Biochimica et Biophysica Acta, 649 (1981) 709–716
Elsevier/North-Holland Biomedical Press

BBA 79517

HUMAN ERYTHROCYTE GALACTOSYLTRANSFERASE

CHARACTERIZATION, MEMBRANE ASSOCIATION AND SIDEDNESS OF ACTIVE SITE

FRANCIS J. HESFORD and ERIC G. BERGER *

Medizinisch-Chemisches Institut der Universität Bern, Bühlstrasse 28, Postfach, CH-3000 Bern 9 (Switzerland)

(Received June 24th, 1981)

Key words: Galactosyltransferase; Active site; Sidedness; (Human erythrocyte membrane)

Human erythrocyte UDPgalactose : 2-acetamido-2-deoxy-α-D-galactopyranosylpeptide galactose β(1 → 3) transferase (Galactosyltransferase) has been characterized in terms of detergent and metal ion requirements, Michaelis constants for donor and acceptor substrates, inhibition constant for *N*-acetylgalactosamine, pH optimum and ionic strength effects. The assay thus optimized permits initial velocity measurements. Galactosyltransferase was shown to be membrane-bound by demonstrating its association with erythrocyte ghosts after high and low ionic strength treatments to remove weakly-associated proteins. In the absence of detergents, no activity was detectable in sealed ghosts and inside-out vesicles derived from erythrocyte membranes. Enzyme activation by detergents paralleled solubilization of membrane proteins. Both latency and solubilization studies indicated a substrate-inaccessible active site for the enzyme in situ in the membrane. Galactosyltransferase activity in resealed ghosts, leaky ghosts and inside-out vesicles was resistant to the action of trypsin, chymotrypsin or pronase applied as single agents. A mixture of these proteases, however, strongly reduced the enzyme activity in inside-out vesicles and leaky ghosts, indicating a cytosolic orientation for the active site of the galactosyltransferase.

Introduction

An erythrocyte galactosyltransferase (EC 2.4.1.-), transferring galactose from uridine 5′-diphosphate galactose to desialylated ovine submaxillary mucin (asialo-mucin) has previously been described [1]. This galactosyltransferase has been shown to be absent in the Tn-transformed erythrocytes from patients affected by permanent mixed-field polyagglutinability [1–3]. The molecular basis of the Tn-transformation consists in the specific absence of sialic acid and galactose in the *O*-glycosyl carbohydrate side-chains of glycophorin [4,5]. The linkage type and anomeric configuration of the product formed using asialo mucin as acceptor substrate has recently been confirmed as being identical to that in glycophorin, namely, Galβ(1 → 3)GalNAcα(1→)*O*-protein [6]. It was also shown that galactose can be incorporated into asialo-agalacto-glycophorin of Tn-erythrocytes by the galactosyltransferase activity associated with normal human erythrocytes [6].

Erythrocyte membrane glycosyltransferases may offer a useful model system for the study of some aspects of glycosyltransferase enzymology, since these membranes are readily prepared and well characterized. One of the unsolved problems regarding membrane glycosyltransferases is the location of the active site. This appears particularly relevant considering the body of information available on *ecto*-glycosyltransferases [7], the activity of which has tacitly been assumed to face the exterior of the cell.

We conducted the present study: (1) to demonstrate association of the enzyme with the erythrocyte membrane, (2) to establish assay conditions permitting initial velocity measurements, (3) to determine accessibility of the enzyme active site to substrates by investigating the effects of detergents and (4) to determine membrane active site orientation, based on

* To whom correspondence should be addressed.

the susceptibility of the transferase activity to proteolytic treatment of membrane vesicles with defined orientation.

Materials and Methods

Chemical and reagents

All chemicals and reagents other than those listed below were of analytical reagent grade from Merck, Darmstadt, F.R.G. or from Fluka, Buchs, CH. Uridine diphospho-D-[6-^{3}H]galactose ammonium salt (spec. act. 13.1 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, U.K. and Dextran-[^{14}C]carboxyl, mol. wt. 20000 (spec. act. 2.76 mCi/g) from New England Nuclear, Dreieck, F.R.G. Butyl-PBD, routinely used for scintillation counting, was from Ciba-Geigy, CH, whereas aqueous samples were counted in Rotiszint 22, from Roth, Karlsruhe, F.R.G. Bovine pancreatic trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) were from Serva, F.R.G.; Pronase P (type VI, *Streptomyces griseus*) (EC 3.4.22.10), Tween 20 and Tween 80 from Sigma, U.S.A.; Neuraminidase (EC 3.2.1.18) from *Vibrio comma* (*cholerae*) from Behringwerke AG, Marburg, F.R.G. Azocoll protease substrate, octyl-β-D-glucopyranoside (octylglucoside), Zwittergent 3-14, sodium cholate and deoxycholate were obtained from Calbiochem, Luzern, CH.

Biological material

Erythrocytes from blood group A or O individuals were used exclusively as source of the galactosyltransferase activity. Erythrocyte membranes (referred to as 'leaky ghosts') were prepared as described previously [6], basically according to Hanahan and Eckholm [8]. 0.02% sodium azide was added to the final suspension in 11 mM Tris-HCl buffer, pH 7.6 (buffer A), which was stored at 4°C.

Impermeable ghosts were prepared by resealing leaky ghosts, essentially according to Steck and Kant [9], except that 0.172 M Tris-HCl buffer, pH 7.6 (buffer B) was substituted for phosphate-buffered saline. Membranes prepared the day before were resuspended in 40 vol. of buffer B and incubated for 40 min at 37°C to induce resealing. The resealed impermeable ghosts were then pelleted and washed twice more in buffer B. These preparations contained approx. 10% unsealed membranes [9]. Sealed inside-out vesicles were prepared also within 24 h from leaky ghosts, again essentially according to Steck and Kant [9], except that 1 mM Tris-HCl buffer, pH 8.0 (buffer C) was substituted for 0.5 mM phosphate buffer, pH 8.0. These preparations contained approx. 10% unsealed or right side-out membranes [9]. The quality of all three membrane preparations, as judged by sidedness assays, namely sialic acid accessibility to neuraminidase; acetylcholinesterase accessibility; and glyceraldehyde-3-phosphate dehydrogenase accessibility, was comparable to that reported [9].

Galactosyltransferase assay

For the determination of galactosyltransferase activity, desialylated ovine submaxillary mucin (asialo-mucin), prepared by the method of Carlson et al. [10] and desialylated by weak acid hydrolysis, was used as the acceptor substrate as described previously [11]. The standard assay (method 1) included the following: 100 μg membrane protein, 3 μmol $MnCl_2$, 150 μmol sodium cacodylate, pH 7.2, 150 μg asialo-mucin, 37.5 μmol UDP-[^{3}H]galactose (spec. act. 53 mCi/mmol) and 15 mg Triton X-100 in 150 μl total volume. Incubation was for 4 h at 30°C, and was terminated by the addition of 2 ml of icecold phosphotungstic acid 5% in 2 M HCl. The precipitate formed was retained on glass-fibre filters (Whatman GF/A), which were washed with cold ethanol, dried, and counted in a scintillation counter in the presence of 0.8% Butyl-PBD in toluene. 2000 cpm per 100 μg erythrocyte membrane protein were routinely measured using this method.

Method 2 was used when it was necessary to avoid contamination with haemoglobin, which led to low counting efficiency (brown precipitate) after the above-mentioned assay termination procedure: In place of soluble asialo-mucin, 100 μl of a 1 : 1 slurry of packed Sepharose 4B-immobilized asialo-mucin in water was used. Other conditions were the same. Asialo-mucin was immobilized at approx. 3 mg/ml by the procedure of March et al. [12]. Incubation was terminated by the addition of 0.5 ml 10 mM EDTA. The beads were then washed five times by centrifugation and aspiration with 8 ml 0.5 M NaCl containing 1% (w/v) Triton X-100, and finally once with distilled water. 1 ml 2 M HCl was then added and the tubes heated for 2 h at 100°C. The tube contents were then counted after the addition of 10 ml Rotiszint 22.

Removal of weakly associated membrane proteins

High ionic strength wash. A 5-ml aliquot of an erythrocyte membrane suspension (protein: 4 mg/ml) in buffer A was mixed with 20 ml of 50 mM Tris-HCl, pH 8.0 containing 0.5 M NaCl and centrifuged immediately at 70 000 × *g* and 4°C for 30 min. Supernatant was aspirated and the pellet washed twice more by resuspension and centrifugation in the same buffer.

Low ionic strength wash. The pellet from the high ionic strength wash was resuspended and centrifuged as before, except that wash buffer consisted solely of 0.1 mM EDTA, disodium salt, pH 8.0 and that incubation for 30 min at 37°C was performed after each addition of this buffer prior to centrifugation. The resulting pellet was transferred back to buffer A by resuspension and centrifugation (two times). This final pellet was then suspended in 5 ml buffer A, assayed for galactosyltransferase activity and protein and galactosyltransferase specific activity calculated.

Protease treatment of membrane preparations

To 0.9 ml membrane preparation (leaky or impermeable ghosts or sealed inside-out vesicles) containing approx. 4 mg/ml protein, was added 0.1 ml protease mixture (1 mg/ml each of trypsin, chymotrypsin and pronase) in the buffer (A, B or C) appropriate to the particular membrane preparation. The contents of the tube were then vortex-mixed and incubated for 2 h at 37°C. In order to remove protease from the treated membranes, the tube contents were applied to columns packed with 9 ml Sepharose 4B and previously equilibrated with the same buffer at 4°C. After sample application (1 ml), an additional 2 ml of the appropriate buffer was applied and the eluate up to this point discarded. The treated membranes were then eluted and collected within a total of 10–15 min by the addition of a further 3.5 ml buffer. They were free of protease activity as judged by the Azocoll method [13]. The eluted membranes were then concentrated by centrifugation at 25 000 × *g* for 30 min, the supernatant aspirated and the pellet resuspended in buffer A to an approximate volume of 1 ml by weighing (1 g).

Analytical methods

Protein was measured by the method of Lowry et al. [14], except in the case of solutions in non-ionic detergents, where the method of Sims and Carnegie [15] was used. Lactate dehydrogenase was determined according to Wroblewski and La Due [16] and haemoglobin according to Drabkin and Austin [17]. Phospholipid determinations were carried out by the method of Rouser et al. [18], after extraction according to Renkonen et al. [19]. Protease activity was assessed using the protein-bound dye, Azocoll [13], and sialic acid was determined according to Skoza and Mohos [20].

Results

Detergent effects on erythrocyte galactosyltransferase activity

The detergents in Table I were tested for their effectiveness in activating galactosyltransferase. Modification of the standard assay (method 1) permitted the testing of all detergents up to 10-times their respective critical micelle concentrations, with the exception of Tween 20, which was too viscous. As can be seen from Table I, the non-ionic detergents Triton X-100, octylglucoside and Triton N-101 were most effective in activating the erythrocyte galactosyltransferase.

No galactosyltransferase activity could be detected in leaky ghosts, inside-out vesicles or sealed right side-out ghosts in the absence of detergent. Therefore, latency, as defined by the presence of a membrane

TABLE I

THE ACTIVATING EFFECT OF VARIOUS DETERGENTS UPON HUMAN ERYTHROCYTE GALACTOSYLTRANSFERASE

Detergent	% of maximum activity [a]	Concn. [b]
Triton X-100	100	3 mM
Octylglucoside	99	125 mM
Triton N-101	84	0.85 mM
Zwittergent 3-14	64	3 mM
Tween 80	62	65 mg/ml
Tween 20	55	60 mg/ml
Brij 35	46	1 mM
Sodium cholate	2	7.5 mM
Sodium deoxycholate	2	0.3 mM

[a] % of maximum activity = % of activity using Triton X-100 at ten times critical micelle concentration (=3 mM).
[b] Concentration at which maximum activity was obtained.

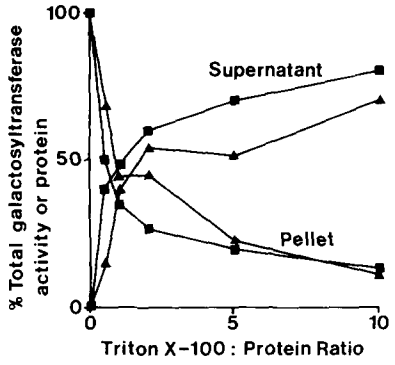

Fig. 1. Solubilization of galactosyltransferase (▲——▲) and protein (■——■) from erythrocyte membranes as a function of the Triton X-100: protein ratio used for solubilization (10 : 1 = 2% (w/v) Triton X-100). Preincubation time and temperature prior to centrifugation were 5 min and 4°C, respectively. Galactosyltransferase activities were measured by the standard assay procedure (method 1), at an assay concentration of 2% (w/v) Triton X-100.

permeability barrier, was not responsible for the observed detergents effects.

Thus, 'activation' of the galactosyltransferase was thought to be most probably ascribable to solubilization of the enzyme, and this was therefore investigated for Triton X-100, Triton N-101 and Brij 35. Membranes (1 mg protein) were mixed by vortexing with the corresponding detergent and distilled water to give the required detergent: protein ratio (up to 10 : 1 w/w) in 0.5 ml final volume. The mixture was then centrifuged at 100 000 × *g* for 1 h. Supernatants and pellets were then assayed for protein [14] and galactosyltransferase activity at concentrations of detergent in the assay corresponding to the highest used for solubilization. Preincubation times and temperatures prior to centrifugation were varied from 0 to 24 h and from 0 to 37°C. Fig. 1 shows that for Triton X-100, galactosyltransferase activity in supernatants and pellets parallels protein concentration. The two other closely-related non ionic detergents, Triton N-101 and Brij 35, gave very similar results. Preincubation time and temperature are not critical in the solubilization process, since essentially the same enzyme activities and protein concentrations were observed between 0 and 37°C and up to 24 h. However, repeated extraction of the pellet at the highest Triton X-100 concentration employed did not extract more than a total of 80% of the enzyme activity in a soluble form.

Requirements for optimized assay for solubilized galactosyltransferase

Freshly-prepared erythrocyte membranes were solubilized as above, at 4°C, without preincubation and at a Triton X-100 concentration of 10 mg/ml (1% (w/v) detergent : protein ratio 5 : 1). Triton X-100 was chosen, since it gave optimal results in terms of both activation and solubilization. The soluble supernatant fraction was then used to determine metal ion requirement, apparent K_m values for donor and acceptor substrates, the inhibition constant for *N*-acetylgalactosamine, pH optimum and the effect of ionic strength on enzyme activity, using appropriate modifications of the standard assay procedure (method 1).

Of the cations tested at 10 mM concentration (Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and Ca^{2+}), only Co^{2+} produced an activating effect, 10% of that using the same concentration of Mn^{2+}. The apparent K_A for Mn^{2+} was found to be 25 mM, K_m for UDPgalactose 0.12 mM and for asialo-mucin 0.67 mg/ml. *N*-Acetylgalactosamine was shown to be a competitive inhibitor with respect to asialo-mucin (see Fig. 2). In contrast, *N*-acetylglucosamine did not inhibit.

High ionic strength (using NaCl) was shown to exert a reversible inhibitory effect upon enzyme

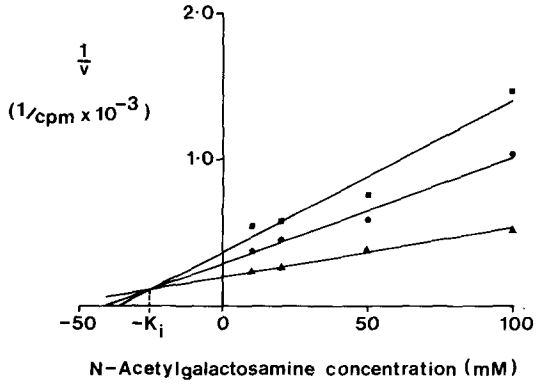

Fig. 2. Dixon [30] plot of $1/v$ versus inhibitor concentration for inhibition of galactose transfer to asialo-mucin by *N*-acetylgalactosamine. Asialo-mucin concentrations: 0.25 mg/ml (■——■); 0.5 mg/ml (●——●); 1 mg/ml (▲——▲), K_i = 25 mM.

TABLE II

COMPARISON OF GALACTOSYLTRANSFERASE (GT) AND LACTATE DEHYDROGENASE (LDH) ACTIVITIES IN WASHED ERYTHROCYTES, HAEMOLYZATE AND MEMBRANE FRACTIONS

Fraction	Total activity (μmol · min^{-1})		Specific activity (μmol · min^{-1} · g^{-1} non-haem protein)	
	GT	LDH	GT	LDH
Washed erythrocytes	$2.91 \cdot 10^{-6}$	96	$1.60 \cdot 10^{-5}$	529
Haemolyzate	$<10^{-8}$	92.3	$<10^{-7}$	495
Erythrocyte membranes	$4.33 \cdot 10^{-6}$	0.44	$2.56 \cdot 10^{-4}$	26

activity: The enzyme had maximum activity at ionic strength, $I < 0.07$ M and only 15% of this activity at an added salt concentration of 0.3 M (not shown). This effect was reversible between pH 6 and pH 8.5 at 4°C and up to 24 h, after buffer exchange on Sephadex G-25 columns. Low ionic strength buffers were therefore used for the determination of the pH optimum. A single optimum at pH 7.2 was found.

The solubilized enzyme was stable for at least 1 month at 4°C in the presence of 0.02% sodium azide, and for 24 h at 37°C. Its activity was reduced by half after 3 h at 42°C. At 56°C enzyme activity was immediately abolished.

Using asialo-mucin as acceptor, the above determinations and optimization of assay conditions, resulting in the standard assay (method 1) described above were possible. Attempts to measure initial velocity by using *p*-nitrophenyl-GalNAc as acceptor substrate [3], were not successful.

Membrane association of erythrocyte galactosyltransferase

Human erythrocyte membranes were prepared as described previously [6] and washed erythrocytes, haemolyzate and membranes were analyzed. Total protein and haemoglobin were determined and non-haem protein calculated as the difference of the two. Galactosyltransferase (method 2) and lactate dehydrogenase activities were measured and specific activities based on non-haem protein calculated. The results are shown in Table II. A 16-fold increase in galactosyltransferase specific activity and a 20-fold decrease in lactate dehydrogenase specific activity is observed in membranes, compared with whole erythrocytes.

The membranes were then subjected to successive washes at high and low ionic strengths (see Methods) in order to remove components weakly associated with the membrane, such as bands 1, 2, 5 and 6 [21]. This procedure resulted in a 1.5-fold increase in specific activity (method 1) of galactosyltransferase.

Sidedness of active site

Galactosyltransferase activity towards asialo-mucin as acceptor substrate was expressed only in the presence of detergents. Since these detergents also disrupt membrane structure, an indirect approach was necessary in order to determine sidedness of the galactosyltransferase active site. The method employed was similar to that used by Frei and Zahler [22] for sheep erythrocyte membrane phospholipase, namely, proteolytic degradation of the enzyme activity in leaky ghosts, sealed impermeable ghosts and sealed inside-out vesicles.

Preliminary experiments indicated that the galactosyltransferase is highly resistant to proteolytic attack, no significant decrease in activity being measured in prolonged incubations with either trypsin, chymotrypsin or pronase alone. A mixture of all three enzymes was, however, capable of reducing galactosyltransferase activity significantly and was used as such for sidedness determination of its active site. In presence of Triton X-100, however, trypsin alone was capable of rapidly degrading galactosyltransferase.

After incubation of leaky ghosts, sealed impermeable ghosts and sealed inside-out vesicles, respectively, with the protease mixture for 2 h at 37°C, protease was removed by gel filtration on small Sepharose 4B columns in order to prevent possible degradation of

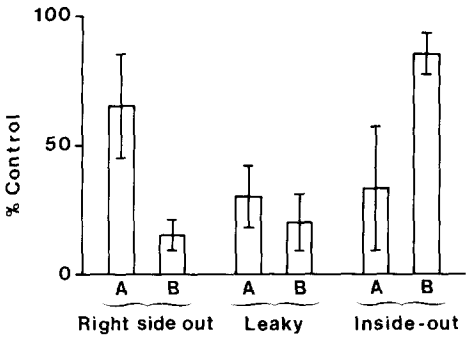

Fig. 3. Effect of proteolysis upon galactosyltransferase activity and sialic acid content of sealed (=right side-out) and leaky erythrocyte ghosts and sealed inside-out vesicles prepared from human erythrocytes. A: Galactosyltransferase specific activity (based on phospholipid) remaining after protease treatment, expressed as percentage of control incubation (without protease) of the same preparation. B: Sialic acid content per mg phospholipid remaining after protease treatment, expressed as percentage of control incubation value (without protease). Vertical bars indicate ±1 S.D. ($n = 5$).

acceptor substrate or the galactosyltransferase in the (detergent-containing) enzyme assay.

Galactosyltransferase activity (standard assay, method 1) and phospholipid were determined. Specific activities based on phospholipid were calculated and compared with control incubations where membrane preparations were treated in identical fashion, except that buffer in place of protease was added. Sialic acid determinations and dodecyl sulphate-polyacrylamide gel electrophoresis served as qualitative checks on proteolysis.

The results of the proteolytic degradation study are summarized in Fig. 3. Leaky ghosts and sealed inside-out vesicles both demonstrated a more significant drop in galactosyltransferase activity compared with control than did sealed impermeable ghosts, indicating that the galactosyltransferase activity is more accessible to proteolytic attack on the cytosolic side of the membrane. The results also correlated inversely with decreases in sialic acid content, mainly due to proteolytic removal of sialic acid-containing glycopeptides from the external face of the erythrocyte [23].

The 40% drop in galactosyltransferase activity observed in sealed impermeable ghosts after protease treatment may be ascribed either to a partial *ecto*-orientation of the transferase active site or an increase in permeability to proteases after proteolytic treatment.

In order to check whether permeability is affected by proteolytic treatment, leaky ghosts were resealed in the presence of Dextran-[^{14}C]carboxyl, mol. wt. 20000 and incubated under the same conditions. It could be shown that this particular membrane preparation lost approx. 30% of the occluded radioactivity compared to a loss of only 6% for the control (same preparation) incubation without protease. We therefore suggest increased permeability of the resealed ghosts to protease upon proteolytic treatment as an explanation for the observed 40% drop in galactosyltransferase activity.

Discussion

This study on human erythrocyte uridine 5′-diphosphate galactose: 2-acetamido-2-deoxy-α-D-galactopyranosylpeptide galactose $\beta(1 \rightarrow 3)$ transferase [6], describes optimized conditions for its assay, produces evidence for its membrane location and indicates that its active site may be partially buried within the membrane structure, but appears to be oriented towards the cytoplasmic side.

The assay described is more suited to the further study of this enzyme than other assays so far described [3], since it involves much shorter incubation times, is more sensitive and permits the measurement of initial velocities, a basic requirement for kinetic studies.

In contrast to lactate dehydrogenase, galactosyltransferase remained associated with erythrocyte membranes during their preparation from whole erythrocytes (Table II). Treatment of the membranes at high and low ionic strengths to remove weakly-associated proteins [21] led to an increase in galactosyltransferase specific activity. The human erythrocyte galactosyltransferase thus appears to be firmly associated with the membrane, a conclusion further supported by its solubilization behaviour with detergents and its relative resistance to proteolysis.

Activation of the transferase by detergent seems to be related to its solubilization (Fig. 1), but the fact that measurable galactosyltransferase activity still remained associated with the pellet, even after repeated extractions with detergent, would indicate

that it is not solubilization per se which is important in activating the enzyme. More likely, it is the removal of membrane components from its immediate vicinity which otherwise interfere (possibly sterically) with its interaction with substrate(s). This is also supported by the relative resistance of activity to protease. The nature of the detergent employed is important, best results being obtained with Triton X-100 or closely-related non-ionic detergents (Table I). This may be due to the fact that such detergents are able to substitute for the natural lipid environment without producing denaturing effects [24]. The nature of the protein-lipid interactions in the case of galactosyltransferase remains to be established. The activation and solubilization data obtained thus lend support to the notion that galactosyltransferase is an intrinsic membrane protein with a cryptic active site. A similar mucin galactosyltransferase was shown to be Golgi-associated by Andersson and Eriksson [25] and to be active in the absence of detergents, in contrast to the galactosyltransferase reported here. The concept of membrane flow implies that Golgi vesicles fuse with plasma membrane [26]. In the case of the mucin galactosyltransferase, transfer from the Golgi to the plasma membrane with subsequent disappearance of enzyme activity could imply masking of the active site by plasma membrane components. As proposed by Morré et al. [26], this could be just one physiological mechanism of enzyme inactivation ('flow-differentiation').

The molecular mechanism of glycosylation at the membrane remains unclear. A few studies on sidedness of glycosylation have been carried out by Eggens and Dallner [27], Kuhn and White [28] and Fleischer [29], using isolated microsomal or Golgi fractions. It is, however, not entirely clear how these disrupted membrane fractions are oriented, even though glycoprotein remains trapped within the presumed lumen in a large percentage of the fractionated membranes. Erythrocyte ghosts offer an alternative system for studies on sidedness, since sealed inside-out vesicles and impermeable ghosts can easily be obtained and can be assayed for their sidedness and impermeability (see Ref. 9 and this paper, Methods). Using proteolytic degradation of galactosyltransferase activity in this system, we have obtained statistical evidence for a predominant *endo*-orientation of the enzyme active site (cf. Fig. 3). It is noteworthy that the galactosyltransferase appeared to be quite resistant to proteolytic attack, since neither trypsin, chymotrypsin nor pronase alone was able to significantly reduce its activity in the absence of detergent.

In conclusion, human erythrocyte membrane galactosyltransferase is membrane-associated. Its active site is apparently oriented towards the cytoplasm and is inaccessible to exogenous high molecular weight substrates. Further work is required to determine if its plasma membrane localisation and *endo*-orientation are of functional significance.

Acknowledgement

The authors would like to thank Professor H. Aebi for continuous support, Professor Zahler and Brodbeck for helpful suggestions and Miss R. Aebi for typing the manuscript. This work was supported by Grant No. 3.355-0.78 from the Swiss National Science Foundation.

References

1 Berger, E.G. and Kozdrowski, I. (1978) FEBS Lett. 93, 105–108
2 Dahr, W., Gielen, W., Pierce, S. and Schaper, R. (1979) in Glycoconjugates. Proceedings of the Fifth International Symposium Kiel, September 1979. (Schauer, R., Boer, P., Buddecke, E., Kramer, M.F., Vliegenthart, J.F.G. and Wiegandt, H. eds.) 272–273, Georg Thieme Publishers Stuttgart.
3 Cartron, J.P., Andrev, G., Cartron, J., Bird, G.W.G., Salmon, C. and Gerbal, A. (1978) Eur. J. Biochem. 92, 111–119
4 Sturgeon, P., Luner, S.J. and McQuiston, D.T. (1973) Vox Sang. 25, 498–512
5 Dahr, W., Uhlenbruck, G., Gunson, H.H. and Van der Hart, M. (1975) Vox Sang. 29, 36–50
6 Hesford, F.J., Berger, E.G. and Van den Eijnden, D.H. (1981) Biochim. Biophys. Acta 659, 302–311
7 Shur, B.D. and Roth, S. (1975) Biochim. Biophys. Acta 415, 473–512
8 Hanahan, D.J. and Eckholm, J.E. (1974) in Methods in Enzymology, vol. 31 (Fleischer, S. and Packer, L., eds.), pp. 168–172, Academic Press, New York
9 Steck, T.L. and Kant, J.A. (1974) in Methods in Enzymology, vol. 31 (Fleischer, S. and Packer, L., eds.), pp. 172–180, Academic Press, New York
10 Carlson, D.M., McGuire, E.J., Jourdian, G.W. and Roseman, S. (1973) J. Biol. Chem. 248, 5763–5773
11 Berger, E.G., Kozdrowski, I., Weiser, M.M., Van den Eijnden, D.H. and Schiphorst, W.E.C.M. (1978) Eur. J. Biochem. 90, 213–222

12 March, S.C., Parikh, I. and Cuatrecasas, P. (1974) Anal. Biochem. 60, 149–152
13 Azocoll, Calbiochem Document No. 3805 (1972) Calbiochem, 10933 N. Torrey Pines Rd., La Jolla, California 92037, USA
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
15 Sims, N.R. and Carnegie, P.R. (1975) Anal. Biochem. 65, 578–580
16 Wroblewski, F. and La Due, J.S. (1955) Proc. Soc. Expt. Biol. Med. 90, 210
17 Drabkin, D.L. and Austin, J.H. (1935) J. Biol. Chem. 112, 51
18 Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496
19 Renkonen, O., Kosunen, T.U. and Renkonen, O.V. (1963) Ann. Med. Exp. Biol. Fenn. 41, 375–381
20 Skoza, L. and Mohos, S. (1976) Biochem. J. 159, 457–462
21 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617
22 Frei, E. and Zahler, P. (1979) Biochim. Biophys. Acta 550, 450–463
23 Steck, T.L., Fairbanks, G. and Wallach, D.F.H. (1971) Biochemistry 10, 2617–2624
24 Coleman, R. (1974) Biochem. Soc. Trans. 2, 813–816
25 Andersson, G.N. and Eriksson, L.C. (1980) Biochim. Biophys. Acta 600, 571–576
26 Morré, D.J., Kartenbeck, J. and Franke, W.W. (1979) Biochim. Biophys. Acta 559, 71–152
27 Eggens, I. and Dallner, G. (1980) FEBS Lett. 122, 247–250
28 Kuhn, N.J. and White, A. (1977) Biochem. J. 168, 423–433
29 Fleischer, B. (1981) J. Cell Biol. 89, 246–255
30 Dixon, M. (1953) Biochem. J. 55, 170