A QUANTITATIVE DIFFERENCE IN THE ACTIVITY OF BLOOD GROUP A-SPECIFIC N-ACETYLGALACTOSAMINYLTRANSFERASE IN SERUM FROM A_1 AND A_2 HUMAN SUBJECTS

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Received September 3, 1971

SUMMARY A soluble glycosyltransferase which can trasfer N-acetylgalactosamine from UDP-N-acetylgalactosamine to exogenous acceptors is present in the serum of human subjects with the blood group A gene. The enzyme is in the serum of secretors and non-secretors of group A and AB but is not in the serum of group O or B subjects. The activity of serum from A_1 subjects is 5 to 10 times higher than that from A_2 subjects. Activity was lower in two group A_1 pregnant women. There was low activity in the serum of a hematopoietic chimera whose A^1 gene is restricted to her "grafted" blood cells.

INTRODUCTION The N-acetylgalactosaminyltransferase determined by the blood group A gene has been found in soluble form in human milk (1,2), and as a membrane-bound enzyme in human submaxillary gland (3), human stomach mucosa (4,5), hog submaxillary gland (6,7) and hog stomach mucosa (8-10). In these assays, the acceptors were low molecular weight oligosaccharides isolated from human milk (1-3), water-soluble high molecular weight H-substance (4,10) and procine submaxillary mucin isolated from A-negative hogs (6,7); these substances all contain the H-specific determinant, α-L-fucosyl-(1→2)-β-D-galactosyl-R. Recently, various glycosyltransferases involved in the synthesis of plasma glycoproteins have been found in human, rat and pig serum (11,12). The present communication describes the finding in human serum of N-acetylgalactosaminyltransferase determined by the A gene. The activity of this enzyme is appreciably higher in A_1 than

in A_2 subjects; the activity is lower in the serum of pregnant women.

MATERIALS AND METHODS

Mucin acceptor. Extracts from 13 A-negative porcine submaxillary glands (supplied by Canada Packers, Toronto) were pooled (150 gm wet weight); mucin was prepared by cetyltrimethylammonium bromide precipitation (13) and stored frozen as a 0.5% aqueous solution. The mucin concentration was determined by assaying for bound sialic acid with the resorcinol procedure (14), assuming a sialic acid content of 20% (15).

Oligosaccharide acceptor. Lacto-N-fucopentaose I (LNF-I) was prepared from the milk of a group O, Le(a-b-) secretor, as described by Kobata et al. (16,17). The concentration of this oligosaccharide was estimated by the reducing sugar method, using glucose as the standard (18).

Radioactive compounds. UDP-N-acetyl-D-galactosamine-1-^{14}C, 50μCi per μmole (from New England Nuclear Corp.), was diluted to a specific activity of 2 x10^7 cpm per μmole with non-radioactive UDP-N-acetylgalactosamine prepared as previously described (19).

Preparation of human serum. Serum was obtained from healthy subjects whose ABO and Lewis phenotype and secretor status had been determined, using standard agglutination and inhibition techniques (20). In some instances, the genotype could be deduced from the results of testing the subject's parents or children (21). One subject was a blood chimera, whose circulation contained a mixture of O and A_1 red cells; the precursors of her A_1 cells were acquired in utero from her twin brother (22). Two subjects were pregnant women.

Serum was collected on the day of the test and kept at 4°C until used (within 5 hrs.). In one experiment, packed red cells (washed 10 times in buffered 0.9% NaCl) were suspended in an equal volume of serum, lysed by freezing and thawing, and centrifuged at 3000 rpm for 10 minutes; the supernatant was assayed for transferase activity.

Enzyme assay. The incubation mixture used for assaying N-acetylgalactosaminyl-

transferase activity with porcine submaxillary mucin as acceptor is shown in Table I. When LNF-I was used as the acceptor, the incubation mixture (in a final volume of 45µl) contained: 0.33 µmole LNF-I; 0.010 µmoles UDP-N-acetyl-D-galactosamine-1-^{14}C ($2x10^7$ cpm per µmole); 1.25 µmoles MES, pH 5.5; 0.5 µmoles $MnSO_4$; and 5µl, 10µl or 20µl enzyme (usually crude serum). Incubations were carried out at 37°C for 2 to 4 hours and the reaction was stopped by the addition of 20µl 0.25 M EDTA-2% sodium tetraborate. Aliquots (50µl) of the resulting solutions were subjected to high voltage electrophoresis on Whatman 3MM paper in 1% sodium tetraborate at pH 9 at 3000 volts for 40 minutes to remove excess radioactive nucleotide-sugar from the origin. After drying, the papers were washed overnight by descending chromatography with 80% ethyl alcohol to remove from the origins of the papers any radioactive N-acetylgalactosamine that may have been formed during the incubations. The papers were scanned for radioactivity with a Packard Model 7201 radioscanner; mucin product remained at the origin after the overnight alcohol washing, whereas the oligosaccharide product moved about 2 to 3 inches from the origin. All products were measured by subjecting the appropriate areas of the paper to liquid scintillation counting (23).

Each serum was assayed with and without exogenous acceptors; the activity obtained without exogenous acceptor was subtracted from the values obtained with exogenous acceptor, thus correcting for transfer of radioactivity to endogenous acceptors, and for trapping of radioactivity at the origin of the paper.

RESULTS

Mucin acceptor. Serum samples from group A human subjects were found to transfer N-acetylgalactosamine (GalNAc) from UDP-GalNAc to A-negative porcine submaxillary mucin. Since this activity is relatively low in crude serum, the enzyme was concentrated 5-fold by ammonium sulphate fractionation. The 35-55% ammonium sulphate fraction contained 88% of the activity, and was used in all studies with the mucin acceptor. As shown

Table I. Requirements for N-acetylgalactosaminyltransferase activity in human serum: Serum from one A_1 donor (J.Cr.) was fractionated with ammonium sulphate; the 35-55% fraction was assayed for glycosyltransferases using mucin as acceptor. The complete incubation mixture (final volume 55µl) contained:
0.1 mg porcine submaxillary mucin
5 nmoles UDP-GalNAc-1-^{14}C (2×10^7 cpm per µmole)
1.25 µmoles MES [2-(N-morpholino) ethanesulphonate] pH 5.5
0.5 µmoles $MnSO_4$; 20µl of 35-55% ammonium sulphate fraction

Incubation mixture	% Activity
Complete mixture	100
- mucin	1-3
- $MnSO_4$	2
+ EDTA, 2.5 µmoles	0
+ Triton X-100, 0.25µl	100

in Table I, the N-acetylgalactosaminyltransferase requires an exogenous acceptor and is absolutely dependent on the presence of cation. There is a fairly sharp pH optimum at pH 5.5. Enzyme activity is unaffected by varying the Mn^{++} concentration from 0.005 M to 0.1 M. The enzyme is soluble and is not activated by the addition of detergent (Triton X-100). Under the assay conditions shown in Table I, product formation is linear with enzyme concentration(up to 20µl of the 35-55% fraction) and with time (up to 4 hours).

When serum from 13 subjects was assayed with mucin acceptor, an N-acetylgalactosaminyltransferase was detected in all the group A and group AB subjects, but not in the group O or group B subjects.

Mucin product identification. The hydrolysate of the product showed only a single radioactive peak in three paper chromatographic systems; this peak co-chromatographed with standard D-galactosamine in all three systems and was separated in at least one of these systems from D-glucosamine, D-galactose, L-fucose, D-mannose and D-glucose. These experiments establish that the serum N-acetylgalactosaminyltransferase incorporates N-acetylgalactosamine into porcine submaxillary mucin (i.e., into a high molecular weight acceptor).

Oligosaccharide acceptor. Table II shows the results of assays on crude serum, using LNF-I as acceptor. Again, N-acetylgalactosaminyltransferase

Table II. N-acetylgalactosaminyltransferase in serum from normal human subjects. All values (cpm/3hrs. at 37°C) corrected for endogenous activity; LNF-I is the acceptor.

Sex	Donor	Genes			Serum, µl 5	10	20
M.	D.Cr.	A^1 O	Le	Sese	1210	2520	
F.	J.Ma.	A^1	Le	Sese		3600	4330
F.	R.Ra.	A^1	Le	Se		2160	2480
*F.	M.Te.	A^1	Le	Se		833	741
*F.	M.Ma.	A^1	Le	Se	365	585	
F.	C.Ti.	A^1 O	lele	Sese		2070	2640
M.	I.Cr.	A^1 O	Le	sese	967	2310	
M.	J.Cr.	A^1	Le	sese	929	2180	3880
F.	H.Fa.	A^1	lele	sese		2070	2450
F.	L.Ae.	A^1 B	Le	Se		1910	
F.	M.Ri.	A^1 B	lele	Se		1730	
F.	T.Bo.	A^2	Le	Se		128	652
F.	C.Ca.	A^2	Le	Se		122	690
F.	L.Cu.	A^2	Le	Se		124	505
F.	C.Hu.	A^2	Le	Se		121	457
F.	J.La.	A^2	Le	Se		129	750
F.	A.Ra.	A^2	Le	Se		125	545
F.	C.Me.	A^2	lele	sese		87	467
F.	E.Co.	A^2 B	Le	Se		156	580
M.	H.Sc.	B	Le	Se			5
F.	M.Ch.	B	lele	Se			4
M.	W.Ma.	B	Le	sese			0
M.	J.Wh.	B	Le	sese			29
F.	M.Cr.	OO	Le	Sese			39
M.	J.El.	OO	Le	Se			0
F.	J.Le.	OO	lele	Se			0
ψF.	A.De.	OO	Le	Sese		117	264
Lysed A_1 cells in O serum							0
Lysed O cells in O serum							35

* pregnant 30 weeks; ψ blood chimera with 50% A_1 red cells.

was present only in subjects who have the A gene. The enzyme was present in the serum of secretors (Se Se or Se se) and non-secretors (se se) and was independent of the Le gene. The enzyme activity was 5-10 times greater in serum from A_1 subjects than in serum from A_2 subjects. The activity in

the serum of two pregnant A_1 women was significantly less than in the serum of the non-pregnant A_1 women.

The assay was linear with time up to 4 hours, but product formation was proportional to serum volume only over a limited range (between 1000-2000 cpm per 3 hours). With A_1 serum, there was inhibition at high enzyme concentrations due to the presence in serum of an unidentified inhibitor. With A_2 serum, there was a lag at low enzyme concentrations. The endogenous activity was low: < 100 cpm/20µl/3 hrs.

DISCUSSION The present study shows the presence in human serum of a blood group A-dependent N-acetylgalactosaminyltransferase similar to the enzyme previously described in human milk (1,2), submaxillary gland (3) and stomach (4,5), and in porcine stomach (8-10) and submaxillary gland (6,7). The enzyme is present in the serum of secretors and non-secretors Similarly, the enzyme in submaxillary glands and milk is independent of the Se gene (2,3).

The three most interesting observations concerning the level of activity of N-acetylgalactosaminyltransferase in serum are: the higher activity in A_1 than in A_2 subjects; the lower activity in pregnancy; the low, but significant, activity in the serum of a hematopoietic chimera whose A_1 antigens are restricted to her "grafted" blood cells.

It has been suggested (24) that the structural basis for the difference between the A_1 and A_2 antigens depends on whether the A determinant, N-acetylgalactosamine, is linked to a Type 1 oligosaccharide side chain [galactosyl-(β,1→3)-N-acetylglucosamine] or to a Type 2 chain [galactosyl-(β,1→4)-N-acetylglucosamine]. The A_1 antigen is believed to contain GalNAc linked to both Type 1 and Type 2 chains, whereas the A_2 antigen may contain GalNAc linked only to Type 2 chains. This theory is supported by the present serum assays which showed a significantly greater incorporation of GalNAc into LNF-I(a Type 1 oligosaccharide) by A_1 than by A_2 subjects.

The low N-acetylgalactosaminyltransferase activity in the serum of

two group A_1 pregnant women could be caused by hormones analagous to the steroids which inhibit glucuronyltransferases (25).

The lack of activity in the hemolysate of A_1 cells (see Table II) indicates that the red cells in the peripheral circulation are not a source of serum N-acetylgalactosaminyltransferase. The hematopoietic cells in the bone marrow, however, do contribute some enzyme, since activity (one-tenth that of A_1 subjects) was present in the serum of a blood chimera whose A^1 gene, and A_1 antigen, appear to be restricted to the blood cell precursors acquired *in utero* from her A_1 twin. Since half of the red cells in this chimera are A_1, the low enzyme activity could imply that only about one-fifth of the N-acetylgalactosaminyltransferase in the serum of A_1 subjects is derived from the bone marrow.

Recent developments have indicated the possible involvement of glycoproteins and glycolipids in the processes of cell-cell interaction such as aggregation and differentiation (26,27). One can speculate that soluble glycosyltransferases may be present not only in serum but also in tissue fluid bathing the organs of the body (28) and may function *in vivo* to modify the surfaces of cells during processes such as embryogenesis which require a changing pattern of cell-cell interaction.

Acknowledgments. We are indebted to Miss Judith A. Shindman for preparing lacto-N-fucopentaose-I, and to Dr. Eloise R. Giblett for helpful advice. This investigation was supported by grants from the Medical Research Council of Canada (Grants MT-3285 and MT-696) and the Bickell Foundation.

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