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HUMAN LIVER GLYCOPROTEIN SIALYLTRANSFERASE

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Summary

Sialyltransferase (CMP-*N*-acetylneuraminate:D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase, EC 2.4.99.1) has been studied using the two glycoprotein substrates asialofetuin and asialo-ovine submaxillary mucin (A-OSM) in fresh and frozen human adult and fetal livers. Enzymatic activity for both substrates is stimulated by Mg^{2+} (10—25 mM) and Triton X-100 (0.4—0.6%, v/v). Freeze-thaw, heat denaturation, stability on storage, additive and substrate competition studies suggest the presence of at least two forms of human liver glycoprotein sialyltransferase. Subcellular fractionation studies indicate that the major parts of both sialyltransferase activities are associated with membraneous fractions but that a greater percentage of A-OSM than asialofetuin activity is associated with these fractions. Subcellular fractionation studies using 0.8% (v/v) Triton X-100 in the extracting buffer greatly diminishes the percentage of both asialofetuin and A-OSM sialyltranferase activity found in the mitochondrial and microsomal pellets. Kinetic characterization of sialyltransferase in a liver homogenate, resuspended 48 200 × *g* pellet and 48 200 × *g* supernatant suggested the possibility of two asialofetuin sialyltransferases. The pH optimum for homogenate and supernatant enzymes with respect to asialofetuin was 7.2 with a suggested second optimum at 8.5. The pellet enzyme had only one optimum at pH 7.2. Michaelis constants with regard to asialofetuin were 0.08, 0.06 and 0.22 mM in homogenate, resuspended pellet and supernatant, respectively. The homogenate had a K_m of 5 μM for CMP-*N*-acetylneuraminic acid (CMP-NANA). Isoelectric focusing indicated that at least eight isoelectric forms of sialyltransferase with p*I* values ranging from pH 5.0 to 8.6 are present in the homogenate; several isoelectric forms were preferentially associated with the pellet or supernatant. All the data suggest at least two forms of liver glycoprotein sialyltransferase. One form is thermolabile, loses activity on freezing and storage, and is associated with membraneous fractions and A-OSM activity. The second form is less thermolabile, more stable to freezing and storage, less associated with membraneous fractions and associated more with asialofetuin

activity. Furthermore, kinetic characterization of sialytransferase activity in liver homogenate, resuspended pellet and supernatant suggests the presence of two asialofetuin sialyltransferases.

Introduction

Sialyltransferase (CMP-*N*-acetylneuraminate:D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase, EC 2.4.99.1) catalyzes the transfer of sialic acid from its nucleotide derivative cytidine monophosphate-*N*-acetylneuraminic acid (CMP-NANA) to various oligosaccharide, glycoprotein and glycolipid acceptors. Several sialyltransferases exist and have been shown to differ with respect to the molecular weight of their preferred substrates and their substrate specificity [1,2].

Many mammalian tissues including rat liver [3—8], muscle [9], brain [10], and mammary gland [1], pork liver [11], sheep submaxillary gland [1], guinea pig brain [12], goat, cow and human colostrum [1] and human serum [13—16] and liver [16,17] have been shown to contain sialyltransferase activity. Although many studies have been done on sialyltransferase [1,3—17], few have been concerned with the human enzymes [13—17]. Furthermore, none of the studies on human sialyltransferases has systematically characterized the enzyme(s) with regard to subcellular location and kinetic properties. Investigations of this nature are important since sialylation of molecules and whole cells has been shown to be very important for their biological function. The presence of sialic acid on some glycoproteins (e.g. intrinsic factor) protects them against proteolytic attack [18]. Similarly, the half-life of certain cells (erythrocytes) and macromolecules (plasminogen, α_1-glycoprotein, ceruloplasmin) in the plasma appears to be regulated by the presence of sialic acid residues [2, 19—21]. The presence of sialic acids in glycoproteins appears to be responsible, at least in part, for the binding and transport of molecules, for the masking of cellular antigens and for the surface charge, aggregation and shape of cells [2, 22]. In this latter regard, sialic acid residues on cell surfaces are probably important in membrane-related cellular phenomena such as malignant transformation, contact inhibition and cellular migration [2,22]. The level of sialyltransferase activity may be important with regard to its correlation with, or possible involvement in, various pathological states including muscular dystrophy [9], α-1-antitrypsin deficiency [16], arthritis [14], cystic fibrosis [13,16] and cancer [14,23—26].

In the present communication we report findings on the activity, subcellular distribution and kinetic properties of sialyltransferases in human livers with regard to the macromolecular glycoprotein acceptors asialofetuin and asialoovine submaxillary mucin (A-OSM).

Methods

General. Protein was determined by the Lowry et al. method [27]. All procedures were carried out at 0—4° C unless otherwise stated. Cytidine 5′-monophosphate [G-^{3}H]sialic acid, specific activity of 2.33 Ci/mmol, lot number 919-

032, was purchased from New England Nuclear.

Preparation of glycoprotein substrates. Fetuin (lot No. 84C-0106) was purchased from Sigma Chemical Co. Asialofetuin was prepared by hydrolyzing 1.0 g fetuin in 400 ml, 0.0125 M H_2SO_4 at 80°C for 1 h [28] followed by dialysis for 2 days against distilled water (four changes of 9 l/change) in the cold room. Assay of the lyophilized retentate for sialic acid by the Warren method [29] indicated 0.6% (w/w) sialic acid in the asialofetuin preparation. This asialofetuin was used for the sialyltransferase assays.

Ovine submaxillary mucin (OSM) was prepared from 83.4 g defatted ovine submaxillary glands according to the method of Murphy and Gottschalk [30]. Asialo-OSM (A-OSM) was prepared by hydrolysis in 270 ml, 0.01 M HCl at 80°C for 100 min [31], followed by dialysis for 2 days against distilled water (three changes of 8 l/change) in the cold room. Assays of the lyophilized retentate for sialic acid [29] indicated that no sialic acid was present in the A-OSM preparation. This A-OSM was used for the sialyltransferase assays.

Sialyltransferase assay. The complete incubation medium contained the following components in a final volume of 0.300 ml: 1.0 mg of asialofetuin (or A-OSM); CMP-*N*-[^{3}H]acetylneuraminic acid ($1.3 \cdot 10^5$ dpm); 100 mM Tris · HCl, pH 7.0, buffer containing 16 mM $MgCl_2$ (10 mM for A-OSM) and 0.6% Triton X-100 (0.4% for A-OSM); and 50—100 μl of a 1 : 5 (w/v) liver homogenate (1.1—5.4 mg protein). Incubations were carried out in duplicate at 37°C for 60 min and the reactions were stopped by adding 0.5 ml ice-cold 1% phosphotungstic acid in 0.5 M HCl (w/v). The precipitate was collected on a Whatman GF/c glass fiber filter in a Gooch crucible, and washed with two, 1.0-ml aliquots of cold 1% phosphotungstic acid in 0.5 M HCl [3]. The precipitate was dried, solubilized in 2 ml 2.5 M NH_4OH and counted in 10 ml Riafluor (New England Nuclear) in a Beckman LS-250 Liquid Scintillation Counter. Control incubations without asialofetuin or A-OSM were carried out for all assays and the incorporation was substracted from the incorporation in their presence. These conditions of assay for human liver were chosen for maximum incorporation of CMP-[^{3}H]NANA into asialofetuin and A-OSM after running curves for sialyltransferase activity versus concentration of $MgCl_2$, Triton X-100, asialofetuin and A-OSM.

Product identification. The standard enzyme incubation was scaled up 4-fold, incubated at 37°C for 1 h, and centrifuged at 48 000 × *g* for 30 min. Polyacrylamide gels (5%) were run at pH 8.9 at 3 mA/gel for 3 h on the reaction product supernatant and on authentic fetuin and asialofetuin. The gels were either stained for protein with Coomassie Blue or sliced and assayed for radioactivity by liquid scintillation counting.

Tissues and tissue preparation. Human fetal livers were obtained from aborted fetuses and stored at —20°C until used. Gestational age was determined by the crown to rump lengths [32]. Postnatal human livers which appeared normal on gross pathological inspection were obtained from autopsied individuals and were processed immediately (for fresh tissue studies) or stored at —20°C until used. The procedures which involved human tissues were approved by the Committee on Investigations/Activities Involving Human Subjects of the School of Medicine, University of California, San Diego.

Liver homogenates were prepared as follows: 0.2—1 0 g of liver were homog-

enized in 0.1 M, pH 7.0 Tris · HCl buffer (1 : 5, w/v) containing 5 mM $MgCl_2$ using ground glass homogenizers.

Fractionation of human liver homogenates by differential centrifugation. Human liver homogenates (1 : 5, w/v) prepared as described were centrifuged at 10 000 × *g* for 10 min to yield a mitochondrial pellet, P_1 [33], and a postmitochondrial supernatant, S_1. The supernatant, S_1, was centrifuged at 102 000 × *g* for 2 h to yield a microsome pellet, P_2, and a postimitochromosomal supernatant, S_2 [33]. The distribution of activity and the specific activity of sialyltransferase with regard to asialofetuin and A-OSM was determined in the homogenate, S_1, P_1, S_2, and P_2. A similar experiment was done using 0.8% (v/v) Triton X-100 in the homogenizing buffer to determine if sialyltransferase activity could be dissociated from the membraneous fractions (P_1 and P_2).

In a separate experiment, a human liver homogenate was centrifuged at 48 200 × *g* for 30 min. The asialofetuin sialyltransferase activity in the homogenate, resuspended pellet and supernatant was characterized kinetically (pH optima and K_m values) and subjected to isoelectric focusing to help determine if more than one sialyltransferase is present in human liver.

pH optimum. pH optimum curves of asialofetuin sialyltransferases activity in crude liver homogenates, and 48 200 × *g* supernatant and resuspended pellets were determined in 0.1 M Tris · HCl buffers (pH 6.4—8.8) which were 22 mM in $MgCl_2$ and 0.7% (w/v) in Triton X-100. Duplicate tubes were made up and the final pH values determined. The reaction was initiated with CMP-NANA and incubated for 60 min at 37° C.

Kinetic studies. Apparent Michaelis constants (K_m values) were determined for sialyltransferase in the crude liver homogenate and in the 48 200 × *g* pellet and supernatant by the Lineweaver-Burk method [34] using asialofetuin and CMP-NANA as substrates. For these studies, sialyltransferase was incubated in substrates of varying concentrations in 0.1 M, Tris · HCl pH 7.0, buffer. In determining the K_m for asialofetuin, $1.6 \cdot 10^{-6}$ M CMP-NANA was used, and in determining the K_m for CMP-NANA $7.5 \cdot 10^{-5}$ M asialofetuin or $3.2 \cdot 10^{-6}$ M A-OSM was used. Non-radioactive CMP-NANA was prepared as described by Kean and Roseman [35] and used to dilute the radioactive CMP-NANA.

Isoelectric focusing. Isoelectric focusing was performed according to the method of Haglund [36] using an LKB 8101 (110 ml) isoelectric focusing apparatus. 2% ampholytes (pH 3.5—10.0) were used in a gradient of 0—67% (w/v) sucrose. The temperature was maintained at 2—4° C with a circulating water bath (Brinkmann Lauda K-2/R). The starting amperage was 3.5 mA and 200 V. After 15 h the voltage was adjusted to 500 V. Electrofocusing was conducted for 40—41 h after which 0.7-ml fractions were collected. The pH of each fraction was measured at 0—2° C with a Beckman digital pH meter and 100-µl aliquots of each fraction were assayed for 2 h for asialofetuin sialyltransferase activity using $4.3 \cdot 10^5$ dpm CMP-NANA/assay. Isoelectric focusing was performed on 5.4 ml of crude human liver homogenates (1 : 5, w/v) made in 0.1 M Tris · HCl, pH 7.0, buffer and on the supernatant (7.5 ml) and resuspended pellet (5.4 ml) after centrifugation of 13 ml of a 1 : 5 (w/v) homogenate at 48 200 × *g* for 30 min. Isoelectric focusing was also done on a supernatant after homogenizing (in the above buffer containing 0.8% (v/v) Triton X-100) and centrifuging at 10 000 × *g* for 30 min.

Inhibition studies. Various salts at concentrations ranging from 0.25 to 25 mM (Mn^{2+}, Ca^{2+}, Hg^{2+}, Zn^{2+}), 0.2—1.5 mM dithiothreitol, 7.0—14.0 mM β-mercaptoethanol and 0.6—3.8 mM ethylenedinitrilotetraacetic acid (EDTA) were incubated with sialyltransferase (50—100 μl of a 1 : 5 (w/v) homogenate) to determine their effect on sialyltransferase activity. Incubations were carried out in duplicate at 37°C for 60 min.

Stability studies. Sialyltransferase activity was assayed (on 50—100 μl of 1 : 5 (w/v) homogenates) in fresh and frozen liver homogenates, in dialyzed homogenates, in homogenates stored with and without β-mercaptoethanol (7.0 and 14.0 mM) for several days at 4°C, and in homogenates which had been stored frozen and later thawed and assayed. Thermal stability studies were performed on human liver sialyltransferase (50—100 μl of 1 : 5 (w/v) homogenates) which had been preincubated at 37°C for 30, 60, 90, 120 and 150 min before the addition of substrates and subsequent assay for 60 min.

Substrate competition studies. Human liver (50 μl of 1 : 5 (w/v) homogenates) was assayed for sialyltransferase activity for 1 h using 1.0 mg asialofetuin alone, 1.0 mg A-OSM alone or the two substrates combined (1.0 mg asialofetuin + A-OSM), to help determine if two distinct glycoprotein sialyltransferase enzymes are present in liver homogenates.

Results

In order to determine the optimal conditions for the assay of human liver sialyltransferase with regard to the acceptor substrates asialofetuin and A-OSM, curves of sialyltransferase activity versus the concentration of asialofetuin, A-OSM, $MgCl_2$, and Triton X-100 were run. Substrate inhibition appears to be exhibited for both asialofetuin and A-OSM at concentrations greater than 1.5 mg/300 μl and 1.0 mg/300 μl, respectively. The reason for the unusually large inhibition exhibited for high concentrations of asialofetuin is not presently known but may be due to a toxic contaminant in the asialofetuin preparation. $MgCl_2$ stimulated sialyltransferase activity approx. 50% for both asialofetuin and A-OSM between 10 and 25 mM (Fig. 1). Triton X-100 also stimulated both asialofetuin and A-OSM sialyltransferase activity 1.5—2.5-fold at 0.4 and 0.6% (v/v), respectively (Fig. 2). From these preliminary studies, the conditions for the human liver sialyltransferase assay were determined (see Methods). The assay was linear with respect to time (0.5 and 1.0 h) and enzyme amount (25, 50, and 100 μl of a 1 : 5 (w/v) homogenate). Polyacrylamide gel electrophoresis of the reaction product, when using asialofetuin as substrate, indicated that the radiolabelled product migrated as a single, broad protein band coincident with the single protein band of authentic fetuin used in preparing asialofetuin.

A large range of normal sialyltransferase activity for both substrates was found in our studies: 1.0—20.0 pmol NANA transferred/h per mg protein for asialofetuin and 0.2—6.1 pmol NANA transferred/h per mg protein for A-OSM. In each of the nine normal livers studied, the sialyltransferase specific activity with regard to the asialofetuin substrate is three to seven times that for the A-OSM-substrate. In four fetal livers studied, the specific activity of sialyltransferase with regard to both substrates falls within the normal range. The sialyltransferase activity does not appear to be related to either the sex or age at

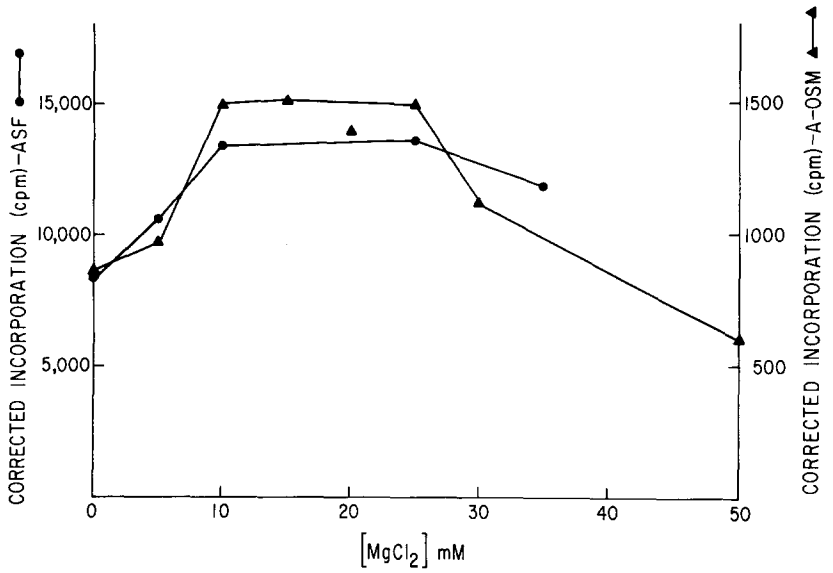

Fig. 1. Human liver sialyltransferase activity (asialofetuin (ASF) and A-OSM) versus $MgCl_2$ concentration. Enzyme (50—100 μl of 1 : 5 (w/v) homogenate) was incubated for 60 min at 37°C.

death of the donor's liver, but our data are too limited for any definite conclusions. For one liver which we obtained fresh at autopsy, the sialyltransferase specific activity for both the fresh and frozen liver was determined. The asialofetuin sialyltransferase specific activity was not appreciably affected by freezing but the A-OSM sialyltransferase specific activity decreased by about 30% after freezing. This suggests that there are at least two human liver sialyltransferases, one that is stable to freezing and acts primarily on asialofetuin and another that loses activity on freezing and acts primarily on A-OSM.

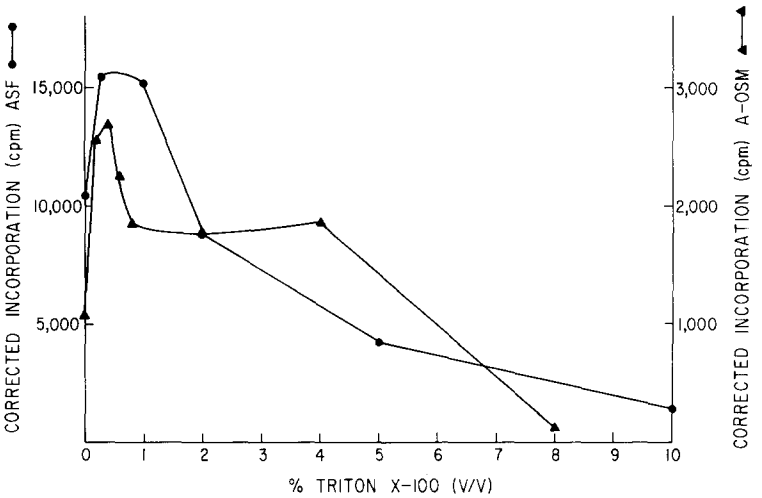

Fig. 2. Human liver sialyltransferase activity (asialofetuin (ASF) and A-OSM) versus Triton X-100 concentration. Enzyme (50—100 μl of 1 : 5 (w/v) homogenate) was incubated for 60 min at 37°C.

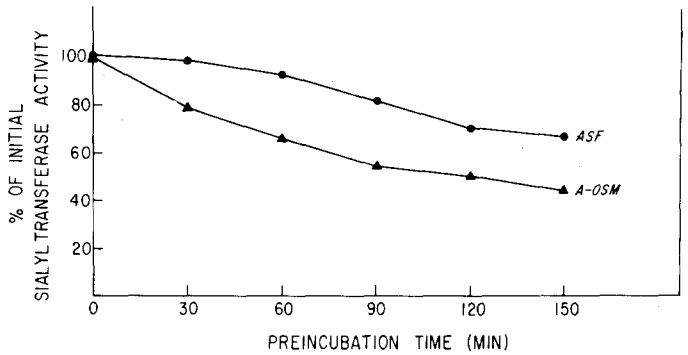

Fig. 3. Heat denaturation curves for asialofetuin (ASF) and A-OSM human liver sialyltransferase activity. See Methods for details.

Fig. 3 depicts typical heat denaturation curves for human liver homogenate sialyltransferase using asialofetuin and A-OSM as substrates. The asialofetuin sialyltransferase was more thermostable than the A-OSM sialyltransferase. In three normal livers that were studied, asialofetuin sialyltransferase retained 67

TABLE I

EFFECTS OF ADDITIVES ON LIVER SIALYLTRANSFERASE ACTIVITY

	Concentration of additive (mM)	Effect on asialofetium sialyltransferase activity (%)	Effect on A-OSM sialyltransferase activity (%)
EDTA	1	+50	—
	2	—10	—
	3.7	—72	—
$MnCl_2$	5	+10	no effect
	25	—50	— 76
	50	—80	— 95
	100	—92	—100
$CaCl_2$	5	no effect	— 12
	25	—56	— 78
	50	—83	— 95
	100	—97	—100
$HgCl_2$	0.5	— 8	— 41
	1.0	—50	— 80
	2.5	—87	—100
$ZnCl_2$	0.25	— 5	no effect
	1.0	—31	— 10
	2.5	—69	— 72
β-Mercaptoethanol	7.0	—25	—
	14.0	—44	—
Dithiothreitol	0.2	—27	— 50
	0.5	—74	— 85
	1.5	—81	— 93

± 1% and A-OSM sialyltransferase retained 44 ± 8% of the original activity after 150 min preincubation at 37° C.

Several salts were added to liver sialyltransferase to test their effects on enzymatic activity with regard to both asialofetuin and A-OSM (Table I). EDTA stimulated asialofetuin sialyltransferase activity at 1 mM and was inhibitory at higher concentrations. $MnCl_2$ and $CaCl_2$ were highly inhibitory at concentrations from 25 to 100 mM and heavy metals ($HgCl_2$ and $ZnCl_2$) were inhibitory, at low concentrations (0.1—2.5 mM). The sulfhydryl reagents β-mercaptoethanol and dithiothreitol were both inhibitory. In general, the additives had a similar but larger inhibitory effect on A-OSM sialyltransferase activity than on asialofetuin sialyltransferase activity.

Stability studies performed on human liver sialyltransferase under various conditions of storage for 24 h are summarized in Table II. Asialofetuin sialyltransferase retains all its activity when stored as a frozen homogenate at −20° C or when stored as fresh intact liver tissue at 4° C. In contrast, storage as a crude homogenate at 4° C or as tissue at −20° C leads to small (11%) losses of activity. Both dialysis and storage in β-mercaptoethanol (0.5 and 1.0%, v/v) lead to large losses of asialofetuin sialyltransferase activity. Considerably larger losses were found for A-OSM sialyltransferase activity under the same storage conditions used for asialofetuin sialyltransferase.

When a liver homogenate was assayed for sialyltransferase activity using the combined substrates (asialofetuin + A-OSM), the activities were additive rather than competitive. This suggests that there are two distinct enzymes for asialofetuin and A-OSM in liver homogenates.

In order to determine whether the sialyltransferases are located in different subcellular fractions, liver homogenates were subjected to differential centrifugation (Fig. 4). The five resulting fractions (H, S_1, P_1, S_2, P_2) were assayed for sialyltransferase activity with respect to both asialofetuin and A-OSM. Table III summarizes the data on the fractionation of human liver sialyltransferase activity for both substrates and gives the percentage of activity found and the specific activity of sialyltransferase in each fraction. The values given are the

TABLE II

STABILITY OF HUMAN LIVER SIALYLTRANSFERASE TO VARIOUS STORAGE CONDITIONS

Conditions of storage	Percentage of original activity after 24 h storage	
	Asialofetium	A-OSM
1) Crude liver homogenate at 4° C in 0.1 M Tris—HCl, pH 7.0	89	60
2) Crude liver homogenate at 4° C in 0.1 M Tris—HCl, pH 7.0, plus:		
(a) 0.5% (v/v) β-mercaptoethanol	75	—
(b) 1.0% (v/v) β-mercaptoethanol	67	—
3) Dialyzed for 20 h against 2000 volumes 0.1 M Tris—HCl, pH 7.0	53	—
4) Fresh liver tissue at 4° C and new homogenate made in 0.1 M Tris—HCl, pH 7.0	100	81
5) Tissue stored at −20° C and new homogenate made	89	65
6) Homogenate at −20° C	100	—

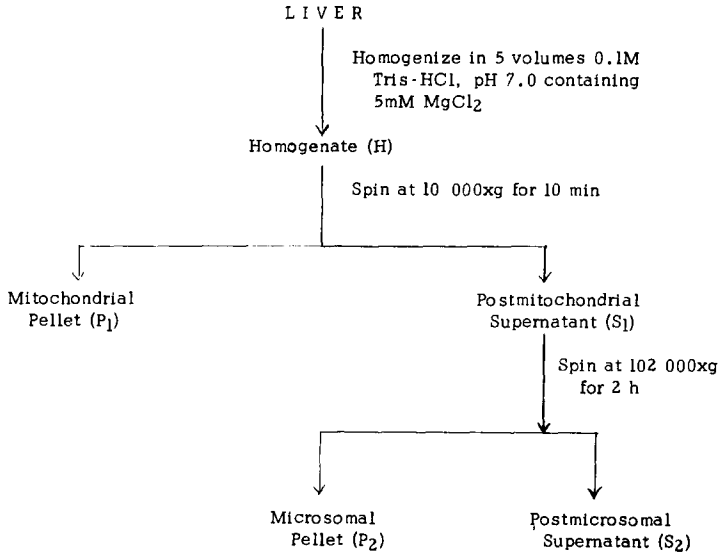

Fig. 4. Fractionation of human liver by differential centrifugation. See Methods for details.

average for two determinations. For both the asialofetuin and A-OSM the largest percentage of sialyltransferase activity and the highest specific activity was found in the two pellets. However, for A-OSM sialyltransferase there was a greater percentage of activity found in the pellets than for asialofetuin sialyltransferase. These data provide additional evidence for two human liver sialyltransferases, the A-OSM sialyltransferase being associated more with membraneous fractions (P_1, P_2) than the asialofetuin sialyltransferase. When a similar fractionation was done using Triton X-100 (0.8%, v/v) in the homogenizing buffer, almost all of the asialofetuin and A-OSM sialyltransferase activity was released into the supernatant fractions (S_1, S_2) (Table III).

A second liver was homogenized in 0.1 M Tris · HCl, pH 7.0, containing 5 mM $MgCl_2$, and a portion of the homogenate was centrifuged for 30 min at 48 200 $\times g$. pH optima curves were run on the homogenate, the resuspended 48 200 $\times g$ pellet and the 48 200 $\times g$ supernatant using asialofetuin as substrate. The homogenate sialyltransferase has a pH optimum of 7.2 with a suggested second optimum near 8.2. The 48 200 $\times g$ pellet sialyltransferase has a broad pH optimum of pH 7.2 with little suggestion of a second optimum. The 48 200 $\times g$ supernatant sialyltransferase has a major pH optimum at pH 7.2 with a second minor, but clearly evident and reproducible pH optimum at pH 8.5. The supernatant sialyltransferase has much less activity than either the homogenate or the pellet enzymes. The pH curves suggest that the liver homogenate contained at least two sialyltransferases, the bulk of the activity being represented by the more neutral form (pH 7.2) most of which pellets out at 48 200 $\times g$ leaving an enriched amount of the more basic form (pH 8.5) in the supernatant.

Apparent Michaelis constants (K_m values) for asialofetuin were run on the sialyltransferases in the homogenate, resuspended 48 200 $\times g$ pellet and supernatant (Fig. 5). Analysis of the Lineweaver-Burk double reciprocal plots indicates that the supernatant sialyltransferase has a K_m for asialofetuin (0.22 mM)

TABLE III

FRACTIONATION OF HUMAN LIVER SIALYLTRANSFERASE ACTIVITY BY DIFFERENTIAL CENTRIFUGATION

	Asialofetium		A-OSM	
Fraction	Asialofetium sialyltransferase activity (percent of recovered activity)	Asialofetium sialyltransferase specific activity (pmol NANA transferred/h per mg protein)	A-OSM sialyltransferase activity of recovered activity)	A-OSM sialyltransferase specific activity (pmol NANA transferred/h per mg protein)
Homogenate (H)	100	8.0	100	1.9
Mitochondrial pellet (P_1)	54.5 ± 0.5 (7) *	9.3	67.0 ± 1.0 (3) *	2.6
Postmitochondrial supernatant (S_1)	45.5 ± 0.5 (93) *	2.3	33.0 ± 1.0 (97) *	0.9
Microsomal pellet (P_2)	26.5 ± 1.5 (7) *	9.4	21.9 ± 0.5 (8) *	1.9
Postmicrosomal supernatant (S_2)	19.0 ± 1.0 (86) *	2.3	11.1 ± 0.5 (89) *	0.4

* Numbers in parentheses are for fractionation when 0.8% Triton X-100 (v/v) was used in the homogenizing buffer.

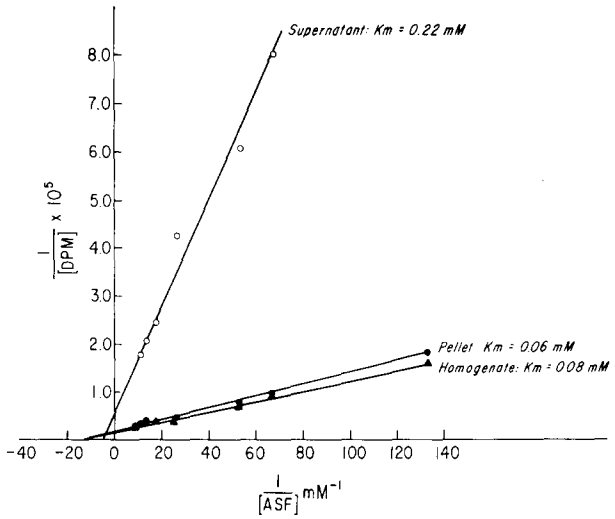

Fig. 5. Lineweaver-Burk plot of liver sialyltransferase activity (homogenate, resuspended 48 200 × *g* pellet and 48 200 × *g* supernatant) versus asialofetuin (ASF) concentration. See Methods for details.

which is somewhat higher than the K_m values found for the pellet and homogenate sialyltransferase (0.06 and 0.08 mM, respectively). The apparent K_m values for CMP-NANA were determined for homogenate sialyltransferase activity using both asialofetuin and A-OSM and found to be the same (5 μM) for both glycoprotein substrates.

Isoelectric focusing of three human liver homogenates indicated the presence of up to ten peaks with asialofetuin sialyltransferase activity (Fig. 6). Eight major forms had isoelectric points (p*I* values) ranging from pH 5.0 to 8.6. Isoelectric focusing of a resuspended 48 200 × *g* pellet (Fig. 6B) and the corresponding supernatant (Fig. 6C) yielded isoelectric profiles which were different from each other and different from that of the homogenate (Fig. 6A). The resuspended pellet and supernatant each contained forms which were found in the homogenate. However, the pellet was enriched in forms with p*I* values between pH 6 and 7, whereas supernatant was enriched in forms with p*I* values between pH 5 and 6 and between pH 7 and 8. The more basic forms (pH 7.5—8.6) which are well resolved and clearly evident in the homogenate were nearly absent in the pellet and supernatant profiles, perhaps indicating inactivation. Except for these basic forms, combination of the profiles for pellet and supernatant accounted for all of the forms found in the homogenate. Isoelectric focusing of a human liver supernatant which was obtained by centrifuging a liver homogenized in Tris · HCl buffer containing 0.8% (v/v) Triton X-100 gave an isoelectric profile (not shown) which contained all the isoelectric forms seen in liver homogenates (Fig. 6A) except that the forms between p*I* values 7.0 and 8.0 were enhanced in relative amounts.

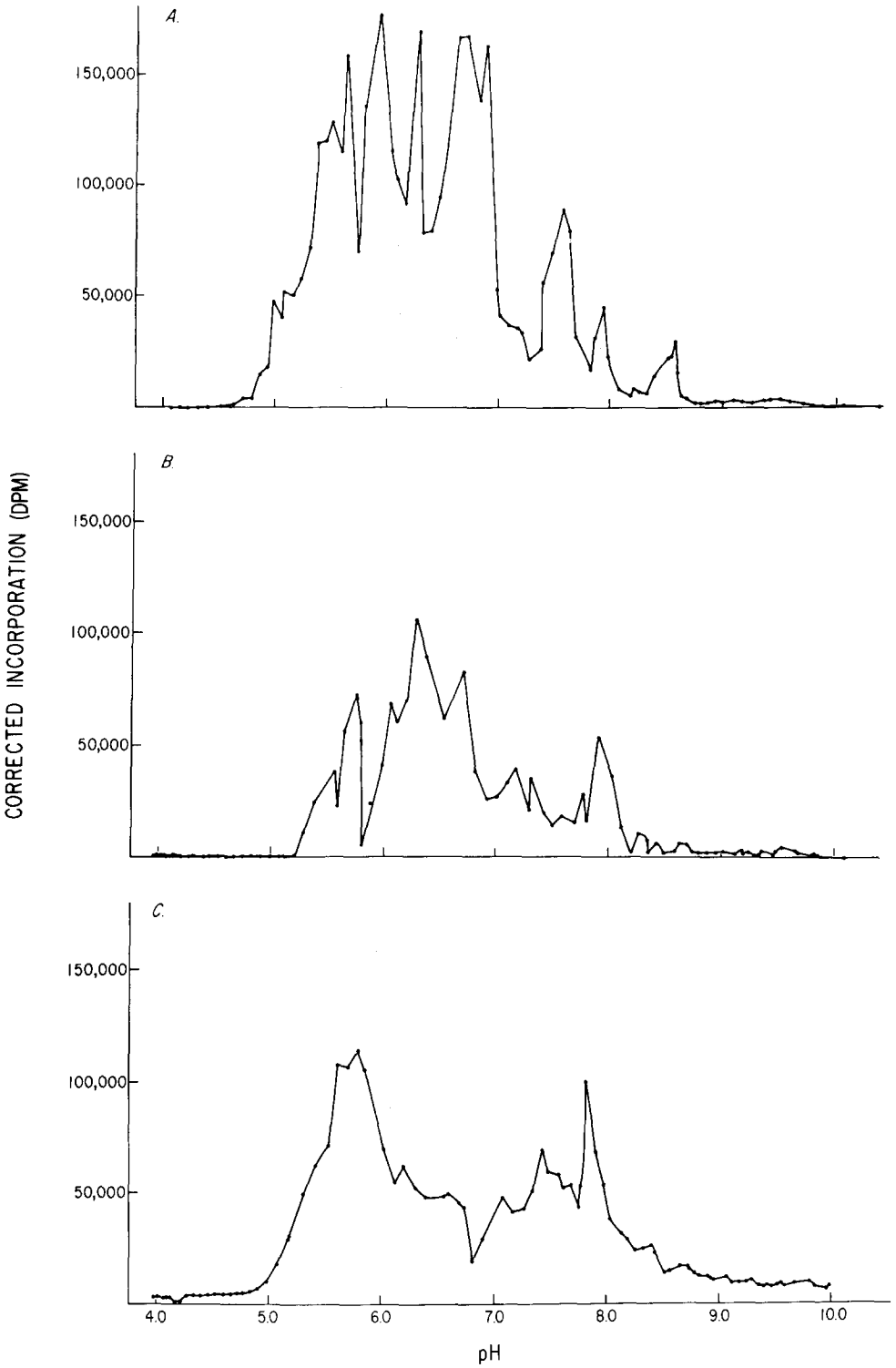

Fig. 6. Isoelectric focusing (pH 3.5—10.0) of normal adult human liver asialofetuin sialyltransferase activity. See Methods for details. (A) Homogenate: 5.4 ml of 1 : 5 (w/v) homogenate, made in 0.1 M Tris · HCl, pH 7.0, buffer. (B) Resuspended 28 200 × *g* pellet; pellet resuspended in 5.4 ml of Tris · HCl buffer after centrifugation of 13 ml of 1 : 5 (w/v) homogenate. (C) 48 200 × *g* supernatant: 7.5 ml after centrifugation of a 1 : 5 (w/v) homogenate.

Discussion

In this paper we have studied the properties of human liver sialyltransferase with regard to the two glycoprotein substrates asialofetuin and asiolo-ovine submaxillary mucin. Like several other investigators [8,37,38], we found that $MgCl_2$ and Triton X-100 stimulated liver sialyltransferase activity (Figs. 1 and 2), and these were incorporated in the assay at optimal concentrations. We have studied the specific activity of sialyltransferase in thirteen human livers including four fetal livers and found a large variability in specific activities for both asialofetuin and A-OSM. Each liver exhibited three to seven times higher activity for asialofetuin than for A-OSM. In one liver which we obtained fresh at autopsy, asialofetuin sialyltransferase activity was stable to freezing whereas A-OSM sialyltransferase activity lost about 30% of its activity on freezing. These results, plus heat denaturation studies (Fig. 3), studies on stability to various storage conditions (Table II), additive studies (Table I) and substrate competition studies all suggest the presence in human liver of at least two glycoprotein sialyltransferases as has been found for pork liver [11]. Similar to rat liver [8], heavy metals (Hg^{2+}, Zn^{2+}) at concentrations of 1.0 and 2.5 mM were found to greatly inhibit human liver sialyltransferase activity.

Subcellular fractionation studies (Fig. 4 and Table III) indicated that the majority of both sialyltransferase activities is loosely associated with membraneous fractions (mitochondrial and microsomal pellets), but that a greater percentage of A-OSM than asialofetuin activity is associated with these fractions. Triton X-100 (0.8%, v/v) causes dissociation of almost all asialofetuin and A-OSM sialyltransferase activity from these membraneous fractions (P_1 and P_2) into the corresponding soluble fractions (S_1 and S_2). Previous subcellular localization studies on rat liver by differential centrifugation and sucrose density gradient centrifugation have indicated that sialyltransferase activity is primarily found in smooth-surfaced microsomes [4]. Other investigators have also found sialyltransferase activity concentrated in microsomes in rat [6,7,37] and pork liver [11].

Evidence suggesting two forms of liver asialofetuin sialyltransferase was obtained by fractionating a human liver and studying the kinetic properties of the enzyme in the homogenate, the resuspended 48 200 $\times g$ pellet and the 48 200 $\times g$ supernatant. These kinetic data should be interpreted with care since it is possible that one membrane-bound enzyme exists and that its properties are altered when released into the supernatant. The pH optima profiles of the homogenate and supernatant are bimodal, whereas the resuspended pellet has a broad, unimodal profile. These profiles suggest that two forms of sialyltransferase may be present in the resuspended pellet fraction. All three fractions have the major pH optimum at or centered around pH 7.2. This is similar to the pH optimum of 7.0 found for human and pork liver [17], and rat liver [3,8] sialyltransferase activity. Apparent Michaelis constants (K_m values) for asialofetuin were determined (Fig. 5) for the three liver fractions. Although these K_m values were not determined using optimal CMP-NANA concentrations, they are still useful for comparison purposes. The K_m values of the homogenate and pellet sialyltransferase were very close (0.08 and 0.06 mM, respectively) but lower than the 0.22 mM value found for the supernatant.

Since the majority of human liver sialyltransferase activity is associated with membraneous fractions (Table III), it is reasonable that the K_m values for asialofetuin of the homogenate and resuspended pellet are in close agreement. There are no literature values on K_m values for asialofetuin in liver sialyltransferase(s) with which to compare our results. An apparent K_m for CMP-NANA was determined on a human liver homogenate to be 5 μM when using either asialofetuin or A-OSM. This is close to the 2 μM value found in rat liver microsomes [7] but lower than the value (40 μM) found for pork liver microsomes [11].

Isoelectric focusing was performed on the three human liver fractions. The homogenate (Fig. 6A) contained at least ten isoelectric forms with regard to asialofetuin activity. The isoelectric profiles for asialofetuin sialyltransferase activity in the resuspended pellet (Fig. 6B) and supernatant (Fig. 6C) each exhibited multiple forms and were different from each other. However, when taken together the profiles appear to account for most, if not all, of the forms found in the liver homogenate. The relationship of the two forms of liver asialofetuin sialyltransferase to the multiple forms found on isoelectric focusing is not known but it appears that each major form (particulate and soluble) may be comprised of several isoelectric forms.

These isoelectric focusing studies, although reproducible in the same and in three different normal livers, are difficult to interpret. Since the majority of asialofetuin sialyltransferase activity is membrane bound, the multiplicity of peaks found on isoelectric focusing may be due, at least in part, to the enzyme being associated with different amounts and/or types of membrane fragments or to the formation of micelles of various composition. Isoelectric focusing studies using various concentrations of the non-ionic detergent Triton X-100 might be useful in simplifying the isoelectric profiles and determining what role, if any, membrane fragments and micelles are playing in the asialofetuin sialyltransferase isoelectric profiles.

The data suggest that liver contains at least two glycoprotein sialyltransferases. One is thermolabile, loses activity on freezing and storage and is loosely asssociated with membraneous fractions and associated with A-OSM activity. The second is less thermolabile, more stable to freezing and storage, less associated with membraneous fractions and associated more with asialofetuin activity. Kinetic characterization of this second enzyme in liver homogenate, resuspended pellet and supernatant suggests that it may be comprised of two forms.

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