Immunologic Homology of Human Blood Group Glycosyltransferases and Genetic Background of Blood Group (ABO) Determination

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The human blood groups (ABO) are known to be determined by the terminal carbohydrate residues attached to common carbohydrate chains of the red cell surface. Specific transferases, i.e., N-acetylgalactosaminyltransferase (A enzyme) in blood group A persons and galactosyltransferase (B enzyme) in blood group B persons, are responsible for transferring the carbohydrates to the terminal galactose of the H-substance of the O red cell surface, with both enzymes absent in blood group 0 persons. It has not been clear whether the expression of these transferases is related to allelic structural genes or is under regulatory control. The activities of A and B enzymes were completely neutralized by the anti-A-enzyme antibody, which was obtained from a rabbit immunized with purified A enzyme. When pretreated with a protein component obtained from blood group O plasma, the antibody had no capacity for neutralization of the two enzymes. If the transferases are under regulatory control, no immunologically cross-reactive protein should be produced in blood type O persons. The existence of an immunologically active component in blood group O plasma proves that the genes for A enzyme, B enzyme, and the immunologically cross-reactive protein in O plasma are allelic and excludes the possibility of involvement of regulatory control in blood group expression.

THE BLOOD GROUPS (ABO) are known to be determined by the terminal carbohydrate residues attached to common carbohydrate chains of the red cell surface. A specific N-acetylgalactosaminyltransferase (A enzyme) of blood group A persons transfers N-acetylgalactosamine from UDP-N-acetylgalactosamine to the terminal galactose of H-substance of the O red cell surface, while a specific galactosyltransferase (B enzyme) of blood group B persons transfers galactose from UDP-galactose to the terminal galactose of the O red cell surface.¹ ² Both enzymes are absent in blood group O persons; thus, the terminal galactose residues remain open, exhibiting blood group O specificity. The blood groups A, B, and O, and consequently the expression of the transferases, is highly polymorphic in man.

Although the transmission of A and B enzymes is compatible with mendelian inheritance, it has not been clear whether the expression of these blood transferases is related to allelic structural genes or is under regulatory control. This article attempts to resolve this problem.

MATERIALS AND METHODS

Human Blood Group Glycosyltransferases

Blood group N-acetylgalactosaminyltransferase (A enzyme) was partially purified to about 100,000-fold from plasma of blood group A, by treatment with Sepharose 4B. Blood group galactosyltransferase...
(B enzyme) was partially purified to about 1000-fold from plasma of blood group B by column chromatography with carboxymethyl-Sepharose and gel filtration with Sephadex G-200. The details of the purification procedures have been previously reported. The partially purified enzymes were reconstituted in a volume about 50 times less than the original volume of plasma. Both enzymes were stable at this stage of purification and were suitable for immunoneutralization studies. A enzyme was further purified to apparent homogeneity for producing antibody, as previously described.

**Immunologically Cross-Reacting Material (CRM) of O-Plasma**

An enzymatically inactive, but immunologically cross-reactive protein (O-CRM) was prepared from plasma of blood group O by column chromatography with carboxymethyl-Sepharose and gel filtration with Sephadex G-200. The procedures were identical to those used for purification of B enzyme. The partially purified O-CRM was reconstituted in a volume about 50 times less than the original volume of plasma.

**Antibody Against A Enzyme**

Blood group transferase activities of rabbit plasma were examined, and rabbits associated with neither A nor B enzyme activity were selected. In our limited experience, about 70% of New Zealand albino rabbits had blood group N-acetylgalactosaminyltransferase activity, and others had neither A nor B enzyme activity. Purified, homogeneous human A enzyme (0.18 mg, from 20 liters of A plasma) was dissolved in 0.5 ml of 0.15 M NaCl and mixed with an equal volume of complete Freund's adjuvant. The mixture (about 0.3 ml) was subcutaneously injected into a rabbit every 10 days. The blood was collected 10 days after the third injection. The antibody was salted out with 2 M (NH₄)₂SO₄ and dissolved in 0.01 M Tris-Cl, pH 7.0, in a volume about 10 times less than the original volume of the antiserum.

**Assay of A and B Enzyme Activity**

The transferase activities were assayed by measuring the incorporation of sugar from nucleotide sugars into red cell ghosts. The reaction mixture, 100 μl, for A enzyme assay contained 0.5% of red cell ghosts, 25 μM UDP-N-acetylgalactosamine (H₃G, O.5 μCi), 15 mM MnCl₂, 0.15 M NaCl, 0.2% bovine serum albumin, 0.25% Triton X-100, 1 mM NaN₃, 40 mM cacodylate buffer, pH 6.8, and enzyme. The reaction mixture for B enzyme assay contained 0.5% of red cell ghosts, 25 mM UDP-galactose (H₁G, 1 μCi), 15 mM MnCl₂, 0.15 M NaCl, 0.5% bovine serum albumin, 0.25% Triton X-100, 1 mM NaN₃, 25 mM imidazole buffer, pH 6.5, and enzyme. The reaction mixture was incubated for 16 hr at 37°C with shaking. The red cell ghosts were collected on a Millipore filter (diameter 13 mm, pore size 1.2 μm), washed thoroughly with water, and transferred into a scintillation vial for measurement of radioactivity. Alternatively, the ghosts were washed with saline by centrifugation and treated with B-lectin coupled with horseradish peroxidase. The peroxidase activity adsorbed to the ghosts was measured by the spectrophotometric method, using 4-amino antipyrine and H₂O₂ as substrates. Details of the assay method utilizing lectin-peroxidase will be reported elsewhere.

UDP-N-acetylgalactosamine was synthesized as previously reported. UDP-galactose was purchased from Sigma Chemical Co. UDP-N-acetylgalactosamine (galactosamine-1-H₃) was purchased from New England Nuclear, and UDP-galactose (galactose-6-H₃) was purchased from Amersham Corp. Purity of these materials was checked by column chromatography with Dowex-1 and found to be at least 97%.

The B-specific lectin was prepared from Streptomyces 27S5 as previously described. Horseradish peroxidase was purchased from Sigma Chemical Co.

**RESULTS**

**Neutralization of Blood Group A and B Enzymes by Anti-A-Enzyme Antibody**

When partially purified A enzyme, 20 μl, was incubated with various amounts of antibody in 0.01 M Tris-Cl, pH 7.0 (totaling 50 μl), for 5 hr at 4°C, the transferase activity decreased (Fig. 1). In the presence of larger quantities of antibody, A enzyme activity was completely neutralized. Control globulin preparation obtained from preimmune rabbit serum had no such neutralization capacity.

The anti-A-enzyme antibody also inactivated B enzyme. In contrast to A
enzyme, about 30% of galactosyltransferase activity, when measured by the incorporation of radioactive sugar into O red cell ghosts, remained un-neutralized even in the presence of large quantities of the antibody (Fig. 2). However, a more specific assay for blood group B enzyme activity, i.e., measurement of formation of blood group B substance on O red cell ghosts utilizing B-lectin coupled with peroxidase, revealed that blood group B enzyme was indeed completely neutralized by the anti-A-enzyme antibody (Fig. 2).

The un-neutralized galactosyltransferase activity was due to the non-blood-group galactosyltransferase, which contaminated the partially purified blood group B enzyme preparation. Existence of the non-blood-group galactosyltransferase, which transfers galactose from UDP-galactose into O red cell ghosts but does not produce group B substance, was observed in the O-CRM preparation and in the protein fraction obtained from A, plasma by the same fractionation procedure as that of the partially purified B enzyme preparation. The highly purified A enzyme preparation used for the above immunoneutralization did not contain the non-blood-group galactosyltransferase.
Fig. 3. Adsorption of anti-A-enzyme antibody by the O-CRM protein. An aliquot of the anti-A-enzyme antibody (---O----0--- 0 μl, --x--x-- 1.5 μl, --x--x-- 3 μl) was incubated with various amounts of O-CRM solution (to total 53 μl) in 0.01 M Tris-Cl, pH 7.0, at 4°C for 5 hr. The partially purified A enzyme, 20 μl, was mixed with 30 μl of the above antibody-O-CRM mixture and was incubated at 4°C for 5 hr. A 50-μl aliquot of the substrate solution was added to 50 μl of the enzyme-antibody solution to give final concentrations of substrates and reagents specified in the text, and was incubated at 37°C for 16 hr with shaking. Quantities of N-acetylgalactosamin transferred into O red cell ghosts were determined by measuring radioactivity of the washed O red cell ghosts. The results represent mean values and deviations of duplicate analysis.

Existence of Enzymatically Inactive but Immunologically Cross-Reactive Protein in O Plasma

O-CRM prepared from O plasma had neither blood group A nor B enzyme activity. However, when the anti-A-enzyme antibody was incubated with O-CRM for 5 hr at 4°C in 0.01 M Tris-Cl, pH 7.0, its capacity for neutralizing A enzyme diminished (Fig. 3). Similarly, the antibody pretreated with sufficient amounts of O-CRM preparation completely lost its capacity for inactivating blood group B enzyme. These results indicate the existence of enzymatically inactive, but immunologically cross-reactive, material in O plasma.

It was also observed that the antibody pretreated with B enzyme no longer neutralized A enzyme, and the antibody pretreated with A enzyme could not neutralize B enzyme.

Immunoprecipitation

On a double-gel diffusion test, no precipitin line was observed between the antibody and the purified A enzyme, partially purified A enzyme, partially purified B enzyme, or O-CRM preparation. Judging from the enzyme activity of homogeneous A and B enzyme preparations, the concentration of the enzymes used for the double-gel diffusion was about 1–5 μg/ml. The negative result in the precipitin test might be due to the low concentration of antigen and/or antibody used for the test, but it is also conceivable that the antigen–antibody complexes are nonprecipitable forms. In the above neutralization experiment, the enzyme solution incubated with the antibody lost its enzymatic activity even without removing the enzyme–antibody complex by centrifugation. This would suggest that the antibody binds to the active site of the enzyme, and therefore, the enzyme–antibody complex has no enzymatic activity.

DISCUSSION

Immunologic homology of blood group A and B enzymes and the protein component (O-CRM) of O plasma was clearly demonstrated by this study. Enzyme neutralization (Figs. 1 and 2) and competition (Fig. 3) profiles indicated that
concentrations of the homologous antigenic material are on the same order of magnitude in A, B, and O plasma. Complete inhibition of B enzyme by anti-A-enzyme antibody, and complete immunocompetition between A enzyme and O-CRM, between B enzyme and O-CRM, and between A enzyme and B enzyme suggests a high degree of structural homology among these three proteins.

Crude human plasma, regardless of ABO blood type, contains various glycosyltransferases, i.e., non-blood-group N-acetylgalactosaminyltransferase, non-blood-group galactosyltransferase, fucosyltransferase, and sialyltransferase. These transferases might immunologically cross-react with the anti-A-enzyme antibody. However, all these glycosyltransferases, except for non-blood-group galactosyltransferase, were removed from the O-CRM preparation by the purification procedures used. Consequently, these glycosyltransferases cannot be the O-CRM protein.

Non-blood-group galactosyltransferase, which was present in the B enzyme preparation and the O-CRM preparation, did not cross-react with the anti-A-enzyme antibody (Fig. 2). Therefore, this enzyme, which transfers galactose from UDP-galactose into glycoproteins but cannot produce B substance from H substance, is immunologically entirely different from the blood group B enzyme.

A generally accepted concept for the genetic mechanism of blood group AB expression is that A and B enzymes are controlled by allelic A and B genes located in a common gene locus on chromosome #9. In the blood group O person, either an enzymatically inactive protein is produced or the allele is “silent,” not producing any protein. Although this hypothesis is compatible with the observed mendelian inheritance of ABO blood groups, it has not yet been fully proven in the author’s knowledge, and it includes some problems that should be considered.

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Another possibility is that the genes for A enzyme and B enzyme are not really allelic. The expression of A gene and B gene might be controlled by allelic regulatory factor (α or β) that is located close to the A gene and B gene, in the same chromosome, and activates (or inactivates) the structural genes at cis position. In other words, A locus consists of nonallelic structural genes A and B and a regulatory gene α, and B locus consists of A, B, and β genes. Thus, only the gene for A enzyme is active in persons with α, and only the gene for B enzyme is active in
persons with $\beta$. In the blood group O persons, both A and B genes are shut off, and no protein can be produced by the two genes. This model is perfectly compatible with mendelian inheritance of ABO blood group expression. In order to account for the rare “Cis-AB” blood group expression, i.e., “AB” childbirth from $AB \times O$ parents, a “pseudo allelic” model was previously proposed.$^{12,13}$ The ratio of Cis-AB to total AB was estimated to be about 0.012%, and the gene frequency of the Cis-Ab to be about $1.1 \times 10^{-3}$ in the populations examined.$^{12,13}$ Moreover, in this model the two transferases are assumed to be synthesized by the nonallelic genes; thus, the structure of the A and B enzymes could be markedly different, favoring the distinct difference in substrate specificity of the two enzymes.

This study unequivocally demonstrated the existence of enzymatically inactive but immunologically cross-reactive protein in O plasma and the immunologic homology of A enzyme, B enzyme, and O-CRM. These facts exclude the second possibility described above. It can be concluded that the genes for A and B enzymes and for O-CRM are allelic, that they are located in a common locus, and that regulatory genes are not involved in the expression of blood group A, B, and O. Although it is quite unusual when compared to other mutant gene products, the mutations on this particular locus have induced a complete change of the transferase specificity for sugar donor from A enzyme to B enzyme, or vice versa, and a complete loss of enzyme activity in O-CRM. Previous biochemical studies showed that both A and B enzymes are dimeric forms (i.e., aa for A enzyme and bb for B enzyme), and their molecular weight (about 85,000) and subunit size (40,000-45,000) are similar.$^{5,7}$ Existence of the heterodimer (ab dimer) in heterozygous AB plasma has also been suggested.$^{14}$ These findings are not in contradiction to the above conclusion.

Since O gene produces the enzymatically inactive but immunologically cross-reactive protein, it is feasible to determine ABO genotypes by measuring the ratio between the transferase activity and the immunologic activity of the plasma samples. Such possibilities are currently under study in this laboratory.

REFERENCES


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