Poster Presentations

[MS5-P25] Structural titlation of mobile components in the active site of soybean β-amylase with maltose. Bunzo Mikami, Hirokazu Kawamura, Sousuke Yamada, Aiko Tanabe, Youna Kang, Kimihiko Mizutani and Nobuyuki Takahashi

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Soybean β-amylase catalyzes the liberation of β- anomeric maltose from the non-reducing ends of starch. The active site cleft of the enzyme is situated in the C- terminal side of $(\alpha/\alpha)_8$ barrel [1]. The enzyme has two mobile loops in the active site, a flexible loop (residue 96-103) [2] and an inner loop (residues 340-346) [3]. The flexible loop moves about 11 Å from "open" to "closed" form to make interactions with substrate. The structure of the inner loop changed about 3 Å upon the hydrolysis of the substrate. Except for these two mobile loops, the side-chain of Lys295 changed their directions before and after the substrate binding. In the trigonal β- amylase crystal, these mobile components can move without symmetry interactions. In order to elucidate the relationship between the conformations of these mobile components and the catalytic mechanism of the enzyme, the crystal diffraction data of the wild and several mutant soybean β-amylases (G95A, G96A, D101N, D101E, V99I, T342V, T342S, K295A and the deleted flexible loop mutant) were collected at 1.0-1.5 Å resolutions at SPring-8 after soaking the crystals in the different maltose concentration (0-200 mM). The structures including anisotropic temperature factors were refined with SHELXL. The refined structures enabled the estimations of the fractions of the bound two maltose molecules at subsites -2~-1 and +1~+2, and the extent of conformational changes of the mobile components in the active site of the enzyme. The estimated K_d values of maltose were smaller than that reported by a solution experiments. From the extent of conformational changes of the mobile components, we could estimate the sequential conformational changes of the mobile components during catalysis of the enzyme. The substrate is incorporated into active site (subsites -2~-1 and +1~+2) in the open flexible loop. The hydrolysis of the substrate occurs in connection with the conformational change of the inner loop after closing the flexible loop and conformational change of the side- chain of K295.

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