AN ENZYMATIC BASIS FOR BLOOD TYPE B IN HUMANS

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Glycosyltransferases responsible for the synthesis of milk oligosaccharides are found in milk in a soluble form. The presence of some of these enzymes in individual samples of milk can be correlated with the blood type of the donor indicating that they are also involved in the formation of blood group substances. Thus, milk from individuals who are unable to synthesize soluble blood group substances A, B, or O(H), or who have the blood type Le(a-b-), lacks specific fucosyltransferases (Shen et al, 1968; Grollman et al, 1968); and a specific N-acetyl-D-galactosaminyltransferase is found only in milk from individuals with blood type A (Kobata et al, 1968).

A D-galactosyl residue linked α 1-3 to a second D-galactosyl residue is a determinant for blood type B (Watkins, 1966). This communication describes a D-galactosyltransferase that occurs in milk from individuals with blood type B or AB and is absent in milk from individuals with blood type A or O. The enzyme transfers D-galactose from UDP-D-galactose to 2'-fucosyllactose and lacto-N-fucopentaose I but not to lactose, lacto-difucotetraose, lacto-N-tetraose, lacto-N-fucopentaose II, lacto-N-difucohexaose I, or lacto-N-difucohexaose II (for the structures of these compounds see Kobata et al, 1968). This acceptor specificity is the same as previously reported for the N-acetyl-D-galactosaminyltransferase in milk from individuals with blood type A.

The chromatographic mobilities of the enzymatic products shown in Fig. 1A indicate that a tetrasaccharide (Peak I) was formed when 2'-fucosyllactose was

used as an acceptor and that a hexasaccharide (Peak I') was formed when lacto-N-fucopentaose I was used as an acceptor. The formation of radioactive lactose

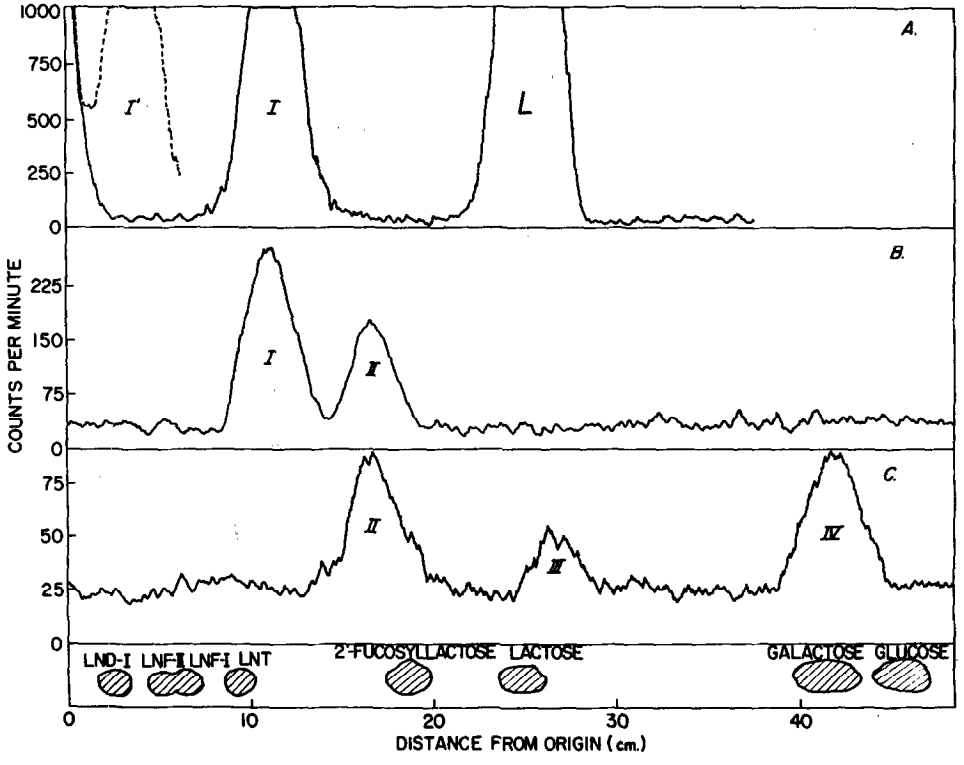

Fig. 1A. Chromatography of radioactive oligosaccharides formed by a galactosyltransferase of human milk using 2'-fucosyllactose (solid line) or lacto-N-fucopentaose I (broken line) as acceptors. The incubation mixture contained 23 mμmoles UDP-D-galactose-^{14}C (2 x 10^7 cpm/μmole as measured with a scintillation counter), 30 mμmoles 2'-fucosyllactose (or lacto-N-fucopentaose I), 125 μmoles $MnCl_2$, 100 μl of milk treated with Sephadex (Shen et al, 1968), and 2 μmoles of Tris buffer, pH 7.5, in a final volume of 110 μl. Toluene, 25 μl, was added and the mixture incubated at 37° for 20 hours. After heating at 100° for 1 min., the mixture was then passed through a column containing 0.5 ml of Dowex 1x8 (Cl^- form) previously washed with 0.02 M lactose followed by H_2O (Babad and Hassid, 1966). The oligosaccharides in the effluent were chromatographed on Whatman 3 MM paper for 30 hours using ethyl acetate - pyridine - H_2O (2:1:2) as the solvent. The resulting chromatogram was scanned for ^{14}C-activity. Fig. 1B. Partial hydrolysis of Peak I. An aliquot of material from Peak I, Fig. 1A (10,000 cpm) was heated at 100° for 40 min. in 0.01 N HCl and the hydrolyzate chromatographed under the conditions given above. Fig. 1C. Partial hydrolysis of Peak II. An aliquot of Peak II, Fig. 1B (2,000 cpm) was heated at 100° for 30 min. in 0.1 N HCl and the hydrolyzate chromatographed under the conditions given above.

The abbreviations used for the standard sugars are as follows: LND-I, lacto-N-difucohexaose I; LNF-II, lacto-N-fucopentaose II; LNF-I, lacto-N-fucopentaose I; and LNT, lacto-N-tetraose.

(Peak L) and the peak at the origin was not dependent on the added acceptors and occurred with all samples of milk regardless of the donor's blood type. Mild acid hydrolysis of the product derived from 2'-fucosyllactose, under conditions which selectively cleave fucosidic linkages, led to the formation of an oligosaccharide with the chromatographic properties of a trisaccharide (Fig. 1B, Peak II). Hydrolysis of Peak II under stronger conditions resulted in the formation of what appeared to be a disaccharide and galactose (Fig. 1C, Peaks III and IV). The labeled galactose was present in an α-linkage since both Peaks II and III were completely hydrolyzed and liberated free, radioactive galactose on treatment with an α-galactosidase prepared from coffee beans (Courtois and Petek, 1966). The unhydrolyzed product (Peak I) was not a substrate for the α-galactosidase, presumably because of interference by the fucosyl residue. The correlation of the occurrence of the enzyme with blood type B shown in Table I suggests that the enzyme is responsible for the formation of B-active structures and that Peak II of Fig. 1 is Gal-α-(1→ 3)-Gal-β-(1→ 4)-Glc and Peak III is Gal-α-(1→ 3)-Gal.

Fig. 2 summarizes the probable reactions catalyzed by four glycosyltransferases of human milk whose occurrence has thus far been correlated with blood type. The presence or absence of these four enzymes would control the formation of the five heterosaccharides that are shown with the serologic activity attributed to each. These findings agree with the proposal by Watkins and Morgan (1959) that differences in blood type among individuals result from the genetically determined presence or absence of specific glycosyltransferases. Thus, the structural determinants of blood type can be considered to be secondary gene products - the direct product of genes that determine blood type would be enzymes and it is these enzymes working in concert that would be responsible for the formation of specific antigenic structures. In the scheme of Watkins and Morgan (see Watkins, 1966), the B gene would produce the D-galactosyltransferase of the present paper, the A gene would produce the N-acetyl-D-galactosaminyltransferase (Kobata et al, 1968), and the H gene (in conjunction with

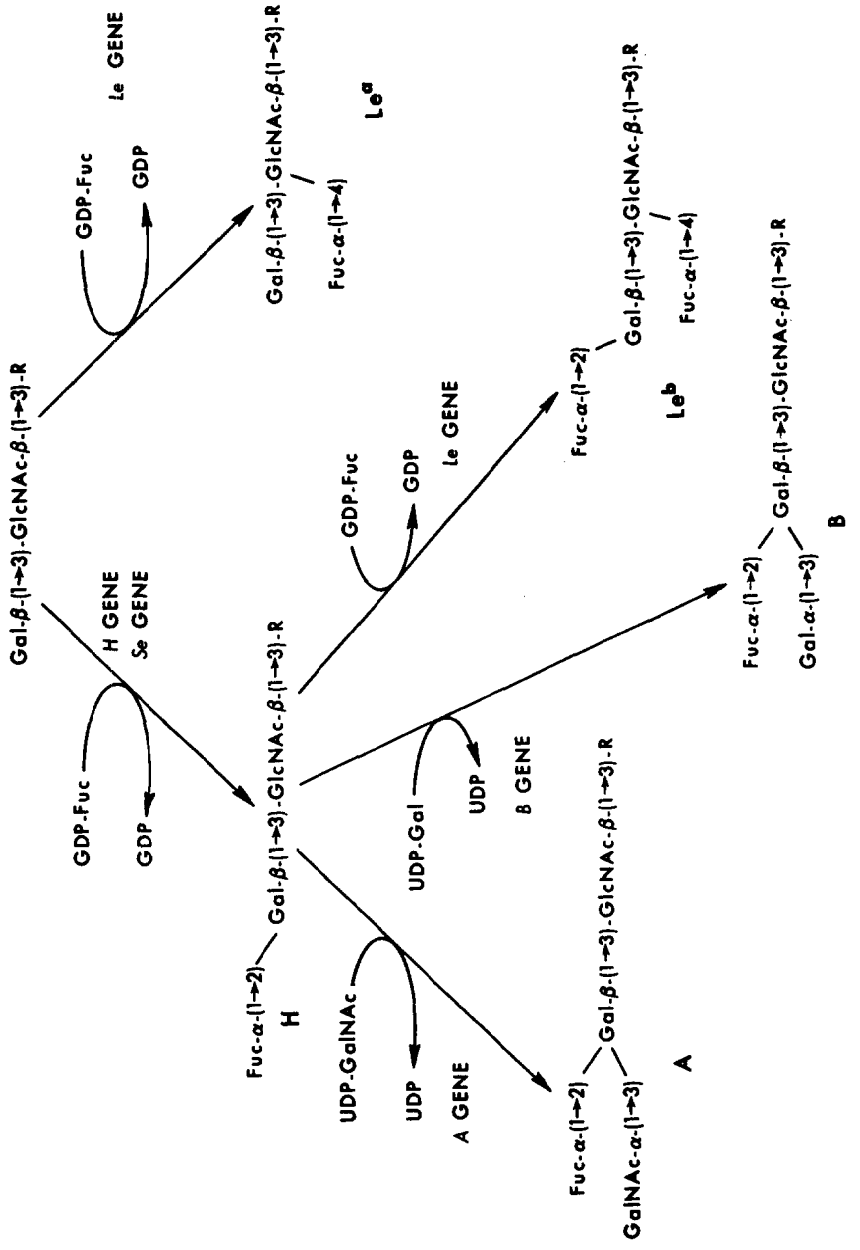

Fig. 2. Probable reactions catalyzed by glycosyltransferases of human milk whose occurrence can be correlated with blood type. R = lactose.

the Se gene) and the Le gene would produce the two different L-fucosyltransferases (Shen et al, 1968; Grollman et al, 1968) as shown in Fig. 2.

Brew, Vanaman and Hill, 1968, have recently reported that a transglycosylase catalyzing the synthesis of N-acetyllactosamine (McGuire et al, 1965) can be converted to lactose synthetase by the presence of α-lactalbumin. It is not known whether similar relationships exist between other transglycosylases such as those of Fig. 2.

Table I

Galactosyltransferase activity of human milk using 2'-fucosyllactose as an acceptor. The conditions of incubation and chromatography are given in the legend of Fig. 1. The ^{14}C-activity incorporated into the oligosaccharide was determined by counting appropriate sections of the chromatogram in a scintillation counter (Shen et al, 1968). These values were corrected for background levels of radioactivity as determined with sections of similar size cut from both sides of Peak I, Fig. 1. A zero in the column below means that the radioactivity in the Peak I area of the chromatogram did not differ significantly from background levels which were approx. 300 cpm in the different experiments.

Donor	Blood Type of Donor	Secretor Status	Galactose-^{14}C Transferred to 2'-fucosyllactose (cpm)
H.G.	B	Secretor	400
M.M.	B	"	1,700
B.O.	B	"	1,000
S.M.	B	"	1,100
S.L.	B	"	13,400
C.J.	A_1B	"	1,100
M.J.	A_1	"	0
K.M.	A_1	"	0
E.K.	A_2	"	0
J.L.	0	"	0
D.C.	0	"	0
S.J.	0	Nonsecretor	0
R.D.	0	"	0

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