

Public Abstract

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Title:UNIVERSAL BLOOD: THE LIFE STREAM

ALL FOUR ONE AND ONE FOR ALL

AN ENZYMATIC METHODOLOGY CREATING A UNIVERSAL BLOOD SUPPLY

$\hat{\pm}$ -NAGA ($\hat{\pm}$ -N-acetylgalactosaminidase) is an exoglycosidase that cleaves a specific carbohydrate, terminal linked 1-3 $\hat{\pm}$ -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 $\hat{\pm}$ -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* $\hat{\pm}$ -NAGA, H225A, was evaluated for activity in vitro and in vivo. *S. linguale* enzyme was characterized in vivo using Type A1 and A2 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* $\hat{\pm}$ -NAGA while the *E. coli* $\hat{\pm}$ -NAGA demonstrated uncompetitive inhibition, K_i 2.6 mM. The *E. coli* $\hat{\pm}$ -NAGA did not appear to convert Type A RBCs. *S. linguale* $\hat{\pm}$ -NAGA appeared to convert Type A RBCs to blood group O with efficiency in glycine or alanine buffers at neutral pH at 4 $\hat{\text{A}}$ ^oC or 25 $\hat{\text{A}}$ ^oC. Pretreating RBCs with glycine solution 24 hours before enzyme conversion appeared to facilitate higher enzyme efficiency. The *S. linguale* $\hat{\pm}$ -NAGA H225A mutant was inactive both in vitro and in vivo.

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