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

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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

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

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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