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Studies on the Purification and Properties of UDP-Galactose–Glycoprotein Galactosyltransferase from Rat Liver and Serum

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1. Rat liver microsomal preparations incubated with 200mM-NaCl at either 0 or 30°C released about 20–30% of the membrane-bound UDP-galactose–glycoprotein galactosyltransferase (EC 2.4.1.22) into a 'high-speed' supernatant. The 'high-speed' supernatant was designated the 'saline wash' and the galactosyltransferase released into this fraction required Triton X-100 for activation. It was purified sixfold by chromatography on Sephadex G-200, and appeared to have a higher molecular weight than the soluble serum enzyme. 2. Rat serum galactosyltransferase was purified 6000–7000-fold by an affinity-chromatographic technique using a column of activated Sepharose 4B coupled with α-lactalbumin. The purified enzyme ran as a single broad band on polyacrylamide gels and contained no sialyltransferase, *N*-acetylglucosaminyltransferase and UDP-galactose pyrophosphatase activities. 3. The highly purified enzyme had properties similar to those of both soluble and membrane-bound galactosyltransferase. It required 0.1% Triton X-100 for stabilization, but lost activity on freezing. The enzyme had an absolute requirement for Mn^{2+}, not replaceable by Ca^{2+}, Mg^{2+}, Zn^{2+} or Co^{2+}. It was active over a wide pH range (6–8) and had a pH optimum of 6.8. The apparent K_m for UDP-galactose was 12.5×10^{-6}M. α-Lactalbumin had no appreciable effect on UDP-galactose–glycoprotein galactosyltransferase, but it increased the specificity for glucose rather than for *N*-acetylglucosamine, thus modifying the enzyme to a lactose synthetase. 4. The possibility of a conversion of higher-molecular-weight liver enzyme into soluble serum enzyme is discussed, especially in relation to the elevated activities of this and other glycosyltransferases in patients with liver diseases.

Glycosyltransferase enzymes occur in animal tissues in membrane-bound and in soluble forms (Hagopian & Eylar, 1969; Mookerjea *et al.*, 1971; Kim *et al.*, 1972*a*,*b*). Much attention has been focused on the potential role of these enzymes in cell–cell recognition and other metabolic processes (Schachter *et al.*, 1970; Roth *et al.*, 1971; Keenan & Morré, 1975; Podolsky & Weiser, 1975*a*). Purification, preparation of antibodies and a precise intramembrane localization of these enzymes will greatly advance our knowledge of their role in intercellular functions. Toward this goal, we have undertaken to purify one of these enzymes, namely UDP-galactose–glycoprotein galactosyltransferase (EC 2.4.1.22) from rat serum and liver, by procedures involving gel filtration and affinity chromatography. Purification of galactosyltransferase was previously achieved from human and bovine milk by using these procedures (Andrews, 1970; Trayer & Hill, 1971; Mawal *et al.*, 1971). A preliminary report of some of this work has already appeared (Fraser & Mookerjea, 1975).

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Materials and Methods

Assay of galactosyltransferase activity

Unless otherwise specified, each assay mixture (total vol. 50μl) contained: 25μl of purified enzyme (60–100ng of protein) or 20μl of serum (1.5–2mg of protein) or 20μl of microsomal suspension (0.3–0.4mg of protein); UDP-[U-^{14}C]galactose, 3nmol (0.01μCi); Mes [2-(*N*-morpholino)ethanesulphonic acid)] buffer, pH6.8, 6.25μmol; $MnCl_2$, 0.625μmol; and 'desialized' and 'degalactosylated' fetuin (DSG-fetuin, described below), 0.125mg. After incubation for 1h at 37°C the reaction was stopped with 1ml of 10% (w/v) trichloroacetic acid/2% (w/v) phosphotungstic acid. The precipitate was filtered by suction through glass-fibre filters (Whatman GF/A) and was washed with 10ml of cold 5% trichloroacetic acid/1% phosphotungstic acid mixture containing 0.5% galactose, with 7ml

of ethanol/ether (1:1, v/v), and then with 5ml of ether. The incorporation of radioactivity into acceptor protein was measured on the dried filter discs in a toluene-based scintillation solution containing 3.92g of PPO(2,5-diphenyloxazole) and 80mg of POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene]/litre. For studies with the purified enzyme, 25μg of bovine serum albumin was added as carrier when the reaction was stopped with the trichloroacetic acid/phosphotungstic acid mixture.

Galactosyltransferase activity was also assayed by using monosaccharides (glucose or *N*-acetylglucosamine) as acceptors instead of DSG-fetuin. These experiments were terminated with 20μl of 0.5M-EDTA in 10% (w/v) sodium tetraborate and the total incubation mixture was subjected to high-voltage electrophoresis in 1% sodium tetraborate at 3000V for 1.5h on Whatman 3MM paper. Radioactive lactose or *N*-acetyl-lactosamine, the disaccharide products of the reaction, remained close to the origin and were monitored by scanning the paper strips in a radiochromatographic scanner (Packard, model 7201). The radioactive areas were cut out and counted in toluene by scintillation spectrometry. For incubation of microsomal suspension and crude serum, ATP (2mM) was included in the assay to prevent UDP-galactose pyrophosphatase action on the radioactive substrate (Mookerjea & Yung, 1975). Triton X-100 (0.5%, v/v) was also included in the assay of membrane-bound enzyme. The enzyme assays were linear with time and with protein concentration in the incubation.

Assay of other enzymes

N-Acetylglucosaminyltransferase and sialyltransferase activities were measured with essentially the same incubation conditions as above, but with UDP-*N*-acetyl[1-^{14}C]glucosamine (6nmol, 0.02μCi) or CMP-[4-^{14}C]sialic acid (10nmol, 0.05μCi) as substrates replacing labelled UDP-galactose. ATP (2mM) was used in the assay of *N*-acetylglucosaminyltransferase. α_1-Acid glycoprotein, depleted of sialic acid, galactose and *N*-acetylglucosamine by enzymic procedures (Schachter *et al.*, 1970), and fetuin, depleted of sialic acid by chemical methods (Spiro, 1964), were used as acceptors for the assay of *N*-acetylglucosaminyltransferase and sialyltransferase activities respectively. $MnCl_2$ was omitted for the sialyltransferase assay. UDP-galactose pyrophosphatase activity was measured by the method described previously (Mookerjea & Yung, 1975) and the enzyme activity was expressed as nmol of galactose 1-phosphate formed/h per mg of protein.

Protein determination

Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. For accurate measurement of protein of the pure enzyme concentrated in the presence of Triton X-100, a modification of the method of Lowry (Wang & Smith, 1975) was used in which the addition of 10% sodium dodecyl sulphate was found to eliminate any spurious optical readings due to Triton.

Purification of the serum galactosyltransferase

Galactosyltransferase was isolated and purified essentially by the method of Andrews (1970). A column (2cm×5cm) of Sepharose–α-lactalbumin was equilibrated with 0.01M-Tris/HCl buffer, pH7.5, containing 0.04M-KCl, 3mM-*N*-acetylglucosamine, 0.1% Triton X-100 and 2mM-β-mercaptoethanol. An equal volume of this buffer mixture at twice these concentrations was added to the serum to be chromatographed. The column was then washed with 3 column-volumes (150–200ml) of buffer and the enzyme then eluted with buffer without any *N*-acetylglucosamine. The eluted enzyme fraction was re-chromatographed on the column for further purification.

Analytical polyacrylamide-gel electrophoresis

Polyacrylamide gels (7%, v/v) were prepared in 0.375M-Tris/HCl buffer, pH8.9, and run in 0.005M-Tris/glycine buffer, pH8.3, for 180min at a current of 2mA/gel (Davis, 1964). Protein was stained with Coomassie Blue. A modified periodic acid/Schiff technique was used to stain for carbohydrates in the glycoproteins after electrophoresis on polyacrylamide gels (Zacharius *et al.*, 1969). For molecular-weight determination, sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described by Neville (1971).

Sephadex column chromatography

Columns (2.5cm×100cm) of Sephadex G-200 were equilibrated in 0.1M-Tris/HCl buffer, pH7.5, containing 0.1M-NaCl and 0.02% NaN_3 and were run with a descending flow by the method of Killander (1964).

Amino acid composition

Amino acid analyses were performed by the method of Spackman *et al.* (1958) on a Beckman 120C automated amino acid analyser. Samples after removal of Triton X-100 (Holloway, 1973) were concentrated by freeze-drying and subsequently hydrolysed in 6M-HCl for 20h at 110°C under vacuum.

Preparation of serum, microsomal fraction and 'saline wash'

Blood was collected from overnight-starved Wistar rats into a syringe by aortic exsanguination under light ether anaesthesia. Clotted blood was centrifuged at 1000*g* for 15min, and serum was obtained.

A microsomal fraction was prepared from rat liver by the method of Mookerjea (1972). The microsomal pellets were suspended in 0.25M-sucrose and a Tris/HCl buffer (35mM, pH7.4) containing 25mM-KCl, 5mM-$MgCl_2$ and 200mM-NaCl. They were incubated at 0 or 30°C for 30min, then centrifuged at 100000*g* in a Spinco model L ultracentrifuge for 1h. The supernatant was drawn off with a Pasteur pipette and designated the 'saline wash'.

Preparation of DSG-fetuin

Sialic acid was removed from foetal-calf serum fetuin by mild acid hydrolysis. Sequential degradation of the monosaccharides of fetuin was performed by periodate oxidation followed by reduction with $NaBH_4$ and mild acid hydrolysis as described by Spiro (1964). When this method is used, only hexosamines and mannose remain attached to the peptide portion of fetuin.

Materials

All radioactive nucleotide sugars were purchased from New England Nuclear Corp. (Dorval, Que., Canada). Triton X-100, α-lactalbumin, fetuin and all nucleotide derivatives were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). CNBr-activated Sepharose 4B and Sephadex G-200 were purchased from Pharmacia (Uppsala, Sweden). Bio-Beads SM-2 were from Bio-Rad Laboratories (Richmond, CA, U.S.A.) and UM-10 and XM-100 membrane filters were from Amicon Corp. (Lexington, MA, U.S.A.).

Results

Comparison of serum and 'saline-wash' enzyme by Sephadex chromatography

Triton X-100 (up to 1%) slightly inhibited the serum galactosyltransferase (results not shown) and also *N*-acetylglucosaminyltransferase (Mookerjea *et al.*, 1971). By contrast, the galactosyltransferase present in the microsomal preparation is stimulated by as much as 15-fold in the presence of 0.5% Triton X-100 (Table 1). This difference is probably due to the fact that the serum enzyme is soluble, whereas the microsomal enzyme is membrane-bound. Although the microsomal enzyme is membrane-bound, as much as 20–30% of it can be 'solubilized' by the simple procedure of incubation in 200mM-NaCl. This 'solubilized' enzyme is certainly not from residual serum left in the liver, because it can also be stimulated by Triton X-100 (Table 1). It is possible that 'saline-wash' fraction retains a fine micellar form of enzyme. It should also be noted that NaCl up to a concentration of 1M in the enzyme incubation mixture had no effect on the enzyme activity. Increasing the NaCl concentration caused no further release of the enzyme. This solubilization of membrane enzyme is probably not linked to any enzymic reaction, as similar amounts of enzyme are released at both 0 and 30°C and for incubation times of up to 1h. About 17% of the galactosyl- and *N*-acetylglucosaminyl-transferase activities were released into the 'saline wash' from the microsomal membrane. By contrast, the release of sialyltransferase and UDP-galactose pyrophosphatase was 2.3 and 10% respectively (Table 2). The galactosyltransferase enzyme obtained by 'saline wash' is probably of high molecular weight, as it could be precipitated by 40% saturated $(NH_4)_2SO_4$ at pH7.0 with about 50% recovery, and it could be concentrated by using an XM-100 Amicon ultrafiltration membrane (results not shown). A comparison of the enzyme specific activity between the Sephadex G-200 column eluate and 'saline-wash' fractions showed that a sixfold purification of galactosyltransferase was achieved by column chromatography (Table 2). The enzyme activity was at the 'front' of the column (Fig. 1) and was enriched in galactosyltransferase over both sialyl- and *N*-acetylglucosaminyl-transferase (Table 2). The enzyme lost most of its activity after freezing, or after a week in the cold-room at 4°C. Compared with the microsomal 'saline-wash' enzyme, the soluble serum enzyme showed a much lower molecular-weight profile on Sephadex G-200 column chromatography (Fig. 2).

Table 1. *Effect of Triton X-100 on galactosyltransferase activity*

The assay for measuring UDP-galactose–glycoprotein galactosyltransferase is described in the Materials and Methods section. The 'saline-wash' fraction was prepared by incubating the microsomal fraction with 200mM-NaCl for 30min at 30°C and then centrifuging at 100000*g* for 1h. The supernatant obtained was designated as 'saline wash'.

	Galactosyltransferase activity (pmol of galactose/h per mg of protein)		
Treatment	Microsomal fraction	Saline wash	Serum
None	112	83	282
Triton X-100 (0.5%)	1710	980	248

Table 2. *Enzyme activities of microsomal fractions*

The assay for measuring enzyme activities is described in the Materials and Methods section. 'Saline-wash' fraction was prepared by incubating microsomal preparation with 200mM-NaCl for 30min at 30°C and then centrifuging at 100000*g* for 60min. The supernatant ('saline-wash') fraction was chromatographed on Sephadex G-200 to obtain the galactosyltransferase-rich 'Sephadex G-200 eluate' peak activity fraction (Fig. 1). Enzyme activities were assayed in 25μl of each fraction. N.M., Not measured.

Fractions	Protein concentration (μg/25μl)	Glycoprotein–glycosyltransferase activity (pmol of sugar/h per mg of protein)			UDP-galactose pyrophosphatase (nmol of galactose 1-phosphate formed/h per mg of protein)
		Galactosyltransferase	Sialyltransferase	*N*-Acetylglucosaminyltransferase	
Untreated microsomal preparation	380	1020	3500	460	26.5
'Saline-wash'	120	540	258	248	9.0
'Sephadex G-200 eluate' peak activity fraction	14	2860	253	630	N.M.

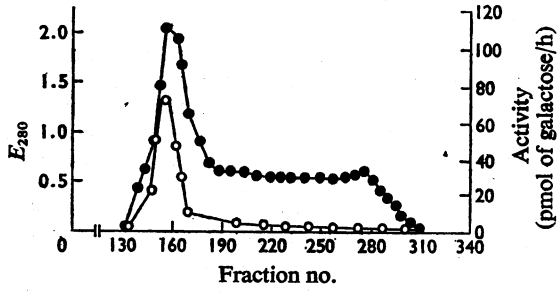


Fig. 1. *Partial purification of galactosyltransferase from microsomal 'saline-wash' fraction*

A column (2.5cm×100cm) of Sephadex G-200 was equilibrated in 0.1M-Tris/HCl, pH7.5, containing 0.1M-NaCl and 0.02% NaN_3. A sample of 'saline-wash' fraction prepared as described in the text was applied to the top of column and the proteins were eluted by a descending flow of column buffer. The eluate fractions (1.3ml/fraction) were analysed for protein (●) (E_{280}), and the enzyme activity (○) was assayed by the method described in the Materials and Methods section.

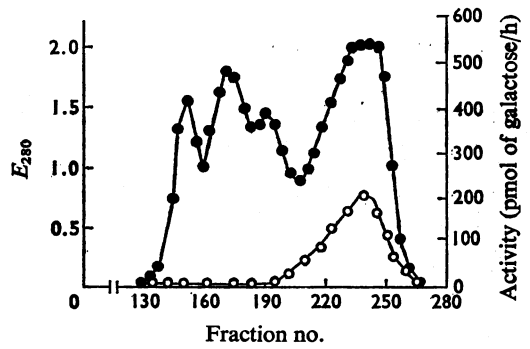
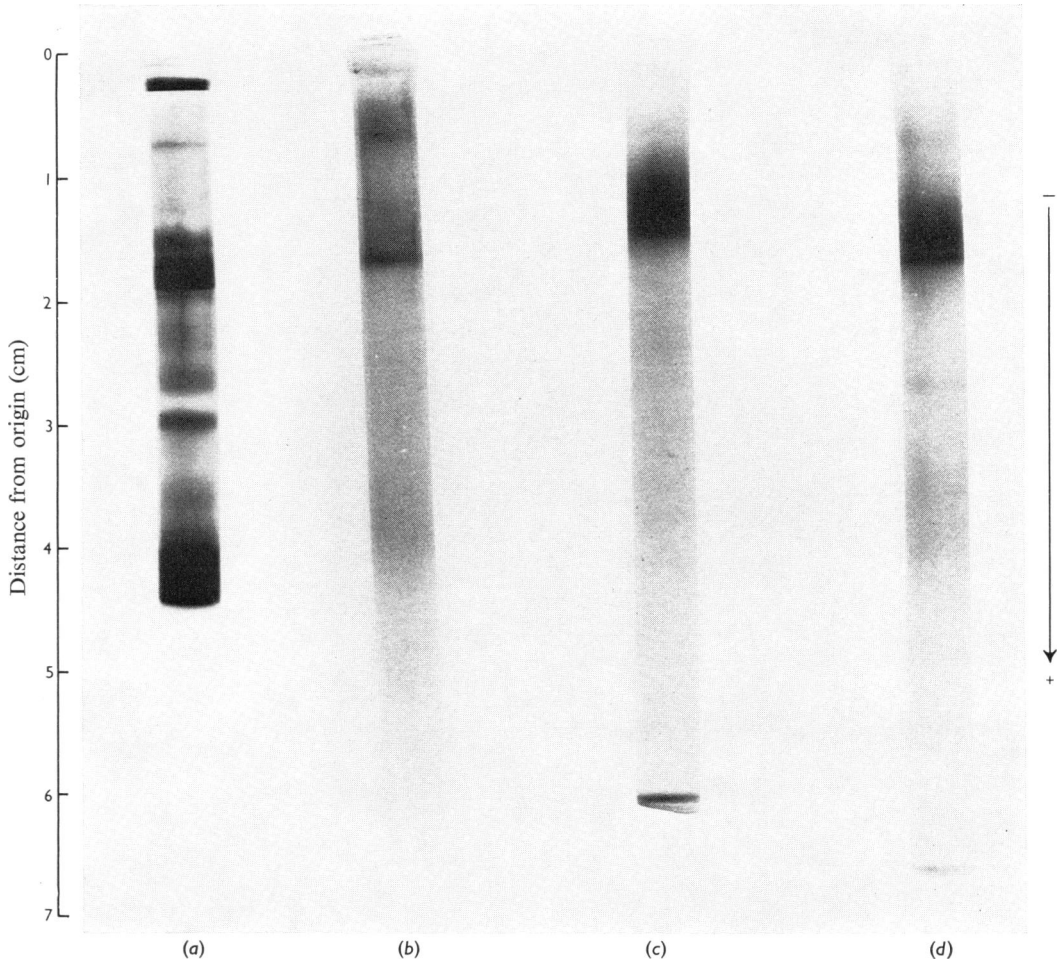


Fig. 2. *Partial purification of rat serum galactosyltransferase by Sephadex column chromatography*

A column of Sephadex G-200 was prepared and used in a way similar to that described for Fig. 1. Protein was monitored at 280nm (●) and enzyme activity (○) was measured in eluate fractions (1.3ml) as described in the Materials and Methods section.

Purification of serum enzyme by affinity chromatography

The serum galactosyltransferase was purified by using the affinity chromatography technique whereby α-lactalbumin was coupled to CNBr-activated Sepharose 4B. The enzyme was retained on such a column in the presence of 3mM-*N*-acetylglucosamine in the buffer and was released from the column when *N*-acetylglucosamine was omitted from the eluting buffer (Fig. 3). We emphasize here that the presence of 0.1% Triton X-100 was essential for the stabilization and recovery of the enzyme from the column. When the columns were run without any Triton X-100 in the buffer, the enzyme was retained on the column, but only 0–10% of the enzyme was recovered in the eluate as against about 80% recovery in the presence of Triton X-100. After re-chromatography of the eluate, a pure enzyme fraction was obtained which gave a single band on analytical polyacrylamide-gel electrophoresis (Plate 1). For disc-gel electrophoresis, the column eluate was concentrated about 100-fold by using an Amicon UM-10 membrane in a 25ml cell. The purified enzyme processed from about 6ml of rat serum was used to obtain the single band as shown in Fig. 4. When the gels were sliced (1mm) and measured for enzyme activity by incubating in the enzyme-incubation mixture containing Triton X-100, the galactosyltransferase corresponded to the single broad protein band on the gel (Fig. 4). Purified galactosyltransferase from serum and erythrocyte membrane was previously electrophoresed on polyacrylamide gels (Podolsky



EXPLANATION OF PLATE 1

Polyacrylamide-gel electrophoresis (pH 8.3) of purified serum galactosyltransferase

The disc-gel electrophoresis method is described in the Materials and Methods section. (*a*) Crude serum; (*b*) first purification, eluate fraction after withdrawal of *N*-acetylglucosamine from the buffer; (*c*) second purification, eluate fraction obtained after re-chromatography and subsequent omission of *N*-acetylglucosamine from the buffer; (*d*) eluate fraction obtained during re-chromatography of eluate (*b*) in the presence of *N*-acetylglucosamine.

et al., 1974; Podolsky & Weiser, 1975*b*) and on cellulose acetate (Kim *et al.*, 1972*b*). None of these studies showed a clear single eluted peak of enzyme activity coincident with the single protein band shown in Fig. 4. In a pure form the enzyme was very unstable and could not be stored frozen without losing most of its activity. Additions of *N*-acetylglucosamine (5mM), dithiothreitol (2mM) and β-mercaptoethanol (2mM) were unable to stabilize the enzyme. Removal of Triton X-100 from the pure enzyme by passing it though a column of SM-2 beads resulted in a considerable loss of activity. The galactosyltransferase was purified about 6000–7000-fold from crude serum with a recovery of about 50–70% (Table 3). Further calculations based on our purification data showed that rat serum contains about 0.9–1.2mg of galactosyltransferase enzyme/100ml, a value close to that reported for milk (1–2mg/100ml) by Andrews (1970).

The purified enzyme preparation contained little or no sialyl- or *N*-acetylglucosaminyl-transferase or UDP-galactose pyrophosphatase activity (Table 3).

Positive periodic acid/Schiff staining of the purified enzyme on polyacrylamide gels (results not shown) indicated that serum galactosyltransferase is

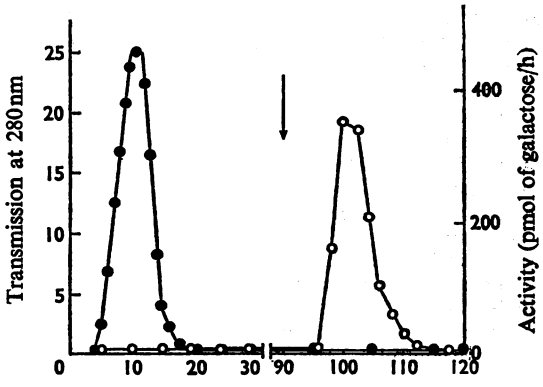


Fig. 3. *Effect of withdrawal of N-acetylglucosamine on the elution of serum galactosyltransferase from α-lactalbumin–Sepharose 4B column*

α-Lactalbumin was coupled to a CNBr-activated Sepharose 4B column and the enzyme was retained on the column owing to its affinity for α-lactalbumin in the presence of 3mM-*N*-acetylglucosamine. The enzyme was released from the column when *N*-acetylglucosamine was withdrawn from the buffer (shown by arrow). The eluate fractions (2.5ml) were analysed for protein (●) (transmission at 280nm) and the enzyme activity (○) was assayed by the method described in the Materials and Methods section.

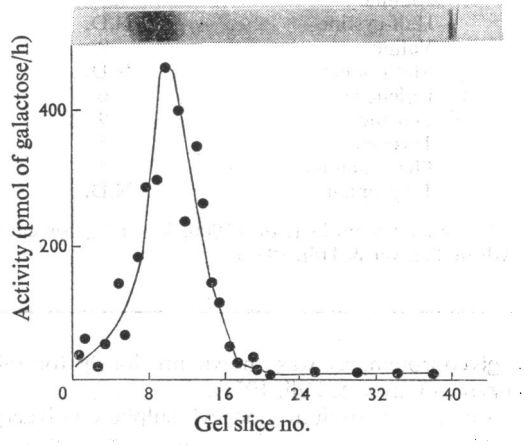


Fig. 4. *Elution of purified serum galactosyltransferase from the polyacrylamide gel*

The band represents enzyme protein purified from an equivalent of 6ml of serum. Slices (1mm) of the gels were incubated in the enzyme-incubation mixture containing Triton X-100, and the enzyme activity was determined as described in the Materials and Methods section.

Table 3. *Enzyme activities of serum fractions*

The assay for measuring enzyme activities is described in the Materials and Methods section. Serum galactosyltransferase was purified by re-chromatography on the column of α-lactalbumin–Sepharose 4B as shown in Fig. 5. Tr., trace.

	Glycoprotein–glycosyltransferase activity				
	Galactosyl transferase		(pmol of sugar/h)		UDP-galactose pyrophosphatase
Fractions	(pmol of galactose/h)	(pmol of galactose/h per mg of protein)	Sialyl-transferase	*N*-Acetylglucos-aminyltransferase	(nmol of galactose 1-phosphate formed/h)
Crude serum	376	570	13.0	9.0	3.0
Purified serum	379	396×10^4	Tr.*	Tr.*	Tr.*

* The purified serum fraction had less than 2% of the original activity found in crude serum.

Table 4. *Amino acid composition of rat serum galactosyltransferase*

Amino acid analyses were run on a Beckman 120C amino acid analyser after digestion in 6M-HCl for 20h under vacuum. Duplicate analysis of a separate enzyme preparation showed essentially similar composition. Abbreviation: N.D., not determined.

Amino acid	Calculated number of residues per molecule*
Lysine	38
Histidine	8
Arginine	3
Aspartic acid	23
Threonine	13
Serine	89
Glutamic acid	50
Proline	10
Glycine	70
Alanine	27
Half-cystine	N.D.
Valine	9
Methionine	N.D.
Isoleucine	6
Leucine	9
Tyrosine	5
Phenylalanine	5
Tryptophan	N.D.

* Based on a mol.wt. of 43000, less 12% for carbohydrate (Trayer & Hill, 1971).

Table 5. *Properties of purified rat serum galactosyltransferase*

Enzyme activity was measured by the method described in the Materials and Methods section; 25μl of enzyme solution purified by re-chromatography on α-lactalbumin–Sepharose 4B column, containing approx. 120ng of protein, was used in each assay.

Requirements	Enzyme activity (pmol of galactose/h)
Complete	430
minus enzyme	1
minus DSG-Fetuin	4
minus Mn^{2+}	1
minus ATP	502
minus ATP, plus EDTA (12mM)	16
minus ATP, minus Mn^{2+}, plus Mg^{2+} (12.5mM)	17
minus ATP, minus Mn^{2+}, plus Ca^{2+} (12.5mM)	10
minus ATP, minus Mn^{2+}, plus Zn^{2+} (12.5mM)	1
minus ATP, minus Mn^{2+}, plus C^{2+} (12.5mM)	108
plus α-lactalbumin (0.2%)	510
plus bovine serum albumin (25μg)	456
plus Triton X-100 (0.5%)	457
plus lysophosphatidylcholine (0.5%)	457
Zero-time control	1

a glycoprotein, as was shown previously for milk enzyme (Trayer & Hill, 1971).

Studies on sodium dodecyl sulphate/polyacrylamide gels and comparison with marker proteins indicated that rat serum enzyme has a mol.wt. of 43000, which was similar to other soluble galactosyltransferases (Andrews, 1970; Trayer & Hill, 1971; Powell & Brew, 1974). The amino acid composition of the pure enzyme is shown in Table 4. The apolar amino acid content was lower than that reported for bovine milk galactosyltransferase (Trayer & Hill, 1971). In contrast with earlier reports, the serine content of the soluble enzyme from rat serum is quite high, which may indicate that this protein has an additional serine-type linkage region for *N*-acetylgalactosamine-containing oligosaccharide. This was previously shown to be true for bovine milk galactosyltransferase (Lehman *et al.*, 1975).

Properties of the pure serum galactosyltransferase

The purified serum galactosyltransferase showed an absolute requirement for Mn^{2+} which could not be replaced by Mg^{2+}, Ca^{2+}, Zn^{2+} or Co^{2+} (Table 5). The enzyme activity was maximum at 12.5mM-Mn^{2+}. Higher concentrations up to 50mM of Mn^{2+} led to a slight decrease in activity; EDTA at 12mM inhibited (95%) the enzyme activity in the presence of Mn^{2+} (Table 5). For the assay of enzyme in crude serum, ATP (2mM) was required to inhibit the UDP-galactose pyrophosphatase activity. The absence of pyrophosphatase in the pure enzyme fraction is further suggested by the fact that ATP is not required for assaying the pure enzyme (Table 5). The pH profile for the enzyme is wide, with a maximum at 6.8 (Fig. 5). A shoulder appears at pH 7.5, which was also seen in galactosyltransferase assay from crude pig serum (Hudgin & Schachter, 1971). The galactosyltransferase activity was proportional with time up to 1h and with enzyme concentration from 25 to 150ng of protein. Addition of albumin or Triton X-100 as carrier on the assay after acid-precipitation of the incubation mixture was beneficial for the linearity of the reaction; albumin was therefore always added as a carrier in assays with pure serum enzyme. Both fetuin, depleted of sialic acid and galactose, and ovalbumin, which has only mannose and *N*-acetylglucosamine in the oligosaccharide chain, served as acceptors for the pure serum galactosyltransferase. Fetuin contains galactose in two different oligosaccharide chains, one containing sialic acid, galactose, mannose and *N*-acetylglucosamine, joined to the peptide molecule through a *N*-acetylglucosamine–asparagine linkage

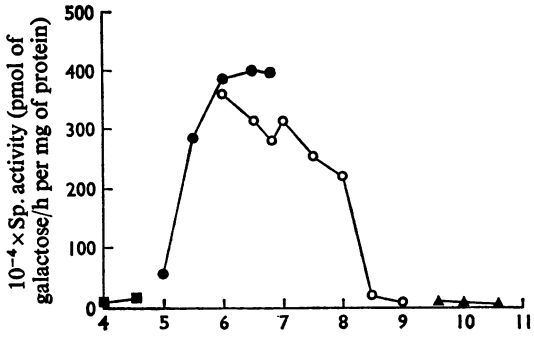


Fig. 5. *Effect of pH on activity of purified serum galactosyltransferase*

Buffers (125mM) used for the assay were: sodium acetate/acetic acid, pH4.0–4.6 (■); Mes/NaOH, pH5.0–6.8 (●); Tris/HCl, pH6.0–9.0 (○); and glycine/NaOH, pH9.6–10.6 (▲). The enzyme-assay method is described in the Materials and Methods section.

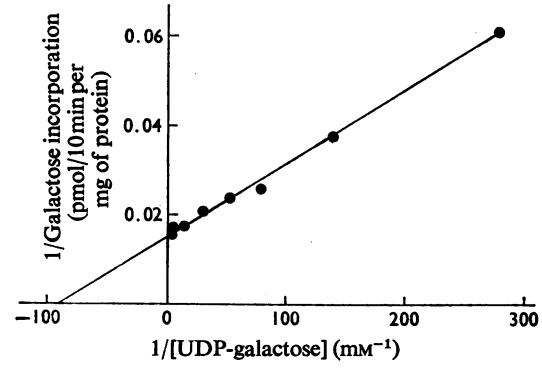


Fig. 6. *Effect of different UDP-galactose concentrations on activity of purified serum galactosyltransferase*

The enzyme-assay method is described in the Materials and Methods section. The data was plotted by the method of Lineweaver & Burk (1934). The apparent K_m value of the enzyme for the substrate is 12.5×10^{-6}M.

that is stable to mild alkali treatment, and the second a minor component containing sialic acid, galactose and *N*-acetylgalactosamine, joined through the alkali-labile linkage *N*-acetylgalactosamine-serine or -threonine (Spiro, 1972). We have tested for the alkali lability of the reaction product (Jato-Rodriguez & Mookerjea, 1974) and found that it was not alkali-labile; this suggested that the purified serum enzyme incorporated galactose into an alkali-stable asparagine–oligosaccharide-type linkage only. The apparent

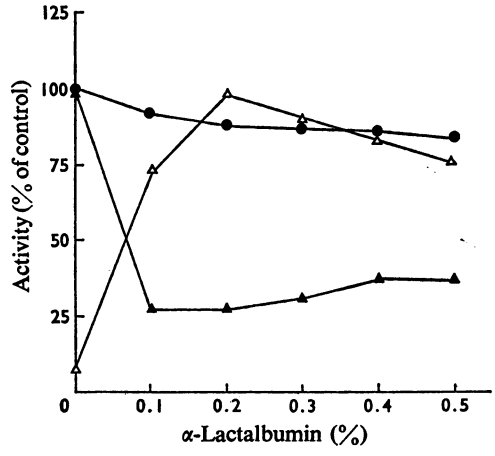


Fig. 7. *Effect of α-lactalbumin on the ability of pure serum enzyme to transfer galactose to DSG-fetuin, glucose and N-acetylglucosamine*

Enzyme was assayed by the method described in the Materials and Methods section, except that for *N*-acetyl-lactosamine synthetase activity the pure enzyme showed an absolute requirement for albumin (125μg per assay) as a stabilizer. Transfer of galactose from UDP-galactose into glucose (△), *N*-acetylglucosamine (▲) and DSG-fetuin (●) is shown as a function of the concentration of α-lactalbumin.

K_m of the pure enzyme for UDP-galactose is 12.5×10^{-6}M (Fig. 6). This is close to the value of 7.5×10^{-6}M established for galactosyltransferase from human serum (Kim *et al.*, 1972*a*). Wagner & Cynkin (1971) reported a higher value of 29.5×10^{-6}M for rat serum enzyme, which has probably resulted from the use of large amounts of substrate in the assay, owing to the presence of UDP-galactose pyrophosphatase in the crude serum (Mookerjea & Yung, 1975). It is also noteworthy that crude human serum contains little, if any, pyrophosphatase (Mookerjea & Yung, 1975). Fig. 7 shows the enzyme activity towards DSG-fetuin, *N*-acetylglucosamine and glucose as acceptors in the absence or in the presence of α-lactalbumin. Addition of α-lactalbumin modified the enzyme such that it made lactose from UDP-galactose and glucose and inhibited its ability to synthesize *N*-acetyl-lactosamine. Thus this enzyme is quite similar to lactose synthetase A protein from bovine milk (Brew *et al.*, 1968). Further, the α-lactalbumin had little effect on the ability of DSG-fetuin to act as acceptor. For the assay of *N*-acetyl-lactosamine synthetase activity, the pure enzyme showed a requirement for albumin (125μg) as a stabilizer for the enzyme. *N*-Acetyl-lactosamine synthesis could not be detected even with 25mM-*N*-acetyl-glucosamine as acceptor when albumin was not

present. However, rat serum (125 μg of protein) and fetuin (125 μg) could substitute for albumin for this assay. For assaying the enzyme with DSG-fetuin and glucose as acceptors, this addition was not required.

Discussion

Serum glycosyltransferases at least partly originate from the liver. This has been suggested from observations that activities of serum *N*-acetylglucosaminyl-, sialyl- and galactosyl-transferases were elevated in patients with alcoholic and other liver disorders (Mookerjea *et al.*, 1972; Kim *et al.*, 1972*b*), whereas they remained at normal values in patients with a variety of non-hepatic diseases. Studies leading to a clear understanding of the properties and functions of these enzymes and their relationship to the membrane environment have been limited by the lack of purified enzyme preparations. The purification procedure described here provides a very simple means of isolating galactosyltransferase from rat serum in good yield. It did not appear necessary to process the serum through the initial steps of $(NH_4)_2SO_4$ precipitation and gel filtration on DEAE-Sephadex or Bio-Gel P-200, which have been shown to result in a considerably lower yield of the milk enzyme (Andrews, 1970; Trayer & Hill, 1971). However, two passages through the Sepharose–α-lactalbumin column as recommended by Trayer & Hill (1971) were required to obtain a homogeneous preparation of the enzyme (Plate 1 and Fig. 4).

The presence of *N*-acetylglucosamine in the Sepharose–α-lactalbumin column was preferred to glucose for retarding the passage of the enzyme protein, as was also found by Andrews (1970). The properties of the pure serum enzyme were essentially similar to those reported for the crude human and rat serum (Kim *et al.*, 1972*a*; Wagner & Cynkin, 1971), with the exception that the apparent K_m for UDP-galactose was much lower, owing to the fact that the purified preparation had virtually no UDP-galactose pyrophosphatase activity.

Although it has been assumed that soluble milk enzyme is derived from mammary gland, the properties of the membrane-bound mammary-gland enzyme and the mechanism of its release into milk are not well understood. The membrane-bound liver enzyme, which could be partly released by incubation with saline, had a higher molecular weight than the serum enzyme, indicating an important difference between the two. We have been able to purify the liver enzyme by a method with slight modifications from that used for serum, which still retained a higher-molecular-weight form (I. H. Fraser & S. Mookerjea, unpublished work). It appears reasonable to suggest that the liver enzyme is a 'precursor' form of the serum enzyme, and possibly there is some mechanism in the liver to convert one form into another. The elevated activity of this enzyme in serum of patients with liver disease may suggest an increased conversion and secretion due to disease. This suggestion is plausible also in view of the work of Judah *et al.* (1973) showing clear evidence of a higher-molecular-weight form of 'precursor' albumin in the liver and their hypothesis that the existence of 'pro-albumin' may assist in albumin secretion in a coupled process of conversion/secretion. Further, it was shown that cytochrome b_5, another microsomal membrane-bound protein, in 'membrane-form' has an extra segment rich in hydrophobic amino acids which is essential for its binding with the membranes, detergents and phosphatidylcholines (Robinson & Tanford, 1975). The activation and secretion of glycosyltransferases by detergents and lysophosphatidylcholine (Mookerjea & Yung, 1974) are probably due to the presence of similar 'extra segments' on the membrane-bound enzymes. Purification of the membrane-bound and soluble glycosyltransferase enzymes is essential for gaining further insights into the mechanisms of enzyme secretion in health and disease.

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