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N-Acetyl-D-Galactosaminyltransferase in Human Serum and Erythrocyte Membranes

(blood group A/mucin receptor/cell-surface antigens)

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ABSTRACT **This study demonstrates the presence of an *N*-acetyl-D-galactosaminyltransferase in human serum and in erythrocyte membranes. This enzyme catalyzes the transfer of *N*-acetyl-D-galactosamine from UDP-*N*-acetyl-D-galactosamine to a mucin receptor and 2′-fucosyllactose that have blood group H activity and may be responsible, therefore, for blood group A antigenicity. It was present in the serum of individuals with blood group A or AB but was absent from those with blood group B or O. The activity measured in the erythrocyte membrane was low and did not show clear-cut separation among donors of different blood groups. The specificity of this enzyme in serum was suggested by the ability of 2′-fucosyllactose to act as an acceptor, as well as desialyzed porcine submaxillary mucin, while lactose and desialized fetuin failed to accept *N*-acetyl-D-galactosamine. The catalytic properties of the *N*-acetyl-D-galactosaminyltransferase from serum and from erythrocyte membranes were similar.**

Although human ABO blood-group specificity is thought to be determined by the specific oligosaccharide sequences at the terminal residues of glycolipids of the erythrocyte membranes (1, 2), most of the structural studies of human blood-group substances were carried out on blood-active glycoproteins and oligosaccharides isolated from ovarian cyst fluids (3), gastric mucosal extracts (4), and human milk (5). The glycosyltransferases (6), a multienzyme system catalyzing the sequential synthesis of the oligosaccharide chains in glycoporteins, mucin, or gangliosides have been found in colostrum, milk (7), and various tissues such as liver (8), submaxillary gland (9), gastric and small intestinal mucosa (10, 11), and thyroid gland (12). These enzymes are usually found associated with various cellular membranes of these tissues (8, 9), but are present as a soluble enzyme in milk and colostrum (6, 7). The studies of soluble glycosyltransferases in human milk (7) and the membrane-bound enzymes in pig and human submaxillary glands (9, 10, 13) confirmed the earlier proposal by Watkins and Morgan (14) that the blood-group specificities are determined by the synthesis of specific glycosyltransferases required for their formation. In the present study, serum and erythrocyte membranes obtained from persons with various ABO blood groups were examined for *N*-acetyl-D-galactosaminyltransferase activity.

MATERIALS AND METHODS

Human erythrocyte membranes were prepared from fresh blood by the method of Dodge *et al.* (15). UDP-*N*-acetyl-[1-^{14}C]D-galactosamine (43 Ci/mol) was purchased from New England Nuclear Corp. Mucin was isolated according to the method of de Salegui and Plonska (16) from porcine submaxillary glands whose aqueous extracts did not inhibit human A–anti A hemagglutination and possessed human blood group H activity. The acceptor used for the standard assay in this study was prepared from this mucin by treatment with sialidase (*Vibrio cholerae*, Calbiochem), heat inactivation of the sialidase, dialysis, and lyophilization (17). The fetuin acceptor was prepared by treatment of fetuin (Calbiochem) with sialidase in a similar manner. The incubation conditions for the *N*-acetyl-D-galactosaminyltransferase are given in Table 1. The standard incubation was for 2 hr at 37°C, after which 5 ml of 1% phosphotungstic acid in 0.5 N HCl was added to terminate the reaction. The precipitates were washed three times with the phosphotungstic acid solution and dissolved by heating at 50°C with 1 ml of NCS solubilizer (Amersham/Searle). The dissolved samples were mixed with Spectrafluor (Amersham/Searle) and were counted in a Packard Tri-Carb liquid scintillation spectrometer. Control incubations were performed in all instances in the absence of either exogenous acceptor or enzyme, and the amounts of radioactivity incorporated in both controls were subtracted from the total radioactivity in the calculation of enzyme activities. Unless stated otherwise, the results are reported as total radioactivity incorporated into the acceptor per 2 hr of incubation.

When either 2′-fucosyllactose or lactose was used as an acceptor, 0.05 μmol of the sugar was added instead of desialyzed porcine submaxillary mucin, otherwise the incubation mixture was the same as that described in Table 1 except that 125,000 dpm of UDP-*N*-acetyl-[1-^{14}C]D-galactosamine was used. After incubation for 24 hr, the reaction was stopped by boiling the mixture for 30 sec. The precipitated protein was separated by centrifugation and the supernatant was passed through a column (9 × 20 mm) containing Amberlite MB-3 resin previously washed with 2 ml of 0.02 M lactose and 5 ml of water. The precipitate was washed twice with distilled water and the washings were passed through the column. The combined effluent was evaporated in a Rotary Evapomix (Buchler Instruments). The dried samples were dissolved in 50 μl of 50% ethanol and applied to Whatman 3 MM paper for chromatography. Paper chromatography was conducted for 50 hr at 25°C in ethyl acetate–pyridine–water 2:1:2. A 5-cm-wide strip was cut for each sample ap-

TABLE 1. *Requirements for human serum and erythrocyte membrane N-acetyl-D-galactosaminyltransferase*

Incubation mixture	Enzyme activity (dpm/2 hr)	Percent change
Serum enzyme		
Complete system*	2060	
− enzyme	10	−95.5
− acceptor	27	−98.7
− ATP	1710	−17.0
− Mn^{++}	12	−99.4
− Mn^{++} + 8.5 μmol of EDTA	27	−98.7
− Mn^{++} + 13.2 μmol of Mg^{++}	77	−96.3
+ 0.33 mg of Triton X-100	2130	+3.4
Erythrocyte membrane enzyme		
Complete system*	377	
− enzyme	10	−97.4
− acceptor	40	−89.4
+ 0.33 μmol of ATP	324	−14.1
− Mn^{++}	8	−97.9
− Mn^{++} + 8.5 μmol of EDTA	28	−92.6
− Mn^{++} + 13.2 μmol of Mg^{++}	10	−97.4
− Triton X-100	141	−62.6

* Complete incubation mixture contained the following components in a final volume of 0.33 ml: sialidase-treated porcine submaxillary mucin, 0.4 mg of protein (theoretical acceptor sites, 0.35 μmol); cacodylate–acetate buffer, pH 6.4, 66 μmol; UDP-*N*-acetyl-[1-^{14}C]D-galactosamine, 0.53 nmol (50,000 dpm); $MnCl_2$, 14.3 μmol; enzyme (serum, 50 μl or erythrocyte membrane protein, 0.5 mg); Triton X-100, 0.33 mg for the assay of erythrocyte-membrane enzyme only; and ATP, 0.33 μmol for the assay of serum enzyme only. Incorporation into the phosphotungstic-acid precipitate was determined. Enzyme activity is expressed as radioactivity incorporated per 2 hr of incubation.

plied to the paper, and the strip was divided into 2.5-cm-long sections. Each section was cut into small squares and counted in a Packard Tri-Carb liquid scintillation spectrometer with toluene phosphor. The 2′-fucosyllactose was a gift of Dr. Adeline Gauhe.

For the identification of the labeled sugar incorporated into protein, the reaction product was precipitated and hydrolyzed in 3 N HCl under nitrogen at 100°C for 4 hr. The hydrolysate was filtered through glass wool and HCl was evaporated at 50°C. The dried hydrolysates were dissolved in water and chromatographed on Whatman 3 MM paper in ethyl acetate–pyridine–water 12:4:4, for 15 hr at 25°C. Radioactive areas on the paper chromatograms were determined as described above. The standard monosaccharides used were D-galactosamine, D-glucosamine, D-galactose, D-glucose, D-mannose, and L-fucose. Reducing sugars on paper chromatograms were detected by the silver nitrate reagent (18).

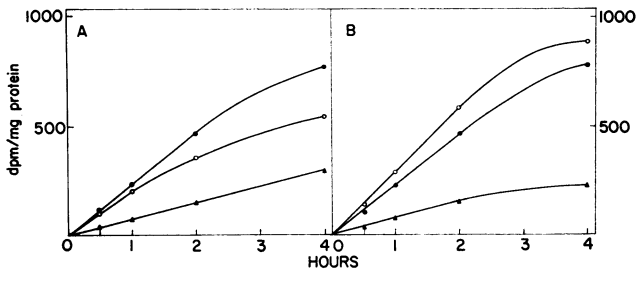

FIG. 1. Effect of ATP on *N*-acetyl-D-galactosaminyltransferase activity in serum (*A*) and erythrocyte membrane (*B*): 0.33 μmol of ATP added (●——●); 3.3 μmol of ATP (▲——▲); no additions (O——O). Incubation conditions as in Table 1. The enzyme activity is corrected for the endogenous values.

RESULTS

The complete incubation mixtures are shown in Table 1. The optimal conditions for the assay of the *N*-acetyl-D-galactosaminyltransferase in erythrocyte membranes and in serum are similar. Triton X-100 was required for maximal activity of the erythrocyte membrane enzyme but had little effect on the activity of the soluble enzyme in the serum.

The optimal pH values for the *N*-acetyl-D-galactosaminyl transferase from erythrocyte membranes and from serum were 7.3 and 6.4, respectively. Of three metal ions studied (Mn^{++}, Mg^{++}, and Ca^{++}), the enzyme activity was dependent only on Mn^{++}. Optimal Mn^{++} concentration and temperature were 40 mM and 38°C, respectively, for the activity of the enzyme both from erythrocyte membranes and from serum.

Fig. 1 shows the effects of ATP and incubation time on the enzyme activity. The enzyme activity was linear with time up to 2 hr. ATP at 1 mM stimulated both the rate and extent of the transfer of *N*-acetyl-[1-^{14}C]D-galactosamine in the serum but inhibited at 10 mM; the enzyme from erythrocyte membranes was inhibited by ATP at both concentrations. Similar results were obtained with GTP. The incorporation of *N*-acetyl-D-galactosamine was markedly inhibited by UTP even at low concentration (1 mM); only 5% incorporation was observed compared to that obtained with added ATP or GTP. The incorporation of *N*-acetyl-D-galactosamine into the acceptor under the standard incubation conditions was linear with regard to enzyme concentration (Fig. 2). Hydrolysis and chromatography of the reaction product yielded a single radioactive peak corresponding to D-galactosamine indicating that conversion to other sugars did not occur during the reaction.

Table 2 shows that *N*-acetyl-D-galactosaminyltransferase activity is present in the serum of persons with blood groups A or AB but is not detected in that of blood group B or O. A similar distribution of the serum enzyme was observed among donors of different blood groups when 2′-fucosyllactose was used as an acceptor (Table 3). Lactose and desialyzed fetuin incorporated only a trace amount of *N*-acetyl-D-galactosamine. The erythrocyte membranes from persons of all blood groups showed low enzyme activity with the porcine mucin receptor. Although no clear-cut separation of the enzyme activity was observed among donors of different blood groups with this

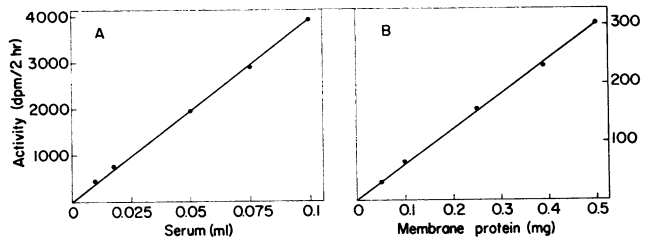

FIG. 2. Effect of enzyme concentration on the transfer of *N*-acetyl-[1-^{14}C]D-galactosamine by serum enzyme (*A*) and by erythrocyte-membrane enzyme (*B*). Incubation conditions as in Table 1.

TABLE 2. *N-Acetyl-D-galactosaminyltransferase activity in human erythrocyte membranes and serum*

Donor	Blood type	Activity (dpm/2 hr)*	
		Serum	Erythrocyte membranes
D.M.	A	2610	248
J.P.	A	836	160
N.Y.	A	1650	—†
E.Y.	A	910	210
R.C.	A	3250	—†
J.W.	B	52	78
Z.P.	B	44	15
J.V.	B	44	8
A.S.	B	70	25
W.F.	B	37	6
M.N.	AB	2590	350
Y.K.	AB	1730	120
E.B.	AB	2440	—†
J.N.	O	44	80
W.K.	O	37	57
A.B.	O	30	33

* Conditions of incubation as in Table 1.
† Not tested.

receptor, the enzyme activity tended to be higher in the erythrocyte membranes from A and AB donors than in those from B or O donors.

DISCUSSION

A *N*-acetyl-D-galactosaminyltransferase is probably responsible for the blood group A activity by virtue of forming the *O*-α-D-GalNAc-(1 → 3)-*O*-β-D-Gal structure that occurs in erythrocyte glycolipids. This blood group A determinant also has been described in glycoproteins isolated from mucous secretions (19) and from submaxillary gland (13), and in oligosaccharides of the milk of women with blood group A activity (20). *N*-Acetyl-D-galactosaminyltransferase activity has been detected in the milk from women of blood group A and AB (7, 9), in human and porcine submaxillary glands (21, 9, 13), and in rat small intestinal mucosa (22).

The following reaction is catalyzed by this enzyme and was used for the assay of the enzyme in the present study.

UDP-GalNAc + Fuc-Gal-mucin →

GalNAc-(Fuc)Gal-mucin + UDP.

In this study, an *N*-acetyl-D-galactosaminyltransferase was found to be present in serum from group A and AB donors but absent from those from the blood group O and B donors. This finding suggests that the serum enzyme may be responsible for the synthesis of the A determinant groups of glycolipids of erythrocyte membranes. The specificity of this enzyme reaction is indicated by the observation that the sera from A and AB donors, but not those from B and O donors, catalyzed the transfer of *N*-acetyl-D-galactosamine both to desialyzed porcine submaxillary mucin and to 2′-fucosyllactose. Both of these receptors possess H activity and have terminal galactose residues. The fact that lactose and desialyzed fetuin—with terminal galactose residues, but without fucose linked in α1–2 linkage to galactose—did not accept *N*-acetyl-D-galactosamine with sera from A and AB donors indicates that only substances having H activity will act as receptors. In the study of *N*-acetyl-D-galactosaminyltransferase in human milk from donors of blood group A or AB, Kobata and Ginsburg (20) found that this enzyme is responsible for the formation of the structural determinants of blood group A by adding *N*-acetyl-D-galactosamine to 2′-fucosyllactose. In contrast to a clear-cut separation of the serum enzyme activity among different blood group donors, the enzyme activity in the erythrocyte membrane was low and the difference among various blood group types was not significant. Further studies on the erythrocyte membranes are in progress. In the present study, however, the temperature optimum and metal ion requirement for this enzyme were similar irrespective of its source. The different effect of a low concentration of ATP and GTP on the assay of the enzyme in serum and in erythrocyte membranes suggests that pyrophosphatase, which degrades the nucleotide sugar substrate, may be present in the serum but absent from erythrocyte membranes. The decreased rate of incorporation observed with higher levels of ATP and GTP (probably too high to be physiologically significant) may be the result of nonspecific binding of these nucleotide phosphates to the glycosyltransferase. The inhibition caused by low concentrations of UTP, however, may be a result of the uridine moiety competing with the sugar nucleotide for the transferase.

TABLE 3. *N-Acetyl-D-galactosaminyltransferase in human serum as tested by transfer to 2′-fucosyllactose*

Donor	Blood type	Activity (cpm/2 hr)*
D.M.	A	32,450
N.Y.	A	23,490
Y.K.	AB	25,107
J.W.	B	118
A.S.	B	49
J.N.	O	34
Y.W.	O	61

* The enzyme was assayed as described in *Methods*.

Recently, a soluble galactosyltransferase was reported to be present in embryonic and adult chicken serum, amniotic fluid, and vitreous humor and in human spinal fluid (23). The data presented in this communication provides evidence of the presence of a *N*-acetyl-D-galactosaminyltransferase in human serum and in human erythrocyte membranes. The source of this enzyme in the serum is not clear at present. The enzyme may arise from cellular secretion or degradation of cells, a process which may result in the release of cellular constituents into the bloodstream. The high serum concentration of the enzyme suggests, however, that this enzyme may play an active role in the formation or maintenance of blood-group-A activity of glycoproteins and glycolipids of the cell surface.

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