PERMANENT MIXED-FIELD POLYAGGLUTINABLE ERYTHROCYTES LACK GALACTOSYLTRANSFERASE ACTIVITY

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1. Introduction

Permanent mixed-field polyagglutinability (PMFP) of human erythrocytes is a rare condition characterized by the presence of Tn antigen on the cell surface [1]. The carbohydrate moiety of Tn antigen is a *O*-glycosidically linked α-*N*-acetylgalactosamine (GalNAc) [2]. In normal individuals the α-GalNAc residue is substituted with galactose (gal) in a β(1→3) linkage forming the Thomsen-Friedenreich (T) antigen [3], which is considered to be a precursor of the M and N blood-groups [4]. Based upon serological evidence, Sturgeon postulated a biosynthetic defect in PMFP consisting of a block in the transfer of D-galactose to the GalNAc [1]. We have recently described a soluble galactosyltransferase activity in human serum which catalyzes a gal β(1→3) GalNAc linkage [5] using sialic acid free ovine submaxillary mucin (Sf-OSM) as an exogenous substrate. We now present evidence of a similar membrane-bound galactosyltransferase: Sf-OSM activity from normal A or O erythrocytes of types M, MN or N, and the absence of this enzyme in Tn transformed erythrocyte membranes from a patient (R.R.) in whom this condition was first observed in 1972 and last confirmed in November 1977 (Dr M. N. Metaxas, personal communication).

2. Material and methods

ACD blood with blood type A and O from healthy donors was obtained from the Swiss Red Cross of Berne and Zürich. Tn erythrocytes were obtained from a patient whose PMFP was described in [1]. The blood was kept at 4°C till further processing within 5 days. Plasma was obtained by low speed centrifugation, the erythrocytes were washed several times with NaCl 0.9% (w/v). Membranes were obtained as in [6].

2.1. *Galactosyltransferase assay*

The standard assay of galactosyltransferase activity using Sf-OSM, prepared as in [7], or ovalbumin (Sigma) included 100 μg erythrocyte membrane protein in 150 μl, 20 mM $MnCl_2$, 70 mM Na-cacodylate, pH 7.35, Triton X-100 1 μg/μl, 4 mg/ml Sf-OSM or 5.3 mg/ml ovalbumin, respectively and UDP-Galactose 0.2 mM (Sigma) to which 1 μCi UDP-[^{3}H] galactose (Amersham, 16.3 Ci/mmol) has been added. The mixture was incubated for 4 h at 30°C and stopped by adding an excess of ice-cold phosphotungstic acid 2% (w/v) in 2 N HCl. The precipitate was filtered over glass fiber filters and washed with cold ethanol. The filters were dried, placed in vials to which 10 ml toluene–butyl-PBD (Ciba-Geigy) 0.8% (w/v) was added. Radioactivity was determined in a liquid scintillation counter. Acetylcholinesterase was measured as in [8].

2.2. *Product identification*

Product identification of the label incorporated into Sf-OSm by membrane galactosyltransferase A activity was carried out by alkaline cleavage: The assay was performed with excess amounts of enzyme (2.4 mg), UDP-galactose (5 μmol, 10 μCi/μmol UDP-[^{3}H]galactose) and exogenous acceptor (10 mg

Sf-OSM) under suitable conditions as described above. The incubation lasted 48 h at 30°C; NaN_3 (0.02% w/v) was added to prevent microbial growth. After the incubation, the unreacted UDP-galactose was separated from the protein by extensive dialysis at 4°C. The insoluble proteins (membranes) were sedimented thereafter (45 000 × g, 60 min) and the supernatant, containing the enzymatically labeled Sf-OSM was recovered and subjected to a modified alkaline-borohydride treatment as in [9]. This procedure (KOH 0.1 N, $NaBH_4$ 2 M, 45°C for 16 h) cleaved nearly 100% label. Samples of the cleaved material were then subjected to acid hydrolysis (2 N HCl, 90 min, 100°C), deionized over a mixed-bed ion-exchange column (BioRad). The product was identified in 2 systems, by thin-layer chromatography as indicated below and on high-voltage electrophoresis (1% Na-tetraborate w/v, 45 V/cm, 70 min). 55% of the label could be identified as galactose in these systems.

2.3. *Thin layer chromatography*

This was performed on pre-coated silica gel plates (Merck) in ethanol/ammonium acetate 1 M, pH 6.9, for 6 h at 20°C. Standards included galactose-1-phosphate (Boehringer), unlabeled UDP-galactose (Sigma), labeled UDP-[^{3}H]galactose (Amersham) and galactose (Merck) as in [10].

3. Results

Erythrocyte membranes were assayed for UDP-galactose: Sf-OSM galactosyltransferase (GT-A), UDP-galactose:ovalbumin galactosyltransferase (GT-B; EC 2.4.1.38) and acetylcholinesterase (EC 31.1.1.7). Mean values of these enzyme activities are given in table 1. Since erythrocyte membrane GT-A has not been described before, some requirements for maximum activity, stability and the app. K_m values for Mn^{2+}, UDP-galactose and Sf-OSM have been determined. They are given in table 2.

As can be noted from table 1, the normal ranges for GT-A activity is relatively narrow compared to the range of GT-B. None of these enzymes appeared to correlate with M, MN and N blood types. The range determined for GT-B corresponds approximately to [11] where sialic-acid–galactose-free fetuin was used as a substrate for GT-B [11]. Tn erythrocytes had normal GT-B activity. However, GT-A activity was found to be very low, whereas acetylcholinesterase activity was within the normal range.

Figure 1 shows the dependency of GT-A product formation on time and amount of erythrocyte membrane protein obtained from a normal individual and the PMFP patient. As can be seen from the graphs and from table 1, the PMFP sample had low but measurable GT-A, compatible with the 7% of normal erythrocytes found in this patient [1]. The arrows on both graphs indicate the standard assay conditions. The low amount of endogenous acceptors present in normal erythrocytes may explain the deviation from linearity in the lower range of the curve presenting GT-A activity as a function of the amount of enzyme [12].

It was concluded from serological evidence that Tn erythrocytes exhibit terminal α-GalNAc residues [2];

Table 1
Galactosyltransferase activity in normal and Tn membranes

Enzyme activity	Controls (mean ± SD)	Tn membranes
UDP-galactose: Sf-OSM galactosyltransferase (GT-A) (pmol h^{-1} mg^{-1})	77.25 ± 12.5 (n = 12) (50.8 – 110.0)	6.1
UDP-galactose:ovalbumin galactosyltransferase (GT-B) (pmol h^{-1} mg^{-1})	25.5 ± 8.8 (n = 11) (7.9 – 35.7)	24.3
Acetylcholinesterase (IU mg^{-1})	1.81 ± 0.73 (n = 22) (1.06 – 3.53)	1.08

Table 2
Erythrocyte membrane galactosyltransferase: requirements and kinetic parameters

A.	Complete system[a]	100 %
	Complete system minus Sf-OSM	11.7%
	Complete system minus Triton X-100	6.9%
	Complete system minus Mn^{2+}	16.4%
	Complete system minus Mn^{2+} plus Mg^{2+} (20 mM)	8.4%
	Complete system minus Mn^{2+} plus Ca^{2+} (20 mM)	9.0%
B.	K_m Mn^{2+}	8 mM
	K_m UDP-galactose	0.14 mM
	K_m Sf-OSM (GT-A)	2.3 mg/ml
	K_m Ovalbumin (GT-B)	2.0 mg/ml
	K_m Tn membranes	0.07 mg/ml
C.	Stability[b]	
	7 days kept at 4°C: 16% loss of activity	
	7 days kept at −20°C: 22% loss of activity	

[a] The complete assay is described in section 2
[b] With respect to GT-A

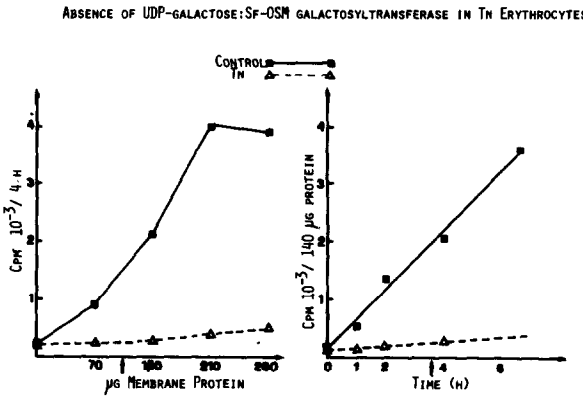

Fig.1. Erythrocyte membrane GT-A activity as function of membrane protein (left) and time of incubation (right). The details of the assay are given in section 2. The arrows on both graphs indicate the standard assay conditions.

thus, Tn membranes should act as exogenous acceptors if incubated with membrane galactosyltransferase from a normal control. Tn membranes indeed serve as acceptors for galactose to a sizable extent. Interestingly the app. K_m was by 1–2 orders of magnitude lower than for Sf-OSM, indicating a higher affinity for Tn erythrocyte membranes than for Sf-OSM (cf. table 2).

Linearity of galactose transfer was observed within 6 h and the range of enzyme concentration tested; this excludes possible interference of nucleotide pyrophosphatase activity [13,14] or extensive chemical breakdown of UDP-galactose [15] under the conditions chosen. In order to quantify for these effects we incubated normal and Tn membranes under standard conditions but without exogenous acceptor. At zero time and after 4 h aliquots were subjected to thin-layer chromatography as described above. Under the conditions of our incubation no increase of label corresponding to gal-*1*-phosphate was observed either in the control or in Tn erythrocyte membranes after 4 h. However, up to 50% of the initial label of UDP-[^{3}H]galactose co-chromatographed with free galactose, indicating chemical breakdown of the sugar nucleotide to an extent, though, which did not affect the substrate saturation of the transferase.

4. Discussion

We describe here a galactosyltransferase activity in normal erythrocytes which incorporates galactose into alkali-labile carbohydrate moieties of Sf-OSM (GT-A); this enzyme clearly differs from GT-B which is specific for GlcNAc residues as shown [11]. They also measured GT-A but detected only very low

activity; since they did not indicate the assay conditions, we are not able to explain this discrepancy.

Tn erythrocytes lack GT-A activity and the presumed product of this enzyme, the T antigen. Sf-OSM which uniformly exposes GalNAc residues [7] is a very potent inhibitor of the Tn antigen [4]. Thus, Sf-OSM appeared to be a suitable acceptor for the corresponding galactosyltransferase. The absence of both galactosyltransferase A and its presumed product in Tn erythrocytes strongly suggests that this enzyme is indeed involved in the biosynthesis of the T antigen.

Moreover, we have found GT-A activity in the serum (unpublished results) of the individual whose erythrocytes lack this enzyme; therefore we assume that GT-A deficiency is confined to the erythroid cell line, perhaps as a consequence of a somatic mutation, as already proposed [1]. The availability of this enzyme deficiency model suggests that galactosyltransferase A is involved in the biosynthesis of the M and N antigen precursors [16]. Sf-OSM as an exogenous substrate renders the study of this enzyme easy and may form a useful approach for further studies on biosynthesis of cell surface antigens.

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References

[1] Sturgeon, P., Luner, S. J. and McQuiston, D. T. (1973) Vox Sang. 25, 498–512.
[2] Dahr, W., Uhlenbruck, G., Gunson, H. H. and Van der Hart, M. (1975) Vox Sang. 29, 36–50.
[3] Dahr, W., Uhlenbruck, G. and Bird, G. W. G. (1975) Vox Sang. 28, 133–148.
[4] Springer, G. F. and Desai, P. R. (1975) Carbohydr. Res. 40, 183–192.
[5] Berger, E. G., Kozdrowski, I., Weiser, M. M., Van den Eijnden, D. H. and Schiphorst, W. E. C. M. (1978) 4th Int. Symp. Glycoconjugates (Jeanloz, R. W. and Gregory, J. D. eds) Academic Press, New York, in press.
[6] Dodge, J. T., Mitchell, C. and Hanahan, D. J. (1963) Arch. Biochem. Biophys. 100, 119–130.
[7] Carlson, D. M., McGuire, E. J., Jourdian, G. W. and Roseman, S. (1973) J. Biol. Chem. 248, 5763–5773.
[8] Ellman, G. L., Courtney, D. K., Andres, V. and Featherstone, R. M. (1961) Biochem. Pharmacol. 7, 88–95.
[9] Carlson, D. M. (1968) J. Biol. Chem. 243, 616–626.
[10] Bretz, R. and Stäubli, W. (1977) Eur. J. Biochem. 77, 181–192.
[11] Kim, Y. S., Perdomo, J. and Whitehead, J. S. (1972) J. Clin. Invest. 51, 2024–2032.
[12] Segel, I. H. (1975) Enzyme Kinetics, pp. 89–96, John Wiley and Sons, New York.
[13] Deppert, W., Werchau, H. and Walter, G. (1974) Proc. Natl. Acad. Sci. USA 71, 3068–3072.
[14] Keenan, T. W. and Morré, D. J. (1975) FEBS Lett. 55, 8–13.
[15] Nunez, H. A. and Barker, R. (1976) Biochemistry 15, 3843–3847.
[16] Dahr, W., Uhlenbruck, G. and Knott, H. (1975) J. Immunogenet. 2, 87–100.