α-N-ACETYLGALACTOSAMINYL- AND α-GALACTOSYLTRANSFERASES IN HUMAN OVARIAN CYST EPITHELIAL LININGS AND FLUIDS

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Summary

Ovarian cyst linings and fluids were examined for glycosyltransferases associated with the biosynthesis of the A and B blood group active glycoproteins. An α-N-acetylgalactosaminyltransferase that transferred N-acetylgalactosamine from UDP-N-acetylgalactosamine to low molecular weight acceptors containing the H-active structure at the non-reducing terminal was detected in both linings and particle-free fluids from ovarian cysts removed from group A subjects. An α-galactosyltransferase with the same acceptor specificity occurred in cysts from group B subjects. β-Galactosyltransferase activity was detected in both cyst linings and fluids irrespective of the ABO group of the donor.

The fluids from human ovarian cysts are a rich source of blood group A and B substances [1]. Glycosyltransferases concerned with the biosynthesis of the blood group active structures are therefore to be expected in the epithelial linings containing the mucous secreting cells [cf.2] . This paper describes the particle-bound α-N-acetylgalactosaminyl- and α-galactosyltransferases in cyst linings from subjects of the appropriate blood group and also the finding of soluble transferases in the fluid contents of the cysts. The α-galactosyltransferase that occurs only in group B subjects is distinguished from a β-galactosyltransferase that is present in both cyst linings and fluids irrespective of ABO group.

Materials and Methods UDP-N-acetyl-D-[^{14}C]-galactosamine (43 mCi/mmole) was purchased from the New England Nuclear

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Corporation, Frankfurt and UDP-D-[^{14}C] galactose from the Radiochemical Centre, Amersham. O-α-L-fucosyl-(1→2)-galactose, 2'-fucosyllactose (O-α-L-fucosyl-(1→2)-O-β-D-galactosyl(1→4)-D-glucose), lacto-N-fucopentaose 1 (O-α-L-fucosyl-(1→2)-O-β-D-galactosyl-(1→3)-O-β-(N-acetyl)-D-glucosaminyl-(1→3)-O-β-D-galactosyl-(1→4)-D-glucose) and lactodifucotetraose (O-α-L-fucosyl-(1→2)-O-β-D-galactosyl-(1→4)-[O-α-L-fucosyl-(1→3)]-D-glucose) were supplied by Dr. A. Gauhe. 3-Fucosyllactose (O-β-D-galactosyl-(1→4)-[O-α-L-fucosyl-(1→3)]-D-glucose) was a gift from Dr. J. Montreuil.

The cyst walls and fluids were chilled to +4° as soon as possible after removal from the patient and transported to the laboratory at this temperature. When visual examination of the cyst lining revealed a surface resembling a mucosal layer this area was scraped away from the underlying tissue and homogenised. In other instances where no such layer was discernible the whole tissue was homogenised. The particulate enzyme was prepared as described previously [3]. The pellet sedimenting at 100,000g from 10gm wet tissue was suspended in 1 ml of tris-HCl buffer pH 7.2 (containing 0.05M mercaptoethanol). When the particulate fraction was to be kept it was converted to an acetone powder as described in [4]. The cyst fluids were stored at +4° or dried from the frozen state. The freeze dried materials were reconstituted to the original concentration in tris-HCl buffer pH 7.2 (containing 0.05 M mercaptoethanol). Both the fresh and reconstituted freeze dried fluids were centrifuged at 100,000g for 60 minutes to remove insoluble material before inclusion in the incubation mixtures.

Paper chromatography was carried out in ethyl acetate--pyridine-water (2:1:2 by vol. upper phase; solvent a) on Whatman No. 40 paper. Radioactive peaks were detected with a

7201 Packard Radiochromatogram Scanner and counted in a Nuclear Chicago Scintillation Counter Series 720.

Details of the reaction mixtures are given in the Tables. At the end of the incubation time the neutral sugars were separated from the charged compounds by electrophoresis as described previously [3] and chromatographed in solvent a. Lactose was included as the reference standard.

The anomeric linkage of the transferred N-acetylgalactosamine was determined by treatment with an α-N-acetylgalactosaminidase from Trichomonas foetus that was free from β-activity [5]. The linkage of the transferred galactose was determined by treatment with purified α- and β-galactosidases from T.foetus [6, 7].

Results and Discussion The transfer of N-acetyl-[^{14}C]-D-galactosamine from UDP-N-acetyl-[^{14}C]-D-galactosamine to 2'-fucosyllactose by enzyme preparations from cyst walls and fluids is shown in Table 1. When the tissues were from group A patients a radioactive compound was formed that had an $R_{lactose}$ value of 0.5 in solvent a and corresponded in its chromatographic properties to the tetrasaccharide previously synthesised with this acceptor when particle-bound enzymes from group A submaxillary gland and stomach mucosal preparations were used as the enzyme source [8]. The labelled N-acetylgalactosamine was completely removed from the tetrasaccharide by treatment with an α-N-acetylgalactosaminidase, thus confirming that the sugar was transferred in α-linkage. Transferase activity was demonstrated in the particle-bound fraction from the cyst walls, in an acetone-powder prepared from a particulate fraction and also in the cyst fluids. Centrifugation of the fluids at 100,000g before inclusion in the incubation mixtures ensured that the activity detected was not attributable to microsomal particles

Table 1. α-N-Acetylgalactosaminyltransferase activity in ovarian cyst fluids and walls tested with 2'-fucosyllactose as acceptor.

Cyst No.	Blood group and secretor status of donor	Cyst fraction	Incorporation of [^{14}C]- GalNAc	
			c.p.m.	% of added radioactivity
639	A secretor	Fluid (fresh)	9,100	3.6
640	A secretor	Wall (100,000g pellet)	58,000	23
653	A non-secretor	Fluid (fresh)	6,500	2.6
652	A secretor	Fluid (fresh)	53,000	21
		Fluid (freeze dried)	77,000	31
		Wall (100,000g pellet)	7,300	3.4
658	A non-secretor	Fluid (fresh)	57,000	23
		Fluid (freeze dried)	60,000	24
		Wall (acetone powder of 100,000 pellet)	200,000	80
654	B secretor	Fluid (freeze dried)	0	0
655	O secretor	Fluid (fresh)	0	0

Abbreviation: [^{14}C]-GalNAc, N-acetyl-[^{14}C]galactosamine.

Reaction mixture: UDP-[^{14}C]GalNAc (250,000 c.p.m.) 5nmole; ATP, 1.5 μmoles; $MnCl_2$, 4.5 μmoles; Tris-HCl pH 7.2, 3.7 μmoles; sugar acceptor, 0.5 μmole; enzyme fraction, 75 μl cyst fluid (fresh or reconstituted) or 25 μl particulate suspension. Total volume 200 μl. The mixtures were incubated for 17 hours at 37°.

suspended in the fluid. The activity of the fluid was stable to freeze drying and the dried material retained activity for several months on storage at -18°. The N-acetylgalactosaminyl-transferase activity was demonstrable in cyst walls and fluids from both secretors and non-secretors of group A. The relative activities of the particle-bound and soluble preparations varied with different ovarian cysts but in some experiments areas of the cyst lining containing relatively few mucous secreting cells may have been selected. Considerable variability in activity was also observed from one cyst fluid to another but despite this all the fluids from group A subjects that were tested soon

Table 2. α-Galactosyltransferase activity in ovarian cyst fluids and walls tested with 2'-fucosyllactose as acceptor.

Cyst No.	Blood Group and Secretor status	Cyst fraction	Incorporation of [^{14}C]-Gal	
			c.p.m.	% of added radioactivity
654	B secretor	Fluid (fresh)	13,500	4.5
		Fluid (freeze dried)	19,400	6.4
		Wall (100,000g pellet)	27,000	9.0
		Wall (acetone powder of 100,000g pellet)	19,600	6.3
		Wall (supernatant from 100,000g pellet)	2,700	0.9
652	A secretor	Fluid (freeze dried)	2,600	0.9
		Wall (acetone powder of 100,000g pellet)	2,400	0.8
655	O secretor	Fluid (fresh)	0	0

Abbreviation: [^{14}C] Gal, D-[^{14}C]-galactose

Reaction mixture: UDP-[^{14}C] galactose (300,000 c.p.m.), 1 nmole; $MnCl_2$, 2.0 μmoles; ATP, 1.0 μmole; sugar acceptor, 0.5 μmole; tris-HCl buffer pH 7.2, 7.5 μmoles; enzyme fraction, 100 μl. Total volume 200 μl. The mixtures were incubated for 17 hours at 37°.

after removal from the patients had some α-N-acetylgalactosaminyltransferase activity whereas no activity was detectable in cyst fluids from group O or B patients.

The cyst fluid from the group B patient that was free from α-N-acetylgalactosaminyltransferase activity had appreciable α-galactosyltransferase activity when tested with UDP-D-[^{14}C]-galactose as sugar donor and 2'-fucosyllactose as acceptor (Table 2). The product had an $R_{lactose}$ of 0.4 in solvent a and co-chromatographed with the tetrasaccharide identified as O-α-D-galactosyl-(1→3)-[O-α-L-fucosyl-(1→2)-] O-β-D-galactosyl-(1→4)-D-glucose [4]. The radioactive galactose was hydrolysed by treatment with α-galactosidase and was not liberated by the corresponding β-enzyme. The α-galactosyl transferase activity of the fluid was retained on freeze drying and no appreciable deterioration in activity has been observed after eight months

storage of the dried cyst at -18°. A particulate preparation from the cyst wall, and an acetone powder prepared from this particulate fraction, also had α-galactosyltransferase activity. The supernatant obtained by centrifuging the homogenised tissue at 100,000g had only weak transferase activity. Slight incorporation of labelled galactose into a compound that co-chromatographed with the tetrasaccharide was also observed when the cyst wall and fluid from a group A subject was tested in this system. Similar weak α-galactosyltransferase activity has been observed occasionally with enzyme preparations from group A stomach and submaxillary glands (C. Race and W.M. Watkins, unpublished results) and may indicate that the α-N-acetylgalactosaminyltransferase does not show absolute specificity for UDP-N-acetylgalactosamine but can use UDP-D-galactose to a very limited extent. If, as has been proposed [cf.2] the α-N-acetylgalactosaminyl and α-galactosyltransferases are the products of the allelic A and B genes, then some slight degree of overlapping specificity would not be entirely surprising. No incorporation of galactose into the tetrasaccharide was observed with the cyst fluid from a group O patient.

The acceptor specificity of the α-galactosyltransferase in the ovarian cyst fluid from the group B patient was tested with a number of different oligosaccharides (Table 3). The results agree with those obtained earlier [9] for stomach mucosal and submaxillary gland enzymes, in that galactose was transferred in α-linkage only to those acceptors that had a terminal β-galactosyl residue substituted at the 2-position with α-L-fucose, that is, O-α-L-fucosyl-(1→2)-D-galactose, 2'-fucosyllactose, and lacto-N-fucopentaose 1 [cf.10] . When the lactose backbone was substituted with a second fucosyl residue, as in lacto-difucotetraose, the oligosaccharide no longer functioned as an

Table 3. Acceptor specificity of α-galactosyltransferase in ovarian cyst fluid.

Cyst No. Blood group and secretor status	Acceptor sugar	*R_{lac} of product	Incorporation of [^{14}C]Gal c.p.m.	Hydrolysis by galactosidases α	β
654 Group B Secretor	α-Fuc-(1→2)-Gal	0.8	13,900	+	-
	2'-fucosyllactose	0.4	19,400	+	-
	3-fucosyllactose	-	0		
	Lacto-N-fucopentaose 1	0.2	12,500	+	-
	Lactodifucotetraose	-	0		
	N-acetyl-D-glucosamine	1.4	174,000	-	+
	β-Gal-(1→4)-GNAc	-	0		

Abbreviations: Fuc, L-fucopyranose; GNAc, N-acetyl-D-glucosamine

Reaction mixture: As in Table 2.

*$R_{lactose}$ value in solvent a.

acceptor. Similarly a β-galactosyl residue not substituted with L-fucose, as in the disaccharide O-β-D-galactosyl-(1→4)-N-acetylglucosamine, was ineffective as an acceptor. Essentially similar results were obtained with the different oligosaccharide acceptors when the particle-bound enzyme from a group A cyst wall was used as the source of α-N-acetylgalactosaminyltransferase. These results therefore support to the proposal that an H-active structure is an essential prerequisite for the formation of A and B active structures and demonstrate the similarity in properties of the transferases found in cyst walls and fluids with those occurring as particle-bound enzymes in stomach mucosal tissue and submaxillary glands [8, 9] and as soluble enzymes in human milk [11, 12].

Examination of N-acetylglucosamine as a galactose acceptor (Table 3) indicated that this sugar was a more avid acceptor than any of the others tested. However, the radioactive product co-chromatographed with the disaccharide O-β-D-galactosyl-(1→4)-N-acetylglucosamine and was completely hydrolysed by β-galactosidase and not by α-galactosidase. This sugar therefore

revealed the presence of a β-galactosyltransferase in the group B cyst fluid. When group A and O cysts were tested for β-galactosyltransferase activity, with N-acetylglucosamine as acceptor, strong activity was detected in the fluids and in the particulate preparation from the cyst walls.

Soluble α-N-acetylgalactosaminyl- and α-galactosyl transferases associated with the A and B blood groups, respectively, occur in human milk [11, 12] and recently have been reported in human serum [13, 14]. The soluble transferases in ovarian cyst fluids may arise from the serum or as breakdown products of the cells in the epithelial tissue that synthesise the blood group active glycoproteins. Large volumes of fluid are sometimes obtained from single ovarian cysts and these pathological secretions, therefore, constitute a potentially valuable source from which to isolate and purify the transferases.

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