Sialyltransferase Activity in Regenerating Rat Liver

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Liver microsomal fractions catalyse the transfer of sialic acid from CMP-*N*-acetylneuraminic acid to various exogenous acceptors such as desialylated fetuin, desialylated human Tamm–Horsfall glycoprotein and desialylated bovine submaxillary-gland mucin. An increase in the rate of incorporation of sialic acid into desialylated glycoproteins was found after a lag period (7 h) in regenerating liver. The increase was maximum 24 h after partial hepatectomy for all acceptors tested. At later times after operation the sialyltransferase activity remained high only for desialylated fetuin. No soluble factors from liver or serum of partially hepatectomized animals influenced the activity of the sialyltransferases bound to the microsomal fraction. The sensitivity of sialyltransferases to activation by Triton X-100, added to the incubation medium, was unchanged in the microsomal preparation from animals 24 h after sham operation or partial hepatectomy. The full activity of sialyltransferases towards the various desialylated acceptors showed some differences. Human Tamm–Horsfall glycoprotein was a good acceptor of sialic acid only when desialylated by mild acid hydrolysis. After this treatment, but not after enzymic hydrolysis, a decrease in molecular weight of human Tamm–Horsfall glycoprotein was observed. Further, the sialyltransferase activity as a function of incubation temperature gave different curves according to the acceptor used. The relationship between the biosynthesis of glycoproteins by regenerating liver and the sialyltransferase activity of microsomal fraction after partial hepatectomy is discussed.

The extremely rapid regeneration of liver which follows partial hepatectomy offers a suitable model for studying the metabolic activity of dividing cells compared with the cells of quiescent liver. The function of hepatocytes in the synthesis of glycoproteins has been well documented (Robinson *et al.*, 1964; Macbeth *et al.*, 1965). Some glycoproteins synthesized by liver are secreted and in the main discharged into the bloodstream; some remain in the cell and they are associated with cellular membranes. A number of intracellular glycoproteins are located in the outer surface of the plasma membranes and they have been supposed to be implicated in the mechanism of cell–cell recognition and cell adhesion (see the review by Nicolson, 1976). Several studies have demonstrated that changes in the surface glycoproteins occur in malignant cells (Meezan *et al.*, 1969; Buck *et al.*, 1971). The glycosyltransferase activities which regulate chain elongation of the oligosaccharide prosthetic groups of glycoproteins undergo changes when the cells are transformed by oncogenic viruses (Bosmann, 1972; Grimes, 1973), during the growth stage in the mitotic cycle of the cell (Bosmann, 1971) and in tissues during embryonic development (Carlson *et al.*, 1973; Jato-Rodriguez & Mookerjee, 1974). Warren *et al.* (1972) found an increase of specific sialyltransferases both in transformed and in dividing cells.

In a previous study, Serafini-Cessi (1976) showed that the overall rate of biosynthesis of serum glycoproteins was decreased in the early period of liver regeneration and that the rate returned to normal 3 days after partial hepatectomy. Bauer *et al.* (1976) investigated galactose metabolism in regenerating rat liver, and found that the UDP-galactose 4-epimerase and the UDP-galactose–glycoprotein galactosyltransferase activities increase after partial hepatectomy. There is also an increased amount of UDP-*N*-acetylglucosamine–asparagine sequon *N*-acetylglucosaminyl transferase activity in preparation of rough endoplasmic reticulum isolated from regenerating rat liver (Khalkhali *et al.*, 1977).

The experiments described in the present paper were designed to measure the sialyltransferase activity located in the microsomal fraction of rat liver at various times after partial hepatectomy. Since the glycosyltransferase reactions require suitable acceptors, the sialyltransferase activities of regenerating and quiescent liver were measured in the presence of various exogenous acceptors. Desialylated fetuin, desialylated human Tamm–Horsfall glycoprotein and desialylated bovine submaxillary-gland mucin were used. Tamm–Horsfall glycoproteins isolated from urine of different mammalian species and treated by mild acid are good acceptors of sialic acid from CMP-*N*-[¹⁴C]neur-

aminic acid in the reaction catalysed by the microsomal fraction from liver (Serafini-Cessi & Marshall, 1975).

Experimental

Materials

Animals. Male Wistar rats weighing 180–200 g were used. Partial hepatectomy by the technique described by Higgins & Anderson (1931) and sham operation consisting of laparotomy and palpation of the liver were performed under diethyl ether anaesthesia. At 7, 24, 48 and 72 h after operation the animals were killed by decapitation and the liver was immediately removed and placed in ice.

Chemicals. CMP-*N*-[¹⁴C]acetylneuraminic acid (214 Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Fetuin and bovine submaxillary-gland mucin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Human Tamm-Horsfall glycoprotein was prepared from human urine by the technique of Tamm & Horsfall (1950, 1952). Neuraminidase from *Clostridium perfringens* (from Sigma) contained 0.5 unit/mg of protein with *N*-acetylneuraminyl-lactose as substrate. All other chemicals were reagent grade.

Methods

Preparation of microsomal fractions. Microsomal preparations were isolated from livers of sham-operated and partially hepatectomized rats by the procedure of Keller & Zamecnik (1956). The pellets were suspended in 0.01 M-Tris/HCl, pH 6.6, at a protein concentration of 10 mg/ml. Protein was determined by the procedure of Lowry *et al.* (1951) with bovine serum albumin as standard. Sialyltransferase activities were measured within 1 h of preparing the microsomal fractions.

Preparation of desialylated acceptors. Sialic acid was removed from glycoproteins by acid hydrolysis as described by Grimes (1970). Removal of sialic acid by neuraminidase from fetuin and human Tamm-Horsfall glycoprotein was performed as follows: 20 mg of glycoprotein was incubated with 0.5 mg of neuraminidase at 37°C for 10 h in 0.5 ml of a solution containing 0.2 M-sodium acetate, 0.15 M-NaCl, 2 mM-CaCl₂ and 1% (v/v) toluene, titrated to pH 6.9 with acetic acid (Morell *et al.*, 1966). After incubation the solution was heated for 2 min in a boiling-water bath to inactivate the enzyme. More than 95% of the sialic acid was removed after the 10 h enzymic hydrolysis. Analysis of sialic acid was by the thiobarbituric acid method of Warren (1959). After acid and enzymic hydrolyses the desialylated glycoproteins were neutralized, dialysed against distilled water and freeze-dried.

Measurement of sialyltransferase activity. The incubation mixture contained 5 μ l of 0.2 M-Tris/HCl buffer, pH 6.6, 3 μ l of 1% Triton X-100, 2 μ l of 0.1 M-MnCl₂, 10 μ l of microsomal preparation, 0.03 μ Ci of CMP-*N*-[¹⁴C]acetylneuraminic acid, 20 μ l of desialylated glycoprotein acceptor (10 mg/ml in 0.1% Triton X-100) and water to a final volume of 50 μ l. Incubation was usually done at 26°C for 1 h and the reaction was stopped by the addition of 0.5 ml of 1% phosphotungstic acid in 0.5 M-HCl. The precipitate was collected on glass-fibre filter and washed with 3 $\times$ 25 ml of ice-cold 1% phosphotungstic acid in 0.5 M-HCl and once with 5 ml of ice-cold 5% (w/v) trichloroacetic acid. The filter was dried, placed in a counting vial containing 5 ml of methylCellosolve and 10 ml of scintillation fluid [0.05% 1,4-bis-(5-phenyloxazol-2-yl)benzene and 0.4% 2,5-diphenyloxazole in toluene] and counted for radioactivity in a Packard Tri-Carb liquid-scintillation spectrometer.

Polyacrylamide-gel electrophoresis. Glycoproteins and desialylated glycoproteins were incubated in 0.1% sodium dodecyl sulphate for 1 h before electrophoresis. The procedure used was that of Marshall & Zamecnik (1969) in the presence of 0.1% sodium dodecyl sulphate. The gels were stained with Coomassie Blue.

Results

Microsomal preparations of liver catalyse the transfer of *N*-[¹⁴C]acetylneuraminic acid from CMP-*N*-[¹⁴C]acetylneuraminic acid to acid-treated glycoproteins such as fetuin, bovine submaxillary-gland mucin and human Tamm-Horsfall glycoprotein (Serafini-Cessi & Marshall, 1975); to measure the sialyltransferase activity, the incubation temperature used in the initial experiments was 37°C. The results reported in Fig. 1 demonstrate that the microsomal preparation from rat liver gave better incorporation of sialic acid into acid-treated glycoproteins when the incubation is performed at 26°C. The difference between incubation at 26 and 37°C was particularly strong when desialylated bovine submaxillary-gland mucin was used as acceptor. Similar results were obtained with microsomal preparation from the livers of sham-operated and partially hepatectomized rats.

Table 1 shows the rate of incorporation of sialic acid into desialylated glycoproteins (which had been prepared by acid treatment) catalysed by microsomal preparation from sham-operated and partially hepatectomized animals. No increment of the sialyltransferase activity appeared at an early time after operation (7 h). The rate of incorporation of sialic acid into all desialylated glycoproteins reached a maximum 24 h after partial hepatectomy. At later times (48, 72 h) the sialyltransferase activity remained high only when desialylated fetuin was used as substrate.

Fig. 1. Effect of incubation temperature on incorporation of sialic acid into acid-treated glycoproteins

Incubation mixtures were prepared as described under 'Methods'. Microsomal fractions of control rats were used. Acceptors: ●, acid-treated fetuin; ○, acid-treated bovine submaxillary-gland mucin; ▲, acid-treated human Tamm-Horsfall glycoprotein. Each point represents the mean ± S.E.M. of three determinations.

A plot of the activity of sialyltransferase expressed as c.p.m. of [¹⁴C]sialic acid incorporated per mg of microsomal proteins into acid-treated fetuin as a function of the length of incubation at 26°C is shown in Fig. 2. The sialyltransferase activity of the microsomal fraction prepared from regenerating liver was higher than that from quiescent liver. The effect of soluble factors present in regenerating liver which might influence the sialyltransferase activity of microsomal fractions was investigated. The addition of postmicrosomal supernatant or serum from partially hepatectomized (24 h) animals did not change the incorporation of sialic acid catalysed by the microsomal fraction of quiescent liver.

Incorporation of sialic acid into desialylated glycoproteins is stimulated by the addition of Triton X-100. According to Grimes (1970) the optimum of the detergent concentration in the incubation mixture is 0.1%. At this concentration the enzyme was stimulated but not solubilized. To investigate whether the increase of sialyltransferase activity in regenerating liver might depend on a different sensitivity to the detergent, the incorporation of sialic acid into acid-treated fetuin was measured in the presence of various amounts of Triton X-100 (Fig. 3). The curves obtained show that the highest incorporation of sialic acid into acid-treated fetuin was reached at the

Table 1. Incorporation of sialic acid from CMP-N-[¹⁴C]acetylneuraminic acid into glycoproteins catalysed by liver microsomal preparations from sham-operated and partially hepatectomized rats

The microsomal fractions were prepared from rats 7, 24, 48 and 72 h after sham operation or partial hepatectomy. Sialyltransferase activity was measured within 1 h of preparing the microsomal fraction. The acceptor glycoproteins were treated under conditions of mild acid hydrolysis in order to remove the sialic residue. For further details see the Experimental section. Values are the means ± S.E.M. of four determinations. Levels of significance are shown by **P* < 0.01; ***P* < 0.02.

Time after operation (h) ...	7			24			48			72		
	Sham-operated	Partially hepatectomized		Sham-operated	Partially hepatectomized		Sham-operated	Partially hepatectomized		Sham-operated	Partially hepatectomized	
Glycoprotein acceptor												
None	2850 ± 210	2720 ± 189		3130 ± 273	3850 ± 412		3080 ± 382	3420 ± 398		4150 ± 394	3170 ± 331	
Acid-treated fetuin	47 870 ± 5910	40 140 ± 3250		50 250 ± 2490	75 330 ± 5880*		47 140 ± 3440	65 420 ± 3210*		45 150 ± 4410	61 130 ± 7150	
Acid-treated human Tamm-Horsfall	25 730 ± 2220	22 310 ± 1980		22 840 ± 795	38 710 ± 5310**		24 800 ± 2410	32 920 ± 2410		30 020 ± 3250	29 650 ± 2460	
Acid-treated bovine sub-maxillary mucin	34 680 ± 3090	31 420 ± 1750		35 470 ± 1310	56 660 ± 6080**		37 820 ± 2890	41 880 ± 2850		35 790 ± 4190	39 030 ± 1810	

same concentration of Triton X-100 both in regenerating and quiescent liver.

Serafini-Cessi & Marshall (1975) found that mild acid hydrolysis (0.05M-H₂SO₄; 85°C; 1h) caused a change in molecular weight of Tamm-Horsfall glycoprotein isolated from the urine of the hamster without any change in the sugar composition, and it was suggested that the product of lower molecular weight might be a better substrate for sialyltransferase than was the original glycoprotein. Human Tamm-Horsfall glycoprotein and fetuin, desialylated in each case either by mild acid hydrolysis or by the action of neuraminidase, were analysed by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. Whereas both enzyme- and acid-treated

fetuin gave the same electrophoretic patterns as untreated fetuin, the human Tamm-Horsfall glycoprotein treated under mild conditions of acid hydrolysis gave a faster-migrating band than those for the untreated or enzymically hydrolysed human Tamm-Horsfall glycoprotein. The original human Tamm-Horsfall glycoprotein was confirmed as yielding a single band of mol.wt. of about 80000 (Fletcher *et al.*, 1970); the product obtained after acid hydrolysis had half the original molecular weight. Carbohydrate analysis performed by g.l.c. (Dunstan *et al.*, 1974) of acid-treated human Tamm-Horsfall glycoprotein did not show any modification of the number of sugar residues compared with the original glycoprotein,

Fig. 2. Rate of incorporation of sialic acid into acid-treated fetuin catalysed by liver microsomal fractions. Incubation temperature was 26°C; other conditions were described under 'Methods'. ●, Microsomal preparation 24h after partial hepatectomy; ○, microsomal preparation 24h after sham operation.

Fig. 3. Incorporation of sialic acid into acid-treated fetuin as a function of Triton X-100 concentration

The composition of incubation mixtures was as indicated in the text except for the Triton X-100 and for acceptor. The acceptor glycoprotein was dissolved in water, and to each sample (50 μ l final vol.) 5 μ l of a solution of Triton X-100 at a concentration ten times that to be tested was added. ●, Microsomal fraction 24h after partial hepatectomy; ○, microsomal fraction 24h after sham operation.

Table 2. Incorporation of sialic acid into fetuin and human Tamm-Horsfall glycoprotein that had been desialylated in each case either by mild acid hydrolysis or by action of neuraminidase

The microsomal fractions were prepared from rats 24h after partial hepatectomy and sham operation. For experimental details see the text. For each experiment different microsomal preparations were used.

Glycoprotein acceptors	Sialic acid incorporation (c.p.m./h per mg of microsomal protein)			
	Sham-operated		Partially hepatectomized	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Acid-treated fetuin	49090	45220	71580	77120
Neuraminidase-treated fetuin	30230	33920	49650	50910
Acid-treated human Tamm-Horsfall	24510	25840	45250	43100
Neuraminidase-treated human Tamm-Horsfall	5150	4810	9160	7830

apart from the lack of sialic acid (F. Serafini-Cessi & R. D. Marshall, unpublished work).

Table 2 shows the results of sialic acid incorporation into glycoproteins desialylated by enzymes or acid. Whereas the amount of sialic acid accepted by neuraminidase-treated fetuin was about 65% of that accepted by acid-treated fetuin, the neuraminidase-treated human Tamm-Horsfall glycoprotein incorporated less than 20% of the sialic acid incorporated by acid-treated human Tamm-Horsfall glycoprotein. Similar results were obtained when microsomal fractions of regenerating liver or quiescent liver were used to catalyse the incorporation of sialic acid.

Discussion

Several different desialylated glycoproteins are suitable acceptors of sialic acid for the sialyltransferases bound to the microsomal fraction of liver. However, the effect of temperature on incorporation of sialic acid into acid-treated bovine submaxillary-gland mucin is different from that seen with the other substrates used. It should be emphasized that the terminal non-reducing sugar moiety of the carbohydrate chains of desialylated bovine submaxillary-gland mucin is *N*-acetyl- α -D-galactosamine (Gottschalk, 1960), instead of the β -D-galactose residue which occurs in desialylated fetuin and desialylated human Tamm-Horsfall glycoprotein (Spiro, 1964; Dunstan *et al.*, 1974). The presence of different sugars in the terminal position of the carbohydrate moiety of substrates might influence the activity of the sialyltransferase system from CMP-*N*-acetylneuraminic acid to various acceptors. The dependence of sialyltransferase activity on the molecular structure of the acceptor was also demonstrated by the results of the experiments in which acid- or enzyme-treated glycoproteins were used. Human Tamm-Horsfall glycoprotein yielded a product that was shown to be a good acceptor of sialic acid only when the former had been prepared by treatment with acid. The decrease of molecular weight after this treatment may have arisen by the cleavage of an aspartic acid-proline linkage of the peptide chain, which is labile under mild acid conditions (Piskiewicz *et al.*, 1970). Changes in the molecular weight of the polypeptide acceptor have been reported to influence the acceptor ability for sugars of the substrate (Van Den Eijnden & Van Dijk, 1975).

The results on the sialyltransferase activity of regenerating liver show that after a lag period the enzyme activity increases significantly after partial hepatectomy. The increase in sialyltransferase activity at 24 h after surgery corresponds to the peak of cell proliferation (Bucher, 1963). An increased activity of many enzymes 1 day after partial hepatectomy has been found and related to the synthesis of enzyme proteins *de novo*; in fact this increase is

blocked by inhibitors of protein synthesis (Akamatsu & Maeda, 1971; Bauer *et al.*, 1976). In the present studies it was found that the changes in sialyltransferase activity of regenerating liver are not due to a different sensitivity to detergent compared with that of quiescent liver, nor to the presence of soluble activating factors in partially hepatectomized animals. It seems likely therefore that the rise in enzymic activity is due to an increase in the amount of sialyltransferases in the microsomal fraction of regenerating liver.

The correlation between the increase of sialyltransferase activity and the decrease in incorporation of D-[1-¹⁴C]glucosamine into proteins of liver microsomal fractions observed 24 h after partial hepatectomy (Serafini-Cessi, 1976) is not easily interpretable. Most of the glycoproteins synthesized by the liver are extracellular glycoproteins discharged into the bloodstream, and according to Braun *et al.* (1962) this biosynthesis is delayed in the early stages of liver regeneration. On the other hand, the intracellular glycoproteins might be synthesized very actively during hepatocyte proliferation. The high values for the transfer of sialic acid into acid-treated submaxillary-gland mucin and Tamm-Horsfall glycoprotein observed in the early period of liver regeneration might indicate a specific metabolic pathway for intracellular glycoproteins which is activated after partial hepatectomy.

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