Bacterial glycosidases for making universal blood group O – the Holy Grail of Blood Transfusion

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The ABO system of blood groups was discovered by Karl Landsteiner in 1901 for which he was awarded the Nobel Prize for Physiology and Medicine¹. The basis of this classification has been the type of antigen on the red blood cells². The critical monosaccharides that determine the specificity of blood group A and B antigens are α -1,3 linked *N*-acetylgalactosamine and α -1,3-galactose, respectively. O group RBCs lack both. Theoretically, a simple hydrolytic cleavage of the glycosidic linkage of group A and B antigens would convert to blood group O type. This was conceptualized nearly 25 years back. The feasibility of these ideas, too, was demonstrated³. A number of glycosidases have been discovered from time to time but all lacked the efficiency of catalysis and the desired pH optimum, i.e., near neutrality⁴. In the absence of the technology to convert group A, B or AB to group O, the transfusion centers face a number of problems like human error in labeling resulting in mismatching of transfusion and consequent adverse reactions, even fatality. In addition, there are sudden heavy demands for the A, B, and AB type blood group in the event of accidents or other emergency situations where it is desirable to use only the universal blood group type for transfusion. Thus, highly competitive research has been underway to look for the right type of glycosidases with high catalytic efficiency and capacity to work at neutral pH. Such Holy Grail has been found recently by the group led by Qiyong P Liu of ZymeQuest Inc., Massachusetts, USA⁴ involving a multi-location international program with laboratories in France, the USA, Sweden and Denmark⁴. The authors report the discovery of bacterial glycosidase gene families that provide very efficient enzymes for removal of A and B antigens at neutral pH. The enzymatic conversion has the potential for producing universal RBCs which in turn would enhance the safety of blood transfusions⁴. The novel idea in these studies was the use of a different substrate (blood group A or B tetrasaccharide labeled with 7-amino-4-methyl-coumarin,

AMC) for screening the microbes for the desired enzymes. The previous efforts had been made using the monosaccharide *p*-nitrophenyl substrates. The latter substrates failed to identify the enzymes with selective and exclusive substrate specificities for more complex blood group A and B antigenic structures. The authors screened 2500 fungal and bacterial isolates for the glycosidase activities. Five of these isolates showed α -galactosidase activity with the B tetrasaccharide-AMC substrate and not with the monosaccharide Gal α -pNP substrate. Two isolates showed α -*N*-acetylgalactosaminidase activity with the A tetrasaccharide-AMC substrate as well as the monosaccharide GalNAc α -pNP substrate. Both activities exhibited neutral pH optima! The α -*N*-acetylgalactosaminidase activities identified in the screen were similar to those of *Elizabethkingia meningosepticum*. An N-terminal truncated construct (amino acids 18–444) of the *E. meningosepticum* was expressed efficiently in *Escherichia coli* with a yield of 1 g/l, purified to homogeneity and used for the studies⁴. The enzyme uses NAD⁺ as a cofactor. The two glycosidase families described in this study contain enzymes that have highly suitable kinetic properties for enzymatic removal of A and B antigens from the surface of RBCs. The efficiency of the conversion processes was demonstrated by Fluorescence-Activated Cell Sorting Analysis (FACS) using anti-A and anti-B reagents⁴.

References

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