# MS5-P32 pH-profile analysis of β-amylase/maltose complex crystal measured at room temperature

Bunzo Mikami[1], Hirokazu Kawamura[1], Kimihiko Mizutani[1], Nobuyuki Takahashi[1]

1. Laboratory of Applied Structural Biology, Graduate School of Agriculture, Kyoto University, Japan

email: mikami@kais.kyoto-u.ac.jp

β-amylase catalyzes the liberation of maltose from the non-reducing ends of α-1, 4-glucan such as starch and glycogen. In contrast to α-amylase, β-amylase produces β-anomeric maltose, and is classified as an inverting enzyme. Though the structural features of β-amylases have been clarified, the detailed enzymatic mechanism of β-amylase action has not been well understood especially in the hydrolytic process including the activation of glycoside linkage to be cleaved. In soybean β-amylae (SBA), the hydrolysis of the α-1, 4-glucosyl linkage is proceeded by two catalytic residues, Glu186 (acid) and Glu380 (base). The enzyme has two mobile loops, flexible loop (residue 96-103) and inner loop (residue 341-345) near the active site. The conformation of these loops change during enzyme action. In this paper, we are intended to determine the structural changes of SBA/maltose complex in a different pH media. In order to control pH correctly, we have determined the crystal structure at room temperature to avoid the undesirable effect of freezing and cryo-protectant such as glycerol. SBA was crystallized by a hanging-drop vapor diffusion (10 ml of 10mg/ml enzyme) against 1 ml of the bottom solution containing 45% saturated ammonium sulfate. The obtained crystals larger than 0.3 mm length were packed in glass capillaries after soaked with 200mM maltose in the different pH buffer, containing 45% saturated ammonium sulfate for 30min at 20˚C. More than ten diffraction data sets were collected at room temperature with a CCD detector at BL26B1 beam-line at SPring-8. The crystal of SBA belonged to a space group of $P3_121$.The crystal data were collected with 98-100 % completeness and $R_{merge}$ of 0.04-0.05 up to 1.58-1.68 Å resolution. The models were refined with SHELXL program including protein anisotropic B-factors. The refined models contains one molecules of SBA comprising 492 amino acid residues, 3-7 sulfate ions and 403-450 water molecules with R = 0.12-0.13 and $R_{free}$ = 0.14-0.16. At pH 4.9-7.8, two maltose molecules were located at the subsites -2~-1 and +1~+2 with α-anomer boat form at subsite -1, whereas α/β-anomer chair forms were clearly found at pH below 4.4 (Fig. 1). This indicates that the glucose residue at subsite -1 is distorted to boat form by the deprotonation of a protein residue in the active site. The elucidation of the structure significance of the distorted sugar form on the catalytic process of this enzyme is now in progress.

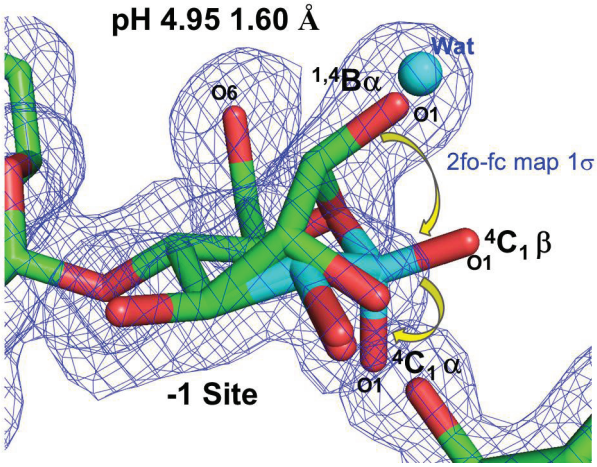


**Figure 1.** The effect of pH on the binding mode of maltose in the active site of SBA.

**Keywords:** beta-amylase, enzyme/substrate complex, enzyme catalytic mechanism