GLYCOSYLTRANSFERASES OF HUMAN PLASMA

T. SAWICKA*
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

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1. Introduction

The enzyme which catalyzes the following reaction:

$$\text{UDP–GalNAc} + \text{Gal}\ldots\ldots \rightarrow \underset{\underset{\text{Fuc}\alpha 1}{|}}{\overset{2}{\text{GalNa}\alpha 1}} \rightarrow \underset{\underset{\text{Fuc}\alpha 1}{|}}{\overset{2}{\text{Gal}}} + (\text{UDP})$$

was previously shown to occur in the soluble form in human milk from donors with A or AB blood type and is under control of the A gene [1, 2]. The A enzyme (*N*-acetyl-D-galactosaminyltransferase) has been found also in the human submaxillary glands [3] and gastric mucosa [4].

The B enzyme (α-D-galactosyltransferase) catalyzes a reaction identical to that catalyzed by the A enzyme except galactose is transferred in place of *N*-acetyl-galactosamine [5]. The B enzyme is present in milk from donors or B of AB blood type and is absent in milk from donors with A or 0 blood type [5]. The occurrence of A and B enzymes in human milk are independent of secretor status of donors. The B enzyme has also been found in gastric mucosa and submaxillary glands of human and baboon [6, 7].

This communication describes the occurrence of A and B enzyme in human plasma from individuals of A and B blood type respectively. Both enzymes occur in plasma of donors with AB blood type and are absent in plasma from individuals of 0 blood type.

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2. Materials and methods

UDP-*N*-acetyl-D-galactosamine labeled with ^{14}C in the acetyl group (5.0 mCi/mM) and UDP-Galactose (Galactose-UL-^{14}C), (31 mCi/mM) were obtained from the International Chemical and Nuclear Corporation (Irvine, California). 2′-Fucosyllactose and Lacto-*N*-fucopentaose-I-COOH with structure**:

$$\underset{\underset{\alpha 1}{|}}{\overset{\text{Fuc}}{\overset{2}{\text{Gal}\beta 1\text{–}3\text{GlcNAc}\beta 1\text{–}3\text{Gal}\beta 1\text{–}4\text{Glc–COOH}}}}$$

(LNF–I–COOH)

were kindly provided by Dr. A. Kobata. The blood type of the donors was determined on samples of blood by Mrs. Mary McGinniss of the blood bank of the National Institutes of Health. Blood was collected using citric acid as anticoagulant. Plasma was prepared freshly for each experiment. The amount of plasma protein used for the assays varied from 0.14 mg to 1 mg per sample. Protein concentration was estimated, using a value of 7.3 g/100 ml as a theoretical concentration of protein in human plasma.

A simple method for assaying A and B enzyme, devised by Kobata [8] was adopted here.

The incubation mixture for the A enzyme assay contained: 0.005 μmole UDPGalNAc (7×10^4 cpm),

** *Abbreviations*:
GalNAc = *N*-acetyl-D-galactosamine
GlcNAc = *N*-acetyl-D-glucosamine
Gal = D-galactose
Fuc = L-fucose
Glc = D-glucose

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0.05 μmole LNF–I–COOH, 1 μmole $MnCl_2$, 1.5 μmole Tris buffer pH 7.5 and plasma in a total volume of 85 μl.

The incubation mixture for the B enzyme assay contained: 0.015 μmole UDPGal (9×10^4 cpm), 0.05 μmole LNF–I–COOH, 0.2 μmole $MnCl_2$, 1.5 μmole Tris buffer pH 7.5 and plasma in a total volume of 85 μl.

One drop of toluene was added and the mixtures incubated at 37° for 16 hr.

The solutions were transferred to Whatman No. 3 MM paper as 3 cm band and subjected to electrophoresis in pyridine/acetic acid buffer pH 5.4 [9] for 110 min at 50 V/cm in a high voltage electrophorator (Gilson Medical Electronics, Middleton, Wisconsin). The section of the chromatogram containing the product was localized using LNF–I–COOH as standard stained with benzidine reagent [10].

Chromatograms were scanned for radioactivity using Vanguard automatic chromatogram scanner. Areas containing radioactive product were cut out and counted in 5 ml Bray's solution [11] in a scintillation counter. Background of ^{14}C activity was determined from the corresponding area of another chromatogram derived from an incubation mixture in which LNF–I–COOH was omitted.

3. Results and discussion

Table 1 summarizes the distribution of the A and B enzymes in human plasma. In washed and hemolysed human erythrocytes no A enzyme activity was found. However, some B enzyme activity was present in hemolysed erythrocytes. Under the experimental condition described here no B enzyme activity was detected in serum, whereas A enzyme was detected. One, from various possible explanations of this phenomenon may be that proteins of B enzyme coagulate during clot formation. The results presented in table 2 show that 2′-fucosyllactose can also serve as acceptor for both glycosyltransferases tested.

Glycosyltransferases of human plasma have properties similar to those described for human milk. This seems very likely, as milk enzymes are derived from plasma. The physiological role of glycosyltransferases occurring in human plasma is so far unknown.

4. Acknowledgements

The author wishes to express her gratitude to Dr. Akira Kobata for supplying oligosaccharide acceptors and for the personal communication of the method of enzyme assay (using LNF–I–COOH) before publication.

Table 1

Distribution of A enzyme (UDP-GalNAc: galactose o <-3-*N*-acetylgalactosaminyltransferase) and B enzyme (UDPGal: galactose o <-3-galactosyltransferase) in human plasma using LNF–I–COOH as acceptor.

Donor	Blood AB0	Secretor status	Sugar transferred to LNF–I–COOH	
			N-Acetylgalactosamine ^{14}C	Galactose ^{14}C
			cpm/16 hr/mg of protein	
F.S.	A	Secretor	879	0
E.R.	A	Secretor	300	22
B.H.	B	Secretor	10	153
A.W.	B	Secretor	3	286
E.K.	AB	Secretor	230	233
C.Q.	AB	Non secretor	242	188
Mc. G.	0	Non secretor	15	25
J.H.	0	Secretor	30	14

Assay conditions as reported in sect. 2.

Table 2
N-Acetylgalactosaminyltransferase and galactosyltransferase activity of human plasma using 2'-fucosyllactose as an acceptor.

Donor	Blood AB0	Sugar transferred to 2'-fucosyllactose	
		N-Acetylgalactosamine ^{14}C	Galactose ^{14}C
		cpm/16 hr/mg of protein	
F.S.	A	1160	–
B.H.	B	–	350
Mc.G.	0	0	0

– = not tested.
Assay conditions as reported under Materials and methods except that 0.5 μmole of 2'-fucosyllactose was used as acceptor in place of LNF–I–COOH. Products were isolated and characterized by paper chromatography according to methods described by Kobata, Grollmann and Ginsburg [1, 5].

Note: During the course of this work a report was published by S. Mookerjea, A. Chow and R.L. Hudgin describing an occurrence of *N*-acetylglucosaminyltransferase in human and rat sera. Can. J. Biochem. 49 (1971) 297.

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