71. On the B Gene-Associated α -Galactosyltransferase in Human Urine

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The α -galactosyltransferase, concerned with the biosynthesis of blood group B specific structure, also existed in serum and saliva of the individuals carrying the B_m phenotype.

The activities of the B_m gene-associated enzyme were lower than those of the normal B gene-associated enzyme, whereas the biochemical properties of these two enzymes were not significantly different.¹⁾

In the present paper, we report on the activities, gel filtration patterns and isoelectric points of the α -galactosyltransferases in urines obtained from blood group B and B_m persons, and compare these results to the previous investigations of serum and saliva.^{1),2)}

Materials and methods. Urines were collected from the same donors described in the previous paper.¹⁾

Freshly obtained urines were dialysed immediately against 0.02 M Tris-HCl buffered saline (pH 7.2) at 4°C overnight, then used as the enzyme source of the reaction mixture.

For the gel filtration, urinary samples were prepared as follows: Urine was dialysed against 0.02 M Tris-HCl buffer (pH 7.2) and freeze-dried. The dried material from 200 ml of urine was dissolved with a small volume of distilled water and dialysed against the gel equilibrating buffer.

Sephacrose CL-4B gel filtration was carried out using two kinds of buffer system, one was 0.02 M Tris-HCl buffer, pH 7.2 (NaCl free buffer) and the other was 0.02 M Tris-HCl buffer, pH 7.2, containing 1% NaCl (NaCl containing buffer).

The enzyme-active fraction from the gel filtration procedure was concentrated with ultrafiltration (Toyo Roshi Co., Ltd., membrane filter UK-10), then analysed with the LKB isoelectric focusing apparatus.

The preparations of serum and saliva samples, and the assay methods of enzyme activity and isoelectric focusing were the same as described in the previous paper.¹⁾

Results. 1) *α -Galactosyltransferase activities in human urine.* In the urines of blood group B, A_1B , B_m and A_1B_m individuals, the enzyme was present which converted, in the presence of UDP-galac-

tose and Mn^{++} , blood group O red cells into blood group B-active cells. When the urines from the individuals of normal B phenotype were used as the enzyme source, converted cells were agglutinated by anti-B human serum (titer 1:128) at a titer of 16 to 32 fold, and when the urines of B_m phenotype were used, converted cells were agglutinated by the same anti-B serum at a titer of 4 to 16 fold.

2) *Gel filtration.* A urinary sample was applied to the Sepharose CL-4B packed column (3.5×67 cm), equilibrated with NaCl containing buffer. A peak of activity of α -galactosyltransferase appeared at an elution volume of 480 ml (V_o and V_t of this column were 180 ml and 600 ml, respectively). When the serum or saliva sample was applied to the same column, the α -galactosyltransferases in both sam-

Fig. 1. Gel filtration on the α -galactosyltransferases from serum, saliva and urine. — Enzyme activity, eluted with NaCl free buffer. - - - Enzyme activity, eluted with NaCl containing buffer.

ples were eluted in the fraction similar to that of the urinary sample, as illustrated in Fig. 1.

In another experiment, the Sepharose CL-4B gel was equilibrated with NaCl free buffer. Then the enzyme-active fraction was shifted to a smaller elution volume, 340 ml in the case of the serum sample and 440 ml in the case of the urinary sample, respectively. The result may indicate that the α -galactosyltransferase molecule exists in aggregated form at low ionic strength as reported by Nagai *et al.*³⁾

3) *Isoelectric focusing.* When the samples obtained from the NaCl free buffer system were analysed with isoelectric focusing, the

Fig. 2. Isoelectric focusing on the α -galactosyltransferases from serum, saliva and urine. --○-- Enzyme activity; samples were prepared by NaCl free gel filtration procedure. —△— Enzyme activity; samples were prepared by NaCl containing gel filtration procedure.

α -galactosyltransferase activities were found in the fractions with the following pH values, pH 4.8 and pH 7.2 to 9.8 (serum sample), pH 7.0 to 9.0 (saliva sample), pH 4.0 and pH 7.0 to 9.0 (urinary sample).

Whereas when the samples obtained from the NaCl containing buffer system were analysed, the α -galactosyltransferase activity existed only in the basic fractions, pH 7.2 to 9.8 (serum sample), pH 7.0 to 9.0 (saliva sample), pH 7.0 to 9.0 (urinary sample), and did not exist in the acidic fraction, as illustrated in Fig. 2.

Therefore, the α -galactosyltransferase with the low pI values may be due to molecular aggregation at low ionic strength.

4) *pI values of urinary α -galactosyltransferase of blood group A_1B_m persons.* The samples were prepared by NaCl free buffer system. Urinary samples from the blood group A_1B_m donors (G. Ka. and A. Ka.) gave two fractions with α -galactosyltransferase activity, one fraction focusing at about pH 4, and the other fraction focusing at pH 7.0 to 9.0. These electrofocusing patterns were almost identical with that of the sample from a blood group A_1B donor (H. T.), as shown in the Table I.

Table I. Isoelectric points of α -galactosyltransferases in urines from A_1B and A_1B_m donors

Urine H.T. (A_1B)				Urine A.Ka. (A_1B_m)				Urine G.Ka. (A_1B_m)			
Fr. no.	pH range	Enzyme activity		Fr. no.	pH range	Enzyme activity		Fr. no.	pH range	Enzyme activity	
		4	8 16 32*			4	8 16 32			4	8 16 32
1	<2.0-2.3	—	— — —	1	<2.0-2.5	—	— — —	1	<2.0-2.3	—	— — —
2	3.8-4.2	+	+	2	3.0-3.5	—	— — —	2	3.7-4.1	+	— — —
3	4.4-4.8	—	— — —	3	4.0-4.5	±	— — —	3	4.4-4.8	—	— — —
4	5.2-5.6	—	— — —	4	4.8-5.0	—	— — —	4	5.2-5.6	—	— — —
5	6.0-6.4	+	— — —	5	5.3-6.0	—	— — —	5	6.1-6.5	±	— — —
6	6.8-7.2	+	+	6	6.4-6.9	+	+	6	6.9-7.4	+	+
7	7.5-7.9	+	+	7	7.3-7.7	+	+	7	7.7-8.2	+	+
8	8.4-8.7	+	+	8	8.0-8.3	+	+	8	8.5-8.8	—	— — —
9	9.0-9.3	—	— — —	9	8.6-9.1	—	— — —	9	9.1-9.4	—	— — —
10	9.7-10.0	—	— — —	10	9.5-10.0	—	— — —	10	9.7-9.9	—	— — —

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

Discussion. The α -galactosyltransferase activities in the urines of blood group B or A_1B donors are almost identical with the activities in the sera or salivas of the same donors.

The urinary samples from blood group B_m and A_1B_m donors also have the α -galactosyltransferase activities, which were slightly lower than the enzyme activities in the urinary samples from normal B and

A₁B donors. This is in accordance with the results for the serum and saliva enzymes reported in the previous paper.¹⁾

The presence of α -galactosyltransferase in human urine was first communicated by Kogure and Furukawa, at the 21st Annual Meeting of the Japan Society of Human Genetics, 1976.⁴⁾ In that meeting they reported that the α -galactosyltransferase activities in urines from blood group AB donors were slightly lower than those from blood group B donors. While, in our present results, this difference was not found.

The gel filtration patterns of the three α -galactosyltransferases present in serum, saliva and urine, suggested that these enzymes were of homogeneous size in the NaCl containing buffer system.

Concerning the molecular size of the α -galactosyltransferase isolated from human plasma, Nagai *et al.*³⁾ reported that the molecular weight of the enzyme was about 80,000, as estimated by Sephadex G-200 gel filtration in the presence of 0.2 M NaCl, and the enzyme was in a dimeric form, as suggested by sodium dodecylsulfate polyacrylamide gel electrophoresis.

The comparative study of the isoelectric points of the three α -galactosyltransferases, which were prepared by the gel filtration procedure with NaCl containing buffer system, revealed that the urinary and saliva enzymes were focused at a similar pH range, 7.0 to 9.0, and the serum enzyme, which was more positively charged, was focused at a pH range, 7.2 to 9.8. This charge heterogeneity of the enzyme molecule may be induced by a secondary modification which occurred at the enzyme synthesis site.

Summary. (1) The activity of urinary α -galactosyltransferase is nearly equal to that of serum or saliva enzyme. (2) Under NaCl containing buffer condition, purification procedures involving Sepharose CL-4B gel filtration showed the homogeneity of the molecular size in the three α -galactosyltransferases. (3) The pI of the urinary α -galactosyltransferase is similar to that of the saliva α -galactosyltransferase and significantly different to that of the serum α -galactosyltransferase, which is more positively charged.

References

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