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 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, kcat, 33 s⁻¹ (E. coli) and 173 s⁻¹ (S. linguale) were derived from the kinetic assays. Product competitive inhibition, Ki 0.18 mM, was evident with the S. linguale α-NAGA while the E. coli α-NAGA demonstrated uncompetitive inhibition, Ki 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%.