Pre-replicative changes of the rat sinusoidal plasma membrane glycoproteins during hepatic regeneration

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Cell-surface glycoproteins of rat liver sinusoidal plasma membranes from control and regenerating livers were studied. The glycoproteins were labeled using specific methods for sialic acid ($NAIO_4/NaB^3H_4$) and galactosyl/*N*-acetyl galctosaminyl residues (galactose oxidase/NaB^3H_4 and neuraminidase-galactose oxidase (NaB^3H_4) and the solubilized proteins were analyzed by gel electrophoresis. The patterns obtained with regenerating livers were quantitatively different from controls. This shows that cell surface glycoproteins change during liver regeneration.

Sialic acid *Liver plasma membrane* *Liver regeneration* *Cell surface glycoprotein*

1. INTRODUCTION

The mammalian liver is an excellent system for the study of growth regulation in the adult organism. Under different experimental situations, like regeneration or neoplasia, the hepatocytes in the liver of adult animals can easily be induced to enter the replicative cycle, and it can be demonstrated that most cells undergo DNA synthesis and subsequent cell division [1,2].

The key events in the control of the triggering of this process and the initiation of DNA synthesis remain unknown. It is, however, probable that the plasma membrane plays a decisive role as a receptor and transductor structure. In the early pre-replicative events of the process it can be anticipated that the sinusoidal plasma membrane of the hepatocyte is involved, since in this region of the cell surface most of the receptors for peptide hormones, growth factors as well as the molecules involved in ion or metabolite transport across the membrane are located [3].

Here, we have radioactively labeled the oligosaccharide portions of the membrane glycoproteins and analyzed the proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The results show specific changes in the plasma membrane glycoproteins at the pre-replicative phase of liver regeneration.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200–250 g, maintained on a standard rat diet, and under light control were used for all experiments. Partial hepatectomies were always performed between 8 and 10 a.m. Surgery was carried out using the procedure of Higgins and Anderson [4]. Ligation and excision of the median and left lateral lobes of liver constituted partial hepatectomy (70%). The membranes were isolated [5] and labeled as in [6,7]. Polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate was done according to Laemmli [8]. After electrophoresis the gels were fixed, treated for fluorography [9] and exposed to Kodak AR X-Omat films. ^{14}C-labeled standard proteins were obtained from the

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Table 1

Specific activities of rat sinusoidal plasma membrane enzymes from control and regenerating livers

Enzyme	Specific activity in controls		Specific activities at 6 h of regeneration	
	Homogenate	Plasma membranes	Homogenate	Plasma membranes
5′-Nucleotidase	26.25 ± 4.96 (4)	522.13 ± 97.35 (10)	31.06 ± 4.93 (3)	324.71 ± 63.24 (6)
Ca^{2+}-activated ATPase	8.73 ± 2.92 (3)	58.67 ± 10.01 (12)	8.02 ± 2.54 (2)	21.63 ± 5.40 (4)
Mg^{2+}-activated ATPase	4.75 ± 1.58 (5)	40.65 ± 10.89 (10)	9.06 ± 1.79 (6)	14.26 ± 6.06 (4)

The results are expressed as: μmol P_i released/mg protein per h for Ca^{2+}-activated ATPase [13] and for Mg^{2+}-activated ATPase [14] and in μmol inosine/mg protein per h for the 5′-nucleotidase [15]. The numbers in parentheses give the number of experiments. The enzymatic assays were performed in triplicate in each instance

Radiochemical Centre, Amersham. Densitometric scans were obtained using a Double-Beam Recording Microdensitometer (Joyce-Loebl). Protein was determined by the method of Lowry et al. [10].

3. RESULTS

In previous studies it was demonstrated that a few hours after the initiation of the regenerative process, induced by partial hepatectomy, several changes occur at the sinusoidal plasma membrane [11,12]. The changes are most remarkable at 6 h after regeneration [11].

The activities of 5′-nucleotidase, Ca^{2+}-activated ATPase (table 1), alkaline phosphatase and phosphodiesterase did not change in the homogenate [11]. There was some increase in Mg^{2+}-activated ATPase activity (table 1).

When compared with the homogenate, the purified plasma membranes from regenerating livers showed a relatively large increase in the specific activity of 5′-nucleotidase. In contrast, the corresponding increase in the specific activities of Ca^{2+}-activated ATPase and Mg^{2+}-activated ATPase was smaller (table 1). The yield of plasma membranes was 0.46 ± 0.14 mg/g liver and 0.54 ± 0.17 mg/g liver for controls and regenerating livers, respectively.

Several changes in the protein composition were detected, the most important being those found in the glycoproteins resolved by the Schiff staining method (fig.1). The reduction of the PAS 5–6 and the presence of new bands (see arrows at 200, 85, 80 and 40 kDa) were the most obvious.

When sinusoidal membranes were labeled by the $NaIO_4/NaB^3H_4$ method, a decreased labeling of GP 80, GP 70, GP 52, GP 40 and GP 32 was seen

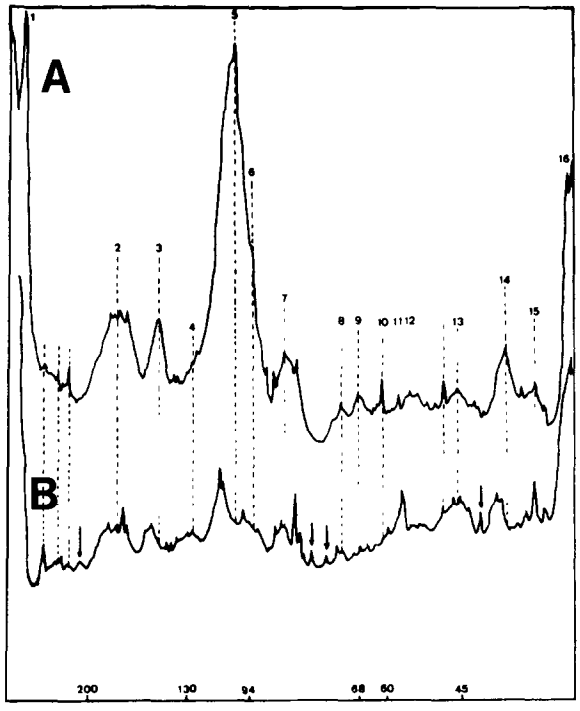

Fig.1. Densitometric scans at 560 nm of PAS-stained polyacrylamide gels after electrophoresis in sodium dodecyl sulfate; 800 μg plasma membrane protein were applied in each sample. (A) Control, (B) 6 h regeneration. The molecular mass scale is based on the electrophoretic migration of reduced standard proteins.

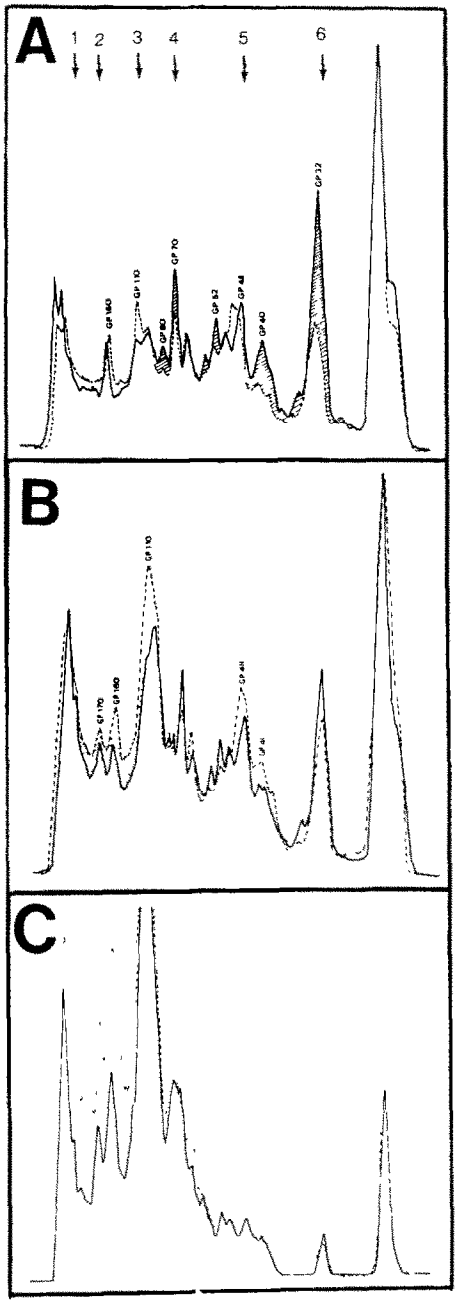

Fig.2. Surface labeling of control (——) and 6 h (– – –) regenerating livers. (A) $NaIO_4/NaB^3H_4$, (B) galactose oxidase/NaB^3H_4, (C) neuraminidase-galactose oxidase/NaB^3H_4. The molecular mass markers are indicated above. 1, myosin (200 kDa); 2, thyroglobulin (168 kDa); 3, phosphorylase *b* (94 kDa); 4, bovine serum albumin (68 kDa); 5, ovalbumin (44 kDa); 6, carbonic anhydrase (30 kDa). The acrylamide concentration was 8% and the densitometric scans were performed from the fluorographs of fig.3.

in regenerating livers. The GP 110 increased in intensity and a change in the GP 48 region was observed (figs 2A and 3).

When the plasma membrane glycoproteins were labeled by the galactose oxidase/NaB^3H_4 method, the densitometric scans of two samples, 6 h and control, show that there was an increased label in several components but especially in the GP 170, GP 160, GP 110, GP 48 and GP 40 indicating that more galactosyl residues were present/accessible at the cell surface.

When the membrane glycoproteins were labeled by the neuraminidase-galactose oxidase/NaB^3H_4 method (fig.2C) it was evident that most bands (GP 250, GP 230, GP 170, GP 160 and GP 48) became more efficiently labeled in the membranes from regenerating cells.

4. DISCUSSION

Hepatic regeneration, obtained after partial hepatectomy, is a suitable model for studies on cell division in adult organisms. The quiescent cell population is activated through a complex sequence of reactions concluding with a burst of DNA synthesis and subsequent cell division. Alterations of cell surface glycoconjugates have been observed in many differentiating and developing systems and the carbohydrate moiety has been implicated to be important in cell differentiation, proteolytic stability, secretion, and recognition [17].

The yields of plasma membranes from control and regenerating livers were similar. On the other hand, the specific activities of the marker enzymes were lower in the isolated membranes from regenerating livers than in those from controls. This could mean that the plasma membranes from regenerating livers are less pure or contain a different ratio of inside-out/outside-out vesicles. This is possible, but the uneven decrease in specific enzymatic activities and the changes in glycoprotein patterns show that molecular changes occur in the plasma membranes.

A significant reduction of sialic acid bound to the sinusoidal plasma membrane was seen in regenerating livers, as well as quantitative changes in the glycoprotein patterns.

From the data obtained it is clear that the labeling of all sialoglycoproteins was not decreased.

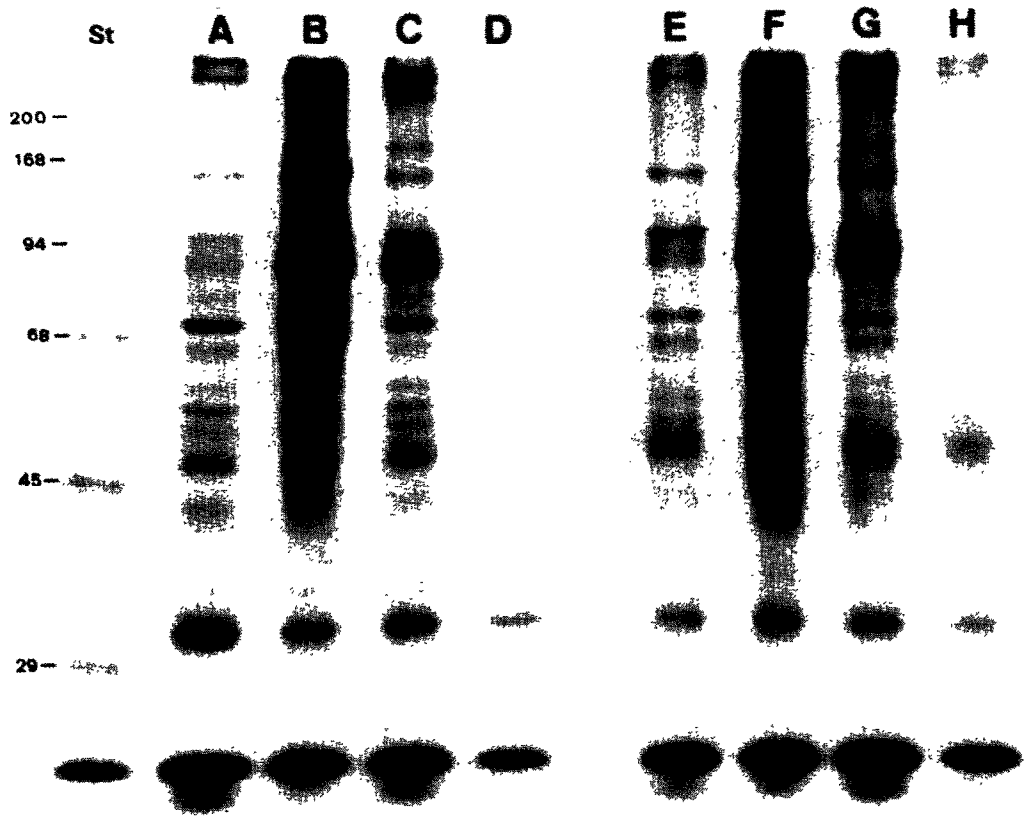

Fig.3. Fluorography of a slab gel of sinusoidal plasma membrane proteins labeled by $NaIO_4/NaB^3H_4$ (A,E); neuraminidase-galactose oxidase/NaB^3H_4 (B,F); galactose oxidase/NaB^3H_4 (C,G). In D and H the membranes were only treated with NaB^3H_4 without oxidation. (A–D) Control samples, (E–H) from plasma membranes after 6 h of regeneration. Molecular mass markers as in fig.2.

The GP 110 in the $NaIO_4/NaB^3H_4$ (fig.2A) pattern shows an increased labeling at 6 h after hepatectomy. Also the Schiff profiles show that at least 4 new components appear, with apparent molecular masses of 200, 85, 80 and 40 kDa (fig.1). Other authors have demonstrated an increased sialylation during the early period of liver regeneration [18].

Although relatively little is known about the hepatic surface glycoproteins [19] it is interesting that whilst regenerating normal liver contains a decreased amount of sialic acids [20], liver cancers seem to contain increased levels [21,22]. It will be important to compare the sialoglycoproteins of growing normal and malignant liver cells.

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REFERENCES

[1] Grisham, J.W. (1962) Cancer Res. 22, 842–849.
[2] Bucher, N.L.R. and Malt, R.A. (1971) in: Regeneration of Liver and Kidney (New England J. Med., Medical Progress Series) pp.17–178, Little, Brown and Co., Boston.
[3] Evans, W.H. (1981) Hepatology 1, 452–457.

[4] Higgins, G.M. and Anderson, R.M. (1931) Arch. Pathol. (Chicago) 12, 186–202.
[5] Wisher, M.H. and Evans, W.H. (1975) Biochem. J. 146, 375–388.
[6] Gahmberg, C.G. and Hakomori, S.-I. (1973) J. Biol. Chem. 248, 4311–4317.
[7] Gahmberg, C.G. and Andersson, L.C. (1977) J. Biol. Chem. 252, 5888–5894.
[8] Laemmli, U.K. (1970) Nature 227, 680–685.
[9] Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Enrich, C. (1983) PhD Thesis, University of Barcelona, Barcelona, Spain.
[12] Enrich, C., Bachs, O., Soriano, M., Serratosa, J. and Domingo, J. (1980) Eur. J. Cell Biol. 22, 267.
[13] Yamaguchi, M. (1979) Endocrinology (Japan) 26, 605–609.
[14] Sulakhe, P.V., Fedelesova, M., McNamara, D.B. and Dhall, N.-S. (1971) Biochem. Biophys. Res. Commun. 42, 793–800.
[15] Ipata, P.I. (1968) Biochemistry 7, 507–515.
[16] Enrich, C., Bachs, O., Rius, E., Serratosa, J. and Domingo, J. (1985) Cell Biochem. Function, in press.
[17] Gahmberg, C.G. (1981) in: Comprehensive Biochemistry, Membrane Structure (Finean, J.B. and Michell, R.H. eds) pp.127–160, Elsevier, Amsterdam, New York.
[18] Köttgen, E., Reutter, W. and Gerok, W. (1978) Eur. J. Biochem. 82, 279–284.
[19] Enrich, C. and Gahmberg, C.G. (1985) Biochem. J., in press.
[20] Kishore, G.S. and Carubelli, R. (1981) Cancer Lett. 13, 281–289.
[21] Mabry, E.W. and Carubelli, R. (1972) Experientia 28, 182–183.
[22] Kloppel, T.M. and Moré, D.J. (1980) J. Natl. Cancer Inst. 64, 1401–1411.