

# Rational design of $\alpha$ -glucosidase for the synthesis of 2-O- $\alpha$ -D-glucopyranosyl-L-ascorbic acid

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## Abstract

$\alpha$ -Glucosidase (AG) is a bifunctional enzyme, it has a capacity to synthesize 2-O- $\alpha$ -D-glucopyranosyl-L-ascorbic acid (AA-2G) from L-ascorbic acid (L-AA) and low-cost maltose under mild conditions, but it can also hydrolyze AA-2G, which leads to low synthesis efficiency of AA-2G. Main Methods and Major Results This study introduces a rational molecular design strategy to regulate enzymatic reactions based on inhibiting the formation of ground state of enzyme-substrate complex. Y215 was analyzed as the key amino acid site affecting the affinity of AG to AA-2G and L-AA. For the purpose of reducing the hydrolysis efficiency of AA-2G, the mutant Y215W was obtained by analyzing the molecular docking binding energy and hydrogen bond formation between AG and the substrates. Compared with the wild type, Isothermal Titration Calorimetry(ITC) results showed that the equilibrium dissociation constant (KD) of the mutant for AA-2G was doubled; the Michaelis constant (Km) for AA-2G was reduced by 1.15 times; and the yield of synthetic AA-2G was increased by 39%. Conclusions and Implications Our work also provides a new reference strategy for the molecular modification of multifunctional enzymes and other enzymes in cascade reactions system.

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