/water (10:10:3, by vol.) from large-scale incubations of the standard reaction mixture described previously (Okamoto & Akamatsu, 1981). [^{14} C]Mannose-labelled oligosaccharide released by mild acid hydrolysis was chromatographed on paper in solvent A for 2 days for desalting and then in solvent B for 5 days. [^{14} C]Mannose-labelled oligosaccharide was separated into two groups, and the one with lower mobility was eluted and used as substrate.

Preparation of ovalbumin glycopeptide V for N-acetylglucosaminyltransferase assay

The glycopeptide fraction was obtained by exhaustive Pronase digestion of ovalbumin (2 g) followed by gel filtration on a Sephadex G-25 (medium grade) column (0.8 cm $\times$ 68 cm) by the method of Huang *et al.* (1970). The glycopeptides were fractionated on a column (1.25 cm $\times$ 114 cm) of Dowex 50W-X2 into sub-fractions I to V as described by Tai *et al.* (1975). The effluents were monitored by use of phenol/ H_2SO_4 reagent (Dubois *et al.*, 1956). The yield of glycopeptide V, Man α 6 (Man α 3)Man α 6 (Man α 3)Man β 4GlcNAc- β 4GlcNAc-Asn (Tai *et al.*, 1975), was 11 μ mol.

Assay of α -glucosidases

[^{14} C]Glc- α -glucosidase activity was assayed by measuring the hydrolysis of [^{14} C]glucose-labelled oligosaccharide in a standard incubation mixture (0.1 ml) containing 2000 d.p.m. of radioactive substrate, 50 mM-sodium phosphate buffer, pH 7.0, 0.2 % Triton X-100 and microsomal fraction (about 1 mg of protein). After incubation at 37 °C for 1 h, the reaction was stopped by adding 0.1 ml of pyridine. Free [^{14} C]glucose was separated from the oligosaccharides by paper chromatography in solvent C for 21 h, and the radioactivity was measured.

PNP- α -glucosidase activity was assayed by incubating microsomal fraction (about 0.3 mg of protein) in 0.2 ml of 50 mM-sodium phosphate buffer, pH 7.0, containing 4 mM-*p*-nitrophenyl α -glucoside at 37 °C for 30 min. The reaction was stopped by adding 1 ml of 0.2 M- Na_2CO_3 , and the absorbance at 400 nm was determined. The absorbance of a blank, in which the substrate and microsomal fraction were incubated separately and mixed after the Na_2CO_3 addition, was subtracted from each value.

Assay of α -mannosidases

[^{14} C]Man- α -mannosidase activity was assayed by measuring the hydrolysis of [^{14} C]mannose-labelled oligosaccharide in a standard incubation mixture (0.1 ml) containing 3000 d.p.m. of radioactive substrate, 50 mM-sodium phosphate buffer, pH 6.1, 0.2 % Triton X-100 and microsomal fraction (about 0.5 mg of protein). After incubation at 37 °C for 1 h, the reaction was stopped by

Fig. 1. Sephadex G-50 column chromatography of glycopeptides

(a) Tissue glycopeptides from neonatal (○) and 2-week-old (●) rat livers; (b) medium glycopeptides from neonatal (○) and 2-week-old (●) rat livers. Vertical dotted lines indicate the elution position of glycopeptides from the medium. Blue Dextran was used to determine V_0 , and mannose was used to determine V_t .

adding 0.1 ml of pyridine. Free [^{14}C]mannose was separated from the oligosaccharides by paper chromatography in solvent C for 21 h, and the radioactivity was measured.

PNP- α -mannosidase activity was assayed by incubating microsomal fraction (about 0.1 mg of protein) as described by Tulsiani *et al.* (1982a).

Assay of N-acetylglucosaminyltransferase I

The assay was done by a modification of the method of Dunphy & Rothman (1983). The standard incubation mixture contained the following: 0.1 M-Mes/NaOH buffer, pH 6.5, 10 mM-MgCl₂, 0.2% Triton X-100, 2 mM-ATP, 20 μM -swainsonine to prevent the action of α -mannosidase II (Tulsiani *et al.*, 1982b) on the reaction product, 0.2 mM-ovalbumin glycopeptide V, 2 mM-UDP-N-acetyl[U- ^{14}C]glucosamine (125 mCi/mol) and microsomal fraction (0.1 mg of protein), in a total volume of 50 μl . After incubation at 37 °C for 30 min, the reaction was stopped by boiling for 5 min. A sample was mixed with 1 ml of phosphate-buffered saline (137 mM-NaCl/2.68 mM-KCl/8.09 mM-Na₂HPO₄/1.47 mM-KH₂PO₄, pH 7.2) and centrifuged for 2 min at 10000 g. The radioactivity in the precipitate was negligible as compared with that in the supernatant. The supernatant was applied to a Con A-Sepharose column (0.48 cm $\times$ 2.8 cm) equilibrated with phosphate-buffered saline. After the column had been washed with 7 ml of phosphate-buffered saline, the fraction eluted with 2.5 ml of 0.1 M-methyl α -D-glucopyranoside in 10 mM-Tris/HCl buffer, pH 8.0, was collected and radioactivity was determined. The radioactivity in this fraction was entirely dependent on the addition of ovalbumin glycopeptide V.

Measurement of radioactivity

Radioactivity in water-soluble samples, acid-insoluble materials containing proteins and paper chromatograms

was determined as described previously (Kato & Akamatsu, 1984).

RESULTS

Characterization of glycopeptides

Tissue and medium glycopeptides were chromatographed on a Sephadex G-50 column (Fig. 1). The elution profiles of tissue glycopeptides from 1-, 3- and 5-week-old rat livers (results not shown) were essentially the same as that from neonatal rat liver, but that from 2-week-old rat liver was slightly different from the others. Tissue glycopeptides from 2-week-old rat liver were relatively rich in higher- M_r components. This difference in the elution profile was consistently observed in four series of experiments. No age-dependent difference in the elution profiles of glycopeptides from the medium was observed.

Each glycopeptide fraction was chromatographed on a Con A-Sepharose column. As representatives, elution profiles of glycopeptides from neonatal and 2-week-old rat livers are shown in Fig. 2. About 72% of tissue glycopeptides from neonatal rat liver were tightly bound to the column and were eluted with 200 mM-methyl α -D-glucopyranoside (Fig. 2a). On the other hand, about 55% of tissue glycopeptides from 2-week-old rat liver were tightly bound to the column (Fig. 2b). Most of the glycopeptides from the medium showed little or no ability to bind to the column, and no significant difference in the elution profile was found (Figs. 2c and 2d).

Each of the Con A fractions [Con A(-), glycopeptides that passed through the column; Con A(+), glycopeptides that were bound to the column and were eluted with 10 mM-methyl α -D-glucopyranoside; Con A(++), glycopeptides that were bound to the column and were eluted with 200 mM-methyl α -D-glucopyranoside] was chromatographed on a Bio-Gel P-6 column. As repre-

Fig. 2. Con A-Sepharose column chromatography of glycopeptides

Tissue glycopeptides from neonatal (a) and 2-week-old (b) rat livers and medium glycopeptides from neonatal (c) and 2-week-old (d) rat livers (fractions 25–42 from the Sephadex G-50 column chromatography in Fig. 1) were separately chromatographed. Recovery of the radioactivity from the column was always above 95%. Arrows (↓) from left to right indicate the exchange of concentration of methyl α -D-glucopyranoside: 10 mM, 200 mM and 500 mM.

sentatives, elution profiles of tissue glycopeptides from neonatal and 2-week-old rat livers are shown in Fig. 3. The elution profiles of Con A(–) and Con A(++) fractions were not significantly different between neonatal and 2-week-old rat livers (Figs. 3a and 3b and Figs. 3e and 3f respectively). In both cases all Con A(++) glycopeptides were susceptible to endo- β -N-acetylglucosaminidase H digestion, whereas Con A(–) glycopeptides were completely resistant. Although a proportion of the Con A(+) glycopeptides were susceptible to endo- β -N-acetylglucosaminidase H digestion in both cases, those from 2-week-old rat liver contained a higher proportion of low- M_r glycopeptides that were resistant (Figs. 3c and 3d). The elution profiles of Con A(–) and Con A(+) fractions from the medium were not significantly different between neonatal and 2-week-old rat

livers (results not shown). Con A(–) glycopeptides from the medium gave the same profiles as those from tissue did, and the Con(+) glycopeptides from the medium were eluted as a single peak at fraction 16 with a shoulder around fraction 20.

Considering Con A specificity (Baenziger & Fiete, 1979; Finne & Krusius, 1982), these results suggest that Con A(–) glycopeptides should be of complex type (tri- or tetra-antennary), Con A(+) glycopeptides should be of complex type (biantennary) and hybrid type, Con A(++) glycopeptides should be of high-mannose type, and tissue Con A(+) glycopeptides should be altered during postnatal development.

Postnatal changes in the affinity of glycopeptides to Con A-Sepharose

Each glycopeptide fraction from the Sephadex G-50 column chromatography was separated into three groups by Con A-Sepharose column chromatography. The results of this separation of the tissue glycopeptides are summarized in Table 1. The percentage of Con A(++) fraction decreased with time until 2 weeks *post partum* and then returned to the neonatal level. Correspondingly, the percentage of Con A(+) fraction increased with time until 2 weeks *post partum* and then returned to the neonatal level. The percentage of Con A(–) fraction increased slightly only at 2 weeks *post partum*. On the other hand, there was no significant postnatal change in the affinity of the glycopeptides from the medium. At each time point the Con A(–) fraction was predominant (about 57%) and the Con A(++) fraction was not more than 6%. Similar changes in tissue glycopeptides were also observed when the standard labelling condition, i.e. 4 h pulse, was replaced by a 2 h pulse and 2 h chase, and also when [^{14} C]glucosamine was used as a radioactive precursor instead of [^{3} H]mannose (results not shown).

A difference in the sugar moiety of oligosaccharide-lipid may result in changes in glycopeptides by affecting the glycosylation of proteins and the subsequent oligosaccharide processing. Therefore [^{3} H]mannose-labelled oligosaccharide obtained from [^{3} H]mannose-labelled oligosaccharide-lipid, which was extracted from liver slices as described previously (Kato & Akamatsu, 1984), was subjected to paper chromatography in solvent A for 2 days and then in solvent B for 7 days. However, no age-dependent difference in the chromatogram was found (results not shown). Thus the possibility mentioned above seems unlikely.

These results indicate that tissue glycopeptides from 2-week-old rat liver contain a higher proportion of complex-type species as compared with those from the other sources and suggest that the oligosaccharide processing of glycoproteins should be altered in liver 2 weeks *post partum*.

Postnatal changes in the activities of enzymes involved in oligosaccharide processing of glycoproteins

Activities of enzymes involved in the oligosaccharide processing of glycoproteins were assayed with liver microsomal fraction obtained from neonatal and 1-, 2-, 3- and 5-week-old rats. Since the amount of microsomal proteins obtained per g wet weight of liver varied depending on the age (results not shown), the enzyme activity per g wet weight of liver was used for the comparison.

Fig. 3. Bio-Gel P-6 column chromatography of each Con A fraction of tissue glycopeptides

Con A(-) glycopeptides from neonatal (a) and 2-week-old (b) rat livers, Con A(+) glycopeptides from neonatal (c) and 2-week-old (d) rat livers and Con A(++) glycopeptides from neonatal (e) and 2-week-old (f) rat livers were chromatographed before (●) and after (○) endo- β -N-acetylglucosaminidase H digestion. Each sample was desalted by Sephadex G-25 column chromatography before and after digestion. Vertical dotted lines indicate the elution position of Con A(-) glycopeptides. Blue Dextran and mannose were used to determine V_0 and V_t (fraction nos. 12 and 45) respectively. [^{3} H]Fucose-labelled asialobiantennary glycopeptides prepared as described previously (Kato & Akamatsu, 1985) were eluted mostly in fractions 20 and 21. No significant radioactivity was detected in fractions 40-45 in each case.

Considering that the [^{14} C]glucose-labelled oligosaccharide used as a substrate was most likely to contain three glucose residues (Oda-Tamai *et al.*, 1985), [^{14} C]Glc- α -glucosidase activity should be mainly due to α -glucosidase I. [^{14} C]Man- α -mannosidase activity should be mainly due to α -mannosidase I, since the substrate used was higher- M_r [^{14} C]mannose-labelled oligosaccharide as described in the Materials and methods section. PNP- α -glucosidase and PNP- α -mannosidase activities are due not to α -glucosidase I (Elting & Lennarz, 1982) and α -mannosidase I (Tulsiani *et al.*, 1982a) but to α -glucosidase II (Burns & Touster, 1982) and α -mannosidase II (Tulsiani *et al.*, 1982a) respectively.

As is evident in Fig. 4, all the enzyme activities reached maximal values at 2 weeks *post partum*. Activities of [^{14} C]Glc- α -glucosidase and PNP- α -glucosidase changed in a similar way. [^{14} C]Man- α -mannosidase activity was already significantly increased at 1 week *post partum*, whereas the change of PNP- α -mannosidase activity was not so evident as compared with that of the other glycosidase activities. Among the enzyme activities determined, N-acetylglucosaminyltransferase I activity changed most prominently and in a similar way to the

proportion of complex-type glycopeptides (Table 1). The activity was higher at 1 and 2 weeks *post partum*, but the changes were not evident at 3 and 5 weeks *post partum*. Although the activities of PNP- α -glucosidase and [^{14} C]Man- α -mannosidase remained at slightly higher levels at 5 weeks *post partum*, the activity of N-acetylglucosaminyltransferase I, which is responsible for the conversion of partially processed high-mannose-type oligosaccharides into complex-type species (Li & Kornfeld, 1978; Harpaz & Schachter, 1980), did not. Thus these changes in the activities of the processing enzymes are consistent with the changes in glycopeptides.

Comparison of tissue glycopeptides from neonatal and 2- and 5-week-old-rat livers

As shown in the preceding sections, the chromatographic behaviour of tissue glycopeptides from 2-week-old rat liver changed distinctly. Therefore the properties of Con A(-) and Con A(+) fractions were compared with those from neonatal and 5-week-old rat livers. The results obtained for 5-week-old rat liver are not shown since they were much the same as those for neonatal rat liver.

Con A(-) and Con A(+) fractions were chromato-

Table 1. Postnatal changes in the affinity of tissue glycopeptides to Con A-Sepharose

Tissue glycopeptides were fractionated by Con A-Sepharose column chromatography as described in Fig. 2. The percentage radioactivity in each fraction is indicated, with the total radioactivity being taken as 100%. Each value is the average of the values obtained from three separate experiments and the standard error was not more than 10 % of the values.

Age of rats (weeks)	Percentage of radioactivity recovered		
	Con A(–)	Con A(+)	Con A(++)
0	13.3	14.5	72.1
1	13.6	19.5	66.9
2	15.0	30.2	54.8
3	12.7	14.2	73.1
5	12.2	14.0	73.8

Fig. 4. Postnatal changes in the activities of processing enzymes

[¹⁴C]Glc-α-glucosidase (●), PNP-α-glucosidase (○), [¹⁴C]Man-α-mannosidase (▲), PNP-α-mannosidase (△) and N-acetylglucosaminyltransferase I (■) activities were assayed. Relative values of each enzyme activity per g wet weight of liver are indicated with the respective neonatal values being taken as 100 %. Each value is the average of the values obtained from three separate experiments and the standard error was not more than 10 % of the values.

graphed on a DE 52 DEAE-cellulose column. With both neonatal and 2-week-old rat livers most of the Con A(–) glycopeptides, which had been eluted with 100 mM-NaCl, could be eluted with 0 mM- and 20 mM-NaCl after neuraminidase digestion (results not shown). On the

Table 2. Comparison of Con A(+) tissue glycopeptides by DE 52 DEAE-cellulose column chromatography

Con A(+) tissue glycopeptides from neonatal and 2-week-old rat livers were separately chromatographed before (–) and after (+) neuraminidase digestion. Equal portion of each sample was applied to the column, and the recovery of radioactivity from the column was always above 95 %. The percentage of the radioactivity in each fraction is indicated, with the total radioactivity being taken as 100 %.

	Neur-aminidase	Percentage of radioactivity eluted		
		With 0 mM-NaCl	With 20 mM-NaCl	With 100 mM-NaCl
Neonatal	–	9.4	53.5	37.1
	+	31.6	64.8	3.5
2-week-old	–	14.1	63.0	22.9
	+	30.0	67.7	2.3

other hand, Con A(+) glycopeptides were adsorbed less strongly than Con A(–) glycopeptides, and a higher proportion of Con A(+) glycopeptides from 2-week-old rat liver were eluted with 0 mM- and 20 mM-NaCl than were those from neonatal rat liver (Table 2). However, after neuraminidase digestion, Con A(+) glycopeptides from each source could mostly be eluted with 0 mM- and 20 mM-NaCl in a similar fashion. When neuraminidase digests of each Con A(+) fraction were chromatographed on a Bio-Gel P-6 column, most of the radioactivity was recovered in lower-*M_r* fractions, in which a higher proportion of radioactivity had been detected in the case of 2-week-old rat liver even before digestion (Fig. 5). Comparing such elution profiles with that of [³H]fucosyl biantennary asialoglycopeptides, it seems likely that the Con A(+) fraction contains biantennary species lacking galactose or N-acetylglucosamine residues in both or either one of the two branches.

These results suggest that Con A(+) glycopeptides from 2-week-old rat liver should be less sialylated than those from neonatal and 5-week-old rat livers, and this could be one of the causes for the higher proportion of low-*M_r* species in Con A(+) glycopeptides from 2-week-old rat liver.

DISCUSSION

By taking advantage of Con A specificity, glycopeptides were fractionated into three groups: Con A(–), multiantennary complex-type species; Con A(+), biantennary complex-type and hybrid-type species; Con A(++), high-mannose-type species. The validity of this grouping was supported by the following findings: Con A(++) glycopeptides were entirely susceptible to endo-β-N-acetylglucosaminidase H digestion (Fig. 3c); Con A(–) glycopeptides were of the highest *M_r* among the three groups and completely resistant to endo-β-N-acetylglucosaminidase H digestion (Fig. 3a), and bound most strongly to the DE 52 DEAE-cellulose column as a consequence of their sialic acid residues; Con A(+) glycopeptides were partly susceptible to endo-β-N-acetylglucosaminidase H digestion (Fig. 3b) and showed sialic acid-dependent binding to the DE 52 DEAE-

Fig. 5. Bio-Gel P-6 column chromatography of Con A(+) tissue glycopeptides

Con A(+) tissue glycopeptides from neonatal (a) and 2-week-old (b) rat livers were chromatographed before (●) and after (○) neuraminidase digestion. Each sample was desalted by Sephadex G-25 column chromatography before and after digestion. Blue Dextran and mannose were used to determine V_0 and V_i respectively. [^3H]Fucose-labelled asialobiantennary glycopeptides prepared as described previously (Kato & Akamatsu, 1985) were eluted mostly in fractions 20 and 21.

cellulose column (Table 2); the major component of the Con A(+) glycopeptides was eluted between Con A(−) glycopeptides and [^3H]fucose-labelled asialobiantennary glycopeptides in Bio-Gel P-6 column chromatography (Fig. 3b). Tissue glycopeptides from 2-week-old rat liver were rich in Con A(+) species and were poor in Con A(++) species as compared with those from the other sources (Table 1). Con A(+) glycopeptides from 2-week-old rat liver contained a higher proportion of low- M_r species (Fig. 3) and appeared to be less sialylated (Table 2 and Fig. 5). It should be noted that such changes in sialylation were not observed in the case of regenerating liver (Kato & Akamatsu, 1985).

The increase in the proportion of complex-type glycopeptides could be caused by the changes in the

sugar moiety of oligosaccharide-lipid, which may affect protein glycosylation and the following oligosaccharide processing. However, this was not the case. Alternatively, it seems possible that the glycoprotein composition in liver should change during the postnatal period, since qualitative and quantitative variations in plasma glycoproteins during the perinatal period are well documented (Sereni & Principi, 1971). However, we found that activities of the processing enzymes changed in a manner consistent with the change in the proportion of complex-type glycopeptides (Fig. 4). This implies that such a qualitative change in the glycopeptides should primarily be due to the change in the activities of the processing enzymes and only secondarily due to possible variations in glycoprotein composition.

Although a large number of studies have indicated correlation between changes in the N-linked oligosaccharides of glycoproteins and cell growth and growth-related cell physiology (Sasak *et al.*, 1982, 1983; Codogno *et al.*, 1983, 1985), the actual changes seem to be dependent on the type of cells and tissue. With regard to liver glycopeptides, there have been some studies with regenerating liver, late-embryonic and neonatal livers and hepatomas (Akasaki *et al.*, 1975; Smets *et al.*, 1975; van Beek *et al.*, 1977). However, the oligosaccharide moieties of the glycopeptides were not characterized in detail. In the present investigation we found that a higher proportion of complex-type glycopeptides, especially of biantennary species, was contained in developing livers during cell growth. In addition, similar results were obtained by our preliminary study with fetal-rat liver at 20 days of gestation, the [^3H]thymidine-labelling index of which was much higher than the postnatal level (Stöcker *et al.*, 1972). These observations, together with those made with regenerating liver (Kato & Akamatsu, 1984, 1985), suggest that such changes in the N-linked oligosaccharides of glycoproteins may be characteristic of normally growing liver cells. Alternatively, it is also claimed that the acceleration of oligosaccharide processing may be necessary for the supplementation of serum glycoproteins, the concentration of which in the blood is significantly lower during liver regeneration (Bresnick, 1971) and postnatal development (Okubo *et al.*, 1976).

The changes in glycopeptides may affect the deposition, turnover and properties of certain glycoproteins such as cell-surface receptors. In terms of insulin receptor, glycosylation-mutant cells bind insulin with different affinities (Podskalny *et al.*, 1984). Since growth-dependent expression of asialoglycoprotein receptor (Theilmann *et al.*, 1983) and transferrin receptor (Hirose-Kumagai *et al.*, 1984) on the cell surface has been reported, postnatal change of these receptors may be a matter of interest.

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