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A kinetic comparison of partially purified rat liver Golgi and rat serum galactosyltransferases

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UDP-galactose : *N*-acetylglucosamine β-1,4-galactosyltransferase was partially purified from rat liver Golgi membranes and rat serum. The kinetic parameters of the two enzymes isolated by affinity chromatography were compared with each other and with those for commercial bovine milk galactosyltransferase. When *N*-acetylglucosamine was the acceptor the K_m values for UDP-galactose were 65, 52 and 43 μM for the rat liver Golgi, rat serum and bovine milk enzymes respectively. The K_m values for *N*-acetylglucosamine were 0.33, 1.49 and 0.5 mM for the three enzymes respectively. The K_m values for UDP-galactose, with glucose as acceptor in the presence of 1 mg of α-lactalbumin, were 23, 9.0 and 60 μM for the three enzymes respectively, and the K_m values for glucose were 2.3, 1.8 and 2.0 mM respectively. The effects of α-lactalbumin in both the lactosamine synthetase and lactose synthetase reactions were similar. The activation energies were 94.0 kJ/mol (22.5 kcal/mol) and 96.0 kJ/mol (22.9 kcal/mol) for the Golgi and serum enzymes respectively. Although some differences in K_m values were observed between the rat liver Golgi and serum enzymes, the values obtained suggest a high degree of similarity between the kinetic properties of the three galactosyltransferases.

The primary role of the enzyme Gal^T is thought to be the addition of galactosyl residues at the non-reducing end of the growing oligosaccharide chains as part of the terminal glycosylation steps in the biosynthesis of glycoproteins. Gal^T was shown to be primarily associated with the hepatocyte Golgi membranes by subcellular fractionation procedures (Schachter *et al.*, 1970) and by immunocytochemical methods (Berger *et al.*, 1981). A soluble form of Gal^T has also been found in several body fluids, including serum (Turco & Heath, 1976; Fraser & Mookerjea, 1976; Fraser *et al.*, 1980; Fujita-Yamaguchi & Yoshida, 1981; Podolsky *et al.*, 1981), milk (Trayer & Hill, 1971; Barker *et al.*, 1972) and ascites fluid (Podolsky & Weiser, 1979). The Gal^T isolated from milk interacts with α-lactalbumin (a modifier protein), changing its acceptor specificity, thus enabling the complex to catalyse the synthesis of lactose (Brew *et al.*, 1968) instead of lactosamine *in vitro*. It was also shown that the membrane-bound Gal^T found in tissues other than the mammary gland had the potential to interact with α-lactalbumin and thus synthesize lactose also (Hudgin & Schachter, 1971; Sturgess *et al.*, 1978). It was then deduced that both forms of Gal^T (membrane-bound and soluble) had to share very similar properties and that the only difference between the two, if any, would be an additional hydrophobic sequence on the membrane-bound form allowing it to be anchored in the lipid bilayer (Fraser & Mookerjea, 1976; Smith & Brew, 1977). If this is the case, one would expect both forms to have similar kinetic parameters. In order to verify this possibility, we undertook the purification of Gal^T from rat serum and rat liver Golgi membranes. We considered it important to isolate the enzyme from purified Golgi membranes rather than whole liver, since this would identify the source of our liver enzyme. Both forms were purified by affinity chromatography on an α-lactalbumin–agarose column and were then compared by several different parameters: K_m for the different substrates, α-lactalbumin modulation, effect of temperature and activation by $MnCl_2$. It was found that the soluble and the membrane-bound Gal^T from the same species were indeed kinetically similar.

Abbreviations used: Gal^T, UDP-galactose : *N*-acetylglucosamine β-1,4-galactosyltransferase; Mes, 4-morpholine-ethanesulphonic acid.

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Materials and methods

Materials

The Triton X-100, *N*-acetylglucosamine, α-lactalbumin, α-lactalbumin–agarose, bovine milk Gal^T, bovine serum albumin (2.8% moisture) and Mes were all purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. The UDP-[^{14}C]galactose was purchased from New England Nuclear Co., Boston, MA, U.S.A., and the Dowex 1-X8 resin was obtained from Bio-Rad Laboratories, Mississauga, Ont., Canada. All the other chemicals used were of reagent grade from standard commercial sources.

Purification of rat liver Golgi membranes

The Golgi-enriched fraction was purified from male Wistar rats weighing 175g by discontinuous-sucrose-density-gradient centrifugation as previously described (Sturgess *et al.*, 1973). The specific activity of Gal^T was used to monitor the purification. A purification factor of 40 was obtained routinely in the Golgi fraction, as compared with the homogenate, with a typical yield of 15%. Throughout the isolation of the Golgi membranes, great care was taken to attain the highest degree of purity possible, to the detriment of the amount of membrane recovered, explaining the low yield of activity obtained. The Golgi-membrane preparation purified from at least 24 rats was pooled and kept frozen at −20°C to provide a uniform source of enzyme. Under these conditions, the Gal^T activity was stable for at least 2 months.

Purification of rat liver Golgi Gal^T

Solubilization of the Golgi membrane. The Golgi membranes from at least 24 rats were thawed and diluted to 50ml in buffer A [15mM-sodium cacodylate buffer, pH7.5, containing 0.1% (v/v) Triton X-100, 5mM-*N*-acetylglucosamine and 3mM-2-mercaptoethanol]. The suspended membranes were sonicated, with a Brinkman probe sonifier at the lowest setting for 1min, and left overnight at 4°C. The suspension thus obtained was centrifuged at 155000*g* ($r_{av.}$ 8.35cm) for 60min at 4°C, and the supernatant, which contained most of the Gal^T activity, was removed and kept at 4°C.

Affinity chromatography on α-lactalbumin–agarose. The solublized Golgi membranes were loaded at a flow rate of 25ml/h at 4°C on a column (1.6cm × 8cm) containing 16ml of α-lactalbumin coupled to agarose (3.5mg/ml of gel), pre-equilibrated with 100ml of buffer A. The column was washed with an additional 150ml of buffer A, and then with 100ml of buffer A containing 0.1M-NaCl. The enzyme activity was eluted from the column in the absence of *N*-acetylglucosamine by washing with 100ml of 15mM-sodium cacodylate buffer, pH7.5, containing 0.1% (v/v) Triton X-100, 0.1M-NaCl and 3mM-2-mercaptoethanol. The active fractions were pooled and concentrated on an Amicon YM-10 ultrafiltration membrane (25mm diameter) at 0.35MPa (50lbf/in^2) above atmospheric pressure. The concentrated enzyme preparation was stored at 4°C and was stable for at least 4 weeks.

Purification of rat serum Gal^T

The serum obtained from the same rats used for the Golgi preparation was diluted 5-fold with buffer so that the final concentrations of buffer, Triton X-100 etc. were identical with those in buffer A. The purification scheme was as described for the Golgi Gal^T.

Assays

UDP-galactose : N-acetylglucosamine galactosyltransferase activity. The Gal^T activity was assayed in the presence of 0.1M-Mes buffer, pH7.5, 10mM-$MnCl_2$, 0.5% (v/v) Triton X-100, 1mM-UDP-[^{14}C]-galactose and 20mM-*N*-acetylglucosamine in a final volume of 50ml. For each determination, two tubes (duplicates) were assayed in presence of the acceptor sugar (*N*-acetylglucosamine) and one tube without acceptor. Routinely, the reaction was carried out at 37°C for 30min and stopped by adding 5μl of 0.25M-EDTA in 2% (w/v) sodium borate in an ice bath. The radioactive product, *N*-acetyl-[^{14}C]lactosamine, was separated from radioactive UDP-galactose by anion-exchange chromatography.

UDP-galactose : glucose galactosyltransferase activity. The conditions of the assay were the same as described above except that glucose (20M) was substituted for *N*-acetylglucosamine, and α-lactalbumin was added to a final concentration of 1mg/ml.

Separation of the product from UDP-galactose by anion-exchange chromatography

The reaction mixture was diluted with 1ml of 1mM-EDTA in 0.008% sodium borate, pH9.2. The incubation mixture was then applied to a column (0.7cm × 5cm) containing fully recycled Dowex 1-X8 (analytical grade) in the Cl^- form. The column was washed three times with 1ml of distilled water each time. A total of 4ml of eluent and wash was collected (starting when the sample was applied) in a scintillation vial. Then 10ml of ACS scintillation fluid was added to the vial, which was then thoroughly vortex-mixed and its radioactivity counted. The possible hydrolysis of UDP-galactose into ^{14}C-labelled galactose and galactose 1-phosphate during the incubation was investigated by submitting the standard assay mixture, incubated with the different enzymes, to high-voltage paper

electrophoresis in the presence of borate. Under these conditions, galactose 1-phosphate and galactose separate from UDP-galactose in 90min, and it was observed that no such hydrolysis of UDP-galactose took place with any of the three enzymes used in the study.

Kinetic analysis

The different kinetic constants of the enzymes were determined by varying the concentration of one substrate while the concentrations of all the others were kept constant at the standard concentration given in the description of the incubation mixture. To obtain the initial velocity, the tubes were incubated for 3min at 37°C and the reaction was quickly stopped by freezing the mixture in a bath of ethanol/solid CO_2. To ensure that the reaction would not proceed any further, 5μl of 0.25M-EDTA was added to the frozen pellet. The constants were obtained by the method of Lineweaver & Burk (1934). The straight line for the double-reciprocal plot was calculated with the least-squares method.

Determination of protein

The protein concentration was determined by the method of Peterson (1977), with bovine serum albumin as standard. When the samples contained interfering substances (e.g. $MnCl_2$), the precipitation step suggested in the Peterson (1977) assay method was performed.

Results

Purification of Golgi and serum galactosyltransferase

In order to compare different parameters of the Gal^T from rat liver and serum, purified enzymes were required. The Golgi membranes obtained by differential sucrose-density-gradient ultracentrifugation were solubilized overnight in buffer A containing 0.1% Triton X-100. The 155000*g* supernatant routinely contained over 80% of the original Gal^T activity present in the Golgi membranes. The Gal^T was then purified on an α-lactalbumin–agarose affinity column as described in the Materials and methods section. A typical elution pattern is shown in Fig. 1. After the sample was loaded (arrow A), a large amount of protein present in the solubilized fraction did not bind to the column. However, almost no Gal^T activity was detected in these fractions. A 0.1M-NaCl wash (arrow B) removed some non-specifically bound protein from the column. Here again, only a small amount of Gal^T activity was eluted. When a buffer containing 0.1M-NaCl (without *N*-acetylglucosamine) was applied, a very sharp peak of Gal^T activity was eluted containing a relatively small amount of protein. The purification of Gal^T from

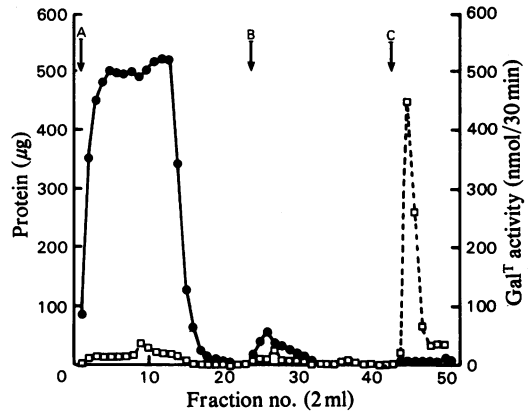

Fig. 1. *Purification of Golgi Gal^T by affinity chromatography on α-lactalbumin–agarose*

Rat liver Golgi membranes solubilized in buffer A were loaded on the column (arrow A) in the presence of 5mM-*N*-acetylglucosamine. Most of the protein was not adsorbed. Non-specifically bound protein was eluted with 0.1M-NaCl in buffer A (arrow B), and the enzyme activity was then eluted by omitting *N*-acetylglucosamine from the last buffer (arrow C). ●, Protein; □, Gal^T activity.

solubilized Golgi membranes on the α-lactalbumin–agarose column gave a yield of 33% (compare the total activity between the solubilized Golgi and the α-lactalbumin–agarose steps in Table 1). The Gal^T activity passing through the column during sample loading and during the 0.1M-NaCl wash cannot account for the loss of activity observed in this step. Therefore the loss of activity most probably represents partial denaturation of the enzyme that may occur before the enzyme is concentrated by ultrafiltration on the Amicon membrane.

The serum enzyme was purified in a similar manner in the presence of Triton X-100 as described in the Materials and methods section. It may be noted that when serum was chromatographed on the α-lactalbumin–agarose column the total Gal^T activity increased 2-fold (Table 1). This increase in activity is possibly due to the separation of the serum Gal^T from inhibiting substances present in the serum.

The purification of Gal^T from both the serum and the Golgi membranes is summarized in Table 1. The Gal^T from the solubilized Golgi membranes was purified 600-fold compared with the homogenate, with an overall yield of 4.7%. The serum Gal^T was enriched 2300-fold over the serum after this one-step purification scheme. The specific activities of the purified Golgi Gal^T and serum Gal^T were 0.168 and 0.04μmol of galactose transferred/min per mg of protein respectively. In an

Table 1. *Purification of rat liver Golgi Gal^T and rat serum Golgi Gal^T*

The Golgi membranes were purified by sucrose-density-gradient centrifugation as described in the Materials and methods section. The Golgi membranes were solubilized in 0.1% (v/v) Triton X-100 and subjected to affinity chromatography on α-lactalbumin–agarose as shown in Fig. 1. The serum was applied to the same affinity column after being diluted 5-fold, under the same conditions as described for the Golgi Gal^T. Gal^T activity was assayed as described in the Materials and methods section: 1 unit is 1 μmol of galactose transferred/min at 37°C.

Fraction	Volume (ml)	Total protein (mg)	Total Gal^T activity (munits)	Sp. activity (munits/mg of protein)	Purification (fold)	Yield (%)
Golgi Gal^T						
Homogenate	200	6640	1830	0.275	1	100
Solubilized Golgi membranes	50	19.48	274	14.1	53.7	15
α-Lactalbumin–agarose column fraction	1.76	0.511	86.2	168	611	4.71
Serum Gal^T						
Serum	50	4710	81.1	0.017	1	100
α-Lactalbumin–agarose column fraction	1.69	3.96	159.5	40.3	2340	197

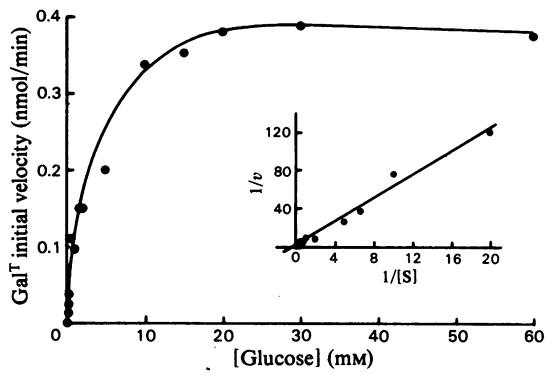

Fig. 2. *Initial velocity of Golgi Gal^T as a function of the glucose concentration*

The purified enzyme was incubated with increasing concentration of glucose in the presence of 0.1 M-Mes buffer, pH 7.5, 10 mM-$MnCl_2$, 0.5% (v/v) Triton X-100, 1 mM-UDP-[^{14}C]galactose and 1 mg of α-lactalbumin/ml for 3 min at 37°C. The double-reciprocal plot is shown in the inset.

attempt to purify both Gal^T further, the enzyme obtained after the α-lactalbumin–agarose step was loaded on a UDP-hexanolamine affinity column (results not shown). It was found that both Gal^T enzymes bound to this affinity column in presence of $MnCl_2$ (25 mM) and were specifically eluted from the column on the removal of the $MnCl_2$ from the buffer and addition of 25 mM-EDTA and 5 mM-*N*-acetylglucosamine. After this second affinity column, purification factors of 7900 and 140000 (specific activities 2.19 and 2.46 μmol of galactose transferred/min per mg of protein) were obtained for the Golgi Gal^T and the serum Gal^T respectively. The respective yields were 1.8% and 66%. However, it was observed that both Gal^T enzymes were highly unstable after this second affinity-column, losing as much as 50% of the original activity in 2 h at 4°C, therefore prohibiting the use of such highly purified enzymes for kinetic studies.

Comparative analysis of rat serum and rat liver Golgi Gal^T kinetic parameters

The apparent K_m values for the two substrates (the nucleotide sugar donor and the acceptor) were calculated from double-reciprocal plots of standard *v*-versus-[S] curves. Fig. 2 shows the curves obtained from a typical experiment. The K_m values are summarized in Table 2. When glucose was the acceptor, the K_m values for glucose were 2.3 mM and 1.8 mM for the rat Golgi and serum enzymes respectively. This is similar to a K_m of 2.0 mM for the bovine milk Gal^T. The K_m values for UDP-galactose of the Golgi and serum enzymes were 23 μM and 9 μM respectively, compared with 60 μM for the bovine milk enzyme.

When *N*-acetylglucosamine was used as acceptor, the K_m values for UDP-galactose were 65 μM and 52 μM for the rat liver Golgi and serum enzymes respectively, similar to the 43 μM obtained with the bovine milk enzyme. The K_m values for *N*-acetylglucosamine were 0.33 mM for the rat liver Golgi enzyme, 1.49 mM for the rat serum enzyme and 0.5 mM for bovine milk Gal^T.

Effect of α-lactalbumin

The effect of increasing concentrations of α-lactalbumin on the ability of the two transferases to transfer galactose from UDP-galactose to glucose in the lactose synthetase assay is shown in Fig. 3(*a*).

Table 2. *Apparent K_m values of rat liver Golgi, rat serum and bovine milk Gal^T enzymes for different substrates*

The apparent K_m values were calculated from the double-reciprocal plots of standard v-versus-[S] curves. The straight lines were the best fit obtained by the least-squares method. The standard conditions were: 0.1 M-Mes pH 7.5, 10 mM-$MnCl_2$, 0.5% (v/v) Triton X-100, 1 mM-UDP-[^{14}C]galactose and 20 mM of the acceptor. When glucose was the acceptor, α-lactalbumin was added to a final concentration of 1 mg/ml. The concentration of the substrate under study was varied while the other reagents were kept constant at the concentration given above.

Acceptor	Substrate tested	K_m (μM) Rat Golgi Gal^T	Rat serum Gal^T	Bovine milk Gal^T
N-Acetylglucosamine	UDP-galactose	65	52	43
N-Acetylglucosamine	*N*-Acetylglucosamine	330	1490	500
Glucose*	UDP-galactose	23	9	60
Glucose*	Glucose	2300	1800	2000
Glucose	α-Lactalbumin	6.93	5.85	11.53

* In the presence of 1 mg of α-lactalbumin/ml.

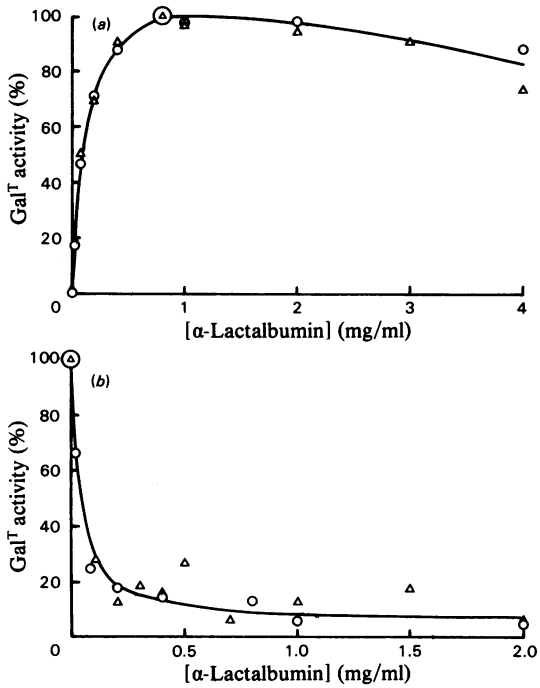

Fig. 3. *Modulation of purified Golgi Gal^T (○) and serum Gal^T (△) by α-lactalbumin*

The enzymes were incubated with increasing concentrations of α-lactalbumin for 30 min at 37°C with glucose (*a*) and *N*-acetylglucosamine (*b*) as acceptor. The conditions were as described in the legend to Fig. 2. The activities are expressed as percentages of total activity to allow a comparison of the serum and the Golgi Gal^T enzymes.

The responses of both enzymes were the same. The K_a values for α-lactalbumin when glucose was the acceptor were 6.93 μM, 5.85 μM and 11.53 μM for the rat liver, the rat serum and the bovine milk enzymes respectively. Even though it is known that both the serum Gal^T and the Golgi Gal^T can interact with α-lactalbumin (as shown by their binding to the α-lactalbumin–agarose affinity column), this similarity in K_a values is surprising, since α-lactalbumin and lactose synthetase are restricted to the mammary gland; nevertheless α-lactalbumin appears to have similar effects on the rat liver Golgi and serum enzymes.

The inhibition of transfer of galactose from UDP-galactose to *N*-acetylglucosamine in the presence of α-lactalbumin is shown in Fig. 3(*b*). Again, the responses of both enzymes were similar. A rapid decrease in enzyme activity occurred at low α-lactalbumin concentrations (below 100 μl/ml) and then remained low over the rest of the concentration range.

Activation by Mn^{2+}

The activating effect of Mn^{2+} on the two enzymes was studied by increasing the concentration of Mn^{2+} in the assay mixture in the range 0–50 mM. The results for the Golgi Gal^T are shown in Fig. 4. Maximum activity was observed when the Mn^{2+} concentration was 3–5 mM in the assay medium. The activation by Mn^{2+} was similar for the serum Gal^T.

Effect of temperature on the activity of rat liver Golgi and rat serum Gal^T

The effect of temperature on enzymic activity can be studied by Arrhenius plots, in which the logarithm of the activity is plotted as a function of temperature. The data for the two enzymes are shown in Fig. 5. For both enzymes straight lines were obtained, with correlation coefficients of −0.996 and −0.986 for the Golgi and serum

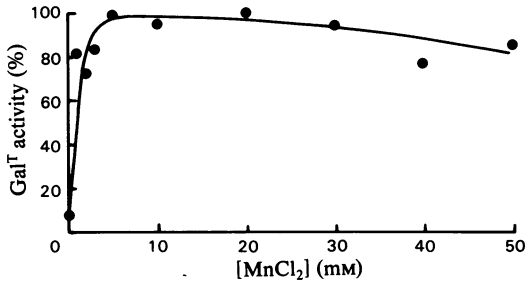

Fig. 4. *Mn^{2+}-dependency of purified Golgi Gal^T*
The enzyme was incubated with various concentrations of $MnCl_2$ for 30min at 37°C. The conditions were as described in the legend to Fig. 2. The activities are expressed as percentages of total activity.

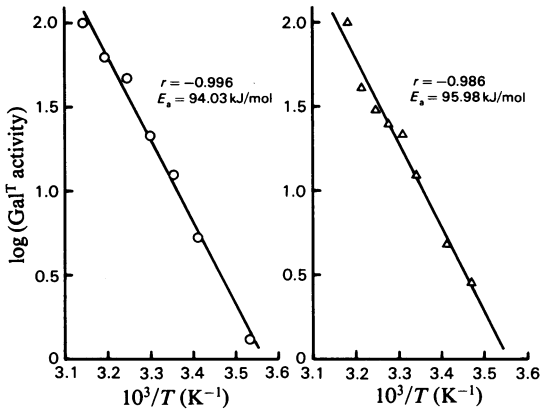

Fig. 5. *Arrhenius plots of purified Golgi Gal^T* (○) *and serum Gal^T* (△)
The enzymes were incubated in a standard assay mixture (see the legend to Fig. 2) at different temperatures for 30min. The activation energies were calculated from the equation given in the text.

enzymes respectively. The energies of activation E_a calculated from the relationship:

$$E_a = \frac{4.56 \times T_1 T_2 (\log k_2 - \log k_1)}{T_2 - T_1}$$

were 94.0kJ/mol (22.5kcal/mol) and 96.0kJ/mol (22.9kcal/mol) for Golgi and serum enzymes respectively.

Discussion

In the present study we have compared different kinetic parameters of two Gal^T enzymes. The origin of our Golgi Gal^T was ensured by the use of highly purified Golgi membranes (Sturgess *et al.*, 1973) as starting material. The enzyme was purified by affinity chromatography with, as a ligand, a protein that is known to interact specifically with the Gal^T enzymes. No *N*-acetylglucosaminyl transferase or sialyltransferase activities could be detected in our enzyme preparation (M. R. Pâquet & M. A. Moscarello, unpublished work).

The apparent K_m values obtained for the different substrates were very similar for both the Golgi and the serum Gal^T enzymes and were in good agreement with the published values (Fujita-Yamaguchi & Yoshida, 1981; Bouchilloux, 1979). The protein α-lactalbumin modulated the activity of both Gal^T enzymes in a very similar way, whether glucose or *N*-acetylglucosamine was used as the acceptor. The almost identical K_a values of α-lactalbumin for the Gal^T enzymes purified from the two sources further stress the kinetic similarity between the two. Lastly, the activation energies of the Gal^T enzymes purified from the different sources were calculated from Arrhenius plots, and these results again suggest that the two enzymes act on their substrates in very similar fashions.

The results obtained in the present study strongly suggest that the Gal^T found in the Golgi membranes is kinetically identical with the Gal^T purified from serum. However, these two Gal^T enzymes seem to differ on at least two points. Firstly, their solubilities are quite different. The serum Gal^T is readily soluble in water, whereas the Golgi Gal^T needs detergent to be released from the membrane and appears to be an intrinsic membrane protein, and thus more hydrophobic. Secondly, differences in M_r values of Gal^T enzymes from different sources have been reported before: the Gal^T purified from pig thyroid microsomal membranes (Bouchilloux, 1979) and from sheep mammary-gland Golgi membranes (Smith & Brew, 1977) have M_r 70000–74000 and 69000 respectively, whereas the Gal^T enzymes purified from soluble sources always show a smaller M_r: human serum, 49000 (Fujita-Yamaguchi & Yoshida, 1981); foetal-calf serum, 47800 (Turco & Heath, 1976); rat serum, 43000 (Fraser & Mookerjea, 1976); bovine milk, 54000 (Barker *et al.*, 1972), 44300 (Trayer & Hill, 1971) and 42000 and 58000 (Magee *et al.*, 1974).

The two Gal^T enzymes studied in the present work may be related to one another in other possible ways. Further work will be necessary in order to determine if the two forms are derived from a same protein through post-translational modifications (e.g. partial hydrolysis, glycosylation) or if they are different proteins altogether.

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References

Barker, R., Olsen, K. W., Sharper, J. H. & Hill, R. L. (1972) *J. Biol. Chem.* **247**, 7135–7147

Berger, E. G., Mandel, T. & Schilt, U. (1981) *J. Histochem. Cytochem.* **29**, 364–370

Bouchilloux, S. (1979) *Biochim. Biophys. Acta* **569**, 135–144

Brew, K., Vanaman, T. C. & Hill, R. L. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **59**, 491–497

Fraser, I. H. & Mookerjea, S. (1976) *Biochem. J.* **156**, 347–355

Fraser, I. H., Wadden, P. & Mookerjea, S. (1980) *Can. J. Biochem.* **58**, 878–884

Fujita-Yamaguchi, Y. & Yoshida, A. (1981) *J. Biol. Chem.* **256**, 2701–2706

Hudgin, R. L. & Schachter, H. (1971) *Can. J. Biochem.* **49**, 838–846

Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666

Magee, S. C., Mawal, R. & Ebner, K. (1974) *Biochemistry* **13**, 99–102

Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356

Podolsky, D. K. & Weiser, M. M. (1979) *J. Biol. Chem.* **254**, 3983–3990

Podolsky, D. K., McPhee, M. S., Alpert, E., Warshaw, A. L. & Isselbacher, K. J. (1981) *N. Engl. J. Med.* **304**, 1313–1318

Schachter, H., Jabbal, I., Hudgin, R. L., Pinteric, L., McGuire, E. J. & Roseman, S. (1970) *J. Biol. Chem.* **245**, 1090–1100

Smith, C. A. & Brew, K. (1977) *J. Biol. Chem.* **252**, 7294–7299

Sturgess, J. M., Katona, E. & Moscarello, M. A. (1973) *J. Membr. Biol.* **12**, 367–384

Sturgess, J., Moscarello, M. A. & Schachter, H. (1978) *Curr. Top. Membr. Transp.* **11**, 15–105

Trayer, I. P. & Hill, R. L. (1971) *J. Biol. Chem.* **246**, 6666–6675

Turco, S. J. & Heath, E. C. (1976) *Arch. Biochem. Biophys.* **176**, 352–357