Protein engineering of selected residues from conserved sequence regions of a novel Anoxybacillus α-amylase

ABSTRACT

The Ŭ-amylases from Anoxybacillus species (ASKA and ADTA), Bacillus aquimaris (BaqA) and Geobacillus thermoleovorans (GTA, Pizzo and GtamyII) were proposed as a novel group of the Ŭ-amylase family GH13. An ASKA yielding a high percentage of maltose upon its reaction on starch was chosen as a model to study the residues responsible for the biochemical properties. Four residues from conserved sequence regions (CSRs) were thus selected, and the mutants F113V (CSR-I), Y187F and L189I (CSR-II) and A161D (CSR-V) were characterised. Few changes in the optimum reaction temperature and pH were observed for all mutants. Whereas the Y187F (t1/2 43 h) and L189I (t1/2 36 h) mutants had a lower thermostability at 65°C than the native ASKA (t1/2 48 h), the mutants F113V and A161D exhibited an improved t1/2 of 51 h and 53 h, respectively. Among the mutants, only the A161D had a specific activity, kcat and kcat/Km higher (1.23-, 1.17- and 2.88-times, respectively) than the values determined for the ASKA. The replacement of the Ala-161 in the CSR-V with an aspartic acid also caused a significant reduction in the ratio of maltose formed. This finding suggests the Ala-161 may contribute to the high maltose production of the ASKA.

Keyword: Protein engineering; Conserved sequence regions; Novel Anoxybacillus Ŭ-amylase