ISOELECTRIC POINTS OF THE HUMAN BLOOD GROUP $\underline{A}^1$, $\underline{A}^2$ and $\underline{B}$ GENE-ASSOCIATED GLYCOSYLTRANSFERASES IN OVARIAN CYST FLUIDS AND SERUM

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SUMMARY: The isoelectric points of the blood group $\underline{A}^1$,$\underline{A}^2$ and $\underline{B}$ gene-associated glycosyltransferases in human ovarian cyst fluids were found by isoelectric focusing to be in the pH range 9.5-10. The $\underline{A}^1$ and $\underline{B}$ transferases in serum had isoelectric points similar to those of the enzymes in cyst fluids but $\underline{A}^2$ transferases in serum had considerably lower isoelectric points, in the pH range 6-7. The difference in the pI values of the $\underline{A}^1$ and $\underline{A}^2$ transferases in the serum of a donor of the genotype $\underline{A}^1\underline{A}^2$ enabled the two enzymes to be preparatively separated by the isoelectric focusing technique. The dissimilarity in the pI values of the $\underline{A}^2$ transferases in ovarian cyst fluids and serum samples indicates that the isoelectric point arises from a post-translational modification of the enzyme protein.

INTRODUCTION. The α-N-acetylgalactosaminyltransferases associated with the human $\underline{A}^1$ and $\underline{A}^2$ genes, and the α-galactosyltransferase associated with the $\underline{B}$ gene, at the blood group ABO genetic locus (1) occur in soluble form in milk (2,3), serum (4,5,6) and ovarian cyst fluids (7). Until now no information has been available on the isoelectric points of these glycosyltransferases. In this paper the isoelectric points of the soluble $\underline{A}^1$, $\underline{A}^2$ and $\underline{B}$ gene-associated glycosyltransferases in ovarian cyst fluids and serum are reported. The isoelectric points of the peptidyl:N-acetylgalactosaminyltransferase (8) and N-acetylglucosamine:ß-galactosyltransferases (9,7) present in the same sources are included for comparative purposes.

MATERIALS AND METHODS. UDP-D-[U-^{14}C]galactose (250 mCi/mmole) and UDP-N-acetyl-D-[1-^{14}C]galactosamine (45 mCi/mmole) were purchased from the Radiochemical Centre, Amersham. 2'-Fucosyllactose (O-α-L-fucosyl-(1→2)-O-ß-D-galactosyl-(1→4)-D-glucose) was a gift from Dr. A. Gauhe. The glycopeptide acceptor for the

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peptidyl: N-acetylgalactosaminyltransferase assays was prepared from purified blood group Le^a substance as described by Donald et al.(10) and treated with an α-N-acetylgalactosaminidase from Lumbricus terrestris (11) to remove the major part of the residual N-acetylgalactosamine glycosidically linked to the peptide.

Ovarian cyst fluids were chilled to 4°C as soon as possible after removal from the patient and transported to the laboratory at this temperature. The fluids were freeze-dried and stored at -20°C until required for use. Blood samples (20 ml) taken by venapuncture were allowed to clot at room temperature for 2 hours and the serum was then separated, centrifuged for 10 minutes at 2000 r.p.m. and the supernatant either used immediately or stored at -20°C.

The proteins and glycoproteins in the ovarian cyst fluids were separated by density gradient centrifugation (12,13). The contents of the freeze-dried cyst fluid were reconstituted in distilled water to their original concentration and the solution was centrifuged at 35,000 g for 1 hour at 4°C to remove insoluble material. Caesium chloride was added to the reconstituted cyst fluid to give a density $\rho = 1.40$ g/ml. The solution was spun at 230,000 g for 24 hours at 4°C; at the end of this time the protein and transferase activities were located in the top 4 ml of the 13.5 ml of fluid present in the centrifuge tubes. The caesium chloride was removed from this fraction by dialysis for 18 hours against 2 litres of 0.1 M Tris-HCl buffer pH 7.5 containing 0.1% 2-mercaptoethanol (Buffer a). The dialysed solution was fractionated with ammonium sulphate and the material that precipitated between 20-40% saturation was dissolved in 2-3 ml of Buffer a and dialysed for 18 hours at 4°C against the same buffer. Serum samples were similarly fractionated with ammonium sulphate and the 30-40% precipitate was dissolved in, and dialysed against, Buffer a.

Isoelectric focusing (14) was performed in an LKB Uniphor apparatus fitted with a glass column 40 x 2.5 cm. The column and buffer chambers were maintained at 4°C. A gradient of sucrose (40-0%), containing the enzyme sample (25-35 mg of the 20-40% ammonium sulphate fraction of the ovarian cyst material or 50-60 mg of the 30-40% ammonium sulphate fraction of serum) and Ampholines (LKB) in the pH range 3-10 (at a concentration of 1% for the cyst fractions and 2% for the serum fractions) was layered onto the anode solution. Focusing was continued for 72 hours at 3 watts with a maximum voltage of 1000 V. The column was eluted by displacement with cathode solution and the pH of each fraction (2 ml) was determined at 4°C. For transferase assays the fractions were pooled in groups of five, dialysed for 18 hours at 4°C against Buffer a, and concentrated to 1 ml by ultra-filtration.

The A^1, A^2 and B gene-associated transferases were assayed by transfer of radioactively labelled sugars from UDP-[^{14}C] N-acetylgalactosamine or UDP-[^{14}C]galactose to the low molecular weight acceptor 2'-fucosyllactose (15). β-Galactosyltransferases were assayed by the transfer of [^{14}C]galactose from UDP-[^{14}C]galactose to N-acetylglucosamine (7). The peptidyl:N-acetylgalactosaminyltransferase activity was assayed with UDP-[^{14}C]N-acetylgalactosamine as sugar donor and the glycopeptide

prepared by degradation of human blood group substance as the acceptor (16). The anomeric linkages of the transferred sugars were determined by means of purified glycosidases as described previously (7,15).

RESULTS. The recovery of α-N-acetylgalactosaminyl- and α-galactosyltransferase activity following isoelectric focusing was low and, in order to obtain sufficient enzyme for the assay procedures, pooling of fractions was necessary. This step precluded precise determination of the isoelectric point but even allowing for this fact the eluted peaks of activity were broad and minor peaks of transferase activity were frequently detected at pH values other than those recorded in Table I. Nevertheless, the major peaks of A^1 gene-associated α-N-acetylgalactosaminyltransferase activity in cyst fluids from two group A_1 persons, and in serum samples from an A_1 and an A_1B donor, focused between pH 9.5 and 10.2 . The B gene-associated α-galactosyltransferases in the cyst fluid and serum from an A_2B person (No.680, Table I), and in serum samples from a group B and group A_1B donor similarly focused between 9.1 and 10.2 . The α-N-acetylgalactosaminyltransferases in serum samples from group A_2 donors, however, focused at much lower pH values than the corresponding transferases in ovarian cyst fluids. The major peaks of transferase activity in the cyst fluids from the two patients carrying A^2 genes focused at pH 10.0 and pH 9.5 whereas the transferases in five serum samples from group A_2 or A_2B persons focused between pH 6.0 and 6.8 (Table I).

The pH optimum of the B gene-associated α-galactosyltransferase in both unfractionated cyst fluids and serum from group B donors is pH 6.5 (C. Race, unpublished observations). This optimum was unchanged after purification of the enzymes by isoelectric focusing. Schachter et al. (17) observed that the A^1

TABLE 1

Isoelectric points of glycosyltransferases in ovarian cyst fluids and serum determined by isoelectric focusing

Source of enzyme	Blood Group of donor	Isoelectric point of transferase				
		A-gene[a] α-GalNAc	B-gene[b] α-Gal	ß-Gal[c] Peak 1	ß-Gal[c] Peak 2	Peptidyl-[d] GalNAc
Cyst 679	A_1	10.0	-	5.2	6.5	7.0
" 705	A_1	9.5	-	4.7	6.4	7.5
" 721[e]	A_2	10.0	-	-	-	-
" 680[f]	A_2B	9.5	9.5	4.9	7.1	7.4
Serum MT	A_1	10.0	-	-	-	-
" MG (Exp 1)	A_2	6.2	-	-	-	7.2
" MG (Exp 2)	A_2	6.0	-	5.0	n.p.	6.7
" PT	A_2	6.2	-	4.6	n.p.	6.3
" 721[e]	A_2	6.8	-	-	-	-
" BP	A_2	6.3	-	-	-	7.3
" 74	A_1B	10.2	10.2	4.7	n.p.	6.8
" 680[f]	A_2B	6.2	9.7	-	-	-
" MC	B	-	9.1	5.2	n.p.	6.8

a UDP-N-acetylgalactosaminyl:α-L-fucosyl(1→2)-D-galactose N-acetylgalactosaminyl transferase

b UDP-D-galactose:α-L-fucosyl-(1→2)-D-galactose α-D-galactosyl transferase

c UDP-D-galactose:N-acetylglucosamine ß-D-galactosyl transferase

d UDP-N-acetylgalactosaminyl:peptide α-N-acetylgalactosaminyl transferase

e Cyst fluid and serum from same A_2 donor

f Cyst fluid and serum from same A_2B donor

\- not tested n.p. not present

and A^2 gene-associated α-N-acetylgalactosaminyltransferases in serum differ in their pH optima; the A^1 transferase has a lower

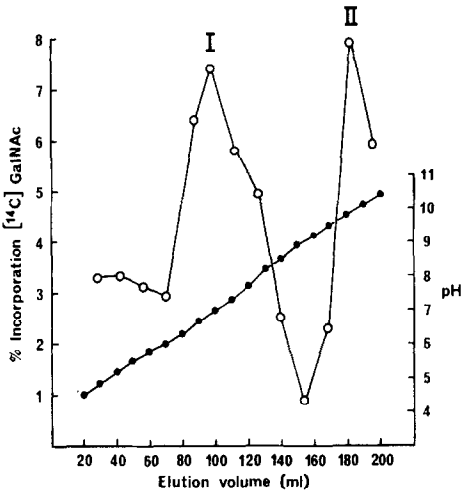

Fig. 1. Isoelectric fractionation of the α-N-acetylgalactosaminyltransferases in the serum of a person (F.B.) of the blood group genotype A^1A^2. o-o α-N-acetylgalactosaminyltransferase activity; ●-● pH.

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and narrower pH optimum than the A^2 enzyme. The A^1 transferase in the serum from the AB donor 74 (Table I) had a pH optimum of 6.5 before and after isoelectric focusing. The pH optima (7.0-8.0) of the A^2 transferases in both the cyst fluids and sera from donors 680 and 721 were unchanged after isoelectric focusing. Serum from a donor (F.B.), known from family studies to be of the genotype A^1A^2, gave two major peaks of α-N-acetylgalactosaminyltransferase activity on isoelectric focusing (Fig. 1). The pH curve for the transferase in unfractionated serum gave a broad optimum extending from pH 6.5 to 8.0. The two transferases resolved by isoelectric focusing had much sharper pH optima; peak I, focusing at pH 6.9 and presumed to be the A^2 transferase, had a pH optimum of 7.5-8.0 whereas peak II, focusing at pH 9.9 and presumed to be the A^1 transferase, had a pH optimum at pH 6.5

Two major peaks of ß-galactosyltransferase activity with differing pI values were detected in the focused products from

cyst fluids but only the one with the lower pI value was found in serum products (Table I). The ß-galactosyltransferases in either source were separated by isoelectric focusing from the B gene associated α-galactosyltransferase. The peptidyl:α-N-acetylgalactosaminyltransferases in both cyst fluids and serum focused in the region of pH 7 (Table I); this enzyme was therefore separable from the A^1 and A^2 transferases in the cyst fluid samples but in serum samples there was some overlap between the peaks of A^2 transferase activity and the peak of peptidyltransferase activity. The purified peptidyl:α-N-acetylgalactosaminyltransferase from serum had a pH optimum of 7.0-8.0 .

Isoelectric focusing of the protein fraction from cyst fluid 680 on a pH 7-10 gradient of Ampholines (LKB) yielded an A^2 enzyme that was purified 500-fold compared with the original cyst fluid and a B enzyme that was purified 200-fold.

DISCUSSION. A comparison of the properties of the A^1, A^2 and B gene-associated glycosyltransferases is of considerable interest because these enzymes are believed to be the protein products of alternative alleles at the ABO genetic locus (1,18). Differences in some kinetic properties of the α-N-acetylgalactosaminyltransferases in the serum of blood group A_1 and A_2 persons have been reported (17) and the results given in this paper reveal further dissimilarities. The isoelectric point of the major peak of α-N-acetylgalactosaminyltransferase activity in serum from A_1 persons differed widely from the major peak of activity in serum from A_2 persons. However, the differing isoelectric points of the enzymes in serum are not reflected in the pI values of the corresponding transferases in ovarian cyst fluids. That this difference is related to the tissue from

which the enzyme is obtained and not to variations in the properties of the transferases derived from different A_2 persons is demonstrated by the fact that the major peaks of α-N-acetylgalactosaminyltransferase activity in serum samples from the patients with ovarian cyst No 680 (group A_2B) and No 721 (group A_2) focused at pH 6.2 and pH 6.8, respectively, whereas the corresponding transferases in the cyst fluids focused at pH 9.5 and 10.0. These results indicate that the isoelectric point of the $\underline{A}^2$ transferase in different tissues arises from secondary modifications of the enzyme and is not a direct result of the coded amino acid sequence of the protein (see 19).

Isoelectric focusing of the serum from a person of the genotype $\underline{A}^1\underline{A}^2$ revealed two major peaks of α-N-acetylgalactosaminyltransferase activity which, on recovery, were shown to have the pH optima expected for $\underline{A}^1$ and $\underline{A}^2$ transferases (17). These results indicate that two distinct, and separable, enzyme species are produced in persons of this genotype.

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