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Human Plasma Uridine Diphosphate Galactose–Glycoprotein Galactosyltransferase

PURIFICATION, PROPERTIES AND KINETICS OF THE ENZYME-CATALYSED REACTION

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The soluble galactosyltransferase of human plasma catalysed the transfer of galactose from UDP-galactose to high- and low-molecular-weight derivatives of *N*-acetylglucosamine, forming a β-1–4 linkage. The enzyme was purified by using $(NH_4)_2SO_4$ precipitation and affinity chromatography on an α-lactalbumin–Sepharose column. The galactosyltransferase was maximally bound to this column in the presence of *N*-acetylglucosamine, and the enzyme was eluted by omitting the amino sugar from the developing buffer. The molecular weight of the enzyme was estimated to be 85000 by gel filtration. The assay conditions for optimum enzymic activity was 30°C and pH7.5. Mn^{2+} ion was found to be an absolute requirement for transferase activity. The K_m for Mn^{2+} was 0.4mM and that for the substrate, UDP-galactose, was 0.024mM. The K_m for the acceptors was 0.21mM for α_1-acid glycoprotein and 3.9mM for *N*-acetylglucosamine. In the presence of α-lactalbumin, glucose became a good acceptor for the enzyme and had a K_m value of 2.9mM. Results of the kinetic study indicated that the free enzyme reacts with Mn^{2+} under conditions of thermodynamic equilibrium, and the other substrates are added sequentially.

The presence of glycosyltransferases in the blood of humans (Kim *et al.*, 1971*a*,*b*; Mookerjea *et al.*, 1971) and other mammals (Hudgin & Schachter, 1971*a*,*b*,*c*) has been reported. These glycosyltransferases are a family of related enzymes responsible for the formation of glycoproteins and glycolipids. Studies of a galactosyltransferase in human serum (Kim *et al.*, 1972*a*) and the alteration in the amount of this transferase during liver disorders (Kim *et al.*, 1972*b*) have been reported.

Galactosyltransferases from bovine (Trayer *et al.*, 1970; Trayer & Hill, 1971) and human milk (Andrews, 1970) and from calf serum (Turco & Heath, 1976) were purified by an affinity-chromatography column consisting of α-lactalbumin covalently coupled to Sepharose 4B. Since the galactosyltransferase from human plasma interacted with α-lactalbumin as shown previously (Kim *et al.*, 1972*a*) in a manner similar to that observed for the milk enzyme, an α-lactalbumin–Sepharose column was utilized in the purification of the enzyme from plasma. The present paper reports the purification of this galactosyltransferase by using affinity chromatography and describes the kinetic and physicochemical properties of the purified protein. A preliminary report of some of this work has appeared (Bella & Kim, 1972*a*).

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Experimental

Materials

Outdated blood was obtained in unit quantities from Irwin Memorial Blood Bank, San Francisco, CA, U.S.A. Pooled outdated plasma used in the preparative-scale purification of the enzyme was given by the American National Red Cross Fractionation Center, Bethesda, MD, U.S.A. $(NH_4)_2SO_4$ and Tris (enzyme grade) were purchased from Schwartz/Mann, Orangeburg, NY, U.S.A. Sepharose 4B was a product of Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A. α-Lactalbumin was prepared (Brodbeck *et al.*, 1967) and purified to the Bio-Gel P-30 step. Whole cow's milk used in the preparation of α-lactalbumin was kindly provided by Challenge Milk Co., Berkeley, CA, U.S.A. CNBr was obtained from

K & K Laboratories, Hollywood, CA, U.S.A. UDP-[^{14}C]galactose (204 μCi/μmol) was purchased from New England Nuclear, Boston, MA, U.S.A., and its purity was checked by paper chromatography in solvent B. Only one radioactive spot was detected. The specific radioactivity was adjusted to 2 μCi/μmol by the addition of unlabelled UDP-galactose obtained from Calbiochem, La Jolla, CA, U.S.A. Other chemicals were obtained from the following sources: Bio-Gel P-30, P-150 and P-200 and Dowex AG-1, Bio-Rad, Richmond, CA, U.S.A.; *N*-acetylglucosamine, Sigma, St. Louis, MO, U.S.A.; Pipes* and UDP, Calbiochem. All other chemicals used were reagent grade.

Methods

Preparation of glycoprotein acceptors. The α_1-acid glycoprotein was prepared (Schmid, 1953) from freeze-dried Cohn Supernatant V obtained from Connaught Medical Research Lab, Toronto, Canada. Sialic acid was removed from α_1-acid glycoprotein and from fetuin by treatment with neuraminidase (*Vibrio cholera*) (Kim *et al.*, 1971*c*). Complete release of sialic acid was obtained in the treatment. Galactose was removed from the desialylated protein by periodate oxidation similar to published procedures (Spiro, 1962): 95% of the galactose was removed by this treatment.

The concentration of the modified glycoprotein is expressed (unless otherwise specified) as the number of potential acceptor sites available for sugar attachment. This was calculated from the amount of sugar residues released by the specific treatment in the preparation. Sheep and ox submaxillary mucins were gifts of Dr. W. Pigman, N.Y. Medical College, NY, U.S.A. The method used to remove sialic acid from these mucins has been described previously (Bella & Kim, 1971).

Paper chromatography. Descending paper chromatography was performed with Whatman no. 1 paper in the following solvent systems: (A) butanol/ethanol/water (10:1:2, by vol.); (B) ethanol/1 M-ammonium acetate, pH 5.0 (7:3, v/v).

Sugars were located with $AgNO_3$ reagent (Trevelyan *et al.*, 1950). Compounds that were radioactively labelled were located by taking 5 cm wide strips along the length of the paper chromatogram, cutting them at 1.3 cm intervals and counting the radioactivity of each section in a liquid-scintillation counter by using 5 ml of Omnifluor (New England Nuclear) 'cocktail' mixture.

Preparation of α-lactalbumin–Sepharose affinity column. The coupling of α-lactalbumin to Sepharose 4B was conducted by using a procedure based on previous methods (Trayer *et al.*, 1970; Trayer & Hill, 1971; Andrews, 1970; Cuatrecasas, 1970). For analysis 1 ml of the settled-bed α-lactalbumin–Sepharose 4B was hydrolysed for 24 h at 100°C with 6 M-HCl, and the amino acids were analysed in a Beckman 120C analyser. The amount of α-lactalbumin bound to the Sepharose 4B was determined to be 4.8 mg/ml of settled bed.

Assay of galactosyltransferase. The enzyme assay was done at 30°C for 15 min in a total volume of 0.10 ml containing 10 μmol of UDP-[^{14}C]galactose (2 μCi/μmol), 5 μmol of Pipes, pH 7.5, 4 μmol of Mn^{2+} and 0.4 mg of α_1-acid glycoprotein (minus sialic acid, minus galactose). The reaction was terminated by the addition of 1.0% phosphotungstic acid in 0.5 M-HCl and the mixture was processed as previously described (Bella & Kim, 1971). When *N*-acetylglucosamine was used as an acceptor, 1 μmol was added and the reaction terminated after 15 min by placing the reaction tube in ethanol/solid CO_2. Cold water (0.2 ml) was added and the mixture applied immediately to a column (0.5 cm × 10 cm) containing 500 mg of Dowex AG-1(X8; Cl^- form). The reaction tube was washed out with 3 × 0.5 ml of water and all the washings were passed through the column. The effluent was collected directly into a glass scintillation vial; 10 ml of scintillation 'cocktail' (Turner, 1968) was added to each vial, which was counted for radioactivity in a Packard Tri-Carb liquid-scintillation counter.

In the assay for lactose synthase 10 μg of α-lactalbumin was included in the standard reaction mixture with 1 μmol of glucose as the acceptor. The reaction mixture was processed in the same way as with *N*-acetylglucosamine as the acceptor.

Steps in enzyme purification. Preliminary purification of the galactosyltransferase was performed by using plasma from recently outdated blood from donors of various blood types. Pooled outdated frozen plasma was used for large-scale preparations of the enzyme. All the procedures were conducted at 4°C unless otherwise stated. The enzyme was precipitated with 25–45%-satd. $(NH_4)_2SO_4$. The precipitate was dissolved in 500 ml of 0.05 M-Tris/HCl buffer, pH 8.0, and was dialysed against the same buffer to remove the $(NH_4)_2SO_4$. The total volume after dialysis was 600 ml. The next step was affinity chromatography on a column containing α-lactalbumin–Sepharose. Application and elution of the enzyme from the column were similar to the method of Andrews (1970). Batches (60–80 ml) of the enzyme solution were applied to a column (40 mm × 45 mm) containing α-lactalbumin–Sepharose which had been previously equilibrated with 0.05 M-Tris/HCl, pH 8.0, containing 5 mM-*N*-acetylglucosamine (buffer 1). The column was washed with buffer 1 at a flow rate of 28 ml/h and fractions were collected every 30 min. Most of the protein in the plasma passed through the

* Abbreviations: Pipes, 1,4-piperazinediethanesulphonic acid; lacto-*N*-biose, *O*-β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-D-glucopyranose; 2′-fucosyllactose, *O*-α-L-fucopyranosyl-(1–2)-*O*-β-D-galactopyranosyl-(1–4)-D-glucopyranose.

column, but the galactosyltransferase was adsorbed. The protein was monitored by reading the A_{280} of the effluent. The adsorbed galactosyltransferase was eluted from the column with 0.05M-Tris/HCl, pH8.0 (buffer 2). The enzyme activity was assayed in the effluent by using *N*-acetylglucosamine as the acceptor. After location of the enzyme fraction, the enzyme was recovered from each tube by adding $(NH_4)_2SO_4$ to 60% saturation and collecting the precipitate. The enzyme was stable at this stage when kept at 4°C. The α-lactalbumin–Sepharose column was re-used repeatedly after washing it with 2M-NaCl and re-equilibrating it with buffer 1. No apparent loss of enzyme-binding capacity was observed with this procedure. A second application of enzyme to the affinity column was also performed under the same conditions of application and elution.

Molecular-weight estimation by Sephadex G-150 and Bio-Gel P-200 chromatography. A column (2.5cm×74cm) of Sephadex G-150 was equilibrated with 0.05M-Tris/HCl/0.1M-KCl, pH7.5. It was calibrated with proteins of known molecular weight and their elution positions were plotted as described by Andrews (1965). The flow rate was maintained at 2.8ml/h per cm^2 with a Buchler peristaltic pump. Fractions were collected every 15min. In a similar manner, a column (1.3cm×85cm) containing Bio-Gel P-200 and a column (1.3cm×75cm) containing Bio-Gel P-150, both equilibrated with 50mM-Tris/HCl buffer, pH7.5, were used for molecular-weight determinations.

Polyacrylamide-gel electrophoresis. Polyacrylamide-gel electrophoresis was carried out by the method of Neville (1971). The protein was stained with Coomassie Brilliant Blue (Weber & Osborn, 1969). Analytical runs were conducted on proteins of known molecular weight, and a plot similar to that of Weber & Osborn (1969) was used in the estimation of molecular weight. Polyacrylamide-disc-gel electrophoresis (7.5% gel) was performed at pH8.9 as described previously (Bella & Kim, 1972*b*).

Acceptor specificity. The standard assay used for *N*-acetylglucosamine was also used for all assays with monosaccharide and disaccharide acceptors. When glycoprotein acceptors were used, the assay was conducted in a manner similar to that with the α_1-acid glycoprotein acceptor. The transferase activity with ichthyocol as the acceptor was assayed as described by Spiro & Spiro (1971).

Characterization of radioactive product formed. To identify the radioactive component transferred to α_1-acid glycoprotein and to *N*-acetylglucosamine, the product formed was treated with β-galactosidase from *Clostridium perfringens* isolated by a published procedure (Chipowsky & McGuire, 1969). To remove unchanged substrate, the α_1-acid glycoprotein product was first dialysed against water before the β-galactosidase treatment and the product with *N*-acetylglucosamine as the acceptor was passed through a column containing 500mg of Dowex 1 (X8; Cl^- form). The β-galactosidase incubations were done at 37°C in 0.05M-sodium acetate buffer, pH4.8, for 2h. After enzymic treatment, the reaction mixtures were passed through a column (0.5cm×5cm) containing a 1:1 (w/w) mixture of Dowex 1 and Dowex 50, concentrated and applied to Whatman no. 1 paper. Descending chromatography was for 5 days in solvent A. The [^{14}C]galactosyl-*N*-acetylglucosamine was also chromatographed together with ^{14}C-labelled α_1-acid glycoprotein which had been partially hydrolysed with 0.05M-H_2SO_4 for 4h at 100°C. Standards of *N*-acetyl-lactosamine and lacto-*N*-biose were also run.

Results

Purification procedure

The purification procedure of the galactosyltransferase from pooled human plasma is summarized in Table 1. Step 1 removed more than half of the plasma protein concomitant with a good recovery of the enzyme activity. Attempts were made to apply the plasma directly to α-lactalbumin–Sepharose, but this procedure was of no advantage, since overloading

Table 1. *Purification procedure for galactosyl transferase*
A unit of galactosyltransferase is defined as 1nmol of galactose incorporated/min.

Step	Fraction	Volume (ml)	Concn. (units/ml)	Total activity (units)	Protein (mg/ml)	Specific activity (units/mg of protein)	Yield (%)	Purification (fold)
	Pooled plasma	2000	1.3	2600	68	0.019	100	
1.	45%-satd.-$(NH_4)_2SO_4$ fraction	600	3.3	1980	62	0.053	76	2.8
2.	First α-lactalbumin–Sepharose column	58	11.5	667	1.1	10.5	26.6	555
3.	Second α-lactalbumin–Sepharose column	15	21.4	320	0.04	535	12.3	28000

of the column bed with a large concentration of protein limited the capacity of the column to bind the enzyme. The fraction obtained from step 1 was applied to the α-lactalbumin–Sepharose column in the presence of 5mM-*N*-acetylglucosamine. When the column was washed with buffer 1, most of the protein passed through the column, with no galactosyltransferase activity detectable in this fraction. However, the enzyme was immediately eluted when the column was washed with buffer 2, containing no *N*-acetylglucosamine. The enzyme fractions were combined and the galactosyltransferase was precipitated by the addition of $(NH_4)_2SO_4$ to 60% saturation. A purification of about 500-fold was achieved by this step. The trailing edge of the protein peak from the buffer-1 wash was always present in the first elution. Further purification by re-application of the enzyme from step 2 to a second α-lactalbumin–Sepharose column resulted in a 28000-fold purification of the galactosyltransferase.

Molecular-weight determinations

The enzyme fractions eluted by steps 2 and 3 were treated with 3% SDS (sodium dodecyl sulphate)/1% mercaptoethanol and subjected to SDS/polyacrylamide-gel electrophoresis. The fraction from step 2 had several protein bands, but the preparation from step 3 had only a broad band, corresponding to mol.wt. of 70000–80000. Since SDS treatment denatures the enzyme, it could not be established if the band had galactosyltransferase activity. A single broad protein band was obtained when the step-3 enzyme preparation was examined by conventional polyacrylamide-gel electrophoresis. Gel filtration on Sephadex G-150, Bio-Gel P-150 or Bio-Gel P-200 gave single peaks of galactosyltransferase activity with mol.wts. of 85000, 84000 and 90000 respectively.

Enzyme specificity

Several potential acceptors were tried, including amino sugars, monosaccharides, oligosaccharides and glycoproteins (Table 2). Only compounds containing terminal *N*-acetylglucosamine were acceptors. Unacetylated glucosamine was not an acceptor.

The specificity was again demonstrated by using glycoprotein acceptors. Galactose was only transferred to those glycoproteins having terminal *N*-acetylglucosamine residues. In addition, no acceptor activity was observed for 2′-fucosyl-lactose, indicating that the galactosyltransferase reported here is not the enzyme responsible for the formation of the blood-group B determinant.

When α-lactalbumin was included in the incubation, glucose became an effective acceptor. Under these conditions the rate of transfer of galactose to *N*-acetylglucosamine-terminating acceptors, in general, decreased slightly.

Table 2. *Activity of galactosyltransferase with low-molecular-weight and glycoprotein acceptors*

All the glycoside acceptors were present at a concentration of 10mM, except for the glycoprotein acceptors which were at 15mM. α-Lactalbumin when added in the reaction mixture was 10μg.

Abbreviations: GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; ManNAc, *N*-acetylmannosamine; Gal, galactose; Fuc, fucose; Glc, glucose; Fuc-Gal-Glc, 2′-fucosyl-lactose.

Acceptor	[^{14}C]Galactose incorporated (nmol/min)	
	No addition	α-Lactalbumin added
$GlcNH_2$	0	0
GlcNAc	67.4	36.1
GlcNAc-GlcNAc	35.8	42.8
GlcNAc-GlcNAc-$(Asp)_n$	13.4	19.6
Gal-GlcNAc	0	0
Fuc-Gal-Glc	0	0
ManNAc	0	0
GalNAc	0	0
GalNAc-$(Thr\text{-}Ala\text{-}Ala)_n$	0	0
Glc	0.3	11.0
Maltose	0.8	1.1
Cellobiose	0.2	0.3
Fetuin (minus sialic acid)	1.2	0.1
Fetuin (minus sialic acid and Gal)	89.0	78.0
α_1-Acid glycoprotein (minus sialic acid)	0	0
α_1-Acid glycoprotein (minus sialic acid and Gal)	77.4	69.4
Sheep submaxillary mucin (minus sialic acid)	0.8	1.2
Ovalbumin	28.4	27.2
Ichthyocol	0	0

Characterization of radioactive products

β-Galactosidase (*Cl. perfringens*) treatment of the ^{14}C-labelled product obtained from the incubation of the galactosyltransferase with either α_1-acid glycoprotein (minus sialic acid, minus galactose) or *N*-acetylglucosamine cleaved all of the ^{14}C label from the product. Paper chromatography showed that all of the radioactivity was galactose. A chromatogram obtained by application of the ^{14}C-labelled α_1-acid glycoprotein product, partially hydrolysed ^{14}C-labelled α_1-acid glycoprotein and the product formed with *N*-acetylglucosamine as the acceptor is shown in Fig. 1. The ^{14}C-labelled α_1-acid glycoprotein remained at the origin, but after partial acid hydrolysis two major radioactive spots were observed, corresponding to galactose and *N*-acetyl-lactosamine. The product with *N*-acetylglucosamine as the acceptor co-chromatographed with *N*-acetyl-lactosamine.

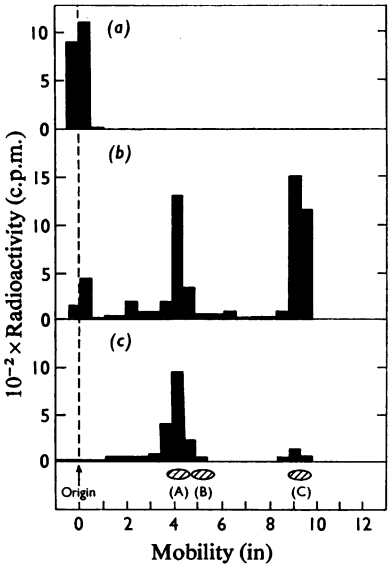

Fig. 1. *Chromatography of the reaction products in solvent A*
The bars represent the radioactivity recorded from the paper after chromatography and the hatched area shows the position of the following sugars: (A) *N*-acetyl-lactosamine, (B) lacto-*N*-biose and (C) galactose. (*a*) Chromatographic pattern of the isolated ^{14}C-labelled product after incubation with α_1-acid glycoprotein (minus sialic acid, minus galactose). (*b*) Chromatographic pattern of the partially hydrolysed ^{14}C-labelled α_1-acid glycoprotein: 30% of the labelled compound was hydrolysed to *N*-acetyl-lactosamine and 50% to galactose. (*c*) With *N*-acetylglucosamine as the acceptor, the product was labelled *N*-acetyl-lactosamine. The reaction mixtures were as described in the text.

Kinetic properties of the galactosyltransferase

The transfer of galactose to the α_1-acid glycoprotein acceptor by the purified galactosyltransferase was essentially linear with time up to 60min of incubation. An incubation time of 15min was used in subsequent assays of the enzyme. Incorporation was also proportional to the amount of enzyme present.

Maximum transfer of galactose to *N*-acetylglucosamine and to the α_1-acid glycoprotein acceptor was observed at pH7.5 and at 30°C. The presence of Mn^{2+} ion in the incubation was an absolute requirement for the enzyme reaction, although the point without Mn^{2+} addition is not shown in Fig. 2, and Mn^{2+} was the only effective metal ion of those tested (Fig. 2). The K_m for Mn^{2+} was 0.4mM. The galactosyltransferase exhibited the same affinity for UDP-galactose with either *N*-acetylglucosamine or α_1-acid glycoprotein as the acceptor. The K_m for UDP-galactose was 24μM; that for the α_1-acid glycoprotein acceptor was calculated to be 0.2mM and for the *N*-acetylglucosamine 3.9μM. The enzyme in the presence of α-lactalbumin had a K_m of 2.9mM for glucose. α_1-Acid glycoprotein (minus sialic acid, minus galactose) was inhibitory at concentrations in excess of 1.4mM with respect to acceptor sites, and *N*-acetylglucosamine was inhibitory at concentrations greater than 10mM.

Initial-velocity studies were conducted to elucidate the mechanism of enzyme reaction. The experimental design was such as to take into consideration a reaction mechanism involving all the reactants, including Mn^{2+}, since it was shown that Mn^{2+} is an absolute requirement for the reaction. Thus to study this 'terractant' mechanism, kinetic investigations of the initial reaction rate were carried out by varying the concentration of one substrate in the presence of different, but fixed, concentrations of another and keeping the concentration of the third substrate constant. Double-reciprocal plots of the initial-velocity data at various concentrations of Mn^{2+} at different concentrations of α_1-acid glycoprotein and at a constant concentration of UDP-galactose yielded a series of lines that intersected to the left of the vertical axis and above the horizontal axis (Fig. 3). A secondary plot of the intercept and slope was linear.

A similar double-reciprocal plot for experiments varying the amount of UDP-galactose but holding the concentration of Mn^{2+} constant at different fixed concentrations of α_1-acid glycoprotein yielded a family of lines which also intersected (Fig. 4).

The initial-velocity pattern obtained for a fixed concentration of α_1-acid glycoprotein but with various amounts of Mn^{2+} at different fixed amounts of UDP-galactose showed a pattern intersecting to the left of the vertical axis (Fig. 5*a*). The secondary plot of the slopes and intercepts against the reciprocal of UDP-galactose was linear, but a plot of the slopes

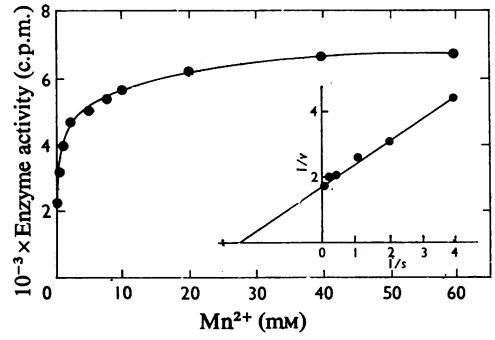

Fig. 2. *Influence of Mn^{2+} concentration on the incorporation of galactose into the α_1-acid glycoprotein acceptor*
The incubations were carried out as described in the standard assay procedure. Inset: Lineweaver–Burk plot of the data shows the K_m to be 0.4mM.

formed a line that passed through the origin. Replotting the data of Fig. 5(*a*) by taking the reciprocal of UDP-galactose at different fixed amounts of Mn^{2+} showed a pattern of lines intersecting on the vertical axis (Fig. 5*b*). A secondary plot of the slope resulted in a line passing through the origin.

Discussion

Purification of the human plasma galactosyltransferase was achieved in three steps. The major steps in the purification were accomplished by taking advantage of the interaction between α-lactalbumin and the galactosyltransferase. This interaction is the same as that observed for lactose synthase found in milk. The galactosyltransferase obtained from human plasma by using this scheme was purified about 28000-fold. No galactosyltransferase bound to the α-lactalbumin–Sepharose in the absence of *N*-acetylglucosamine, suggesting that the interaction of the galactosyltransferase with α-lactalbumin under these conditions is weak or does not occur at all. *N*-Acetylglucosamine was more effective in facilitating binding of the enzyme to the α-lactalbumin–Sepharose than was glucose. This observation was in

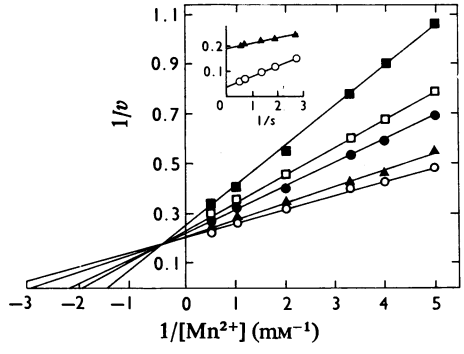

Fig. 3. *Double-reciprocal plot of initial velocity with variable Mn^{2+} concentration at different fixed concentrations of α_1-acid glycoprotein acceptor and a constant concentration of UDP-galactose* (0.1 mM)

The concentrations of the α_1-acid glycoprotein acceptor were: ■, 0.36mM; □, 0.54mM; ●, 0.74mM; ▲, 1.44mM; ○, 1.83mM. Inset shows a secondary plot of the slope (○) and the intercept (▲) versus the inverse of the α_1-acid glycoprotein acceptor concentration (mM).

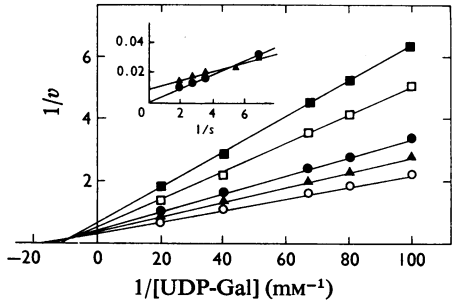

Fig. 4. *Double-reciprocal plot of initial velocity with variable UDP-galactose concentrations at different fixed concentrations of the α_1-acid glycoprotein acceptor and at a fixed concentration of Mn^{2+}* (0.8 mM)

The concentrations of the α_1-acid glycoprotein acceptor were: ■, 0.14mM; □, 0.18mM; ●, 0.28mM; ▲, 0.36mM; ○, 0.54mM. Inset shows a secondary plot of the slope (●) and the intercept (▲) versus the inverse of the α_1-acid glycoprotein acceptor concentration (mM).

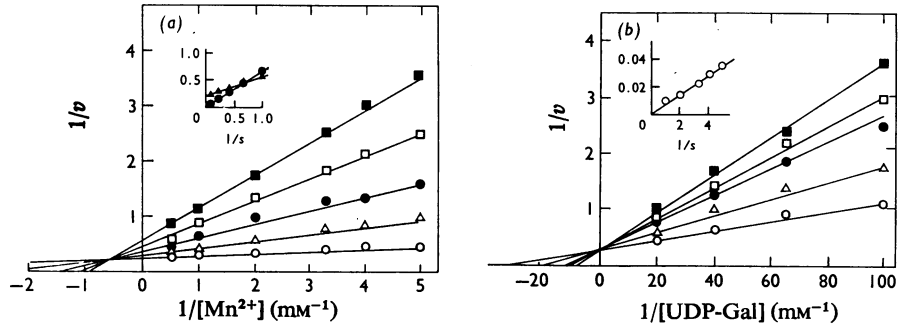

Fig. 5. *Double-reciprocal plots of initial-velocity studies with (a) variable Mn^{2+} concentration at different fixed concentrations of UDP-galactose, both at a constant concentration of the α_1-acid glycoprotein acceptor* (1.4 mM)

In (*a*) the concentrations of UDP-galactose were: ■, 10μM; □, 15μM; ●, 25μM; △, 50μM; ○, 100μM. Inset shows a secondary plot of the slope (●) and the intercept (▲) versus the inverse of the UDP-galactose concentration. In (*b*) the concentrations of Mn^{2+} were: ■, 0.20mM; □, 0.25mM; ●, 0.30mM; △, 0.50mM; ○, 1.0mM. Inset shows a secondary plot of the slope.

accord with a previous report (Andrews, 1970) on studies of human milk lactose synthase.

A mol.wt. of 42000 has been reported for the bovine milk lactose synthase (Trayer & Hill, 1971), and multiple forms of the bovine enzyme mol.wts. 45000 and 58000 have been found (Magee *et al.*, 1972). Although only gel filtration was used in the present study, we obtained a mol.wt. of about 85000 for the human plasma galactosyltransferase. This value must be considered tentative, since molecular weights determined by gel filtration may be in error if the protein contains substantial carbohydrate. The molecular weight obtained by gel filtration was the same as that determined by SDS/polyacrylamide-gel electrophoresis, suggesting that the enzyme may be a single polypeptide chain. Turco & Heath (1976) have purified a galactosyltransferase from foetal calf serum and have determined its molecular weight to be 47800.

Specificity of the galactosyltransferase was also investigated, since glycosyltransferases are involved in the formation of unique carbohydrate structures found in glycoproteins. With a variety of potential acceptors the enzyme exhibited a high degree of specificity, attaching galactose only to acceptors containing terminal *N*-acetylglucosamine residues. However, glucose became an effective acceptor in the presence of α-lactalbumin.

The human plasma galactosyltransferase is distinct from the galactosyltransferase responsible for the synthesis of collagen, which transfers galactose to hydroxylysine, or for the glycosylation of submaxillary mucin, which adds galactose to *N*-acetylgalactosamine, since the purified enzyme did not transfer galactose to ichthyocol, a precursor for collagen, or to sheep submaxillary mucin, which has terminal *N*-acetylgalactosamine residues. This enzyme is also different from the α-galactosyltransferase that is responsible for the formation of the blood-group-B determinant (Kobata *et al.*, 1968; Race *et al.*, 1968), since it did not transfer galactose to 2′-fucosyl-lactose.

Experiments were carried out to establish the mechanism of enzyme action. The double-reciprocal plots in Figs. 3, 4 and 5 resulted in intersecting lines, which suggests a sequential mechanism, that is, one in which all substrates must add to the enzyme before any product is released. The data of Figs. 5(*a*) and 5(*b*) with the secondary plot of the slope going through the origin are diagnostic of an equilibrium ordered reaction (Cleland, 1970). It is apparent then that the mechanism involves the addition of Mn^{2+} at thermodynamic equilibrium, that is, Mn^{2+} must add to some enzyme form or forms before the addition of UDP-galactose. In this case the rate of UDP-galactose combination with the enzyme–Mn^{2+} complex is greater than the rate at which Mn^{2+} dissociates from the same complex. This mechanism is consistent with known Mn^{2+}-dependent enzymic reactions that have been reported that form E–Mn complexes (Cleland, 1970). This mechanism of substrate addition is similar to that found by others (Morrison & Ebner, 1971) for the bovine milk galactosyltransferases. It appears from our preliminary kinetic data that there is an ordered addition of Mn^{2+}, UDP-galactose and α_1-acid glycoprotein, although more kinetic data are required to establish this mechanism firmly (Bell *et al.*, 1976). The mechanism for the release of the products is unknown.

It is apparent that the galactosyltransferases from different sources, goat colostrum (McGuire *et al.*, 1965), bovine milk (Brew *et al.*, 1968), human milk (Andrews, 1970), thyroid (Spiro & Spiro, 1968), pig serum or liver (Hudgin & Schachter, 1971*b*), rabbit gastric mucosa (Ziderman *et al.*, 1967) and rat serum (Fraser & Mookerjea, 1976) are similar to the galactosyltransferase reported here, since each will catalyse the addition of galactose to *N*-acetylglucosamine and each has a requirement for Mn^{2+} ion. The results that we have obtained are comparable with the data from a study of a human milk galactosyltransferase (Khatra *et al.*, 1974), although the enzyme examined here is present in the serum of both male and female subjects.

The source and function of the soluble galactosyltransferase in human plasma are not clearly understood at present, since the biosynthesis of glycoproteins occurs in the smooth membranes within the cell. A previous publication (Kim *et al.*, 1972*a*) suggested that a probable source of this enzyme is the liver, since patients with liver damage have a higher galactosyltransferase activity in the plasma. If the liver is the source of this enzyme, it is probably active in the synthesis of α_1-acid glycoprotein and other serum glycoproteins. The linkage formed when galactose is transferred to the oligosaccharide of α_1-acid glycoprotein terminating in *N*-acetylglucosamine or to free *N*-acetylglucosamine is a β-1–4 linkage, which is the naturally occurring bond in native α_1-acid glycoprotein.

Since the enzyme present in human plasma described herein is very similar to that of the A-protein of lactose synthase present in human milk (Powell & Brew, 1975), yet the molecular weight of the plasma enzyme appears to be nearly twice that of the milk enzyme, the possibility exists that the plasma enzyme may be a precursor for the milk galactosyltransferase. This speculation is supported by the findings (Prieels *et al.*, 1975) that the human milk galactosyltransferase is a heterogeneous population of enzymes with mol.wts. of 38000 to 50000, thought to be a result of proteinase activity. Whether the milk and plasma enzymes are thus related remains to be determined.

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