73. Chemical Effects of A- and B-Decomposing Enzymes on Blood Group Substances from Red Cells

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Activity of blood group substance of red cells has been considered to be present in lipid fraction. However, Kossjakow¹⁾ assumed it to be present in polysaccharide fraction, on the other hand Yamakawa and Iida²⁾ claimed it to be found in glycolipid designated as globoside, consisting of fatty acid, sphingosine, acetylchondrosamine, D-galactose, and D-glucose. Yamakawa *et al.*³⁾ recognized glucosamine in globoside besides the sugars described above, and Yamakawa and Irie⁴⁾ reported that in group A substance, obtained from red cells, was found mucolipid, which contained sialic acid besides hexose and hexosamine. Also Radin⁵⁾ noted A and B activity presumably in glycolipid, extracted from dried red cells. Hakomori and Jeanloz,⁶⁾ who obtained glycolipids, having A and B activity, assumed that difference between A and B is based on the mode of attachment of one unit of galactosamine. Kirita⁷⁾ found the presence of large amount of fucose in glycolipid from O red cells. Also Koscielak and Zakrzewski⁸⁾ noted the presence of fucose in A, B, and O blood group substances from red cells, but could not detect O(H) activity even in substance from O red cells. Ludewig⁹⁾ clarified that carbohydrate of human red cell stroma contained galactose, glucose, mannose, sialic acid, and a small amount of fucose.

As to action of the enzyme on A and B substance from red cells, Morgan¹⁰⁾ reported that an enzyme from *Clostridium welchii* exerted no action on red cells, but acted on blood group substance of red cell stroma, especially on that of group O to abolish its blood group activity. Iseki and Masaki¹¹⁾ reported that A-decomposing enzyme from *Clostridium tertium*, acting on carbohydrate-like substance of A red cells, caused depression of A activity and enhancement of O(H) activity. Howe *et al.*¹²⁾ recognized that this enzyme abolished A antigen of red cells. And according to Iseki and Furukawa,¹³⁾ A-decomposing enzyme from *Clostridium tertium* and B-decomposing enzyme from *Clostridium maebashi*, acting on A and B red cells, caused conversions of blood group activity from A into O(H) and from B into O(H), respectively.

This report deals with chemical effects of A-decomposing enzyme

from *Cl. tertium* and B-decomposing enzyme from *Cl. maebashi* on red cells and blood group active substances derived from them.

Materials and methods. 1) Stroma. Ten volumes of 0.3% acetic acid was added to red cells and after allowing to stand overnight, the sediment was collected. 2) Crude glycolipid. Prepared by the method of Yamakawa *et al.*²⁾ 3) Sugar constituents. Test material was hydrolyzed with 2 N sulphuric acid at 100°C for 45 minutes, neutralized with barium hydroxide, and concentrated to be subjected to qualitative and quantitative determination of sugars by routine methods.

Results. 1. Constituent sugars. Sugars in red cells, stroma, and crude glycolipid of A, B, and O blood groups consisted of hexose, hexosamine, methylpentose and sialic acid, and as hexose were demonstrated galactose, glucose and mannose, as methylpentose, fucose, and as hexosamine, galactosamine and glucosamine. Amount of fucose for stroma was 25–35% lower than that for red cells, and that for glycolipid 96–98% lower.

Table I. Serological changes affected by A- and B-decomposing enzyme, of blood group substances from red cells (Agglutination inhibition test)

Blood group substance	Antiserum	Enzyme action	Antigen dilution				
			2	4	8	16	32
A red cells 50% (v/v)	Anti-A human serum	Before	—	—	—	—	+
		After	+	+	+	+	+
	Anti-O(H) eel serum	Before	—	—	+	+	+
		After	—	—	—	—	+
A stroma 10% (w/v)	Anti-A human serum	Before	—	—	—	—	+
		After	+	+	+	+	+
	Anti-O(H) eel serum	Before	—	+	+	+	+
		After	—	—	+	+	+
A glycolipid 0.4% (w/v)	Anti-A human serum	Before	—	—	—	—	+
		After	+	+	+	+	+
	Anti-O(H) eel serum	Before	±	+	+	+	+
		After	—	±	+	+	+
B red cells 50% (v/v)	Anti-B human serum	Before	—	—	—	—	+
		After	+	+	+	+	+
	Anti-O(H) eel serum	Before	—	—	+	+	+
		After	—	—	—	—	+
B stroma 2.5% (w/v)	Anti-B human serum	Before	—	—	—	+	+
		After	+	+	+	+	+
	Anti-O(H) eel serum	Before	—	+	+	+	+
		After	—	—	+	+	+
B glycolipid 0.65% (w/v)	Anti-B human serum	Before	—	—	—	—	+
		After	+	+	+	+	+
	Anti-O(H) eel serum	Before	—	+	+	+	+
		After	—	—	+	+	+

2. Changes in specific activity of blood group substances from red cells induced by enzyme. The A- and B-decomposing enzymes were respectively applied to A and B substance from red cells, and these were investigated for changes in the blood group specificity. The results are shown in Table I.

In crude glycolipids of groups A and B were respectively recognized A and B activity, but O(H) activity, reacting with anti-O(H) eel serum, was extremely low. When the A- and B-decomposing enzymes were applied to A and B red cells, stroma and glycolipid, respectively, A and B activity diminished while O(H) activity to react with anti-O(H) eel serum was enhanced, though less weakly in stroma and glycolipid than in red cells.

3. Chemical changes in blood group substances of red cells induced by enzyme. When the A- and B-decomposing enzymes were respectively applied to A and B blood group substance of red cells, the results as shown in Table II were obtained.

Table II. Chemical changes induced by A- and B-decomposing enzyme, of blood group substances of red cells

Blood group substance	Enzyme action	Hexose (as galactose)	Methylpentose (as fucose)	Hexosamine (as galactosamine)
A ₁ red cells (50 ml)	Before	5340 μ g	1305 μ g	2672 μ g
	After DP	2833	0	1080
	After NP	2510	1300	1590
A glycolipid (40 mg)	Before	660	122	383
	After DP	547	0	229
	After NP	116	124	92
B red cells (50 ml)	Before	4008	1816	2840
	After DP	2074	0	0
	After NP	1920	1820	2820
B glycolipid (65 mg)	Before	670	97	518
	After DP	515	0	0
	After NP	160	96	520

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

In an incubation of 1 g of the A-decomposing enzyme preparation with 50 ml of A₁ red cells, about 45% of total amount of sugar was liberated, which was consisted of about 50% of original amount of hexose, and about 40% of original amount of hexosamine. As hexose was demonstrated galactose, and as hexosamine was demonstrated *N*-acetylgalactosamine as simple sugar. Besides these were found sialic acid with $R_{\text{galactose}}$ value of 0.33 and a 1→3 (or 1→6) linked disaccharide with $R_{\text{galactose}}$ value of 0.71, consisting of *N*-acetyl-

galactosamine and galactose.

In an incubation of 500 mg of the A-decomposing enzyme preparation with 40 mg of crude glycolipid from A red cells, about 74% of total amount of sugar was liberated, which was composed of about 82.5% of original amount of hexose, and about 60% of original amount of hexosamine. As hexose was demonstrated galactose, and as hexosamine was demonstrated *N*-acetylgalactosamine. Besides these were found assumedly sialic acid with $R_{\text{galactose}}$ value of 0.345 and a 1→3 (or 1→6) linked disaccharide with $R_{\text{galactose}}$ value of 0.76, consisting of *N*-acetylgalactosamine and galactose, and an oligosaccharide with $R_{\text{galactose}}$ value of 0.505, consisting of *N*-acetylgalactosamine and galactose.

When 1 g of the B-decomposing enzyme preparation was applied to 50 ml of B red cells, about 24% of total amount of sugar was liberated, all of which was galactose, estimated at about 51% of original amount of hexose. When 1 g of the B-decomposing enzyme was applied to 65 mg of crude glycolipid from B red cells, about 40% of total amount of sugar was liberated, all of which was occupied by galactose, calculated at about 77% of original amount of hexose.

When either the A- or B-decomposing enzyme was applied to A₁B red cells, O(H) activity was enhanced. The chemical effects in these cases were approximately the same with those described above.

Discussion. In the digestion by A-decomposing enzyme, of A red cells or of crude glycolipid derived from them, there were liberated not only galactose but also *N*-acetylgalactosamine, the end determinant of the A specificity, and oligosaccharide, consisting of *N*-acetylgalactosamine and galactose with assumedly 1→3 linkage. As the result of application of the A-decomposing enzyme, 1,080 μg of galactosamine was liberated from 50 ml of A₁ red cells. From this result, molecule number of this determinant of A specificity per one red cell is computed at about 7,300,000, using Avogadro number, and taking number of red cells per 1 mm³ as 10,000,000. This value is approximate to the number of combining sites of one A red cell, which was estimated by Boyd *et al.*¹⁴⁾ to be 8 or 10 million from the combining capacity to anti-A agglutinin from Sieva lima bean.

When the B-decomposing enzyme was applied to B red cells or crude glycolipid from these, galactose, the determinant of the B specificity, was liberated. And in this way, from 50 ml of B red cells was obtained 2,074 μg of galactose. Molecule number of this determinant of the B specificity per one red cell is computed at about 14,000,000, applying an equation described above. And if the determinant of the B specificity in red cells is α -D-galactopyranosyl-(1→3)-

D-galactose, which result was obtained by Painter *et al.*¹⁵⁾ from water-soluble blood group substance, and if the B-decomposing enzyme liberates a disaccharide consisted of two-molecular galactose, then molecule number of galactose as the terminal determinant of the B specificity will be about 7,000,000. The value, however, is considerably larger as compared with the number of combining sites of one B red cell, which was estimated by Wurmser *et al.*¹⁶⁾ to be 500,000 from the combining capacity to human anti-B agglutinin. Similarly, molecule number per one A₁B red cell of galactosamine as the terminal determinant of A specificity was computed from 167 μ g of this sugar, liberated from 15 ml of A₁B red cells by the action of the A-decomposing enzyme, at about 3,770,000. Further from 391 μ g of galactose, liberated from 15 ml of A₁B red cells by the action of the B-decomposing enzyme, molecule number of galactose as the end determinant of the B specificity was computed to be about 4,400,000.

Summary. 1. Sugars in red cells, stroma, and crude glycolipid of A, B, and O blood groups consisted of galactose, glucose, mannose, fucose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and sialic acid.

2. When the blood group specific enzyme was applied to red cells or crude glycolipid derived from them, disaccharide, consisting of *N*-acetylgalactosamine and galactose, was mainly demonstrated besides such monosaccharides as *N*-acetylgalactosamine and galactose in the case of group A, and monosaccharide, galactose, in the case of group B.

3. From the amount of sugar liberated from red cells as the result of the enzyme action, molecule number of sugar as the end determinant of the blood group specificity per one red cell was calculated.

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