Genetic Mechanism of Cis-AB Inheritance. II. Cases Associated with Structural Mutation of Blood Group Glycosyltransferase

AKIRA YOSHIDA,[1] HIDEO YAMAGUCHI,[2] AND YASUTO OKUBO[2]

SUMMARY

The genetic mechanism of the rare occurrence of Cis-AB expression, that is, AB and/or O offspring from AB × O parents, has not been fully understood. The synthesis of blood group A and B substances are controlled by *N*-acetylgalactosaminyltransferase (A-enzyme) and galactosyltransferase (B-enzyme). Therefore, the genetic mechanism of Cis-AB expression may be elucidated by examining the characteristics of A- and B-enzymes in Cis-AB plasma. In a previous study, we presented evidence that Cis-AB expression in one case examined was due to unequal chromosomal crossing over producing a single chromosome with the genes for A- and B-enzymes, rather than to a structural mutation producing a single abnormal enzyme with bifunctional activity. In contrast to the previous case, the present two Cis-AB plasma contained a single abnormal enzyme that can transfer both *N*-acetylgalactosamine (GalNAc) and galactose (Gal). Moreover, the subjects' plasma also contained an enzymatically inactive, but immunologically cross-reacting material. Therefore, Cis-AB expression in the present two cases is due to a structural mutation in either the *A* or *B* gene producing a single abnormal enzyme with bifunctional activity.

INTRODUCTION

The genetic mechanism of the rare occurrence of Cis-AB expression has not been well understood [1–4]. Since blood group A-enzyme is responsible for synthesis of A substance and blood group B-enzyme is involved in the synthesis of B substance [5, 6],

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[1] Department of Biochemical Genetics, City of Hope National Medical Center, Duarte, CA 91010.

[2] Osaka Red Cross Hospital and Osaka Red Cross Blood Center, Osaka, Japan.

the Cis-AB inheritance could be related to unequal chromosomal crossing over producing a single chromosome with *A* and *B* genes, or to a structural mutation inducing formation of a bifunctional mutant enzyme. In a previous study, we presented evidence that the Cis-AB case examined originated from unequal chromosomal crossing over, not from structural mutation [7]. However, in rare genetic abnormalities, such as Cis-AB, the underlying mechanisms are not necessarily identical in unrelated subjects with similar abnormalities. Therefore, we examined two additional Cis-AB cases in the present study. It was found that, in contrast to the previous case, the present Cis-AB presumably originated from a structural mutation of the blood group transferase.

MATERIALS AND METHODS

Blood Samples

Plasma from fresh blood (ACD anticoagulant) of the Cis-AB subjects (Kat and Sa) and the controls with phenotypes A_1, A_2, A_1B, B, and O were stored at −60°C. No significant decrease in blood group glycosyltransferase activity occurred during storage. The red cell type of subjects Kat and Sa was found to be A_2B_3, that is, weak A and very weak B. Pedigrees of the subjects have been reported [8]. There is no known consanguinity between the two families.

Assay of A- and B-enzymes:

The enzyme activities were assayed by measuring the incorporation of sugar from nucleotide sugars (i.e., UDP-GalNAc-^{3}H for A-enzyme and UDP-Gal-^{3}H for B-enzyme) into fucosyllactose, an analog of the natural sugar acceptor, as reported [7].

Assay of Immunologically Cross-Reacting Material

An aliquot of antibody against A-enzyme, 20 μl, was mixed with 40 μl of the protein samples to be examined (or with 0.01 M Tris-Cl, pH 7.0, as control), and the mixture was incubated at 4°C for 5 hrs with shaking. An aliquot of the mixture (or Tris buffer as control), 20 μl, was mixed with 10 μl of partially purified A_1-enzyme and was incubated at 4°C for 5 hrs with shaking. The incubated enzyme was subjected to an assay of A-enzyme activity. The chemicals and Sepharose 4B (lot no. 17C-0110) were the same as that described previously [7]. Antibody against A_1-enzyme was prepared as described [9].

EXPERIMENTS AND RESULTS

A-enzyme activity of Kat plasma was only 9.8% of that of the average phenotype A_1 plasma, and its B-enzyme activity was 8.4% of that of the average phenotype B plasma at pH 6.5. The A- and B-enzyme activity of Sa plasma was 7.6% and 5.8% of the normal levels, respectively.

Since the plasma enzyme activities were very low, the blood group glycosyltransferases were partially purified from the subjects' plasma by treatment with Sepharose-4B [7] and reconstituted in a volume about 10 times less than the original volume of plasma for the following characterization of their enzymatic properties.

A pH activity profile of A-enzyme from Kat and Sa was distinctively different from that of common A_1- and A_2-enzymes, exhibiting high activity at alkaline pH (fig. 1*A*). The pH activity profile of B-enzyme from Kat and Sa was also entirely different from that of common B-enzyme (fig. 1*B*). These results indicate that the subjects' plasmas contain an abnormal transferase (or transferases), different from common A_1-, A_2-, and B-enzymes.

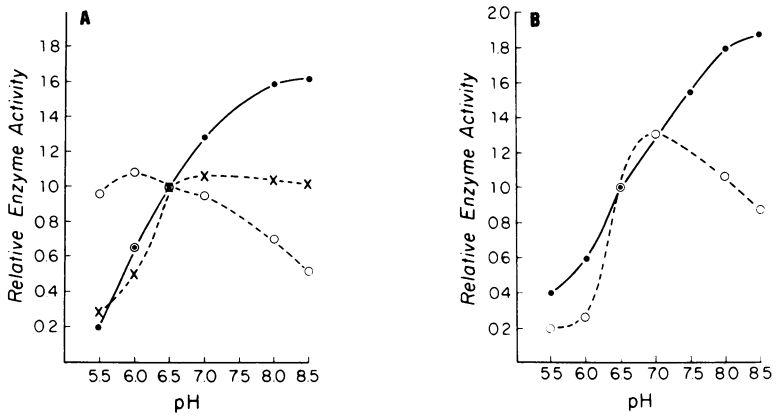

FIG. 1.—Effect of pH on blood group transferase activity. *A*, A-enzyme activity assayed in 40 mM cacodylate buffer at pH specified; o---o = A_1; x---x = A_2; ●—● = Cis-AB. *B*, B-enzyme activity assayed in 25 mM imidazole buffer at pH specified; o---o = B; ●—● = Cis-AB.

To elucidate whether or not A- and B-enzyme activities are associated with two different enzyme molecules, and whether or not the subjects' plasmas contain an enzymatically inactive but immunologically cross-reacting material, the following experiments were performed. Aliquots (10 ml) of the Cis-AB plasma, heterozygous A_1B plasma, and a mixture of A_1 and B plasma (1:1, v/v) were placed in a Sepharose-4B column (bed vol 0.5 ml) washed with H_2O, and the column was then washed with 2 ml of 0.1 M cacodylate buffer, pH 7.0, containing 2 mM $MnCl_2$ and 1 mM EDTA. The eluate was concentrated to about 1 ml by vacuum dialysis, and after dialysis against 0.01 M Tris-Cl, pH 7.0, it was used for assay of enzyme activity and CRM activity. Sepharose 4B was removed from the column and suspended in 1 ml of 0.01 M Tris-Cl, pH 7.0, and used for assay of A- and B-enzyme activities.

The results are summarized in table 1. In contrast to a mixture of A_1 and B plasma and heterozygous A_1B plasma from which only A_1-enzyme was adsorbed by Sepharose 4-B, both A- and B-enzyme activities of Cis-AB plasma were completely adsorbed by Sepharose 4B. The ratio of A-enzyme activity to B-enzyme activity in the original plasma was almost identical with that of the enzyme adsorbed with Sepharose 4B. The A-enzyme activity of a mixture of A_1 and B plasma was completely adsorbed by Sepharose 4B, while about 20% of the A-enzyme activity of heterozygous A_1B plasma was not adsorbed (table 1). Similar results have been reported [10]. The findings suggest the existence of a hybrid enzyme in heterozygous A_1B plasma [10].

The antibody against A_1-enzyme when treated with the concentrated eluate from Sepharose 4B showed diminished capability for neutralizing A_1-enzyme (table 2), indicating that the eluate contained enzymatically inactive, but immunologically cross-reacting material.

DISCUSSION

Recent demonstration of the immunologic homology of blood group A_1- and B-enzyme proteins and the existence of an enzymatically inactive but immunologically

TABLE 1

SEPARATION OF A AND B ENZYMES BY SEPHAROSE 4B

SAMPLES	ENZYME ACTIVITY								
	PLASMA			BOUND TO SEPHAROSE 4B (× 10)			NOT BOUND TO SEPHAROSE 4B (× 10)		
	A	B	A/B	A	B	A/B	A	B	A/B
Kat	0.73	0.74	1.0	6.6	6.2	1.1	< 0.1	< 0.1	···
Sa	0.57	0.51	1.1	4.8	4.2	1.1	< 0.1	< 0.1	···
A_1+ B*	4.2	6.8	0.62	(8.6)	< (0.1)	> 80	< (0.1)	(11.4)	< 0.01
A_1B†	5.6	8.2	0.68	(7.4)	< (0.1)	> 64	(1.6)	(12.2)	0.13

NOTE. —Enzyme activity expressed as % sugar transferred after incubation for 16 hrs (or 3 hrs, in parentheses) under assay conditions using 50 μl of enzyme samples in a total 100 μl of reaction mixture. Enzymes bound and not bound to Sepharose 4B were reconstituted in a volume about 10 times less than original volume of plasma samples.

* Mixture (1:1) of A_1 and B plasma.

† Plasma of heterozygous *AB*.

cross-reacting material in *O*, *AO*, and *BO* plasma, but not in homozygous A_1A_1 and *BB* plasma, proved that the *A*, *B*, and *O* genes are truly allelic [9, 11]. Consequently, the rare Cis-AB inheritance can be a result only of (1) unequal chromosomal crossing over producing a single chromosome with genes for A-enzyme and B-enzyme, or (2) a structural mutation producing a mutant A- (or B-) enzyme that has the capability to synthesize both A and B substances.

In the case of the Cis-AB previously reported, the subject's plasma contained two separable enzyme components, one with kinetic properties similar to those of common A_2-enzyme, and another with kinetic characteristics similar to those of common B-enzyme [7]. Therefore, Cis-AB expression in this case should be due to unequal chromosome crossing over producing a single chromosome with the genes for A_2- and B-enzymes.

It has been known that Sepharose 4B completely adsorbs both A_1- and A_2-enzymes, but not the B-enzyme and immunologically cross-reacting material [9–11]. Thus, the A- and B-enzymes are completely separable by treatment with Sepharose 4B. Utilizing the unique characteristics of A- and B-enzymes, separation of the two enzyme

TABLE 2

CONTENT OF CROSS-REACTING MATERIAL (CRM) IN CIS-AB PLASMA

Antibody	Enzyme activity neutralized by antibody (%)
Treated with buffer (control)	< 5
Treated with CRM fraction from Kat plasma	80
Treated with CRM fraction from Sa plasma	65
Treated with CRM fraction from *O* plasma	72
Treated with *AA* plasma	8

NOTE. —CRM was prepared as described in MATERIALS AND METHODS. An aliquot of antibody, 20 μl, was mixed with CRM (or 0.01 M Tris-Cl, pH 7.0, as control), and mixture was incubated at 4°C for 5 hrs with shaking. An aliquot of each mixture (or Tris buffer as control), 20 μl, was then mixed with 10 μl of partially purified A_1 enzyme and incubated at 4°C for 5 hrs with shaking. Incubated enzyme was subjected to an assay of A-enzyme activity. Results represent mean values of duplicate analysis (maximum deviation was ± 6%).

activities in the Cis-AB plasma was attempted. In contrast to the previous case, the A- and B-enzymes in the present Cis-AB plasma could not be separated by the treatment with Sepharose 4B (table 1).

The enzymatic characteristic, that is, pH optima, of the A- and B-enzymes in the present Cis-AB plasma is distinctively different from that of the common A_1- and A_2-enzymes and the B-enzyme (figs. 1 and 2). This implies that the Cis-AB plasma contains either a single abnormal enzyme with bifunctional activity or two abnormal enzymes, that is, an abnormal A-enzyme and an abnormal B-enzyme that, in contrast to normal B-enzyme, strongly adsorb to Sepharose 4B. However, the existence of immunologically cross-reacting material in the Cis-AB plasma refutes the second possibility. In the second hypothesis, the *O* gene for immunologically cross-reacting material should be located in one chromosome 9 of the Cis-AB subject, and the counter chromosome should contain a gene for an abnormal A-enzyme and a gene for an abnormal B-enzyme. This could occur only in a combination of three rare events: a structural mutation in *A* gene producing mutant A-enzyme; a structural mutation in B gene producing mutant B-enzyme; and an unequal chromosomal crossing over producing a chromosome with two genes, that is, one for abnormal A-enzyme and another for abnormal B-enzyme. Accordingly, one can conclude that the present Cis-AB originated from a structural mutation in the *A* or *B* gene producing abnormal blood group glycosyltransferase that could transfer both GalNAc and Gal.

The abnormalities examined are very similar in the present two Cis-AB cases, suggesting that the identical structural mutation is involved in both Kat and Sa cases.

Several investigators have examined properties of A- and B-enzymes in Cis-AB plasma [12–15]. The enzyme abnormalities differed among the unrelated Cis-AB subjects they examined [12–15], indicating the heterogeneous origin of Cis-AB. Although these investigators could not present any conclusive evidence, some of these Cis-AB cases might also originate from structural mutations in the *A* or *B* gene.

REFERENCES

1. YAMAGUCHI H, OKUBO Y, TANAKA M: "CisAB" bloods found in Japanese families. *Jpn J Hum Genet* 15:193–215, 1970
2. REVIRON J, JACQUET A, SALMON CH: Un exemple de chromosome 'Cis A_1B'. Étude immunologique et génétique du phénotype induit. *Nouv Rev J Hematol* 8:323–338, 1968
3. SALMON CH, LOPEZ M, GEBROL A, BOUGUERRA A, COUTON JP: Current genetic problems in the ABO blood group systems. *Biomedicine* 18:375–386, 1973
4. WATKINS WM: Blood group gene specified glycosyltransferases in rare ABO groups and in leukaemia. *Rev Fr Transfus Immunohematol* 21:201–228, 1978
5. RACE C, ZIDERMAN D, WATKINS WM: An α-D-galactosyltransferase associated with the blood group B character. *Biochem J* 107:733–735, 1968
6. HEARN VM, SMITH ZG, WATKINS WM: An α-*N*-acetylgalactosaminosyltransferase associated with the human blood group A character. *Biochem J* 109:315–317, 1968
7. YOSHIDA A, YAMAGUCHI H, OKUBO Y: Genetic mechanism of Cis-AB inheritance. I. A case associated with unequal chromosomal crossing over. *Am J Hum Genet* 32:332–338, 1980
8. YAMAGUCHI H, OKUBO Y, TANAKA M: "Cis-AB" bloods found in Japanese families. *Jpn J Hum Genet* 15:193–215, 1970
9. YOSHIDA A, YAMAGUCHI YF, DAVÉ V: Immunologic homology of human blood group glycosyltransferases and genetic background of blood group (ABO) determination. *Blood*

54:344–349, 1979
10. NAGAI M, YOSHIDA A: Possible existence of hybrid glycosyltransferase in heterozygous blood group AB subjects. *Vox Sang* 35:378–381, 1978
11. YOSHIDA A: Identification of genotypes of blood group A and B. *Blood* 55:119–123, 1980
12. BADET J: Activités glycosyltransférasiques sériques associées à la biosynthèse des antigènes de groupes sanguins A et B. Application à l'étude de sujets B normaux et Cis AB. *Rev Fr Transfus Immunohematol* 19:105–114, 1976
13. WATKINS WM: Blood group gene specified glycosyltransferases in rare ABO groups and in leukaemia. *Rev Fr Transfus Immunohematol* 21:201–228, 1978
14. SABO BH, BUSH M, GERMAN J, CARNE LR, YATES AD, WATKINS WM: The Cis AB phenotype in three generations of one family: serological, enzymatic and cytogenetic studies. *J Immunogenet* 5:87–106, 1978
15. BADET J, ROPARS C, SALMON CH: α-*N*-acetyl-D-galactosaminyl and α-D-galactosyltransferase activities in sera of CisAB blood group individuals. *J Immunogenet* 5:221–231, 1978