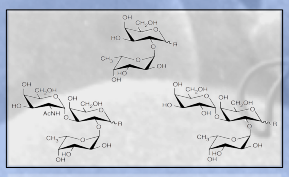
Enzymatic Blood Conversion

From blood group A to O

Guillaume Ponchel[1], Qiyong P. Liu[2], Eric P. Bennett[3], Martin L. Olsson[4,5], Jean Spence[2], Ed Nudelman[2], Yves Bourne[1], Bernard Henrissat[1], Henrik Clausen[2,3] & Gerlind Sulzenbacher[1]

[1]*Architecture et Fonction des Macromolécules Biologiques, UMR6098, CNRS, Universités Aix-Marseille I & II, Case 932, 163 Avenue de Luminy, 13288 Marseille Cedex 9, France.*
[2]*ZymeQuest Inc, 100 Cummings Center Suite 436H, Beverly, MA 01920.* [3]*Departments of Cellular and Molecular Medicine and Oral Diagnotics, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark.*
[4]*Division of Hematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University and University Hospital Blood Center, SE-22185, Lund, Sweden.*
[5]*Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA.*



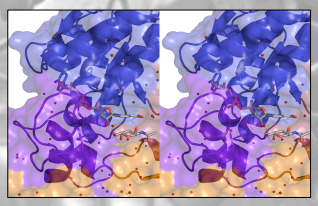
Blood group A (left), B (right) and H antigens. The H antigen identifies group O RBCs.

Blood conversion

The ABO blood group system is the most important to consider in transfusion medicine[1]. The signature of A and B groups are immunodominant monosaccharides, lacking from the O group red blood cell (RBC) surface[2]. Use of universal O group RBCs in every emergency case leads to recurrent blood banks shortage of O group RBCs, while other blood cell units are subject to outdating[3].

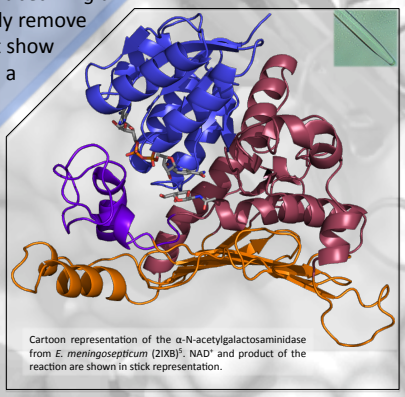
The enzymatic conversion of A and B antigens to the underlying H antigens, was proposed by Goldstein more than 25 years ago[4], but the lack of efficient enzymes prevented the up scaling of this life saving process[5]. Our collaborators from ZymeQuest screened more than 2500 bacteria and funghi to find novel enzymes, specific toward the blood antigens[5]. One of them, isolated from *Elizabethkingia meningosepticum*, remains today the only one proved to completely remove the A monosaccharide from all type A RBCs. As this enzyme did not show sequence homology with known glycosyl hydrolases (GH), it coined a new family, namely GH109, in the CAZy database[6].

Elizabethkingia meningosepticum α-N-acetylgalactosaminidase - Azyme



Stereoscopic view of the NAD^+ cofactor bond to *E.meningosepticum* Azyme.

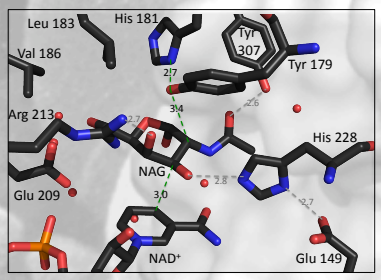
The Rossmann fold is shown in blue and the α-helical bundle closing the NAD^+ binding tunnel in violet.



Cartoon representation of the α-N-acetylgalactosaminidase from *E. meningosepticum* (2IXB)[5]. NAD^+ and product of the reaction are shown in stick representation.

E. meningosepticum Azyme's catalytic pathway[5].

Attempts to gather structural data for the enzyme/substrate complex failed. We have tried soaks and cocrystallization trials of inactive mutants (Y179S & H181S) with A trisaccharide and a derivate; We also conducted the native enzyme inhibitio, using a reducing agent. However, the aglycon part of the bound ligand was not clearly visible, leaving questions about the structural features governing substrate recognition.

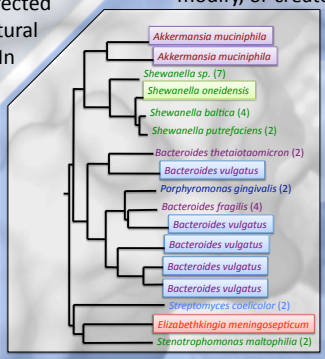


Tyrosine 225, coordinating C3-OH on N-acetylgalactose (NAG), is not shown for clarity.

In order to enhance the catalytic efficiency of Azyme by directed mutagenesis further structural studies will be conducted. In parallel we are investigating other members of GH109.

The GH109 family

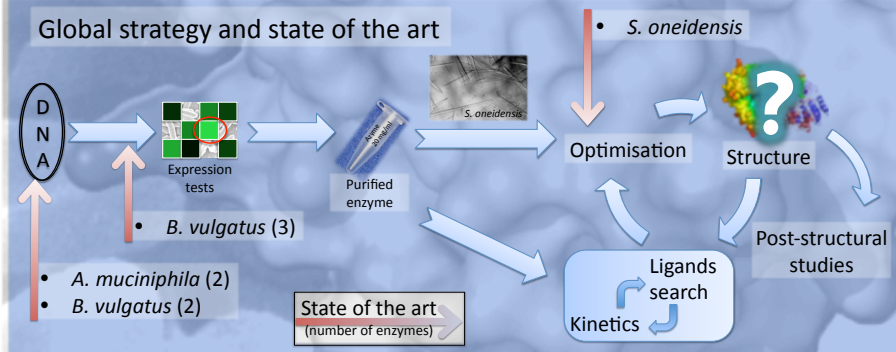
The Carbohydrate-Active enZyme database[6] (CAZy) describes the families of structurally-related enzymes that degrade, modify, or create glycosidic bonds.



Simplified phylogenic tree of GH109 family. Targets for structural and biochemical studies are highlighted.

This Maximum Likehood phylogenic tree was computed with a BLOSUM 62 matrix fed with protein sequences of the targets under study, plus one from each species with at least two members in the GH109 family.

Global strategy and state of the art



From these studies we await the answer to several questions: what are the key elements for the specific binding of complex oligosaccharides, what are the natural substrates for these enzymes produced by bacteria mostly living in soil, and, finally, what is their physiological role.

References. 1:Landsteiner, K. Agglutination phenomena of normal human blood. *Wien. Klin. Wochenschr.* **113**, 768–769 (2001). 2:Watkins, W.M. Biochemistry and Genetics of the ABO, Lewis, and P blood group systems. *Adv. Hum. Genet.* **10**, 1–136, 379–185 (1980). 3:Olsson, M.L. & Clausen, H. Modifying the red cell surface : towqrds an ABO-universal supply. *British Journal of Haematology*, **140**, 3–12 (2007). 4:Goldstein, J., Siviglia, G., Hurst, R., Lenny, L. & Reich, L. Group B érythrocytes enzymatically converted to group O survive normally in A, B, and O individuals. *Science* **215**, 168–170 (1982). 5:Liu, Q.P., Sulzenbcher, G., Yuan, H., Bennet, E.P., Pietz, G., Kristen, S., Spence, J., Nuldelman, E., Levery, S.B., White, T., Neveu, J.M., Lane, W.S., Bourne, Y., Olson, M.L., Henrissat, B. & Clausen, H. Bacterial glycosidases for the production of universal red blood cells. Nat. Biotech. **25**, 454-464 (2007). 6: Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res in press (2008), http://www.cazy.org.