Site-saturation mutagenesis of lysine 47 in cyclodextrin glycosyltransferase from Paenibacillus macerans to enhance its specificity towards maltodextrin

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We performed site-saturation mutagenesis of lysine 47 in cyclodextrin glycosyltransferase (CGTase) of Paenibacillus macerans to improve its specificity towards maltodextrin, which can be used as an inexpensive glycosyl donor for the enzymatic synthesis of 2-O-D-glucopyranosyl-L-ascorbic acid (AA-2G) by CGTase, an enzyme widely applied for the production of the glycosyl derivatives of stevioside, hesperidin, rutin and rhamnose. Compared to the wild-type CGTase, the maltodextrin specificity of the mutants K47F (lysine → phenylalanine), K47L (lysine → leucine), K47V (lysine → valine) and K47W (lysine → tryptophan) was increased by 30.7, 57.6, 47.0, and 29.2%, respectively. In accordance with the enhanced maltodextrin specificity, the AA-2G yield was increased by 35.8, 67.9, 55.2, and 34.3% by the mutants K47F, K47L, K47V, and K47W, respectively. Further investigation revealed that wild-type CGTase had a relatively higher cyclization activity than the four mutants, while the four mutants had a higher disproportionate activity (transfer of part of a linear oligosaccharide to another oligosaccharide) than the wild-type enzyme. Structure analysis using molecular modelling indicated that the enhancement of substrate specificity towards maltodextrin might be due to the weakening or removal of hydrogen-bonding interactions between the side chain of residue 47 and the bent intermediate or substrate. The optimal temperature and pH for AA-2G synthesis by the wild-type and four mutant CGTases were 36oC and pH 6.5, respectively. The optimal substrate ratio (L-ascorbic acid: maltodextrin) for the wild-type and mutants K47F, K47V, and K47W was 1:1, while that for mutant K47L was 5:1. Under optimal conditions, the titer of AA-2G produced by mutant K47L reached 2.99 g/L, which is 64.3% higher than that obtained by the wild-type CGTase (1.82 g/L). Here, we demonstrate that the mutant CGTases, especially K47L, show good potential using maltodextrin as an inexpensive substrate for the large-scale production of AA-2G.

Keywords: Cyclodextrin glycosyltransferase (CGTase), L-ascorbic acid (L-AA), maltodextrin, 2-O-glucopyranosyl-L-ascorbic acid (AA-2G), saturation mutagenesis