



Recent Approach in Conversion of Universal Blood Group by Enzymatic Method

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ABSTRACT

Blood group "O" plays a very important role in blood system. It can be transfused to A, B and AB recipients. By seeing the great demand of O blood group, scientists discovered different methods of blood conversion and making all blood type universal donor. Buffers play a very important role in enzymatic reaction of Blood Conversion. Different buffers were used for blood conversion.

Keywords: Universal Blood Group, Enzymatic conversion, Antigen, Antibodies, RBC.

INTRODUCTION

In the year 1900, Karl Landsteiner at the University of Vienna, discovered, why certain blood transfusions were positive while others could be unsuccessful. Landsteiner discovered the ABO blood group system by mixing the red cells and serum of every of his staff. He showed that the serum of some people followed the red cells of other. From these early experiments, he documented three types, called A, B and C (C was later to be re-named O for the German "Ohne", meaning "without", or "Zero", "null" in English). The fourth less common blood group AB, was discovered a year later. In 1930, Landsteiner acknowledged the Nobel Prize in physiology and medicine for his work¹. The gene that controls human ABO blood type is located on chromosome 9 (9q34.1) and is called ABO *glycosyltransferase*. The ABO locus has three main allelic forms: A, B, and O, as specified above and each of them are responsible for the production of its glycoprotein. It is then the alliance of alleles that are genetic from parents that rules which glycoproteins (antigens) are originate on persons' blood cells and thus their ABO blood type¹.

The ABO Blood System				
Blood Type (genotype)	Type A (AA, AO)	Type B (BB, BO)	Type AB (AB)	Type O (OO)
Red Blood Cell Surface Proteins (phenotype)	A agglutinogens only	B agglutinogens only	A and B agglutinogens	No agglutinogens
Plasma Antibodies (phenotype)	b agglutinin only	a agglutinin only	NONE No agglutinin	a and b agglutinin

Figure 1: ABO Blood Group System

In Transfusion Medicine, the ABO blood group system is considered as one of the most important blood group system². A and B antigens play a very important role in

blood system. Whole system is based on the presence and absence of a and b antigens. Carbohydrates structure which is designated by ABH, are found close to the oligosaccharide chains on glycolipids and glycoproteins on the surface of RBCs^{3,4}.

Blood group A

Blood group A specificity is a terminal α -1,3-linked-N-acetylgalactosamine (GalNAc). Blood group A plasma contains naturally occurring antibodies to the B antigen. The genetic basis of ABO antigens has been clarified; genes defining A and B blood types translate *glycosyltransferases* in which a few amino acid changes result in different substrate specificities: the A enzyme for UDP-GalNAc⁵. Blood group A exists in many subtypes. There are three recognized major subgroups of blood type A. These subtypes are A₁, A₂, A intermediate (A_{int}). There are both quantitative and qualitative differences that differentiate these three subtypes of A blood group. Quantitatively A₁ RBCs are having extra antigenic A sites than A_{int} RBCs which in turn is having more antigenic A sites than A₂ RBCs^{6,7}. Qualitatively, A₁ RBCs are having a dual repeated A structure on a subset of glycosphingolipids, though A₂ cells are having an H structure on a parallel subsets of glycolipids. These variances between A₁ and weak A subtypes are thought to relate to changes in the kinetic properties of blood group A isoenzyme variants responsible for the formulation of A antigens⁸.

Blood group B

Blood group B specificity is α -1,3-linked galactose (Gal). Blood group B individuals contains antibodies to the A antigen.

The genetic basis of ABO antigens has been clarified; genes determining A and B blood types encode *glycosyltransferases* in which a few amino acid replacements result in differing substrate specificities: the

B enzyme for UDP-Gal. the variance of B blood group subtypes are solely to quantitative nature⁵.

Blood group AB

Blood group AB is having both A and B antigens. No antibodies are present in AB blood group. It is also known as universal recipient⁸.

Blood group O

Blood group O cells lack both of these monosaccharides at the ends of their oligosaccharide chains, which instead are terminated with α -1,2-linked fucose (Fuc) residues and selected the H antigen. Blood group O plasma has both antibodies. The gene for the O blood type codes an inactive protein⁵. Blood group O is also known as Universal Donor and can be transfused to A, B and AB recipients. Group O is always in great demand for many reasons, for eg, if the patient is brought in emergency and its blood group is not known, group O is transfused. Further reasons-lacks of blood from a particular blood group, pediatric transfusions, and lack of clarity about the patient's blood group⁸.

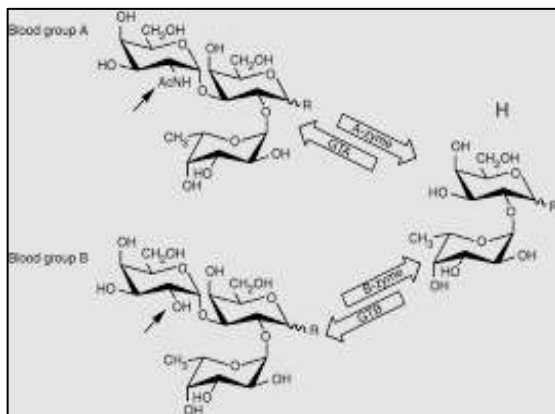


Figure 2: Structural basis of the ABO blood group antigens.

H-antigen

The precursor to the ABO blood group antigens is H-antigen. Irrespective of the ABO system, H antigen is present in all RBC. As H-antigen acts as precursor, its deficiency means the absence of antigen A and B. Though, the persons produce no antibodies to H-antigen as well as to antigens A and B⁹.

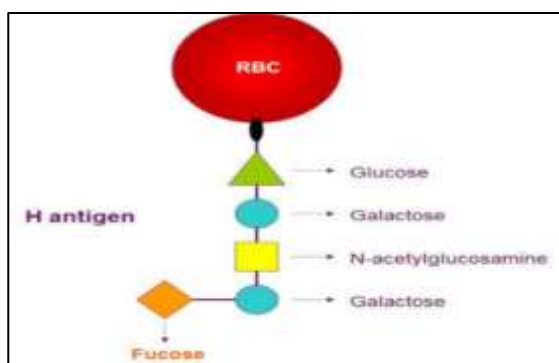


Figure 3: Generalized structure of H-antigen

Rhesus System

After ABO system, Rhesus-system is the second most important blood group system. Presently, the Rh-system comprises of 50 distinct blood group antigens out of which only five are important. RBC surface of an individual may or may not have any Rh factor or immunogenic D-antigen. Thus, the position is specified as either Rh-positive (D-antigen present) or Rh-negative (D-antigen absent). In compare to the ABO system, anti-Rh antibodies are, commonly, not present in the blood of persons with D-negative RBCs, except the circulatory system of these individuals has been exposed to D-positive RBCs. These immune antibodies are immunoglobulin G (IgG) in nature and hence, can cross the placenta⁹.

Current Trends and Future Areas of Research

Enzymatic Conversion of Blood Type

The discovery relates to the enzymatic elimination of type A and B antigens from blood group A, B and AB responsive cells in blood product, and thus changing these to non-A and non B responsive cells. Precisely this invention relates to enzymatically removal of the immunodominant monosaccharides stating the blood group A and B antigens, i.e. α 1,3 -D-acetylgalactosamine, correspondingly. More chiefly, this relates to the usage of exclusive α -N-acetylgalactosamine and α -N-acetylgalactosidase with higher kinetic properties for elimination of the immunodominant monosaccharides of the blood group A and B antigens and better performance in enzymatic conversion of red blood cells¹⁰.

Enzymatic conversion of antigens on RBCs by use of exoglycosidases for discriminatory exclusion of α GalNAc and α Gal residues of the immunodominant A and B trisaccharide antigens, correspondingly, to depict the primary H antigen¹¹. Properties of an *exoglycosidase* suitable for enzymatic conversion of RBCs include the following parameters: (i) high substrate specificity for the blood group antigens to limit the reaction to the immunodominant blood group A and B antigens (ii) reaction conditions appropriate for preservation of RBC reliability and functions (iii) high efficacy in cleavage of antigens on the RBC surface to decrease remaining antigens and enzyme consumption and (iv) possessions to enable enzyme exclusion from the RBCs by routine cell-washing techniques. The glycosidases presented in this study offer all of these characteristics⁸. In particular, the α -galactosidases represent, the only α 3-linkage-specific *exo-galactosidases* reported and their specificity for the branched blood group B trisaccharide structure suggests restricted substrate specificity^{12,13}. Fruitful conversion of group A RBCs has not yet been achieved. Biochemistry of A antigens is more complex. Subgroup A₁ RBCs have five times more A antigens than subgroup A₂[6,7]. Conversion of only the A₂ subgroup to group O was accomplished with a chicken liver α -N-acetylgalactosaminidase using 1g of enzyme per unit of RBCs at pH 5.5¹³. Ana-N-

acetylgalactosaminidase from *Clostridium perfringens* has also been used¹⁴. Purification of an α -N-acetylgalactosaminidase from *Arenibacter latericius* with neutral pH ideal, which seemed to change both subgroup A₁ and A₂ cells after 24 h incubation¹⁵. The ability of the conversion, still, was not confirmed by certified typing methods, and the gene has not yet been recognized. It is, then, not clear whether the difficulties with conversion of the A₁ subgroup relate to the difficulty of A antigens or to the deprived kinetic properties and acidic pH optima of the enzymes used to date^{13,14}.

For conversion of group B RBCs, Goldstein used an α -galactosidase derived from green coffee beans (*Coffea canephora*), has a very low pH optimum (pH 3.5) and required as much as 1–2 g enzyme per unit of group B RBCs for appropriate conversion at acidic pH 5.5. A phase 2 limit trial was completed with full unit (B200–220 ml packed) transfusions of group B RBCs enzyme-converted to group O (B-ECO), in which the converted cells functioned and survived normally in group A and O patients. Though this study clearly showed that enzymatic conversion of RBCs is viable and that B-ECO cells can function as well as ABO-matched natural cells in transfusions, a major difficult to clinical use of this way is the great amounts of enzyme required¹⁶⁻¹⁸.

Enzymatic conversion of blood group A, B and AB RBCs at neutral pH

The two glycosidase families GH109 and GH110 contain enzymes that have highly suitable kinetic properties for enzymatic elimination of A and B antigens from the surface of RBCs. Firstly, they developed a buffer system for enzymatic transformation of RBCs with the help of recombinant *E. meningosepticum*. The recombinant enzyme is having an isoelectric point of 7.6, and study of the electrostatic surface of the crystal structure specified that the protein surface near the catalytic region to some extent positive. So they assumed that the enzyme at neutral pH might interrelate with the durable negative charges of chiefly sialic acids on the surface of RBCs important to native concentration of the enzyme at substrate positions⁸.

Enzymatic conversion of blood group by using glycine buffer

The GH109 α -N-acetylgalactosaminidase family is the recognized source of enzymes which are appropriate for enzymatic conversion of blood group A RBCs.

In enzyme conversion process, the ionic strength was kept very low for glycine buffer with different concentration of NaCl. A antigens were completely removed in 200mM glycine (pH6.8) with 3mM of NaCl to 10mM or more. NaCl concentration increases the conversion efficacy. Knowingly, this featured also allowed a simple and efficient wash removed way of enzyme which involves three wash cycles in PBS. The remaining enzyme load associated with washed cells was reliably fewer than 40 mg/ml of packed RBCs as measured with a

sensitive capture enzyme-linked immunosorbent assay using total detergent lysates of cells. About 60 mg enzyme is necessary to change one unit of blood group A₁ cells. Conversion of the weak blood group A₂ subgroup, however, requires only 15 mg enzyme. The conversion process does not disturb physiologic and metabolic limitations of RBCs, counting osmotic fragility, stages of 2,3-DPG, ATP and met hemoglobin⁸.

The GH110 α -galactosidase family has unique restricted specificity for blood group B substrates. The recombinant *FragA* α -galactosidase was selected because it is having slightly basic isoelectric point like to the *E. meningosepticum* enzyme, the same glycine buffer system was used as for group A RBCs by enzymatic conversion of blood group B RBCs with the *FragA* enzyme. B-ECO RBCs that type as group O by licensed reagents and methods used in clinical blood bank practice. Comparable to results with the A conversion process, the low ionic strength is necessary for efficient conversion, and growing NaCl concentration in the glycine buffer or use of further buffer systems with greater ionic strength like PBS and phosphate-citrate falls the efficacy and has a very low enzyme consumption of 10 mg/ml packed RBCs for 60 min incubation period. Less than 2 mg enzyme are essential to convert one unit of blood group B cells⁸.

For AB blood group conversion, both the enzymes are used which were used for A and B blood group conversion⁸.

Enzymatic conversion of blood group by using Glucose and additional buffers

The buffer plays a major role in the enzymatic reaction involved in blood group conversion. It has been studied that a glycine buffer is appropriate for A to O or B to O blood group conversion. The scientist examined that the use of 5% glucose and additional buffers for A to O or B to O blood group conversion by α -N-acetylgalactosaminidase or α -galactosidase. The A to O conversion efficiency in glucose buffer was similar to that in glycine buffer with the same dose (>0.06 mg/mL pRBC). B to O conversion efficiency in glucose buffer was also similar to that in glycine buffer with the same dose (>0.005 mg/mL pRBC). Most enzymes could bind with RBC in glycine or glucose buffer, but few enzymes could bind with RBC in PBS, PCS, or normal saline^{2,10,16}.

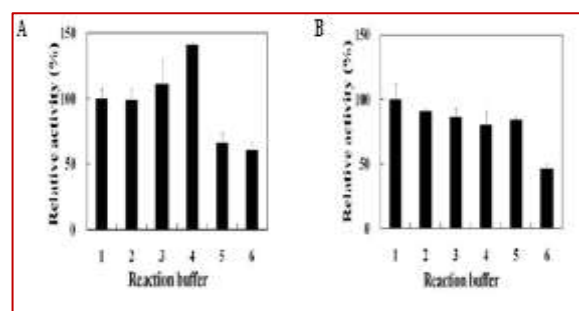


Figure 4: The activity of α -N-acetylgalactosaminidase(A) and α -galactosidase(B) in different buffer at the same

concentration of substrate.(1: control (100 mmol/L NaPO₄, pH 6.8, 50 mmol/L NaCl); 2: normal saline (0.9% NaCl); 3: PCS (77.25 mmol/L Na₂HPO₄, 11.375 mmol/L citric acid, 53.9 mmol/L NaCl, pH 6.8); 4: PBS; 5: glycine buffer (250 mmol/L glycine, 3 mmol/L NaCl, pH 6.8); 6: glucose buffer (5% glucose buffer).¹⁹

There are certain benefits of glucose as a reaction buffer of α -N-acetylgalactosaminidase or α -galactosidase. First, there is viable 5% glucose buffer on the market. Secondly, 5% glucose buffer is cheaper than glycine buffer, which would decrease costs¹⁹.

Glycine buffer was confirmed to be not appropriate for enzyme maintenance because precipitation occurred. Inappropriately, α -N-acetylgalactosaminidase or α -galactosidase also precipitated in glucose buffer signifying that the glucose buffer was not suitable for maintaining the enzymes either. Certain ion strength is necessary for keeping α -N-acetylgalactosaminidase or α -galactosidase activity. This might be because salt ions could prevent the accumulation of enzymes and avoid precipitation¹⁹. α -galactosidase has a latent use for eliminating the xenoantigen epitopes, Gal α 1,3Gal residues, on the surface of animal cells but not on human ones^{14,20}. It might, then, also be used in the production of xenografts, such as pig cruciate ligaments, skin, and bone. Thus, 5% glucose can be appropriate buffer for use in creating xenografts, in addition to being an A/B to O reaction buffer¹⁹.

CONCLUSION

Universal blood group is always in great demand. Enzymes and buffers play a very important role in the conversion process but there were some problems observed in preserving the enzymes. Certain ion strength is necessary for maintaining the enzymes activity which will help in preservation of enzyme for longer time period.

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