Structural Analysis of Thr342 Mutants of Soybean β-Amylase: The Role of Conformational Changes of Two Loops in the Catalytic Mechanism

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Soybean β-amylase has two mobile loops in the active site, a flexible loop (residue 96-103) and an inner loop (residues 340-346). The flexible loop moves about 10 Å from "open" to "closed" form to make interactions with substrate. Though the movement is relatively small (about 3 Å), two different conformations of the inner loop have been found in the enzyme/substrate complexes. In the "product form", the Thr 342 residue creates hydrogen bonds with the Glu 186 (catalytic acid) and with the glucose residues at subsites -1 and +1, whereas most of those interactions are lost in the "apo form". To elucidate the relationship between the structural states of inner loop and the catalytic mechanism, Thr 342 was mutated to Val, Ser, and Ala, respectively, and their crystal structures complexed with maltose were determined together with that of the apo enzyme at 1.27-1.64 Å resolutions. The k_{cat} values of the T342V, T342S, and T342A mutants decreased by 13-, 360- and 1700-fold, respectively, compared to that of the wild-type enzyme. Whereas the inner loops in the wild-type/matose and T342V/maltose complexes adopted the product form, those of the T342S/maltose and T342A/maltose complexes showed the apo form. Structural analyses suggested that the side-chain of Thr 342 in product form plays an important role in distorting the sugar ring at subsite -1, stabilizing the deprotonated form of Glu 186, and grasping the glucose residue of the remaining substrate at subsite +1.

Keywords: amylases, mutations, enzymatic reaction mechanism