Poster Presentation

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High-resolution neutron structure analyses of porcine pancreatic elastase

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Elastase is a serine protease classified in the chymotrypsin family, and is attractive target for studies of structure based drug design (SBDD). The structural information including hydrogen positions and hydration will help us to further elucidate the catalytic mechanism of serine protease. To obtain such structural information, we performed the neutron structure analyses of porcine pancreatic elastase (PPE) with and without its inhibitor using diffraction data obtained at a BIX-3 diffractometer in the research reactor JRR-3. The PPE structure in complex with a peptidic inhibitor, which was used to mimic the tetrahedral intermediate state, was determined to 1.65 Å resolution [1]. His57, Asp102, and Ser195 (chymotrypsin numbering) compose the "catalytic triad" conserved in the active site of serine protease. The complex structure determined by neutron crystallography shows that the hydrogen bond between His57 and Asp102 is essentially short but conventional hydrogen bond, not a low-barrier hydrogen bond. In addition, this neutron structure clearly shows that the oxygen of oxopropyl group of the inhibitor is present as an oxygen anion rather than a hydroxyl group, supporting the role of the oxyanion hole in stabilizing the intermediate in catalysis. The neutron structure of PPE without inhibitor determined to 1.9 Å resolution shows that a water molecule and hydroxyl group of Ser195 block to two backbone amides of Gly193 and Ser195, which form oxyanion hole, respectively. This structural information allows us to understand the role of resting state upon the catalytic reaction. Furthermore, the structural change of the active site residues including hydration structure obtained from the comparison between structures with and without inhibitor may help designing potent inhibitors by SBDD.

[1] *T. Tamada, T. Kinoshita, K. Kurihara et al., J. Am. Chem. Soc., 2009, 131, 11033-11040*

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