Biochem. J. (1985) **229**, 521–528
Printed in Great Britain

Alterations in fucosyl oligosaccharides of glycoproteins during rat liver regeneration

Shigemi KATO and Nobu AKAMATSU
Department of Biochemistry, St. Marianna University School of Medicine, 2095 Sugao, Miyamae-ku, Kawasaki 213, Japan

(*Received 15 February 1985/27 March 1985; accepted 3 April 1985*)

[^{3}H]Fucose-labelled glycopeptides in the slices of liver 24h after partial hepatectomy were fractionated on Sephadex G-50. Glycopeptides from regenerating liver contained a higher proportion of lower-M_r components than did controls. Regenerating liver contained a higher proportion of glycopeptides that were bound to concanavalin A–Sepharose and were subsequently eluted with 20mM-methyl α-D-glucopyranoside than did controls. Concanavalin A-bound glycopeptides from each source were entirely bound to a lentil lectin–Sepharose column. Both the concanavalin A-bound and -unbound fractions from regenerating liver were indistinguishable from the respective controls by Bio-Gel P6 column chromatography and neuraminidase digestion. These results show that fucosyl glycopeptides from regenerating liver contain a higher proportion of biantennary species with core fucose residues than do controls. Glycopeptides from regenerating livers 12h, 72h and 144h after partial hepatectomy were also examined; however, the difference was not significant. These observations suggest that the alterations in fucosyl glycopeptides may be related to rapid growth of hepatocytes 24h after partial hepatectomy. No significant difference was found in either [^{3}H]mannose- or [^{3}H]fucose-labelled glycoproteins from regenerating liver and from controls by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, suggesting that the alteration in glycopeptides should depend on some differences in the late stage of oligosaccharide processing.

Carbohydrate moieties of glycoproteins are implicated in cellular differentiation and interaction, and in intracellular localization of glycoproteins (Olden *et al.*, 1982; Sharon, 1984). We have previously reported that glycoproteins from regenerating rat liver contain a higher proportion of complex-type oligosaccharides than do controls, and that the proportion changes in a time-dependent manner after partial hepatectomy. Moreover, these changes were accompanied by the consistent change in the activities of neutral α-mannosidase and *N*-acetylglucosaminyltransferase, most probably transferase I (Kato & Akamatsu, 1984). These alterations in oligosaccharides of glycoproteins may be related to hypertrophy and hyperplasia of hepatocytes in liver regeneration.

Abbreviations used: Con A, concanavalin A; SDS, sodium dodecyl sulphate.

In this paper we have studied the properties of fucosyl oligosaccharides of glycoproteins synthesized by liver slices using affinity chromatography to get more information about alterations in *N*-linked oligosaccharides of glycoproteins during liver regeneration.

Experimental

Materials

Neuraminidase (EC 3.2.1.18) from *Arthrobacter ureafaciens* was purchased from Nakarai Chemicals, Kyoto, Japan; Pronase P was from Kaken Kagaku, Tokyo, Japan. L-[6-^{3}H]Fucose (16.1Ci/mmol) and D-[2-^{3}H]mannose (12Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Con A–Sepharose, lentil lectin–Sepharose 4B and the electrophoresis calibration kit were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Other chemicals were of special grades available commercially.

Preparation of liver slices and incubation procedure

Liver slices were prepared and incubated with 3.3 μCi of [^{3}H]fucose (0.33 μCi/μl of 0.9% NaCl) as previously described (Kato & Akamatsu, 1984).

Preparation of glycopeptides

Tissue and medium glycopeptides were prepared as described previously (Kato & Akamatsu, 1984).

The Pronase digest was centrifuged at 1000*g* for 10min and the supernatant was used for analyses. The radioactivity in the precipitate was negligibly low compared with that in the supernatant.

Chromatography

The glycopeptides were desalted by passage through a Sephadex G-25 (medium grade) column (1.2cm × 48cm), and used for analyses.

A column of Sephadex G-50 (superfine grade) (1cm × 67cm) was equilibrated with 0.1M-ammonium acetate/0.02% NaN_3. Flow rate was about 9ml/h, and 1ml fractions were collected. The glycopeptide fractions were combined and used for further analyses.

A column of Bio-Gel P-6 (−400mesh) (1cm × 57cm) was equilibrated with 0.1M-$NaHCO_3$/0.02% NaN_3. Flow rate was adjusted to 5ml/h by pumping, and 1ml fractions were collected.

A column of Con A–Sepharose (0.6cm × 3.6cm) was equilibrated with 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl, 1mM-$CaCl_2$, 1mM-$MgCl_2$ and 0.02% NaN_3. The glycopeptides (half of the amount derived from 250mg of liver slices) dissolved in 1ml of the same buffer were applied to the column and eluted stepwise, with 5ml of the buffer, 12ml of 20mM-methyl α-D-glucopyranoside in the buffer, and 7ml of 0.5M-methyl α-D-mannopyranoside in the buffer. Flow rate was about 6ml/h, and 1ml fractions were collected.

A column of lentil lectin–Sepharose (0.6cm × 3.6cm) was equilibrated with 10mM-Tris/HCl buffer, pH8.0, containing 0.15M-NaCl, 1mM-$CaCl_2$, 1mM-$MgCl_2$ and 0.02% NaN_3. The glycopeptides (one-sixth of the amount derived from 250mg of liver slices) dissolved in 1ml of the buffer were applied to the column and eluted in the same way as for Con A–Sepharose column chromatography, except that 10mM- rather than 20mM-methyl α-D-glucopyranoside (Kornfeld *et al.*, 1981) was used.

Enzymic digestions

Pronase digestion was performed as described previously (Kato & Akamatsu, 1984).

Digestion with neuraminidase was performed by incubating the glycopeptides (less than half of the amount derived from 250mg of liver slices) with 0.05unit of the enzyme in 100μl of 0.1M-sodium acetate buffer, pH5.0, under a toluene layer at 37°C for 24h. The reaction was stopped by boiling for 2min.

Preparation of glycoproteins for gel electrophoresis

About 250mg of liver slices was incubated with 30 μCi of [^{3}H]mannose or 10 μCi of [^{3}H]fucose for 4h at 37°C. Media were centrifuged at 100*g* for 5min at 4°C, and the resulting supernatant was mixed with 10% (w/v) SDS and 0.2M-phenylmethanesulphonyl fluoride (solution in methanol) to final concentrations of 1% (w/v) and 2mM respectively. The tissues were homogenized with 5ml of ice-cold 0.15M-Tris/acetate buffer, pH7.4, containing 4mM-$MgCl_2$, and then mixed with 10% SDS and 0.2M-phenylmethanesulphonyl fluoride as above.

Medium and tissue materials were dialysed three times against 50vol. of distilled water at 4°C, freeze-dried and dissolved in the sample buffer solution for electrophoresis as described by Laemmli (1970). Then a one-hundredth of tissue materials and three hundredths of medium materials were reduced by boiling for 2min in the presence of 5% (v/v) mercaptoethanol.

SDS/polyacrylamide-gel electrophoresis

This was performed by the method of Laemmli (1970), with 7.5% (w/v) polyacrylamide for the separation gel and 3% (w/v) polyacrylamide for the stacking gel in tubes (diameter 7mm). Proteins were detected by staining with Coomassie Brilliant Blue R. The unstained gels were sliced (2mm thick), and the radioactivity in each slice was determined.

The following proteins were used as M_r standards: thyroglobulin subunit, 330000; ferritin subunit, 220000; albumin, 67000; catalase subunit, 60000; lactate dehydrogenase subunit, 36000.

Measurement of radioactivity

Radioactivity in water-soluble samples was determined as previously (Kato & Akamatsu, 1984).

Each gel slices was solubilized in 0.5ml of NCS/water (9:1, v/v) for 2h at 50°C and cooled. Then 10ml of 0.6% (w/v) 2,5-diphenyloxazole in toluene/Triton X-100 (2:1, v/v) was added, and radioactivity was measured after dark-adaptation for 2days.

Results

Characterization of [^{3}H]fucose-labelled glycopeptides

Since the glycoprotein synthesis in regenerating rat liver decreased during the first 48h after partial

hepatectomy (Okamoto & Akamatsu, 1977; Okamoto *et al.*, 1978; Oda-Tamai *et al.*, 1985), tissue and medium glycopeptides 24h after the operation were analysed in the following experiments. [^{3}H]Fucose-labelled glycopeptides were chromatographed on Sephadex G-50 (Fig. 1). In all cases, the major radioactive peak had a similar $K_{av.}$ to the complex-type glycopeptides previously labelled with [^{3}H]mannose (Kato & Akamatsu, 1984). The radioactive peak in fractions 35–39, which was detected only for tissue glycopeptides, had a similar $K_{av.}$ to the [^{3}H]mannose-labelled high-mannose-type glycopeptides or asialoglycopeptides (Kato & Akamatsu, 1984). When [^{3}H]mannose was used as a radioactive precursor, no significant radioactivity was detected in either the void-volume fraction or fractions 41–50. Therefore fractions 21–40 were used for analyses as glycopeptides with *N*-glycosyl linkage, and the other fractions were not used.

As shown in Fig. 1, the major component of both tissue and medium [^{3}H]fucose-labelled glycopeptides from regenerating liver contained a lower proportion of higher-M_r components (fractions 26–30) than did controls. In spite of some fluctuation in the total incorporation of radioactivity into both tissue and medium glycopeptide fractions (Table 1), such a difference in the elution profile was always observed with three different pairs of regenerating livers 24h after partial hepatectomy and controls.

Affinity of [^{3}H]fucose-labelled glycopeptides to Con A–Sepharose

The [^{3}H]fucose-labelled glycopeptides were chromatographed on Con A–Sepharose (Table 1). In each case, most of the radioactivity was eluted from the column with the buffer or 20mM-methyl α-D-glucopyranoside. Although a small part of tissue glycopeptides (about 6%) was bound to the column and eluted with 0.5M-methyl α-D-mannopyranoside, they were not characterized further. Both tissue and medium glycopeptides from regenerating liver contained a higher proportion of glycopeptides which were bound to the column and eluted with 20mM-methyl α-D-glucopyranoside (Con A-bound glycopeptides) than in controls. The difference was more evident in medium glycopeptides. Such a difference was also observed with one more pair of regenerating and control livers. In addition, Con A-bound [^{3}H]fucose-labelled glycopeptides from each source were entirely bound to a lentil lectin–Sepharose column and eluted with 10mM-methyl α-D-glucopyranoside and 0.5M-methyl α-D-mannopyranoside (results not shown).

Con A-unbound and Con A-bound glycopeptides from regenerating liver were separately chromatographed on Bio-Gel P-6 (Fig. 2). Con A-bound glycopeptides from both tissue and medium were more retained on the column than were Con A-unbound glycopeptides. Although a greater part of each glycopeptide fraction became more retained on the column after neuraminidase digestion, the difference in size between Con A-bound and Con A-unbound glycopeptides was not eliminated. These results are consistent with the binding specificity of Con A (Baenziger & Fiete, 1979; Finne & Krusius, 1982), and indicate that most of the glycopeptides are sialylated, except Con A-bound tissue glycopeptides (Fig. 2*b*), some of which are unlikely to be sialylated because of their resistance to neuraminidase digestion. Essentially the same results as in Fig. 2 were obtained for glycopeptides from controls. Taken together, it is shown that fucosyl glycopeptides from tissue and medium of regenerating liver contain a higher proportion of Con A-bound and low-M_r species, and each Con A fraction from regenerating liver is not different in either size or sialylation from respective controls.

Time-dependency of alteration in oligosaccharide moieties of [^{3}H]fucose-labelled glycoproteins after partial hepatectomy

Since the difference in fucosyl glycopeptides was found between regenerating livers 24h after partial hepatectomy and controls, it was important to determine the time-dependency of the difference. [^{3}H]Fucose-labelled glycopeptides from tissues and media of regenerating liver 12, 72 and 144h after partial hepatectomy were chromatographed on Con A–Sepharose columns. As shown in Table 2, the ratio of Con A-bound glycopeptides to Con A-unbound glycopeptides was the highest at 24h, when liver growth is most evident (Bresnick, 1971). The difference in the tissue was not significant other than at 24h, whereas the difference in the medium was already evident at 12h, but was not found with time after a maximum at 24h. When one more pair of regenerating livers and controls 12, 72 and 144h after the operation respectively were examined, almost the same results were obtained. The fluctuation of the ratio was not more than that in Table 1.

Analysis of [^{3}H]mannose- or [^{3}H]fucose-labelled glycoproteins by SDS/polyacrylamide-gel electrophoresis

To determine whether the increased proportion of Con A-bound fucosyl glycopeptides in regenerating liver was accompanied by the difference in the glycoproteins synthesized by regenerating liver, [^{3}H]fucose-labelled glycoproteins were analysed by SDS/polyacrylamide-gel electrophoresis. [^{3}H]Mannose-labelled glycoproteins were also analysed to detect glycoproteins with *N*-glycosyl

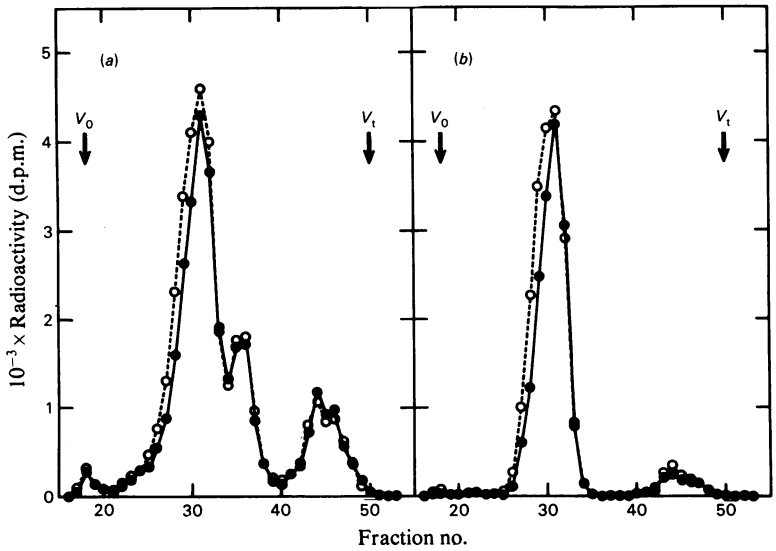

Fig. 1. *Sephadex G-50 column chromatography of [^3H]fucose-labelled glycopeptides*
(*a*) Tissue and (*b*) medium glycopeptides from regenerating liver 24h after partial hepatectomy (●) and controls (○). Blue Dextran was used to determine V_0 (void volume), and mannose was used to determine V_t (total volume).

Table 1. *Incorporation of [^3H]fucose into glycopeptides with N-glycosyl linkage*
[^{3}H]Fucose-labelled glycopeptides (fractions 21–40 from Sephadex G-50 column chromatography in Fig. 1) were designated 'total glycopeptides'. They were fractionated by Con A–Sepharose column chromatography: Con A-unbound glycopeptides, glycopeptides that passed through the column; Con A-bound glycopeptides, glycopeptides that were bound to the column and were eluted with 20mM-methyl α-D-glucopyranoside. Expts. A and B were with two different pairs of regenerating livers 24h after partial hepatectomy and controls.

Expt.		Incorporation of radioactivity into (d.p.m./mg wet wt. of tissue)			
		Total glycopeptides	Con A-unbound glycopeptides (*a*)	Con A-bound glycopeptides (*b*)	Ratio (*b*)/(*a*)
A	Tissue				
	Regenerating liver	950	407	488	1.20
	Control	782	375	363	0.97
	Medium				
	Regenerating liver	3418	1419	1999	1.41
	Control	2336	1220	1116	0.91
B	Tissue				
	Regenerating liver	820	351	418	1.19
	Control	982	469	463	0.99
	Medium				
	Regenerating liver	2563	1114	1449	1.30
	Control	3201	1707	1494	0.88

linkage. The electrophoretogram of tissue glycoproteins 24h after the operation showed little or no difference between regenerating liver and controls, regardless of the radioactive precursor used (Fig. 3). Therefore it is likely that the difference in the oligosaccharide moiety of tissue glycoproteins should be caused by the difference in oligosaccharide processing rather than by the difference in glycoprotein composition.

On the other hand, some differences were observed in the electrophoretogram of medium glycoproteins. As shown in Fig. 4, the radioactivity in the region of M_r about 60000 was markedly decreased when regenerating liver was compared

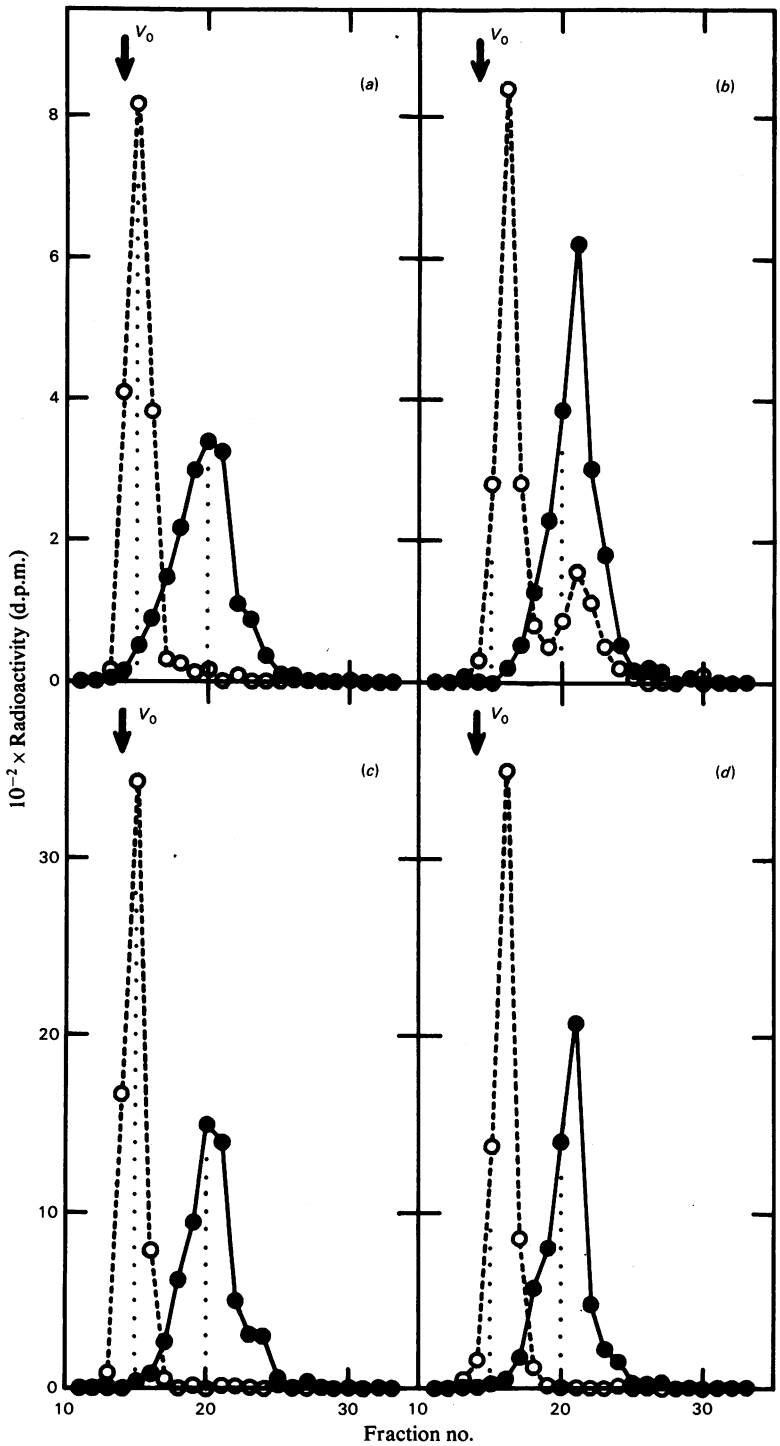

Fig. 2. *Bio-Gel P-6 column chromatography of [^3H]fucose-labelled glycopeptides from regenerating liver 24h after partial hepatectomy*

Con A-unbound tissue glycopeptides (*a*), Con A-bound tissue glycopeptides (*b*), Con A-unbound medium glycopeptides (*c*) and Con A-bound medium glycopeptides (*d*) were chromatographed before (○) and after (●) neuraminidase digestion. Blue Dextran was used to determine V_0, and mannose was used to determine V_t (fraction no. 45). No significant radioactivity was detected in fractions 30–45 in each case.

Table 2. *Time-dependency of increase in affinity of [^{3}H]fucose-labelled glycopeptides to Con A–Sepharose*
[^{3}H]Fucose-labelled glycopeptides obtained from regenerating liver and control were fractionated by Con A–Sepharose column chromatography as shown in Table 1. The ratio of radioactivity in Con A-bound glycopeptides to that in Con A-unbound glycopeptides is indicated. Values in parentheses are relative ones, with each control value as 1.00.

Time after operation (h)	Tissue		Medium	
	Regenerating liver	Control	Regenerating liver	Control
12	1.11 (1.02)	1.09	1.05 (1.28)	0.82
24*	1.19 (1.20)	0.99	1.30 (1.48)	0.88
72	0.94 (1.02)	0.92	0.97 (1.17)	0.83
144	0.99 (1.02)	0.97	0.81 (0.96)	0.84

* Data from Table 1 (Expt. B).

with controls. The difference was observed in both [^{3}H]mannose- and [^{3}H]fucose-labelled glycoproteins. This difference may be one of the causes for the difference in the oligosaccharide moiety of medium glycoproteins.

Discussion

A higher proportion of [^{3}H]fucose-labelled glycoproteins from regenerating liver 24h after partial hepatectomy bound to both Con A–Sepharose (Table 1) and lentil lectin–Sepharose as compared with controls. Con A binds biantennary glycopeptides, but neither tri- nor tetra-antennary glycopeptides (Baenziger & Fiete, 1979; Finne & Krusius, 1982). Lentil lectin binds biantennary glycopeptides with core fucose residue and certain triantennary glycopeptides (Kornfeld *et al.*, 1981). Therefore it should be concluded that the fucosyl glycopeptides from regenerating liver 24h after partial hepatectomy contain a higher proportion of biantennary species with core fucose residue.

Although several studies indicated correlations between the alterations in *N*-linked oligosaccharides of glycoproteins and cell growth (Muramatsu *et al.*, 1976; Hakimi & Atkinson, 1980; Rupar & Cook, 1982; Kato & Akamatsu, 1984), cell adhesion (Sasak *et al.*, 1980), cell density (Sasak *et al.*, 1982) and cell differentiation (Herscovics *et al.*, 1980), little attention was paid to the properties of complex-type glycopeptides. Concerning complex-type glycopeptides, it has been shown that fucosyl glycopeptides of growing cells were larger in size than those of non-growing cells (Buck *et al.*, 1971; Muramatsu *et al.*, 1973). Taking advantage of Con A specificity, cell-surface fucosyl glycopeptides in subconfluent cells were shown to be more highly branched than those in confluent cells. Additionally, biantennary fucosyl glycopeptides were more sialylated in subconfluent cells than in confluent cells (Sasak *et al.*, 1983). On the other hand, by numerous studies on transformed cells, glycopeptides from the surface and intracellular membranes have been shown to have higher M_r values because of more extensive sialylation of the oligosaccharides as compared with their normal counterparts (Warren *et al.*, 1978; Atkinson & Hakimi, 1980). Also, it has been shown that the glycopeptides of transformed cells contain more highly branched oligosaccharides than do those of normal cells (Ogata *et al.*, 1979; Santer & Glick, 1979). As for individual glycoproteins, for example, fibronectins of transformed cells have more highly branched and sialylated oligosaccharides than those of normal cells (Wagner *et al.*, 1981). Our results present a contrast with those observations. The fucosyl glycopeptides from regenerating liver, especially 24h after partial hepatectomy, i.e. growing cells, were of lower M_r (Fig. 1) and contained a higher proportion of biantennary species (Table 1) as compared with controls, i.e. non-growing cells. Thus it remains to be answered whether such growth-dependent alterations of glycopeptides are specific to liver cells under normal growth control. It will be interesting to examine the glycopeptides obtained from hepatoma.

In the endocytosis of asialoglycopeptides, the number of galactose residues per cluster and their branching mode are major determinants of their binding and uptake to hepatocytes (Baenziger & Fiete, 1980; Lee *et al.*, 1983). The more highly branched oligosaccharide of asialoglycopeptides is favourable for endocytosis to occur. Decreased endocytosis of asialoglycoproteins, with concomitant decrease in the number of asialoglycoprotein receptors, has been observed during liver regeneration (Howard *et al.*, 1982). Therefore it is tempting to speculate that during liver regeneration the decrease not only in the number of asialoglycoprotein receptors but also in the degree of oligosaccharide branching of fucosyl glycoproteins

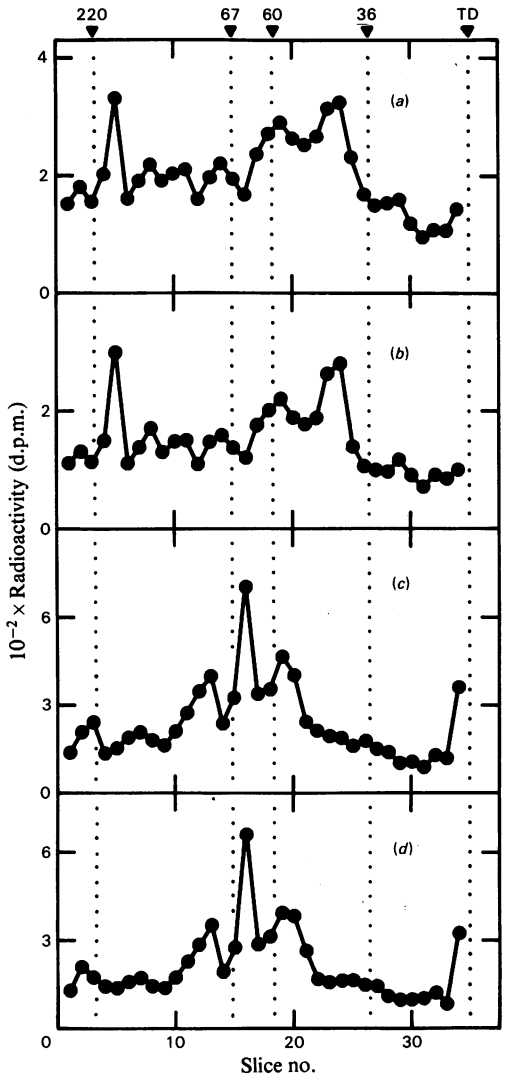

Fig. 3. *SDS/polyacrylamide-gel electrophoresis of tissue glycoproteins*

[^{3}H]Mannose-labelled glycoproteins from regenerating liver 24h after partial hepatectomy (*a*) and from control liver (*b*), and [^{3}H]fucose-labelled glycoproteins from regenerating liver 24h after partial hepatectomy (*c*) and from control liver (*d*), were electrophoresed. The mobilities of the tracking dye (TD) and protein standards ($10^{-3} \times M_r$) are indicated.

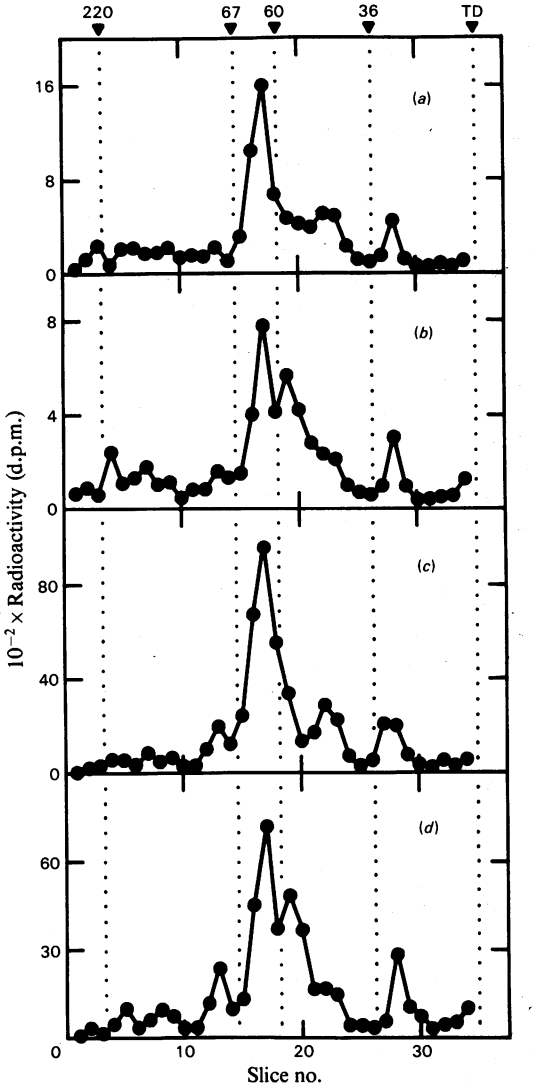

Fig. 4. *SDS/polyacrylamide-gel electrophoresis of medium glycoproteins*

[^{3}H]Mannose-labelled glycoproteins from regenerating liver 24h after partial hepatectomy (*a*) and from control liver (*b*), and [^{3}H]fucose-labelled glycoproteins from regenerating liver 24h after partial hepatectomy (*c*) and from control liver (*d*), were electrophoresed. The mobilities of the tracking dye (TD) and protein standards ($10^{-3} \times M_r$) are indicated.

may contribute to maintain the blood content of plasma glycoproteins (Bresnick, 1971), in compensation for the decrease of glycoprotein synthesis (Okamoto & Akamatsu, 1977; Okamoto *et al.*, 1978; Oda-Tamai *et al.*, 1985). In regenerating liver, the decreased oligosaccharide branching of glycoproteins may be one of the causes for the decreased degradation of plasma-membrane glycoproteins (Tauber & Reutter, 1981) and may affect the deposition and/or turnover of some glycoproteins such as asialoglycoprotein receptor (Howard *et al.*, 1982) and transferrin receptor (Hirose-Kumagai *et al.*, 1984). In relation to these points, it should be mentioned that non-fucosyl oligosaccharides of glycoproteins as well as fucosyl ones were less branched in regenerating liver as com-

pared with controls (I. Ishii, N. Takahashi, S. Kato, N. Akamatsu & Y. Kawazoe, unpublished work).

Substrate specificity of glycosyltransferases involved in the synthesis of oligosaccharide branches should be taken into consideration to understand the structural alteration in oligosaccharides of glycoproteins. The presence of, at most, two galactose residues on branches has been shown to prevent the further action of α-1,6-fucosyltransferase (Schachter *et al.*, 1983), β-1,4-*N*-acetylglucosaminyltransferase (transferase IV) (Schachter *et al.*, 1983) and β-1,6-*N*-acetylglucosaminyltransferase (transferase V) (Cummings *et al.*, 1982). The activity and subcellular compartmentation of these enzymes, including β-1,4-galactosyltransferase, in regenerating liver should be favourable for the production of a biantennary fucosyl oligosaccharides.

We are grateful to S. Oda-Tamai for able technical assistance, and M. Yoshimura for manuscript preparation. Part of this work was supported by a Grant-in-Aid for Scientific Research from The Ministry of Education, Science and Culture, Japan.

References

Atkinson, P. H. & Hakimi, J. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., ed.), pp. 191–239, Plenum Press, New York

Baenziger, J. U. & Fiete, D. (1979) *J. Biol. Chem.* **254**, 2400–2407

Baenziger, J. U. & Fiete, D. (1980) *Cell* **22**, 611–620

Bresnick, E. (1971) *Methods Cancer Res.* **6**, 347–397

Buck, C. A., Glick, M. C. & Warren, L. (1971) *Biochemistry* **10**, 2176–2180

Cummings, R. D., Trowbridge, I. S. & Kornfeld, S. (1982) *J. Biol. Chem.* **257**, 13421–13427

Finne, J. & Krusius, T. (1982) *Methods Enzymol.* **83**, 269–277

Hakimi, J. & Atkinson, P. H. (1980) *Biochemistry* **19**, 5619–5624

Herscovics, A., Bugge, B., Quaroni, A. & Kirsch, K. (1980) *Biochem. J.* **192**, 145–153

Hirose-Kumagai, A., Sakai, H. & Akamatsu, N. (1984) *Int. J. Biochem.* **16**, 601–605

Howard, D. J., Stockert, R. J. & Morell, A. G. (1982) *J. Biol. Chem.* **257**, 2856–2858

Kato, S. & Akamatsu, N. (1984) *Biochim. Biophys. Acta* **798**, 68–77

Kornfeld, K., Reitman, M. L. & Kornfeld, R. (1981) *J. Biol. Chem.* **256**, 6633–6640

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Lee, Y. C., Townsend, R. R., Hardy, M. R., Lonngren, J., Arnarp, J., Haraldsson, M. & Lonn, H. (1983) *J. Biol. Chem.* **258**, 199–202

Muramatsu, T., Atkinson, P. H. & Nathenson, S. G. (1973) *J. Mol. Biol.* **80**, 781–799

Muramatsu, T., Koide, N., Ceccarini, C. & Atkinson, P. H. (1976) *J. Biol. Chem.* **251**, 4673–4679

Oda-Tamai, S., Kato, S., Hara, S. & Akamatsu, N. (1985) *J. Biol. Chem.* **260**, 57–63

Ogata, S. I., Muramatsu, T. & Kobata, A. (1979) *Nature (London)* **259**, 580–582

Okamoto, Y. & Akamatsu, N. (1977) *Biochim. Biophys. Acta* **498**, 272–281

Okamoto, Y., Ito, E. & Akamatsu, N. (1978) *Biochim. Biophys. Acta* **542**, 21–27

Olden, K., Parent, J. B. & White, S. L. (1982) *Biochim. Biophys. Acta* **650**, 209–232

Rupar, C. A. & Cook, G. M. W. (1982) *Biochem. J.* **201**, 377–385

Santer, U. V. & Glick, M. C. (1979) *Biochemistry* **18**, 2533–2540

Sasak, W., De Luca, L. M., Dion, L. D. & Silverman-Jones, C. S. (1980) *Cancer Res.* **40**, 1944–1949

Sasak, W., Herscovics, A. & Quaroni, A. (1982) *Biochem. J.* **201**, 359–366

Sasak, W., Quaroni, A. & Herscovics, A. (1983) *Biochem. J.* **211**, 75–80

Schachter, H., Narasimhan, S., Gleeson, P. & Vella, G. (1983) *Can. J. Biochem. Cell Biol.* **61**, 1049–1066

Sharon, N. (1984) *Trends Biochem. Sci.* **9**, 198–202

Tauber, R. & Reutter, W. (1981) *Eur. J. Cell Biol.* **26**, 35–42

Wagner, D. D., Ivatt, R., Destree, A. T. & Hynes, R. O. (1981) *J. Biol. Chem.* **256**, 11708–11715

Warren, L., Buck, C. A. & Tuszynski, G. P. (1978) *Biochim. Biophys. Acta* **516**, 97–127