CORRELATION BETWEEN SECRETOR STATUS AND THE OCCURRENCE OF 2'-FUCOSYLLACTOSE IN HUMAN MILK

Evelyn F. Grollman and Victor Ginsburg

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland 20014

Received May 25, 1967

2'-Fucosyllactose[1], a component of human milk, was isolated and characterized by Kuhn, Baer and Gauhe (1955). The presence of this trisaccharide was shown to be independent of the bloodgroup type (ABO system) of the mother. The present paper confirms this finding. However, a direct correlation can be seen between the occurrence of the trisaccharide and secretor status as shown in Table I. No 2'-fucosyllactose is found in the milk or colostrum of non-secretors (1 μg/ml would have been detected) while its level in the milk or colostrum of secretors agrees with the published values of 0.30 - 0.33 mg/ml which were determined in pooled samples[2] (Kuhn et al, 1955). Total hexose is normal in all samples.

Eighty percent of the population (secretors) have in body secretions soluble bloodgroup substances (A, B, and O(H)) corresponding to their individual bloodgroup type. Approximately 20% of the population (non-secretors) do not have these substances in their secretions which instead contain Le^a substance (see Kabat, 1956). Unlike bloodgroup substances with A, B, and H specificity, Le^a substance does not contain L-fucose attached to a galactosyl residue in an α 1,2 linkage—the same grouping found in 2'-fucosyllactose.

---

[1] O-α-L-fucopyranosyl-(1 → 2)-O-β-D-galactopyranosyl-(1 → 4)-D-glucose. An isomer of this compound (3-fucosyllactose) in which the fucose is linked to the 3-position of glucose has also been reported to occur naturally (Polonovski and Montreuil, 1954). However, the two isomers can be separated chromatographically and the 6 samples of trisaccharide isolated in the present work are predominantly if not exclusively the 2' isomer.

[2] About 20% of the donors would be expected to be non-secretors (Kabat, 1956).

Table I

Occurrence of 2'-Fucosyllactose in Human Milk and Colostrum

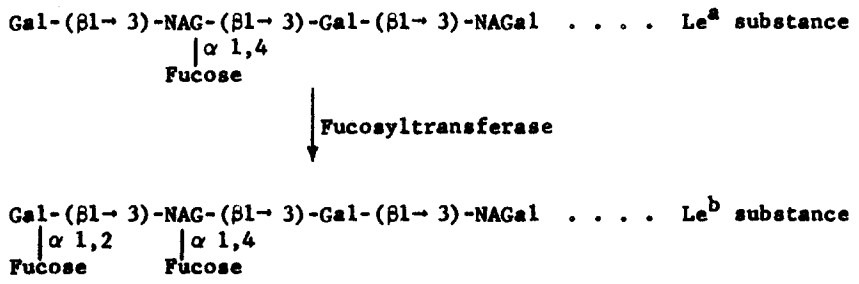
Donor	Blood Type	Secretor Status [a]	Bloodgroup Substance in Saliva [a]	Time Postpartum	2'-Fucosyllactose in milk or colostrum [b]	Total hexose in milk or colostrum [c]
					mg/ml	mg/ml
1	A	Secretor	A,H	1-3 days	0.33	70.2
2	A	"	H	1-3 days	0.24	72.0
3	A	"	A,H	5 weeks	0.46	81.0
4	B	"	B,H	1-3 days	0.36	81.1
5	B	"	B,H	1-3 days	0.34	68.4
6	O	"	H	6 weeks	0.31	44.2
1	A	Non-Secretor	none	8 weeks	< 0.001	76.7
2	A	"	"	1-3 days	"	31.8
3	A	"	"	3 weeks	"	74.0
4	O	"	"	1-3 days	"	64.1
5	O	"	"	1-3 days	"	52.0
6	O	"	"	5 weeks	"	96.4

[a] Secretor status is determined on undiluted saliva using a standard hemagglutination inhibition test (Kabat, 1956) with human anti A and anti B sera, and Ulex europaeus extract. Milk could also be used to determine secretor status (Lawler, 1960).

[b] 2'-Fucosyllactose is isolated and determined colorimetrically using methods previously described (Grollman et al, 1965). Deproteinized, defatted milk or colostrum, 0.5 ml, is passed through a charcoal column (16 x 6 cm) of equal parts of activated carbon (Darco G-60) and Celite No. 535. The column is eluted with 1 liter H_2O, then 2.5 liter 8% ethanol, and finally 1.75 liter 15% ethanol. The last fraction, which contains the trisaccharide, is reduced in volume in vacuo, applied as a 3-inch band on Whatman #1 filter paper and chromatographed for 72 hours using pyridine-ethyl acetate-water (1:3.6:1.15) as the solvent. The 2'-fucosyllactose area of the chromatogram is eluted with H_2O and 2'-fucosyllactose in the eluate determined with the cysteine-sulfuric acid reaction (Dische and Shettles, 1948) using authentic 2'-fucosyllactose (kindly provided by Dr. R. Kuhn and A. Gauhe) as a standard.

[c] Total hexose is determined in defatted, deproteinized milk or colostrum with anthrone reagent (Roe, 1955) using galactose as a standard.

It is this L-fucosyl residue that plays a role in the determination of Le^b as well as H specificity. The specific structures responsible for the Le^a and Le^b specificity of soluble bloodgroup substances and their biosynthetic relationship proposed by Watkins and Morgan (1959) (Watkins, 1966) are as follows[3]:



In their proposal, the H gene would be responsible for the fucosyltransferase that adds L-fucose, presumably from GDP-L-fucose[4], to the terminal D-galactosyl residue of Le^a substance and converts Le^a substance to Le^b substance (as shown above) and that also adds L-fucose in the same linkage to "precursor" substance converting it to H substance. H substance would then be converted to A or B substance by the addition of N-acetyl-D-galactosamine or D-galactose, respectively, to the heterosaccharide chains. The secretory organs of nonsecretors would lack the fucosyltransferase activity coded for by the H gene and would therefore secrete Le^a substance irrespective of their bloodgroup type. The data presented in this paper support this hypothesis.

2'-Fucosyllactose is synthesized by transfer of L-fucose from GDP-L-fucose to the galactosyl moiety of lactose (Grollman et al, 1965). The simultaneous absence in non-secretors of 2'-fucosyllactose and glycoproteins

---

[3] Gal = D-galactose; NAG = N-acetyl-D-glucosamine; NAGal = N-acetyl-D-galactosamine.

[4] Incorporation into bloodgroup substance of L-fucose from GDP-L-fucose (Grollman and Marcus, 1966) as well as N-acetyl-D-galactosamine from UDP-N-acetyl-D-galactosamine (Tuppy and Staudenbauer, 1966) has been observed with preparations of hog gastric mucosa.

containing O-α-L-fucopyranosyl-(1→ 2)-O-β-D-galactopyranosyl structures in secretion makes it likely that the fucosyltransferase responsible for the synthesis of 2'-fucosyllactose is also involved in the synthesis of soluble bloodgroup substances and that its presence or absence determines secretor status.

More L-fucose in low molecular weight material is found in the urine of secretors than in the urine of non-secretors (Evans et al, 1964; Lundblad, 1966). This material, which includes oligosaccharides, has not been characterized.

Acknowledgement

We wish to acknowledge the expert technical assistance of Mrs. Barbara Torain and to thank Dr. Jaye Grollman for supplying the samples of milk.

References

Dische, Z., and Shettles, L. B., J. Biol. Chem., 175, 595 (1948).
Evans, D. A. P., McConnell, R. B., and Donohoe, W. T. A., J. Lab. Clin. Med., 64, 581 (1964).
Grollman, A. P., Hall, C. W., Ginsburg, V., J. Biol. Chem., 240, 975 (1965).
Grollman, A. P., and Marcus, D. M., Biochem. Biophys. Res. Commun., 25, 542 (1966).
Kabat, E. A., Blood Group Substances, Academic Press, Inc., New York (1956).
Kuhn, R., Baer, H. H., and Gauche, A., Chem. Ber., 88, 1135 (1955).
Lawler, S. D., Proc. 7th Congr. europ. Soc. Haemat., London, 1959; part II, p. 1219 (1960).
Lundblad, A., Biochim. Biophys. Acta, 130, 130 (1966).
Polonovski, M., and Montreuil, J., Compt. rend., 238, 2263 (1954).
Roe, J. H., J. Biol. Chem., 212, 335 (1955).
Tuppy, H., and Staudenbauer, W. L., Nature, 210, 316 (1966).
Watkins, W. M., and Morgan, W. T. J., Vox Sang. (Basel), 4, 97 (1959).
Watkins, W. M., Science, 152, 172 (1966).