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# Genetic Mechanism of Cis-AB Inheritance. I. A Case Associated with Unequal Chromosomal Crossing Over

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SUMMARY

In contradiction to the general Mendelian inheritance of blood group ABO expression, the A and B characteristics are inherited together from one parent in the rare Cis-AB phenotype. Since the synthesis of blood group A and B substances are controlled by *N*-acetylgalactosaminyltransferase (A-enzyme) and galactosyltransferase (B-enzyme), the genetic mechanism of Cis-AB expression may be elucidated by examining the characteristics of A- and B-enzymes in Cis-AB plasma. Biochemical study reveals that the examined Cis-AB plasma contains two separable enzyme components: one with kinetic properties similar to those of common $A_2$-enzyme, but differing from $A_1$-enzyme, and another with kinetic characteristics similar to those of common B-enzyme. Therefore, Cis-AB expression, at least in the case examined, is due to unequal crossing over, producing a chromosome with alleles for $A_2$- and B-enzymes, rather than to a structural mutation in *A* or *B* alleles producing a single abnormal enzyme with bifunctional activity.

## INTRODUCTION

The rare occurrence of Cis-AB expression, that is, AB children from AB × O parents, has been known for some time, although early reports of such unusual inheritance of the ABO blood group were attributed to poor blood-grouping or to nonpaternity. However, undisputed reports on the Cis-AB inheritance in various populations has been accumulated [1–3].

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Specific blood group glycosyltransferases are responsible for synthesis of blood group A and B antigens on the red cell surface. A specific A-enzyme of blood group A persons transfers the sugar from UDP-*N*-acetylgalactosamine (UDP-GalNAc) to the terminal galactose (Gal) of the H substance on the O red cell surface, producing A antigen, while the B-enzyme transfers Gal from UDP-Gal to the H substance, producing B antigen on the red cell surface [4–6].

It was not clear whether the genes governing the ABO blood group were truly allelic; thus, to account for the Cis-AB expression, a "pseudo-allelic model" was proposed for the *ABO* locus [7]. However, recent findings of immunologic homology of blood group A- and B-enzyme proteins and the existence of an enzymatically inactive but immunoiogically crossreactive protein in O plasma indicates that the *ABO* genes are truly allelic [8, 9]. The following two major hypotheses are plausible for the mechanism of Cis-AB inheritance: unequal chromosomal crossing over occurs—thus, the alleles for both A- and B-enzymes could be located on the same chromosome; and, alternatively, a structural mutation occurs in the *A* or *B* allele, and a resulting mutant A (or B) transferase can transfer both GalNAc and Gal to the H substance and synthesize both A and B antigens [10, 11].

We examined the biochemical properties of A- and B-enzymes of a subject with Cis-AB expression and found that in this particular subject the Cis-AB expression is due to unequal chromosomal crossing over, rather than to structural mutation in the blood group transferase.

## MATERIALS AND METHODS

### *Blood samples*

Plasma from fresh blood (ACD anticoagulant) of the Cis-AB subject, and from the controls with phenotypes $A_1$, $A_2$, B, and O, were stored at −60°C. No significant decrease in the blood group transferase activity occurred during several months' storage. The phenotype of this Cis-AB subject's red cells was classified as $A_2B_3$ according to the previous definition [12]. Pedigree of the subject is shown in figure 1.

### *Assay of A- and B-Enzyme Activity*

The transferase activities were assayed by measuring the incorporation of sugar from nucleotide sugars into fucosyllactose, an analog of the natural sugar acceptor [13–15]. The reaction mixture, 100 μl, for the A-enzyme assay contained 2 mM 2-fucosyllactose, 25 μM UDP-GalNAc ($H^3$, 0.5 μCi), 1 mM ATP, 15 mM $MnCl_2$, 0.15 M NaCl, 0.2% bovine serum albumin, 0.25% Triton X-100, 1 mM $NaN_3$, 40 mM cacodylate buffer, pH 6.5, and plasma or partially purified enzyme. For the B-enzyme assay, 25 μM UDP-Gal ($H^3$, 0.5 μCi) was used as a sugar donor, and 25 mM imidazole, pH 6.5, was used as a buffer. The reaction mixture was

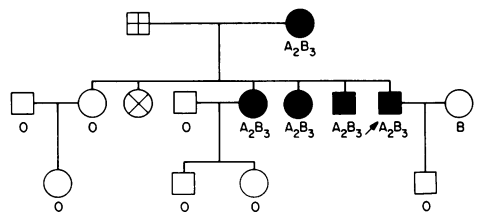


FIG. 1.—Pedigree of Cis-AB subject examined. *Arrow* = propositus; □ = deceased; ⊗ = not examined

incubated for 16 hrs at 37°C. An aliquot (20 μl) of the reaction mixture was applied to an arrow-shaped piece of DEAE-cellulose paper (2 × 7 cm), and eluted with water (total 2 ml). The radioactivity of the eluate was measured by a scintillation counter. To correct for spontaneous and catalytic hydrolysis of the nucleotide sugars, the reaction mixture minus fucosyllactose was also incubated as a control. The radioactivity from the control mixture (less than 10% in the case of crude $A_1$ and B plasma as the enzyme source and less than 3% in the case of partially purified enzymes) was subtracted from that of the complete reaction mixture. The enzyme activity was expressed as per cent of carbohydrates transferred into the acceptor. The enzymes were partially purified from $A_1$, $A_2$, B, and Cis-AB plasma as described [8, 15].

UDP-GalNAc was synthesized as reported [15]. UDP-Gal was purchased from Sigma Chemical (St. Louis, Mo.), UDP-GalNAc (galactosamine-1-$H^3$) from New England Nuclear (Boston, Mass.), and UDP-Gal (galactose-6-$H^3$) from Amersham-Searle (Arlington Heights, Ill.). Purity of these materials was checked by column chromatography with Dowex-1 and found to be at least 97%. Fucosyllactose was prepared from human milk as described [16] and further purified by gel filtration with Biogel P-2. Preparation purity was better than 98% as determined by its sugar composition. Sepharose 4B (lot. no. 17C-0110) was obtained from Sigma Chemical.

## RESULTS

### *Enzymatic Properties of A- and B-Enzyme in Cis-AB Plasma*

A-enzyme activity at pH 6.5 in the Cis-AB plasma is 23% of that in pooled phenotype $A_1$ plasma from four individuals, and B-enzyme activity at pH 6.5 is 38% of that in pooled phenotype B plasma from three individuals. A pH activity profile of A-enzyme in the Cis-AB plasma is distinctly different from that of $A_1$ plasma, but similar to that of $A_2$ plasma (fig. 2).

The rate of A-enzyme reaction as a function of the concentrations of UDP-GalNAc and fucosyllactose indicated the usual Michaelis-Menten relationship. Typical examples are shown in figure 3. The $K_m$'s for the substrates calculated from a Lineweaver-Burk plot are given in table 1. $K_m$'s of A-enzyme in Cis-AB are similar to those of $A_2$-enzyme, but they are significantly higher than those of $A_1$-enzyme.

A pH activity profile of B-enzyme in the Cis-AB plasma is similar to that of usual B-enzyme, both having maximum activity at pH 6.5–7.5 (fig. 4). $K_m$'s for UDP-Gal

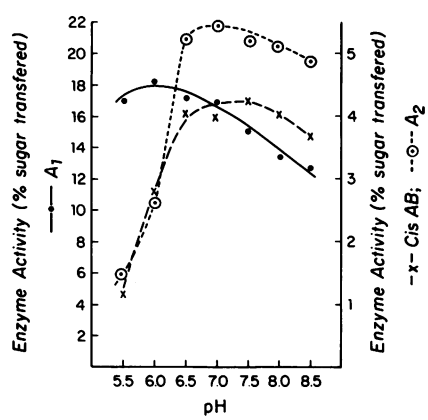


FIG. 2.—Effect of pH on A-enzyme activity. Reaction mixture composition specified in MATERIALS AND METHODS. Activity was assayed in 40 mM cacodylate adjusted pH specified in figure. Crude $A_1$ plasma (50 μl), crude $A_2$ plasma (25 μl), and crude Cis-AB plasma (50 μl) were used as enzyme.

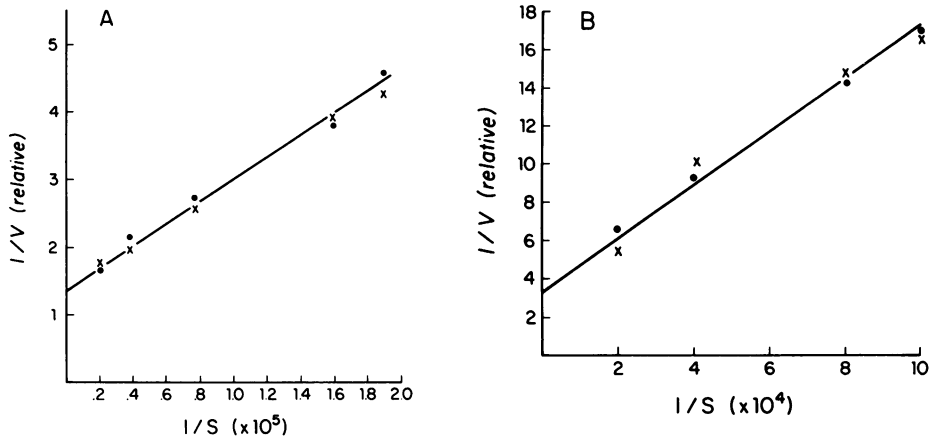


FIG. 3.—Lineweaver-Burk plots of effect of UDP-GalNAc concentration of A-enzyme activity. Reaction mixture contained reagents specified in MATERIALS AND METHODS and various concentrations of UDP-GalNAc. *A* = $A_1$ plasma; *B* = Cis-AB plasma.

and fucosyllactose are also similar in the Cis-AB plasma and common B plasma (table 1). These results indicate that the Cis-AB plasma contains an enzyme (or enzymes) with kinetic properties which are similar, or identical, to $A_2$- and B-enzymes.

*Separation of A- and B-Enzymes from the Cis-AB Plasma*

Sepharose 4B completely adsorbs the A-enzyme present in both $A_1$ and $A_2$ plasma but not the B-enzyme, and the B-enzyme does not interfere with the adsorption of A-enzyme with Sepharose 4B [15, 17]. The adsorbed A-enzyme can be eluted from Sepharose 4B with a buffer containing uridine 5′-disphosphate [15, 17]. A-enzyme adsorbed with Sepharose 4B is fully active, and the transferase activity can be assayed using a suspension of A-enzyme-Sepharose complex without eluting from Sepharose 4B [15]. A- and B-enzyme in the Cis-AB plasma were separated from each other by the following procedure. An aliquot (5 ml) of the Cis-AB plasma was placed in a Sepharose 4B column (bed volume 0.4 ml), washed with $H_2O$, and the column was then washed with 1 ml of 0.1 M cacodylate buffer, pH 7.0, containing 2 mM $MnCl_2$ and 1 mM EDTA. Sepharose 4B was removed from the column and suspended in 5 ml of 0.01 M Tris-Cl, pH 7.0. The eluate (total 6 ml) and the suspension of Sepharose 4B were subjected to assays of A- and B-enzyme activities. The eluate contained B-enzyme activity and weak A-enzyme activity, while the suspension of Sepharose 4B contained A-enzyme activity but not B-enzyme activity (table 2). The results indicate that the Cis-AB plasma contains two separate enzyme proteins; that is, one with $A_2$-like enzyme activity and one with B-like enzyme activity.

*Conversion of the $A_2B_3$ Red Cells to $A_1$ and B in Vitro*

The Cis-AB subject's red cells, which originally had weak A and very weak B antigenic activities, were fully converted to $A_1$ and B types by incubation in vitro with partially purified $A_1$- and B-enzymes together with UDP-GalNAc and UDP-Gal, indicating that the subject's red cells are not structurally unusual.

TABLE 1

$K_m$'S OF A- AND B-ENZYMES IN $A_1$, $A_2$, B, AND CIS-AB PLASMA

| Plasma (enzyme) | UDP-GalNAc ($\mu$M) | UDP-Gal ($\mu$M) | Fucosyllactose ($\mu$M) |
|---|---|---|---|
| $A_1$ ($A_1$-enzyme) | 9 (8–11) | ··· | 19 (16–22) |
| $A_2$ ($A_2$-enzyme) | 60 (45–70) | ··· | 125 (102–148) |
| $A_2$ (A-enzyme) | 55 (52–64) | ··· | 172 |
| Cis-AB (B-enzyme) | ··· | 31 | 77 |
| B (B-enzyme) | ··· | 22 (16–36) | 56 (50–61) |

NOTE.—$K_m$'s were calculated from Lineweaver-Burk plot. Reaction mixture contains various concentrations of UDP-GalNAc, UDP-Gal, or fucosyllactose, in addition to other reagents specified in MATERIALS AND METHODS. The partially purified enzymes were used for the assay. Values in parentheses give range of duplicate or triplicate assays.

## DISCUSSION

A rare Cis-AB phenotype has been found in various populations. Based on a survey of blood samples of Japanese populations, the ratio of Cis-AB to total AB ($A_1B$ and $A_2B$) was estimated to be about 0.012%, and the gene frequency of the Cis-AB, about $1.1 \times 10^{-5}$ [18]. The common characteristic of Cis-AB red cells from different individuals is that A and/or B antigens are weak or very weak on their red cells [1–3]. Recently, several investigators assayed blood group A- and B-enzyme activities of Cis-AB plasma obtained from several unrelated individuals and found that either A- or B-enzyme (or both A- and B-enzyme) activities are weak or very weak [11, 19–22].

The two conceivable genetic mechanisms of Cis-AB expression, i.e., (1) unequal chromosomal crossing over producing a single chromosome with the genes for both A- and B-enzymes, and (2) a structural mutation producing a mutant A- (or B-) enzyme which has a capacity to synthesize both A and B substances in red cells, can be tested by a biochemical approach. If Cis-AB is due to unequal chromosomal crossover, Cis-AB plasma should contain common A- ($A_1$ or $A_2$) and B-enzymes, although the quantities of these enzymes could be reduced because of the unusual arrangements of the two genes in a single chromosome. The kinetic properties of A- and B-enzymes from Cis-AB plasma should be identical to those of common A- ($A_1$ or $A_2$) and B-enzymes. It should be possible to separate A- and B-enzymes present in Cis-AB plasma from each other. In contrast, if Cis-AB is due to a structural mutation in *A* or *B* loci, Cis-AB plasma should contain a single abnormal enzyme with bifunctional

TABLE 2

SEPARATION OF A- AND B-ENZYMES BY SEPHAROSE 4B

| | A-enzyme activity | B-enzyme activity |
|---|---|---|
| Plasma placed on a column (5 ml) | 3.5 | 6.2 |
| Eluate through a column (6 ml) | 0.40 | 4.7 |
| Suspension of Sepharose 4B (5 ml) | 2.9 | < 0.3 |

NOTE.—Cis-AB plasma was treated with Sepharose 4B as described in MATERIALS AND METHODS. An aliquot, 50 $\mu$l, of the plasma, eluate, or suspension of Sepharose 4B-enzyme complex was used for assay of A- and B-enzyme activities. The enzyme activity is expressed as percentage of the sugar transferred into fucosyllactose under the assay condition specified in MATERIALS AND METHODS.

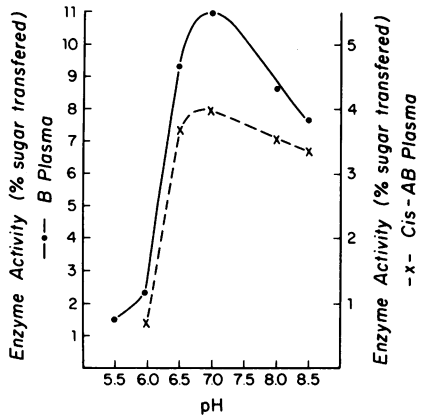


FIG. 4.—Effect of pH on B-enzyme activity. Composition of reaction mixture was specified in MATERIALS AND METHODS. Activity was assayed in 25 mM imidazole adjusted pH specified in figure. Crude B plasma (50 $\mu$l) and crude Cis-AB plasma (50 $\mu$l) were used as enzyme.

activity. The kinetic properties of the postulated mutant enzyme should be significantly different from those of common A- ($A_1$ or $A_2$) and B-enzymes.

This study reveals that the Cis-AB plasma examined contains two separable enzymes, one with kinetic properties similar to those of common $A_2$-enzyme, and another with kinetic characteristics similar to those of common B-enzyme. Therefore, Cis-AB expression, at least in the case examined, is due to unequal chromosomal crossing over, rather than to structural mutation in *A* ($A_1$ and $A_2$) or *B* genes.

The agglutinability of the subject red cells ($A_2B_3$; i.e., weak A and very weak B) is not in proportion with the plasma B-enzyme activity, which is 38% of the normal level. It has been reported that B-enzyme activity in the plasma from subjects with a weak B phenotype was similar to, or moderately weaker than, that of normal B or AB plasma [11, 23]. The B-enzyme activity of weak B plasma may not be linearly related to the enzyme activity in bone marrow tissues and in red cell membranes as previously suggested [11, 23].

In rare expressions, such as Cis-AB, the genetic mechanisms are not expected to be identical in unrelated subjects with the abnormality. Thus, some Cis-AB cases could be due to structural mutations producing bifunctional glycosyltransferases, and other cases, such as the case examined in this study, could be related to unequal chromosomal crossing over.

Several investigators examined kinetic properties of A- and B-enzymes in Cis-AB plasma samples from unrelated families, but the mechanisms of Cis-AB expression in these individuals remained unresolved [11, 20–22]. The genetic mechanisms of Cis-AB expressions in several unrelated Japanese individuals are currently being investigated.

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