76. On a Le^a Substance-Decomposing Enzyme. III
Chemical Effects of the Enzyme on Blood Group
Substances from Red Cells

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Concerning the determinant of Le^a specificity, Watkins and Morgan¹⁾⁻³⁾ estimated that a branched trisaccharide unit, in which α -L-fucosyl unit by 1 $\rightarrow$ 4 linkage and β -D-galactosyl unit by 1 $\rightarrow$ 3 linkage are attached to N-acetylglucosamine, would constitute its important part, because the reaction of anti-Le^a agglutinin was inhibited most strongly by lacto-N-fucopentaose II⁴⁾ and fairly well by lacto-N-difucohexaose II.⁵⁾ It was elucidated by Watkins and Morgan¹⁾ that Le^a activity of lacto-N-fucopentaose II was destroyed by the crude *Trichomonas foetus* enzyme preparation to be broken down into its component sugars.

Watkins⁶⁾ clarified that Le^a-decomposing enzyme from *T. foetus* splits lacto-N-fucopentaose II into lacto-N-tetraose⁷⁾ and fucose. Recently Rege *et al.*⁸⁾ reported that from Le^a substance of human ovarian-cyst fluids was obtained not only 4-O- β -D-galactopyranosyl-N-acetyl-D-glucosamine (N-acetyl-lactosamine), which is believed to be the antigenic determinant of an antigen common to the blood group substance and *Diplococcus pneumoniae* type XIV, but also a trisaccharide, 3-O- β -D-galactopyranosyl-4-O- α -L-fucopyranosyl-N-acetyl-D-glucosamine, which inhibits the agglutination of Le^a erythrocytes by human anti-Le^a serum, to a degree only slightly less than that of lacto-N-fucopentaose II, but not the agglutination of O cells by anti-H reagents. The trisaccharide is therefore considered the Le^a determinant structure.

Previously, Furukawa and Iseki⁹⁾ clarified that Le^a-decomposing enzyme from *Bacillus cereus* Le^a liberated fucose and galactose from Le^a substance. The present paper concerns chemical effects of the enzyme on blood group substances from red cells.

Materials and Methods. 1. Crude glycolipid was prepared by the Yamakawa and Iida's method,¹⁰⁾ and analysis of sugar constituents was performed by a method reported by Fujisawa *et al.*¹¹⁾

2. Le^a-decomposing enzyme was prepared by a method reported by Furukawa *et al.*¹²⁾

Results. The Le^a-decomposing enzyme from *Bac. cereus* Le^a

was incubated with red cells and crude glycolipid of blood group OLe(a + b -) and OLe(a - b +), the mixtures were centrifuged, and both the dialyzable and the non-dialyzable portion were examined for sugar constituents, with the results as shown in the Table.

Table. Chemical effects of Le^a-decomposing enzyme on blood group substances from O red cells

Blood group substances	Enzyme action	Methylpentose (as fucose) (μg)	Hexose (as galactose) (μg)	Hexosamine (as glucosamine) (μg)
OLe (a + b -)				
Red cells (20 ml)	Before	620	1560	1100
	After NP DP	550 52 (8.4%)	1320 210 (13.5%)	1100 0
Glycolipid (200 mg)	Before	435	3860	2150
	After NP DP	390 48 (11.1%)	3650 160 (4.1%)	2100 0
OLe (a - b +)				
Red cells (20 ml)	Before	645	1730	1270
	After NP DP	640 0	1620 92 (5.3%)	1220 0
Glycolipid (200 mg)	Before	467	4100	2260
	After NP DP	467 0	3910 78 (1.9%)	2260 0

DP: dialyzable portion, NP: non-dialyzable portion.

To 20 ml of OLe(a + b -) red cells, the equal volume of 1 % enzyme solution was added, the mixture was incubated at room temperature for 24 hours to abolish the Le^a activity, and then it was subjected to dialysis.

In the dialysate, fucose as methylpentose was demonstrated in an amount of 8.4 % of the original quantity and galactose as hexose in an amount of 13.5 % of the original quantity.

To phosphate buffer, containing 200 mg of crude glycolipid from OLe(a + b -) red cells, the equal volume of 1 % enzyme solution was added, the mixture was incubated at 37°C for 5 days to abolish Le^a activity, and then it was dialyzed.

In the dialysate, fucose was demonstrated in an amount of 11.1 % of the original quantity and galactose in an amount of 4.1 % of the original quantity.

When the enzyme was applied on 20 ml of OLe(a - b +) red cells, the dialysate contained 5.3 % of the original amount of galactose, and when crude glycolipid from OLe(a - b +) red cells was subjected to the enzyme action, the dialysate contained about 1.9 % of the original amount of galactose.

Discussion. Application of the Le^a-decomposing enzyme preparation from *Bac. cereus* Le^a liberated from OLe(a + b -) red cells and

crude glycolipid, extracted from them, similarly as from water-soluble Le^a substance, fucose and galactose, which are assumed to be the determinants of the Le^a specificity,¹⁾⁻³⁾ abolishing as the result the Le^a activity. It can consequently be assumed that the Le^a determinant structure of red cells will be similar to that of the water-soluble Le^a substance. When this enzyme preparation was applied on $\text{OLe}(a - b +)$ red cells and crude glycolipid from them, only galactose was liberated in half the amounts freed from $\text{OLe}(a + b -)$ red cells and their crude glycolipid, respectively, but not fucose.

It is considered that the fucosidase in the enzyme preparation, similarly as that of Le^a -decomposing enzyme from *Trichomonas foetus*, reported by Watkins,⁶⁾ will split α -L-fucosyl unit with $1 \rightarrow 4$ linkage, which is believed to be the determinant of Le^a specificity, but that the galactosidase in the enzyme preparation will liberate not only galactose, a sugar constituent of the Le^a determinant structure, but also galactose, which is unrelated to the Le^a specificity.

From the amount of fucose, liberated from $\text{OLe}(a + b -)$ red cells by the action of Le^a -decomposing enzyme, the molecule number of fucose as the determinant of the Le^a specificity per one red cell was computed to be about 955,000, using Avogadro number and taking number of red cells per 1 mm^3 as 10,000,000. The molecule number of fucose, attached to one red cell as the determinant of Le^b specificity, which was computed from difference between amounts of fucoses, liberated from $\text{OLe}(a - b +)$ red cells by $\text{O}(\text{H})$ - and Le^b -decomposing enzyme and by $\text{O}(\text{H})$ -decomposing enzyme was about 500,000.¹³⁾

Summary. 1. When $\text{OLe}(a + b -)$ red cells or crude glycolipid extracted from them were incubated with Le^a -decomposing enzyme from *Bac. cereus* Le^a , fucose and galactose were liberated, as the result abolishing Le^a activity. In this case, about 8.4 % of the original amount of fucose and about 13.5 % of the original amount of galactose were liberated from the red cells, and about 11.1 % of the original amount of fucose and about 4.1 % of the original amount of galactose from the crude glycolipid. Incubation of $\text{OLe}(a - b +)$ red cells with this enzyme preparation resulted in the liberation of a small amount of galactose unrelated to the blood group specificity.

2. From the amount of fucose liberated from red cells by the enzyme action, the molecule number of fucose attached to one red cell as the determinant of the Le^a specificity was computed.

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