## MS09-P07

### Crystal structures of Bowman-Birk Inhibitor in complex with α-chymotrypsin

Eva Johansson[1], Christian Wenzel Tornøe[1]

1. Department of Protein and Peptide Chemistry 1, Novo Nordisk A/S, Måløv, Denmark

**email: evjh@novonordisk.com**

Bowman-Birk Inhibitor (BBI) protein from soy bean is a serine protease inhibitor of 71 amino acid residues containing seven disulphide bonds. It has two distinct 9 amino acid loops which inhibit α-chymotrypsin and trypsin, respectively. Chemical protein synthesis, using a divergent strategy, was used to prepare analogues of BBI to improve α-chymotrypsin inhibition. Four BBI analogues were prepared, and a four-fold improvement in chymotrypsin inhibition was obtained. Crystal structures of co-crystallised α-chymotrypsin:BBI complexes were determined for both wtBBI from soy bean and synthetic 27L,42T,43F,45I,47P-BBI variant. The crystal structures confirmed the correct protein fold of the synthetic BBI and showed a similar overall structure to the wtBBI.

The improved inhibition of α-chymotrypsin by the modified BBI may be explained comparing the two complex structures. The entire Phe43 amino acid residue is clearly pulled further into the chymotrypsin P1 pocket. This also results in withdrawal of the Thr42 backbone carbonyl group preventing a hydrogen bond formation across the inhibitory loop present in the α-chymotrypsin:wtBBI structure. However, the A42T modification provides the possibility of an alternative hydrogen bond formation utilising the threonine residue side chain hydroxyl group instead of the backbone carbonyl. Furthermore, the introduction of the Pro47 residue makes the structure more rigid and facilitates the hydrogen bond formation mentioned above.

References:

Tornøe, C., Johansson, E. & Wahlund, P.-O. (2017) Synlett, 28, 1901-1906

**Keywords: Bowman-Birk inhibitor, chymotrypsin, chemical protein synthesis**

## MS09-P08

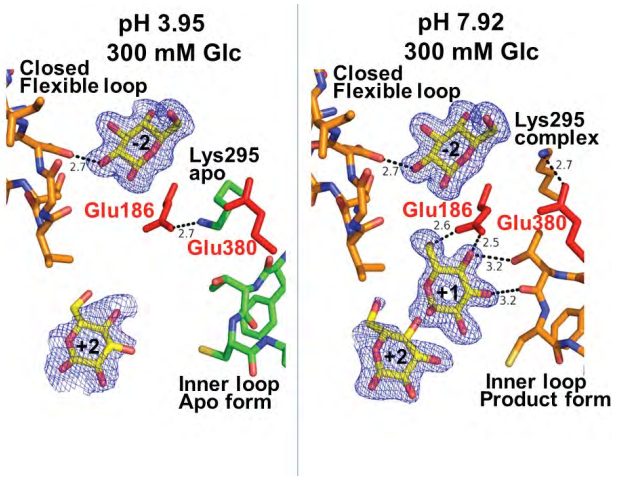
### pH dependent conformational changes of β-amylase/glucose complex crystal measured at room temperature

Bunzo Mikami[1], Xueni Hou[1], Kotaro Mori[1], Adachi Motoyasu[2], Kimihiko Mizutani[1], Nobuyuki Takahashi[1]

1. Graduate School of Agriculture, Kyoto University, Uji, Kyoto, Japan
2. Japan Atomic Agency, Tokai-mura, Ibaraki, Japan

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

β-Amylase catalyzes the liberation of maltose from the non-reducing ends of starch. In contrast to a-amylase, b-amylase produces β-anomeric maltose, and is classified as an inverting enzyme. In soybean β-amylae (SBA), the hydrolysis of the α-1, 4-glycosyl linkage is proceeded by two catalytic residues, Glu186 (acid) and Glu380 (base) where the substrate binding site consists of five subsites (-2, -1, +1, +2 and +3). Near this active site, the enzyme has two mobile loops, flexible loop (residue 96-103) and inner loop (residue 341-345). The conformation of these loops change from open to closed form and from apo to product form, respectively, during enzyme action. The side-chain of Lys295 also changes conformation from apo to complex form. In this paper, we are intended to determine the structural changes of SBA/G1 (glucose) complexes in a different pH media. We have determined the crystal structure at room temperature to avoid the undesirable effect of freezing and cryo-protectant. SBA was crystallized by a hanging-drop vapor diffusion against 1 ml of the bottom solution containing 45% saturated ammonium sulfate, at pH 5.4 and 4˚C. The obtained crystals were packed in glass capillaries after soaked with 0~300mM G1 in the different pH media for 30min at 20˚C. The diffraction data sets were collected at 20˚C with a MX225HE (Rayonix) detector at BL26B1 beam-line in SPring-8. The crystal belonged to $P3_121$ with cell dimensions of a = b = 84-85 and c = 144-145 Å. The crystal data were collected with 98-100 % completeness and $R_{merge}$ of 0.04-0.07 up to 1.6-1.9 Å resolution. The models were refined with SHELXL with R = 0.13-0.14 and $R_{free}$ = 0.15-0.17. At pH 3.95, two G1 molecules were located at the subsites -2 and +2 with open flexible loop, apo inner loop and apo form of Lys295, whereas at pH 7.9, three G1 molecules were found at subsites -2, +1 and +2 with closed flexible loop, product form of the inner loop and complex form of Lys295 (Fig. 1). These results indicate that the conformational change of the inner loop and the side-chain of Lys295 depend on the G1 binding at +1 site. We are now trying to determine the dissociable residue controlling the sugar binding and the conformational changes of the active site




**Keywords: β-amylase, enzyme reaction, enzyme/substrate complex**

## MS09-P09

# Structure analysis of haloalkane dehalogenase DbeA ΔCl variant from *Bradyrhizobium elkanii* USDA94

Tatyana Prudnikova[1], Pavlina Rezacova[1-2], Radka Chaloupkova[3], Jiri Damborsky[3], Ivana Kuta Smatanova[4]

1. Institute of Organic Chemistry and Biochemistry, South Bohemia University , Ceske Budejovice, Czech Republic
2. Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic
3. Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Brno, Czech Republic
4. Faculty of Science, University of South Bohemia , Ceske Budejovice, Czech Republic

**email: talianensis@gmail.com**

A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkanii* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. The DbeA protein was crystallised using the sitting-drop vapour-diffusion method and the crystal structure of a DbeA enzyme has been solved and deposited at Worldwide Protein Data Bank under PDB ID 4k2a. The DbeA wt structure revealed the presence of two halide-binding sites. The first chloride-binding site is located in the active site in between two halide-stabilizing residues. The second halide-binding site is unique to DbeA and has not been previously reported in any other structure of this enzyme family. To elucidate the role of the second halide-binding site, a two-point variant DbeA ΔCl (I44L+Q102H) lacking this site was constructed and biochemically characterized [1]. Elimination of the second halide-binding site decreased the stability and catalytic activity, and dramatically altered the substrate specificity. The two-point substitution resulted in a shift of the substrate-specificity class, which is the first time this has been demonstrated for this enzyme family. Rational design of buried halide-binding sites represents a novel strategy for engineering of enzymes with modified catalytic properties.

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References:

1. Chaloupkova R, et al., Acta Crystallogr. D70, 1884-1897 (2014)

**Keywords: Haloalkane dehalogenase, second halide binding site and halogenated compounds**