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Studies on the mutant G5-forming α-amylase of *Pseudomonas* sp. KO-8940 with regard to maltopentaose production from soluble starch

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Alpha-amylases (EC 3.2.1.1) are able to hydrolyze α-1,4-glucosidic linkages within starch molecules to maltooligosaccharides. The pure forms of separate oligosaccharides such as maltotriose, maltetraose, maltopentaose or maltohexaose can be prepared from hydrolyzed starch products and have potential for use in industries such as food & cosmetics. The price, however, is rather high because of the many difficulties in purification process. In order to construct the G5-*Amy* mutants which would improve the production of maltopentaose level, we used site-directed mutagenesis to substitute amino acid residues Q61, E223 and H290 of G5-*Amy*. These amino acids are corresponding to proposed binding residues of porcine pancreatic amylase. The mutant enzymes, Q61D, Q61E, Q61K, Q61N, E223H, E223D, E223N, H290K, H290R were expressed in *E.coli* using pET system. The level of enzyme exported into the periplasmic space of the E. coli host was very low. A TLC and HPLC analysis of crude extracts from periplasmic space show the mutants Q61D, Q61K and E223H are the most interesting ones regarding G5 formation. They were chosen for purification and characterization. The kinetic parameters of K_{cat} and K_m values of G5-*Amy*, E223H G61D, Q61K, for the soluble starch hydrolysis were 23437 U.min-1.mg-1; 3221 U.min-1.mg-1; 11224 U.min-1.mg-1; 6056 U.min-1.mg-1 and 2.25 mg.ml-1; 9.85 mg.ml-1; 1.71 mg.ml-1; 4.98 mg.ml-1, resp. The other biochemical properties were also determined as pH, temperature optimum for reaction, irreversible inactivation. Using HPLC analysis, the mutant H223 showed significant change in G5 production in comparison with the native enzyme. All data support the hypothesis that E223 in G5-*Amy* plays a role in substrate binding, while Q61 might contribute in conformational stability of the enzyme molecule and less in substrate binding. The mutant E223H might be one of promising candidate for an application.