78. Biochemical and Serological Studies on α -Galactosyltransferases in the Sera and Salivas from Blood Group B_m and A_1B_m Individuals

By Hisao TAKIZAWA, Koichiro KISHI, and Shoei ISEKI, M. J. A.

National Research Institute of Police Science, Tokyo

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In a previous paper,¹⁾ we reported the isoelectric points of the *B*-gene specified α -galactosyltransferase (*B* transferase) in the sera and salivas of individuals with normal *B* and A_1B blood groups, and showed that the two types of *B* transferase, focused at pH 4.8 and pH 8.8, were found in serum samples, whereas in saliva samples, the enzyme activity was found at single isoelectric point, pH 8.1.

The present paper reports on the activities and isoelectric points of *B* transferases in the serum and saliva samples from the persons of B_m and A_1B_m blood groups.

Materials and methods. Serum and saliva samples from 11 donors of B_m and A_1B_m phenotypes were investigated. G. Ka., A. Ka. and S. Ka. are members of Ka. family, and Ma. Iz. and Mi. Iz. are members of Iz. family. B_m phenotypes of both families are inherited as a dominant character unlike the case by a recessive modifying gene *z*.²⁾ Genetic levels of other six donors failed to be defined. Expression of *B* antigen on their red cells is very weak and only detectable by absorption-elution method with anti-*B* isoagglutinin. All of their sera lacked anti-*B* isoagglutinin. Blood group H activities of B_m red cells are expressed within normal range of group O red cells.

Measurements of *B* transferase activity, gel filtration and isoelectric focusing of the enzyme were carried out by the same methods described in a previous paper.¹⁾ Saliva samples were dialyzed against 0.02 M Tris-HCl buffered saline, pH 7.2 and stored in a frozen state.

Results. 1) *Measurements of B transferase activity.* *B* transferase activities in the serum and saliva samples were assayed by the converting ability from group O erythrocytes into *B*, and indicated by the hemagglutination titers of the converted cells with anti-*B* human serum (Table I).

The enzyme action of *B* transferase was observed in the sera of all cases of B_m and A_1B_m donors, and the enzyme activities in the sera (titer: 4–16) were slightly lower than that of normal *B* subject K.K. (titer: 32). On the other hand, *B* transferase activities in the

Table I. α -Galactosyltransferase activities of B_m and A₁B_m sera and salivas tested by converting ability from O red cells into B-reactive cells

Donor	Blood group	Agglutination titer of converted cells with anti-B serum											
		Serum as a source of enzyme						Saliva as a source of enzyme					
		4	8	16	32	64	1	2	4	8	16	32	64
H. H.	A ₁ B _m sec.	+	+	+	—	—	—	—	—	—	—	—	—
M. A.	B _m sec.	+	+	+	—	—	+	+	+	—	—	—	—
K. Mat.	B _m sec.	+	+	+	—	—	+	—	—	—	—	—	—
T. I.	B _m non-sec.	+	+	+	—	—	+	—	—	—	—	—	—
K. Mah.	B _m sec.	+	+	+	—	—	+	+	+	—	—	—	—
Y. K.	B _m sec.	+	+	+	—	—	+	+	+	—	—	—	—
G. Ka.	A ₁ B _m sec.	+	—	—	—	—	+	+	—	—	—	—	—
A. Ka.	A ₁ B _m non-sec.	+	—	—	—	—	—	—	—	—	—	—	—
S. Ka.	B _m sec.	+	—	—	—	—	+	+	—	—	—	—	—
Ma. Iz.	A ₁ B _m sec.	+	+	+	—	—	—	—	—	—	—	—	—
Mi. Iz.	B _m sec.	+	+	+	—	—	—	—	—	—	—	—	—
K. K.	B sec.	+	+	+	+	—	+	+	+	+	+	+	—

Reaction mixture: 0.25 ml of serum or saliva, 0.1 ml of 0.1 M Tris-HCl buffer (pH 7.2) containing 0.1 M MnCl₂, 0.05 ml of 1.6 mM UDP-galactose and 0.025 ml of 50% group O erythrocyte suspension. Incubated at 37°C for six hr. The erythrocytes were washed three times with saline, and then hemagglutination reaction against anti-B human serum (titer 1:128) were examined.

saliva samples of same B_m and A₁B_m donors showed very weak converting ability.

B_m erythrocytes could be converted to B expressed cells, whose agglutinabilities against anti-B serum were similar to those of cells converted from group O acceptor cells, by both transferases in the sera of B_m themselves and normal B (K.K.) (Table II).

2) *Effects of the concentration of UDP-galactose and Mn⁺⁺ ion, and pH in the reaction mixture.* Both serum samples from B_m (K. Mah.) and normal B (K.K.) were incubated at 37°C for six hr with UDP-galactose, at the concentration of 0.75–0.01 mM, in the presence of 23 mM MnCl₂ and group O acceptor cells, at pH 7.2. Agglutination titers of the converted B cells against anti-B serum were 1–8 when B_m serum was used as the enzyme sample and 1–16 when normal B serum was used.

M⁺⁺ ion concentration was also altered at the concentration of 23–0.2 mM, in the presence of 0.19 mM UDP-galactose. In this experiment, agglutination titers of the converted B cells against anti-B serum were 2–16 when B_m serum was used as the enzyme sample and 4–32 when normal B serum was used.

Both serum samples from B_m (K. Mah.) and normal B (K.K.)

Table II. α -Galactosyltransferase activities of B_m and A_1B_m sera tested by converting ability from B_m or A_1B_m red cells into B-reactive cells

Source of enzyme		Acceptor cells		Agglutination titer of converted cells with anti-B serum					
				1	2	4	8	16	32
H. H.	(A_1B_m)	H. H.	(A_1B_m)	+	—	—	—	—	—
H. H.	(A_1B_m)	M. A.	(B_m)	+	+	+	+	+	—
K. Mat.	(B_m)	K. Mat.	(B_m)	+	+	+	+	—	—
T. I.	(B_m)	T. I.	(B_m)	+	+	+	+	+	—
G. Ka.	(A_1B_m)	G. Ka.	(A_1B_m)	+	—	—	—	—	—
G. Ka.	(A_1B_m)	S. Ka.	(B_m)	+	+	+	—	—	—
A. Ka.	(A_1B_m)	A. Ka.	(A_1B_m)	+	—	—	—	—	—
S. Ka.	(B_m)	S. Ka.	(B_m)	+	+	+	—	—	—
Ma. Iz.	(A_1B_m)	Ma. Iz.	(A_1B_m)	+	—	—	—	—	—
Mi. Iz.	(B_m)	Mi. Iz.	(B_m)	+	+	+	+	+	—
K. K.	(B)	S. Ka.	(B_m)	+	+	+	+	+	—
K. K.	(B)	Mi. Iz.	(B_m)	+	+	+	+	+	±

Conditions of the reaction mixture were the same as those described in Table I.

were incubated at pH 6.0–9.0, in the presence of 0.19 mM UDP-galactose and 12 mM $MnCl_2$. Agglutination titers of the converted B cells against anti-B serum were 0–16 in the case of B_m serum and 4–32 in the case of normal B serum.

From the experimental results, it was concluded that the optimal conditions for the assay of B transferase in the serum of B_m phenotype were not significantly different from those in the serum of normal B group.

3) *Gel filtration and isoelectric focusing.* The sera of three B_m and three A_1B_m persons were applied to a Sephadex G-150 column and eluted by the same way described in a previous paper.¹⁾ The B transferase active fraction was found in the same elution volume as that of normal B serum. Those enzyme active fractions from six donors were concentrated to a small volume, then separated and characterized by isoelectric focusing.

All sera of B_m and A_1B_m persons gave two peaks with enzyme activity, which focused in the pH range 4.2–6.0 (pI 4.8) and 6.8–9.6 (pI 8.5) (Table III). These results were similar to those of normal B sera, described in a previous paper.¹⁾

Discussion. All serum samples obtained from 11 persons of blood group B_m or A_1B_m converted both O and B_m erythrocytes into clearly B-active cells in the presence of UDP-galactose and Mn^{++} ion, and these B transferase activities in the sera of B_m phenotype were one half to one fourth of that of normal B serum, when estimated with the hemagglutinability of the converted B cells against anti-B

Table III. Isoelectric points of α -galactosyltransferases in the B_m and A₁B_m sera

Serum H.H. (A ₁ B _m)				Serum M.A. (B _m)				Serum K.Mat. (B _m)			
Fr. No.	pH range	Enzyme activity				Fr. No.	pH range	Enzyme activity			
		4	8	16	32*			4	8	16	32
1	2.0-3.1	—	—	—	—	1	2.0-2.1	—	—	—	—
2	3.8-4.1	—	—	—	—	2	3.5-3.8	—	—	—	—
3	4.4-4.8	+	+	+	—	3	4.2-4.5	+	+	+	—
4	5.1-5.5	—	—	—	—	4	4.8-5.2	+	—	—	—
5	6.0-6.4	—	—	—	—	5	5.6-6.0	—	—	—	—
6	6.8-7.2	+	+	—	—	6	6.4-6.9	—	—	—	—
7	7.7-8.1	+	+	+	—	7	7.4-7.6	+	+	—	—
8	8.4-8.6	+	+	+	—	8	7.9-8.2	+	+	+	—
9	8.9-9.1	+	+	—	—	9	8.5-8.8	+	+	+	—
10	9.4-9.6	—	—	—	—	10	9.0-9.3	+	+	—	—
11	10-12	—	—	—	—	11	9.8-12	—	—	—	—

Serum G.Ka. (A ₁ B _m)				Serum A.Ka. (A ₁ B _m)				Serum S.Ka. (B _m)			
Fr. No.	pH range	Enzyme activity				Fr. No.	pH range	Enzyme activity			
		4	8	16	32			4	8	16	32
1	2.0-3.6	—	—	—	—	1	2.0-3.2	—	—	—	—
2	3.9-4.5	—	—	—	—	2	3.8-3.9	—	—	—	—
3	4.7-5.1	+	+	+	+	3	4.3-4.5	+	—	—	—
4	5.6-6.0	+	—	—	—	4	5.0-5.4	—	—	—	—
5	6.4-6.9	—	—	—	—	5	5.8-6.2	—	—	—	—
6	7.3-7.7	+	—	—	—	6	6.7-7.1	—	—	—	—
7	8.1-8.5	+	+	—	—	7	7.4-7.7	+	—	—	—
8	8.8-9.1	+	+	+	+	8	8.2-8.4	+	+	—	—
9	9.4-9.6	+	—	—	—	9	8.7-9.0	+	—	—	—
10	10-10.7	—	—	—	—	10	9.2-9.4	—	—	—	—
11	11.6-12	—	—	—	—	11	9.6-12	—	—	—	—

* Agglutination titer of converted cells against anti-B serum.

serum.

Kogure and Furukawa³⁾ also reported the weakness of *B* transferase activity in B_m sera, and in another report,⁴⁾ Kogure described that this enzyme activities in B_m or *cis* AB sera were approximately one eighth to one sixteenth of those in normal B sera. Kościelak *et al.*⁵⁾ estimated the activity of *B* transferase in the serum and erythrocyte membrane related to B_m phenotype, and reported that the level of *B* transferase in B_m serum was similar to that in normal B serum, but that in erythrocyte membranes was much lower than that in normal B phenotype. Cartron *et al.*⁶⁾ reported that on a quantitative level, the α -N-acetylgalactosaminyltransferase activities of A_m sera only reached 30-50% of the average value observed for A₁ or

A₂ subjects.

The *B* transferase activities in B_m saliva were weaker than those in B_m sera, while Kogure and Furukawa³⁾ reported that the level of *B* transferase activities in B_m salivas was demonstrated to the same degree as in the normal B sera. This discrepancy may be explained by the fact that *B* transferase in the saliva was more unstable as compared with the enzyme in the serum.

The optimal conditions in the reaction mixture—pH, concentration of UDP-galactose and Mn⁺⁺ ion—for the *B* transferases in the sera of B_m and A₁B_m persons were not significantly different from those of corresponding enzymes in the sera of normal B persons.

The sera of the individuals with B_m phenotype contained two types of *B* transferase on isoelectric focusing, and the isoelectric points were quite similar to those of normal B sera.

From our present experiments, it may be thought that the B_m gene is concerned with the reduced production of α -galactosyltransferase and with the inhibition of the enzyme action in the hematopoietic tissue, so that the biosynthesis of B antigen on erythrocytes might not depend on a circulating serum *B* transferase.

Summary. 1) The activities of serum and saliva α -galactosyltransferases from group B_m subjects were lower than those from normal group B subjects, respectively.

2) B_m erythrocytes could be converted to the agglutinable cells against anti-B serum by their own serum transferases and UDP-galactose.

3) Sera from group B_m subjects gave two major fractions with α -galactosyltransferase activity on isoelectric focusing; one, focusing at pH 4.2–6.0 (pI 4.8) and the other, focusing at pH 6.8–9.6 (pI 8.5).

References

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