122. Chemical Effects of O(H)-Decomposing Enzymes on Blood Group Substances from Red Cells

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(Comm. by Tanemoto FURUHATA, M.J.A., Sept. 12, 1963)

Morgan¹⁾ reported that an enzyme from *Clostridium welchii* exerted no action on red cells, but acted on red cell stroma, especially on that of group O to abolish its blood group activity. Watkins and Morgan,²⁾ investigating on enzyme from *Trichomonas foetus*, and Naylor and Baer,³⁾ investigating on enzyme from *Bacillus fulminans*, reported that they destroyed O(H) antigen on human red cells. Iseki and Furukawa⁴⁾ clarified that when O red cells were acted on by enzyme from *Bac. fulminans*, they lost O(H) antigen which reacts with eel serum, and came to increase an antigenicity common to *Shigella dysenteriae*, and that⁵⁾ when acted on by enzyme from *Bacillus cereus*, they lost O(H) antigen completely, and came to increase an antigenicity common to *Diplococcus pneumoniae* Type XIV.

This report deals with chemical effects of O(H)-decomposing enzymes from *Bac. fulminans* and *Bac. cereus* on red cells and blood group active substance derived from them.

Materials and methods. 1. Preparation of red cell stroma and crude glycolipid, and analysis of sugar composition were made by the methods as described in the previous report.⁶⁾ 2. O(H)-decomposing enzymes from *Bac. fulminans* and *Bac. cereus* were prepared by the method of Iseki *et al.*⁷⁾ The enzyme preparation from *Bac. fulminans* was further subjected to purification using DEAE cellulose column and Sephadex-G-100 column, and 19 fractions were obtained. Fractions 4~13 contained both O(H)- and Le^b-decomposing enzyme, while fractions 14~18 contained only the O(H)-decomposing enzyme.

Results. 1. Changes in specific activity of blood group substances from red cells induced by the enzymes. When a preparation of the O(H)-decomposing enzyme (Fr. 14~18) from *Bac. fulminans* was incubated with red cells, stroma, and crude glycolipid of blood group O, reactivity with anti-O(H) antibody in eel serum was abolished, and the antigenicity common to *Sh. dysenteriae* was enhanced. When a preparation of the O(H)-decomposing enzyme from *Bac. cereus* was incubated with red cells, stroma, and crude glycolipid of blood group O, O(H) activity was abolished and the antigenicity common to *Dip. pneumoniae* Type XIV was enhanced (Table I).

Table I. Serological changes in O(H)-active substances of red cells induced by the O(H)-decomposing enzyme (Fr. 14~18) from *Bac. fulminans* and the O(H)- and Le^b-decomposing enzyme from *Bac. cereus* (Agglutination inhibition test)

O(H) substance	Enzyme action	Antiserum	Antigen dilution			
			4	8	16	32
Red cells 50% (v/v)	Before	Eel anti-O(H)	—	—	—	+
		Chicken anti-Shiga	—	—	+	+
		Chicken anti-Pn. XIV	—	—	+	+
	After <i>Bac. fulminans</i>	Eel anti-O(H)	+	+	+	+
		Chicken anti-Shiga	—	—	—	+
		Chicken anti-Pn. XIV	—	—	±	+
	After <i>Bac. cereus</i>	Eel anti-O(H)	+	+	+	+
		Chicken anti-Shiga	+	+	+	+
		Chicken anti-Pn. XIV	—	—	—	+
Stroma 2.5% (w/v)	Before	Eel anti-O(H)	—	—	—	+
		Chicken anti-Shiga	—	—	+	+
		Chicken anti-Pn. XIV	—	—	+	+
	After <i>Bac. fulminans</i>	Eel anti-O(H)	+	+	+	+
		Chicken anti-Shiga	—	—	—	+
		Chicken anti-Pn. XIV	—	—	±	+
	After <i>Bac. cereus</i>	Eel anti-O(H)	+	+	+	+
		Chicken anti-Shiga	+	+	+	+
		Chicken anti-Pn. XIV	—	—	—	+
Glycolipid 0.5% (w/v)	Before	Eel anti-O(H)	—	—	+	+
		Chicken anti-Shiga	—	—	+	+
		Chicken anti-Pn. XIV	—	—	+	+
	After <i>Bac. fulminans</i>	Eel anti-O(H)	+	+	+	+
		Chicken anti-Shiga	—	—	—	+
		Chicken anti-Pn. XIV	—	—	±	+
	After <i>Bac. cereus</i>	Eel anti-O(H)	+	+	+	+
		Chicken anti-Shiga	+	+	+	+
		Chicken anti-Pn. XIV	—	—	—	+

2. Chemical changes in blood group substances of red cells induced by the enzymes. O red cells and O(H)-active crude glycolipid derived from them were incubated with the preparation of the O(H)-decomposing enzyme (Fr. 14~18) of *Bac. fulminans* and that of the O(H)- and Le^b-decomposing enzyme of *Bac. cereus*, then the materials were subjected to dialysis, and the dialyzable and non-dialyzable portion were examined for sugar composition. The results are shown in Table II.

When 10 ml of O red cells was incubated with 300 mg of the enzyme of *Bac. fulminans*, fucose was demonstrated as simple sugar in the dialyzable portion in an amount of 67.6% of the original quantity in the case of OLe(a—b+) red cells, and 55.8% in the case of OLe(a+b—).

When 100 mg of glycolipid from OLe(a—b+) red cells was incubated with 220 mg of the enzyme of *Bac. fulminans*, fucose was demonstrated as simple sugar in the dialyzable portion in an amount

Table II. Chemical changes in O(H)-active substances of red cells induced by the O(H)-decomposing enzyme (Fr. 14~18) from *Bac. fulminans* and the O(H)- and Le^b-decomposing enzyme from *Bac. cereus*

O(H) substance	Enzyme action		Hexose (as galactose) (μg)	Methylpentose (as fucose) (μg)	Hexosamine (as galactosamine) (μg)
Red cells	Before		801	340	668
OLe(a-b+) (10 ml)	After	DP	0	230 (67.6%)	0
	<i>Bac. fulm.</i>	NP	810	105	665
	After	DP	178 (22.2%)	245 (72.2%)	0
	<i>Bac. cereus</i>	NP	640	90	670
OLe(a+b-) (10 ml)	Before		1090	340	782
	After	DP	0	190 (55.8%)	0
	<i>Bac. fulm.</i>	NP	1100	145	790
	After	DP	151 (13.9%)	203 (59.7%)	0
	<i>Bac. cereus</i>	NP	980	125	790
Glycolipid	Before		2180	238	1210
OLe(a-b+) (100 mg)	After	DP	0	145 (60.6%)	0
	<i>Bac. fulm.</i>	NP	2060	111	1100
	After	DP	194 (8.9%)	94 (39.5%)	0
	<i>Bac. cereus</i>	NP	1980	143	1200

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

of 60.6% of the original quantity.

When 10 ml of O red cells was incubated with 500 mg of the enzyme of *Bac. cereus*, the dialyzable portion contained 72.2% of the original amount of fucose and 22.2% of the original amount of galactose in the case of OLe(a-b+) red cells, and 59.7% of the former and 13.9% of the latter in the case of OLe(a+b-) red cells.

When 100 mg of glycolipid from OLe(a-b+) red cells was incubated with 500 mg of the enzyme of *Bac. cereus*, the dialyzable portion contained 39.5% of the original amount of fucose and 8.9% of the original amount of galactose.

Discussion. The O(H)-decomposing enzyme (Fr. 14~18) from *Bac. fulminans*, acting on O red cells or on O(H)-active crude glycolipid, released fucose, which is the terminal determinant of the O(H) specificity, and the O(H)- and Le^b-decomposing enzyme from *Bac. cereus* released fucose and galactose, which are considered to be the antigenic determinants of the O(H) specificity and assumedly of the Le^b specificity.

The molecule number of fucose, attached to one red cell as the terminal determinant of the O(H) or Le^b specificity, was computed using Avogadro number and taking number of red cells per 1 mm³ as 10,000,000.

From 230 μg of fucose, liberated from 10 ml of OLe(a-b+) red cells by the O(H)-decomposing enzyme from *Bac. fulminans*, the

molecule number of fucose as the terminal determinant of the O(H) specificity per one red cell was computed to be about 8,500,000. And from 245 μ g of fucose, liberated by the O(H)- and Le^b-decomposing enzyme from *Bac. cereus*, the molecule number of fucose as the terminal determinant of the O(H) specificity and assumedly of Le^b specificity per one red cell was computed to be about 9,000,000.

From 190 μ g of fucose, liberated from 10 ml of OLe(a+b-) red cells by the enzyme from *Bac. fulminans*, the molecule number of fucose as the terminal determinant of the O(H) specificity per one red cell was computed at about 7,000,000, and from 203 μ g of fucose, liberated by the enzyme from *Bac. cereus*, the same molecule number was computed at about 7,500,000.

Summary. 1. A purified O(H)-decomposing enzyme from *Bac. fulminans*, when incubated with O red cells or crude glycolipid extracted from them, released fucose, as the result abolishing their reactivity with anti-O(H) antibody in eel serum, and instead enhancing antigenicity common to *Sh. dysenteriae*. The O(H)- and Le^b-decomposing enzyme from *Bac. cereus* released from the same materials fucose and galactose, consequently abolishing the O(H) activity completely, and instead enhancing antigenicity common to *Dip. pneumoniae* Type XIV.

2. From the amount of fucose liberated from red cells as the result of the enzyme action, the molecule number of sugar as the terminal determinant of the O(H) specificity per one red cell was computed.

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