UNIVERSAL BLOOD: THE LIFE STREAM

ALL FOUR ONE AND ONE FOR ALL

AN ENZYMATIC METHODOLOGY CREATING A UNIVERSAL BLOOD SUPPLY

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ABSTRACT

α-NAGA (α-N-acetylgalactosaminidase) is an exoglycosidase that cleaves a specific carbohydrate, terminal linked 1-3 α-N-acetylgalactosamine, from the A antigen. The enzymatic hydrolysis creates the H antigen. This modification transforms the immune response to the blood group from Type A to O. The product of the enzyme treatment produces Type O blood a universally transfusable product for potential medical use.

Purified recombinant α-NAGAs from *E. coli* and *S. linguale* were characterized *in vitro* for molecular mass, substrate specificity, pH and temperature optima, and product inhibition. A mutant of the *S. linguale* α-NAGA, H225A, was evaluated for activity *in vitro* and *in vivo*. *S. linguale* enzyme was characterized *in vivo* using Type A_1 and A_2 RBCs for pH, temperature, and buffer optima. *In vitro* each enzyme appeared to function as a dimer under the conditions tested, was highly active at a neutral pH, and over a range of temperatures. Turnover rate, k_{cat}, 33 s^{-1} (*E. coli*) and 173 s^{-1} (*S. linguale*) were derived from the kinetic assays. Product competitive inhibition, K_i 0.18 mM, was evident with the *S. linguale* α-NAGA while the *E. coli* α-NAGA demonstrated uncompetitive inhibition, K_i 2.6 mM. The *E. coli* α-NAGA did not appear to covert Type A RBCs. *S. linguale* α-NAGA appeared to convert Type A RBCs to blood group O with efficiency in glycine or alanine buffers at neutral pH at 4°C or 25°C. Pretreating RBCs with glycine solution 24 hours before enzyme conversion appeared to facilitate higher enzyme efficiency. The *S. linguale* α-NAGA H225A mutant was inactive both *in vitro* and *in vivo*.

The *S. linguale* α-NAGA appeared to possess desirable attributes to enzymatically convert RBCs. The use of Type A converted RBCs by *S. linguale* α-NAGA could potentially increase the universal blood supply by 40%.