The molecular and enzymic properties of galactosyltransferase from Golgi membranes isolated from homogenates of sheep mammary Golgi membranes have been investigated.

The enzyme appears to be an intrinsic membrane component, not being solubilized by extraction with 0.1 or 1 M NaCl or with EDTA. Many solubilization procedures produce inhibition of the enzyme, whereas low concentrations (1%) of Triton X-100 produce a stimulation of activity together with complete solubilization of the enzyme. The Triton-solubilized enzyme was purified by a combination of gel filtration and affinity chromatography, to give a product with a specific activity similar to those of previously characterized soluble galactosyltransferases. On polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate the purified enzyme showed two protein components, a major glycoprotein component of apparent molecular weight 69,000 and a minor diffuse component of apparent molecular weight 53,000. Gel filtration of the enzyme directly solubilized from Golgi membranes on calibrated columns of Bio-Gel P-150 gave a major activity peak at $M_r = 60,000$ and a shoulder at $M_r = 55,000$. It is concluded that the galactosyltransferase of Golgi membranes is larger by 10 to 13 x $10^6$ daltons than the most intact ($M_r = 50,000$) of previously studied galactosyltransferases. In contrast, the enzymic properties of the Golgi membrane enzyme, assayed in the presence of Triton X-100, closely resemble those of soluble galactosyltransferases. Finally, we suggest that soluble galactosyltransferases may be produced by proteolytic cleavage of the membrane enzyme during membrane turnover in secreting tissues.

Galactosyltransferase (UDP-galactose, N-acetylglucosamine $\beta$-4-galactosyltransferase) is commonly used as an enzymic marker for Golgi membranes from a number of cell types (1-3). In most tissues, the galactosyltransferase function in catalyzing the synthesis of a galactosyl $\beta$-4-GlcNAc' linkage that is present in the oligosaccharide moieties of many secreted glycoproteins (4, 5); in the lactating mammary gland galactosyltransferase acts as the catalytic component of lactose synthase (EC 2.4.1.22). Many secreted glycoproteins (4, 5); in the lactating mammary gland galactosyltransferase acts as the catalytic component.

**MATERIALS AND METHODS**

The sources of most material were reported in previous publications from this laboratory (8-10). UDP-Sepharose and $\alpha$-lactalbumin-Sepharose conjugates were prepared as described previously (8).

**Proteins** – $\alpha$-Lactalbumin from bovine milk was prepared as described previously (8). Carbonic anhydrase was a gift from Dr. P. Whitney of this department. Trypsin was purchased from the Worthington Biochemical Corp. and other proteins from the Sigma Chemical Co.

**Tissue Sources** – Lactating sheep mammary glands were obtained from the Experimental Farm, University of Leeds, Leeds, England. Following slaughter, the glands were immediately removed and stored on ice until homogenization (approximately 1 h). Pregnant

The abbreviations used were: GlcNAc, $N$-acetyl $\beta$-glucosamine; SDS, sodium dodecylsulfate.
Guinea pigs were purchased from the Camden Research Institute, Wayne, N.J.

Isolation of Golgi Membranes—A procedure closely similar to that of Morre (11) was used for Golgi membranes from homogenates of liver or mammary gland. Tissue was freed from extraneous fat and cut into small pieces. Livers were homogenized directly, but, as reported previously (12), for satisfactory homogenization, mammary tissue required pretreatment with a hand mincer to remove connective tissue.

The tissue was homogenized in 10-g batches with 20 ml of ice cold homogenizing fluid (0.5 M Tris/maleate buffer, pH 6.4) containing 0.5 M sucrose, 1.0% (w/v) dextran, 1 mM MgCl₂, and 5 mM 2-mercaptoethanol, using a glass-Teflon homogenizer with a clearance of 0.91 inch. The material was homogenized by three complete passages of the pestle at a speed of approximately 1750 rpm. The homogenate was centrifuged at 1000 × g for 10 min at 4° to sediment unbroken cells and residual connective tissue. The pellet was discarded. The supernatant was centrifuged at 8250 × g for 60 min at 4°. The resulting supernatant was discarded and the inside of the tube were wiped free of fat. The pellet was resuspended in homogenizing medium (3.0 ml/original 10 g tissue) using a small all-glass hand homogenizer. The resuspended material was layered onto 1.5 to 2.0 volumes of 1.25 M sucrose (unbuffered) and centrifuged at 90,000 to 105,000 × g for 60 min at 4°. A membrane fraction derived from the Golgi apparatus collects at the homogenate-1.25 sucrose interface, and was removed using a Pasteur pipette with a wide orifice. This material was diluted with an equal volume of 50 mM sodium cacodylate buffer, pH 7.4, or unbuffered isosonic sucrose. The 2.0 M sucrose was concentrated by centrifuging at 10,000 × g for 60 min at 4°, and the pellet was resuspended in 25 mM sodium cacodylate buffer, pH 7.4, or 0.25 M sucrose to give stock solutions of approximately 10.0 mg of Golgi protein/ml of suspension.

Measurement of Galactosyltransferase Activity—Galactosyltransferase activity was assayed by a radiochemical procedure as described previously (4, 7). Triton X-100 was found to increase the enzyme solubility being failure of enzyme activity to sediment. Triton X-100 was included in assays of membrane fractions and of the membrane enzyme during purification. In assays using the glycoprotein acceptor ovalbumin, reactions were terminated by the addition of 1.0 ml of 20% (w/v) trichloroacetic acid and 0.1 ml of 1% (w/v) bovine serum albumin as carrier. The precipitated protein was collected by centrifugation, washed with 3 × 1.0 ml of 10% trichloroacetic acid, and dissolved in formic acid (0.2 ml), and radioactivity was determined by scintillation counting.

Kinetic data were analyzed by computer fitting to appropriate rate equations as described previously (10).

Characterization of Golgi Membranes—Isolated Golgi membranes were assayed for the marker enzyme 5'-nucleotidase (plasma membrane), glucose-6-phosphatase (endoplasmic membranes), sucinic-2-(p-indophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium-reductase (mitochondria), and thiamin pyrophosphatase (Golgi membranes) as described (11), as well as for galactosyltransferase.

Membranes were also examined by electron microscopy. The pellet of material collected by centrifugation at 10,000 × g for 60 min was fixed overnight with 9.5% (w/v) glutaraldehyde in 50 mM cacodylate buffer, pH 7.4, containing 2% sucrose and 1 mM CaCl₂. The fixed material was postfixed with osmium tetroxide, dehydrated, embedded in Araldite, and stained with ethanolic uranyl acetate and lead citrate. Sections were examined using a Philips EM 300 electron microscope.

Analytical Gel Filtration—Determination of molecular weights was performed using a calibrated column of Bio-Gel P-150 (200 to 400 mesh) (1.5 × 87 cm), equilibrated in 0.01 M sodium cacodylate buffer containing 0.1 M NaCl, 5 mM G6Nac and, 3 mM 2-mercaptoethanol, pH 7.4 at 4°. Molecular weight markers were dissolved in 1.0 ml of equilibrating buffer and applied to the column. The column was calibrated using the following: blue dextran 2000, transferrin, bovine serum albumin, ovalbumin, carbonic anhydrase, myoglobin, and catalase. The molecular weight of unknown samples was determined from a plot of Kₑ against log molecular weight as described previously (8).

RESULTS

Characteristics of Golgi Membranes from Lactating Sheep Mammary Gland

Some enzymic characteristics of Golgi membranes isolated, as described, from homogenates of lactating sheep mammary gland are summarized in Table I. The marker enzymes for Golgi membranes, thiamin pyrophosphatase and galactosyltransferase (11), show a large enrichment in specific activity over the homogenate, while the enzymic markers for other cell membrane fractions are decreased in specific activity.

The specific activity of galactosyltransferase in Golgi membranes from the lactating mammary gland is far higher than values previously reported, and those observed here, for Golgi membranes purified by similar methods from homogenates of other tissues. Thus, we find that the specific activity in rat liver Golgi membranes is 7.2; membranes isolated from a homogenate of the mammary tissue of a guinea pig at a late stage of pregnancy showed a specific activity of 40 units/mg of protein, while Golgi membranes from mammary tissue obtained from a guinea pig in the 2nd day of lactation had a specific activity of 150 units/mg, similar to that found for the sheep.

As the most well characterized soluble galactosyltransferrases are from bovine secretions but lactating bovine mammary tissue was not available to us, lactating sheep mammary Golgi membranes were chosen as the source of enzyme for all subsequent studies, being from a species relatively closely related to the bovine.

Solubilization

A range of procedures were investigated for effectiveness in solubilizing galactosyltransferase, the initial criterion for enzyme solubility being failure of enzyme activity to sediment when centrifuged at 10,000 × g for 60 min at 4°, conditions under which vesicles derived from Golgi membranes are

| EnzymeActivity of Golgi membranes isolated from lactating sheep mammary gland | Specific activities | Relative |
|---|---|---|
| Thiamine pyrophosphatase* | 0.42 | 0.0035 | 12.00 |
| Galactosyltransferase | 176.6 | 2.96 | 59.5 |
| Succinic-INT reductase | 0.0031 | 0.033 | 0.094 |
| Glucose-6-phosphatase* | 0.0066 | 0.047 | 0.13 |
| 5'-Nucleotidase* | 0.027 | 0.028 | 9.96 |

* Activity is expressed as micromoles of phosphate released per min per mg of protein with G6Nac as acceptor.

* Arbitrary units (INT, 2-(p-indophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium).
otherwise quantitatively sedimented. The procedures were also tested for effect on enzyme activity. Subsequently, the only satisfactory procedure by this criterion was subjected to a more rigorous test of solubilization: the investigation of the enzyme size by gel filtration.

Suspension of Golgi membranes in solutions of NaCl (0.1 or 1.0 M) or EDTA (1 mM) as well as treatment with ultrasonic vibrations at 0° for 15 to 60 min produced negligible solubilization of galactosyltransferase (<0.2%). Other extraction procedures, urea (2 M), SDS at several concentrations (>0.1%), as well as pyridine and acetone at a number of concentrations, strongly inhibited enzyme activity. Treatment with trypsin (0.5% w/w protein for 30 min at 20°) or steapsin (5% w/w for 30 min at 20°) resulted in a loss of activity. 1-Butanol at a concentration of 2% v/v (butanol/protein ratio of 4:1) solubilized 70% of galactosyltransferase, but at higher concentrations produced inhibition. Deoxycholate also produced partial solubilization with enzyme inhibition.

In contrast to all these procedures, treatment of Golgi membranes with a 1% (w/v) concentration of Triton X-100 released galactosyltransferase quantitatively into the supernatant. Triton X-100 had a stimulatory (2.5-fold at a concentration of 0.5 to 2.0% (w/v)) rather than inhibitory effect on galactosyltransferase activity in Golgi membranes. The quantitative solubilization of galactosyltransferase by Triton X-100 (1% w/v solution) was not affected by varying in the Golgi protein to detergent ratio (w/w) from 0.1 to 1.0, by ionic strength (0 to 0.125 M NaCl), or by the presence of GlcNAc (20 mM) or 2-mercaptoethanol (3 mM). However, 5 mM GlcNAc and 3 mM mercaptoethanol were included in subsequent solubilization studies to help maintain enzyme stability.

Purification of Galactosyltransferase from Golgi Membranes

A number of procedures were investigated for purifying detergent-solubilized galactosyltransferase from Golgi membranes, and the following procedure was chosen as the most satisfactory on the criteria of effectiveness, yield, and simplicity. Because of a limited supply of Golgi membrane material, the procedure was used only on small batches of material. All steps in the purification procedure were performed at 4°.

Solubilization—Three to five milligrams or less of Golgi membrane protein was suspended in 1 ml of 25 mM sodium cacodylate buffer containing 1% (w/v) Triton X-100, 0.1 M NaCl, 5 mM GlcNAc, and 3 mM 2-mercaptoethanol, pH 7.4. After brief agitation with a vortex mixer, the partially clarified suspension was centrifuged for 60 min at 10,000 g at 4°. Ninety-nine per cent of the galactosyltransferase activity was recovered in the supernatant.

Gel Filtration—The supernatant was loaded on a column of Bio-Gel P-150 (87 × 1.5 cm) equilibrated with 25 mM sodium cacodylate buffer containing 0.1 M NaCl, 5 mM GlcNAc, and 3 mM 2-mercaptoethanol, pH 7.4. The column was developed with the same buffer at a flow rate of 3.2 ml/h, and the effluent was collected in 2.0-ml fractions. The fractions were monitored by absorbance at 280 nm and assayed for galactosyltransferase activity. As shown in Fig. 1, a small proportion of the enzyme emerged in the void volume of the column but most (70% of the applied activity) was retarded, emerging as a peak and shoulder on the trailing edge of the main protein peak.

Affinity Chromatography with UDP-Sepharose—The pooled material was stirred and 0.5 M MnCl₂ was added to a final concentration of 25 mM. The pH was readjusted to 7.4 with dilute NaOH. The material was loaded on a 1-ml column of UDP-Sepharose 4B (2 μmol of UDP/ml of gel) contained in a Pasteur pipette blocked with a plug of glass wool. The column, which had been pre-equilibrated with 25 mM sodium cacodylate buffer containing 25 mM MnCl₂, 5 mM GlcNAc, and 3 mM 2-mercaptoethanol, pH 7.4, was washed with 50 column volumes of equilibrating buffer. The column was eluted with 25 mM sodium cacodylate buffer containing 25 mM EDTA, 5 mM GlcNAc, and 3 mM 2-mercaptoethanol, pH 7.4, the enzyme being recovered in the first 13 ml of elution buffer.

Affinity Chromatography with α-Lactalbumin-Sepharose—Solid NaCl and GlcNAc were added to the solution of enzyme, with stirring, to final concentrations of 1.0 M and 20 mM, respectively. The material was loaded on to a second affinity column composed of 2.5 ml of Sepharose 4B/α-lactalbumin (2 mg of protein/ml of gel), equilibrated with 25 mM sodium cacodylate buffer containing 1.0 M NaCl, 20 mM GlcNAc, and 3 mM 2-mercaptoethanol, pH 7.4. After the loaded column had been washed with equilibrating buffer (30 to 40 column volumes), the enzyme was eluted with the same buffer devoid of GlcNAc. The presence of 1 M NaCl in the elution buffer was found to be essential for the recovery of membrane galactosyltransferase from α-lactalbumin-Sepharose. It was necessary to repeat the final purification step to obtain optimal purification of the enzyme. A typical purification of the galactosyltransferase is outlined in Table II.

Purity and Molecular Size of Membrane Galactosyltransferase

The progressive purification of galactosyltransferase solubilized from Golgi membranes is illustrated by the SDS-polyacrylamide gel electrophoresis patterns shown in Fig. 2. The total Golgi membrane proteins and the fractions of material soluble and insoluble in 1% Triton X-100 have complex patterns, but distinct differences can be observed between the Triton-soluble and -insoluble fractions. Enzyme from the final purification stage, shown in Gel 7, is resolved into two components. Comparison of the mobilities of these components with those of standard proteins of known size gives apparent molecular weight values of 69,000 for the larger, well defined component and a molecular weight range of 53 to 55 × 10⁶ for the lesser, diffuse component. As the
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TABLE II
Purification of galactosyltransferase from Golgi membranes of lactating sheep mammary gland

| Step       | Volume | Protein | Total units | Specific activity | Purification from homogenate | From Golgi Membranes | Yield % |
|------------|--------|---------|-------------|------------------|-----------------------------|---------------------|--------|
| Golgi      | 1.0    | 3.3a    | 581.0       | 176b             | 59.5                        | 1.0                 | "100"  |
| Triton X-100 | 1.0    | 1.88c   | 580.0       | 308.4            | 104.2                       | 1.6                 | 99.8   |
| Bio-Gel P-150 | 22.1   | 0.110c  | 446.4       | 3,670.0          | 1,307                       | 21.8                | 77.3   |
| UDP-column | 14.7   | 0.041c  | 334.4       | 8,156            | 2,755                       | 48.6                | 57.5   |
| α-Lactalbumin | 10.0   | 0.008d  | 271.3       | 133.0            | 16,600b                     | 7,400               | 123.3  |
| α-Lactalbumin | 10.0   | 0.008d  | 133.0       | 16,600b          | 7,400                       | 123.3               | 22.7   |

* Determined colorimetrically by the Lowry procedure.
+ Measured in the presence of 0.5% (v/v) Triton X-100.
, Determined by fluorescent assay using Fluram.
, From amino acid analysis.

Main component is a glycoprotein (indicated by positive staining with periodate-Schiff reagent), the molecular weight value is likely to be an overestimate.

The molecular size of the enzyme was also investigated by analytical gel filtration. While the galactosyltransferase activity of intact Golgi membranes was relatively stable, the solubilized and partially purified enzyme was very unstable. Enzyme directly solubilized from Golgi membranes with Triton X-100 was therefore used for gel filtration. The column of Bio-Gel P-150 was calibrated with globular proteins of known molecular size. The elution position of the main peak of galactosyltransferase corresponded to a molecular weight of 65,000 while a shoulder on the trailing edge corresponded to the elution position of a 55,000 molecular weight species, in reasonable agreement with the mobilities on SDS gels of the components present in the purified enzyme.

The sedimentation coefficient of the solubilized membrane enzyme was also measured, as an additional indication of size, using the method of Yphantis and Waugh (14) as previously described (10), where the depletion of enzyme activity from the upper compartment of a separation cell during centrifugation is determined. The sedimentation coefficient, derived from three separate runs of 30, 45, and 60 min, gave a value for $s_{20, w}$ of 3.63.

Enzymic Properties

Because of the inherent instability of the purified membrane galactosyltransferase, the enzymic properties were investigated using Golgi membranes in the presence of Triton X-100. A separate investigation with soluble galactosyltransferase from sheep colostrum showed that, while a small increase in activity is obtained in the presence of 1% Triton X-100, the apparent $K_m$ values for Mn$^{2+}$, UDP-galactose, and N-acetylglucosamine, each determined at fixed concentrations of the other substrates, were unchanged in the presence of Triton. It would appear that a reasonably valid comparison can be made between the enzymic characteristics of the membrane enzyme in the presence of Triton and those published for the naturally soluble colostrum enzyme, based on experiments performed in the absence of detergent. Under the assay conditions used, enzyme activity was found to increase linearly with time and with the amount of added Golgi protein.

Cation Activation

In the absence of added cations, Golgi membranes galactosyltransferase shows a low, but significant level of activity that is stimulated by the addition of Mn$^{2+}$ or other cations. This can be eliminated by the addition of Triton X-100 and EDTA to final concentrations of 1.0% (v/v) and 0.02 M, respectively, and then dialyzing the preparation against four 1000-ml changes of 25 mM cacodylate buffer, pH 7.4, containing 3 mM β-mercaptoethanol; the enzyme preparation then only shows activity in the presence of added cations. As previously reported for colostrum galactosyltransferase, Zn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, and Cd$^{2+}$ all supported enzyme activity although at lower rates than obtained with Mn$^{2+}$. Mg$^{2+}$ or Ca$^{2+}$ did not support activity but Ca$^{2+}$ did stimulate galactosyltransferase activity in the presence of 20 μM Mn$^{2+}$. These properties are similar to those previously observed with colostrum galactosyltransferase (9).

Kinetic Properties

For the evaluation of the kinetic parameters of the membrane galactosyltransferase, initial velocities were measured at varying concentrations of one substrate or activator and a series of fixed concentration of a second substrate while maintaining other assay components at fixed concentrations. As the purpose of the study is not to accomplish a complete steady state kinetic analysis of the enzyme, but to effect a comparison with previous data, not all possible combinations of substrates and activators were varied for the reactions catalyzed by the enzyme (N-acetyllactosamine synthesis, lactose synthesis in the presence of α-lactalbumin, and transfer of galactose to glycoprotein). Selected pairs of substrates and activators were varied to elucidate specific parameters and kinetic features. The following combinations were used.

1. for transfer to GlcNAc (N-acetyllactosamine synthesis): (a) Mn$^{2+}$ and UDP-galactose at a fixed concentration of GlcNAc (20 mM); (b) UDP-galactose and GlcNAc at a fixed concentration of Mn$^{2+}$ (10 mM). 2. for lactose synthesis: α-lactalbumin and glucose at fixed concentrations of Mn$^{2+}$ (10 mM) and UDP-galactose (0.3 mM). 3. for transfer of galactose to glycoproteins, for which ovalbumin was utilized as a model substrate (8), the concentration of ovalbumin only was varied at fixed concentrations of Mn$^{2+}$ (10 mM) and UDP-galactose (0.3 mM).

The investigation of lactose synthesis and glycoprotein synthesis were insufficiently detailed to give more than apparent kinetic parameters and patterns of Lineweaver-Burke plots. These parameters and patterns can be compared with those previously obtained with soluble galactosyltransferase under similar conditions. The data from the kinetic experi-
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FIG. 2. SDS-polyacrylamide gels of fractions from various stages of the purification of galactosyltransferase. The direction of electrophoresis is downwards. The samples were: 1, standard protein mixture: transferrin (monomer and dimer), pepsin, chymotrypsin A and cytochrome c; 2, total Golgi membrane proteins; 3, Golgi proteins that are insoluble in 1% Triton X-100; 4, Golgi proteins solubilized by Triton X-100; 5, galactosyltransferase fraction from Bio-Gel P-150; 6, enzyme purified by affinity chromatography with UDP-Sepharose; 7, purified material after affinity chromatography with α-lactalbumin-Sepharose.

merits, apart from combination 1(a), which gives nonlinear replots (8), and combination 3, which is a single Lineweaver-Burk plot, were subjected to computer analysis by fitting to appropriate rate equations as described previously. Only the data for varying concentrations of the substrates UDP-galactose and GlcNAc are shown (Fig. 3). These give in double reciprocal plots a set of parallel lines and fit best to the rate equation as found for the soluble galactosyltransferases under these conditions (9, 15).

The values for kinetic parameters obtained from these data are summarized in Table III. For N-acetyllactosamine synthesis, the apparent parameters were corrected in “true” values utilizing the rate equation

\[
v = \frac{VABC}{K_aK_bK_c + K_{a}K_bA + K_{a}K_c} + K_{a}A + K_{b}C + A BC
\]

where: \(A\), \(B\), and \(C\) are concentrations of \(\text{Mn}^{2+}\), UDP-galactose, and GlcNAc respectively; \(K_{a}\) and \(K_{b}\) are dissociation constants for \(\text{Mn}^{2+}\) and UDP-galactose from \(E\cdot\text{Mn}^{2+}\) and \(E\cdot\text{Mn}^{2+}\cdot\text{UDP-galactose}\), respectively; and \(K_{a}\), \(K_{b}\), and \(K_{c}\) are the Michaelis constants for the appropriate substrates. The procedures for correction to the true values have been described previously (15). For lactose synthesis, the \(K_{a}\) value for α-lactalbumin as variable substrate at a series of fixed concentrations of glucose, the lines intersect to the left of the \(1/v\) axis. The values for kinetic parameters obtained from these data are summarized in Table III. For N-acetyllactosamine synthesis, the apparent parameters were corrected in “true” values utilizing the rate equation as found for the soluble galactosyltransferases under these conditions (9, 15). The other patterns are closely similar to those previously reported for human milk (15) and bovine colostrum galactosyltransferase (9).

For lactose synthesis at varying concentrations of α-lactalbumin and glucose, an asymmetric intersecting kinetic pattern was obtained, where Lineweaver-Burk plots with glucose as variable substrate at a series of fixed concentrations of α-lactalbumin intersect precisely on the vertical axis, but for α-

![Fig. 3. Double reciprocal plots for initial velocities at varying concentrations of UDP-galactose and a series of fixed concentrations of GlcNAc. The concentrations of GlcNAc were: A, 2.0 mM; B, 2.5 mM; C, 3.0 mM; D, 5.0 mM; and E, 10 mM.](http://www.jbc.org/)

**TABLE III**

| Substrate          | Kinetic constant | Value    |
|--------------------|------------------|----------|
|                    | Human milk enzyme | Bovine colostrum enzyme | Sheep milk enzyme |
| UDP-galactose (B)  | \(K_a\)          | 0.083    | 0.24    | 0.42    |
|                    | \(K_b\)          | 1.41     | 0.949   | 0.36    |
|                    | \(K_c\)          | 0.705    | 3.40    | 4.38    |
| GlcNAc (C)         | \(K_a\)          | 0.16     | 0.275   | 0.194   |
| Ovalbumin (C)      | \(K_{a}(app)\)   | 0        | 0       | 0       |
| α-Lactalbumin      | \(K_{a}\)        | 0.87     | 0       | 0.95    |
| Glucose (D)        | \(K_{a}\)        | 2.1      | 0.838   | 0.68    |

* From Ref. 17.

b From Ref. 8.
the region of 2.5 mM Mn\(^{2+}\). This may reflect the binding of Mn\(^{2+}\) to the substrate or to other components (Golgi proteins and phospholipids) present in the assay. The kinetic parameters for Mn\(^{2+}\) show the greatest deviation from previously described values, which could reflect binding to these components, but other true and apparent values for kinetic parameters are closely similar to those previously reported.

**DISCUSSION**

This investigation of the nature of the galactosyltransferase present in Golgi membranes was intended to serve as a basis for assessing if the properties observed with soluble forms of the enzyme also apply to the membrane-bound enzyme that functions within the cell. The sheep was chosen as a source of material closely related to the bovine, the source of soluble enzyme for many previous studies. Although galactosyltransferase is present in Golgi membranes from a range of cell types (1-3), membranes from the lactating mammary gland were used because of the high specific activity for galactosyltransferase, reported here. This high level of enzyme activity can be attributed to the function of the enzyme in the biosynthesis of lactose in the lactating mammary gland (6, 7).

The requirement for a detergent for the release of galactosyltransferase from Golgi membranes and the ineffectiveness of high and low salt concentrations and of EDTA in its solubilization confirm the status of galactosyltransferase as an "intrinsic" protein component of Golgi membranes. A considerable proportion of the total protein of Golgi membranes (60%) is also solubilized by Golgi membranes, giving the complex gel electrophoresis patterns shown in Fig. 2. An initial separation by gel filtration, in which detergent was not included in the elution buffer, was found to be essential for the further purification of galactosyltransferase by the affinity chromatography procedures previously developed for the isolation of naturally soluble forms of the enzyme (16).

The specific activity of the purified enzyme (16,000 units/mg) is closely similar to that previously observed for pure soluble colostrum galactosyltransferase (18,500 units/mg) and reasonably similar to that found for galactosyltransferase from the milk fat globule membrane, when assayed in the presence of Triton X-100. Although a major band and a minor component of lower molecular weight are observed in the purified preparation, the identification of both components as forms of galactosyltransferase is supported by the high specific activity of the preparation, as well as the observation of a peak and shoulder of galactosyltransferase activity with appropriate molecular weights on gel filtration of Triton-solubilized Golgi membranes. The nature of the purification procedure (affinity chromatography) also suggests that both components are galactosyltransferase. The molecular weight of the main component, determined either by analytical gel filtration of directly solubilized Golgi membranes or by mobility of the purified enzyme on SDS-polyacrylamide gel electrophoresis, is clearly greater by about 13,000 than that determined for soluble colostrum galactosyltransferase by similar procedures (8), but is closely similar to that of galactosyltransferase from fat globule membranes. While these molecular weight values are by no means rigorously determined, they are nevertheless useful on a comparative basis. This study therefore indicates that the galactosyltransferase that is present in Golgi membranes is considerably larger than the most intact soluble forms previously studied. The minor, more diffuse, component present in the purified enzyme preparation appears to be similar in size to the soluble colostrum enzyme (apparent \(M_r = 55,000\) on SDS gels electrophoresis). The release of galactosyltransferase from the mammary gland into its secretions during lactation therefore appears to be the consequence of proteolytic attack.

In contrast, the results of our enzymic studies, using Triton-solubilized Golgi membranes, fail to show any significant difference between the membrane-derived enzyme and the soluble enzyme forms. The cation activation which indicates the requirement for two metal ions physiologically for activation of Mn\(^{2+}\) and Ca\(^{2+}\) is similar to results recently reported for colostrum galactosyltransferase (9). The kinetic parameters, summarized in Table III, show little difference from those found with bovine colostrum galactosyltransferase. The kinetic patterns obtained for the activation of lactose synthesis by \(\alpha\)-lactalbumin, which have been attributed previously to random, synergistic binding of glucose and \(\alpha\)-lactalbumin with an enzyme-Mn\(^{2+}\):UDP-galactose complex, are closely similar in nature to those previously observed for human and bovine galactosyltransferases (8, 15).

It is therefore reasonable to conclude that the loss of an \(M_r = 13,000\) portion of the membrane galactosyltransferase molecule has little consequence for the kinetic properties of the enzyme.

The premise that this portion of the enzyme is involved in its interaction with membrane components, is supported by studies of the closely similar galactosyltransferase from colostrum and milk fat (17).

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