The research activities in this laboratory are performed for X-ray structural analyses of biological macromolecules and the investigation of the electronic state in materials as follows: The main subjects of the biomolecular crystallography are crystallographic studies on the reaction mechanism of enzymes, the relationship between the multiform conformation and the functional variety of proteins, and the mechanism of thermostabilization of proteins. In the investigation of the chemical state in materials, the characteristics of the chemical bonding in the atom and molecules are investigated in detail using a newly developed X-ray spectrometer with a high-resolution in order to elucidate the property of materials. The theoretical analysis of the electronic states with DV-Xα and WIEN2k, and the development of new typed X-ray spectrometer with ultra high-resolution have also been carried out.

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Research Activities (Year 2005)

Presentations

Structure and Inhibition Mode of Protein Ic in Complex with Carboxypeptidase Y, Hata Y, Hayashida M, Fujii T et al., XX Congress of the IUCr, 30 August.

The Structure of Carboxypeptidase Y Inhibitor Ic in Complex with the Cognate Proteinase and a Novel Mode of the Proteinase-Protein Inhibitor Interaction, Hata Y, Hayashida M, Fujii T et al., Pacificchem2005, 16 December.

Structure of the Carboxypeptidase Y Inhibitor Ic in Complex with the Cognate Proteinase Reveals a Novel Mode of the Proteinase-Protein Inhibitor Interaction, Mima J (Kyoto University), Hayashida M, Fujii T et al., J. Mol. Biol. 346, 1323-1334 (2005).


Grants

Hata Y, Structural Analyses of Gene-products Involved in Protein Structure Formation, Protein 3000 Project, 1 April 2002 - 31 March 2007.


Crystal Structure of the Inhibitor IC in Complex with Carboxypeptidase Y

The proteinous proteinase inhibitor IC, which exists in the cytoplasm of the yeast *Saccharomyces cerevisiae*, specifically forms the 1:1 complex with and then inhibits carboxypeptidase Y (CPY) which is the vacuolar serine carboxypeptidase. The inhibitor belongs to the phosphatidylethanolamine-binding protein (PEBP) family. In order to reveal the inhibitory mode of IC against CPY, we determined the 2.7 Å crystal structure of the IC–CPY complex by the MR method of X-ray crystallography. This represents the first structure of a natural serine carboxypeptidase inhibitor complexed with the cognate proteinase and also that of a PEBP member complexed with its macromolecular binding partner.

The final structure model of the IC–CPY complex contains 204 amino acid residues of IC consisting of 219 amino acid residues. Residues Lys73–Ala78 and Glu122–Ala130 of IC are excluded in the model because they are invisible in the electron density map. The overall structure of the complex is a compact ellipsoidal structure (Fig 1). The structure of the complex contains two binding sites of IC toward CPY: the N-terminal inhibitory reactive site and the secondary CPY-binding site, which interact with the S1 substrate-binding site of CPY and the hydrophobic surface flanked by the active site of the enzyme, respectively. The structure of IC in complex with CPY consists of one major β-type domain and an N-terminal helical segment (Fig 2). The N-terminal segment is the helical region 1 (HR1), which is formed by one 310-helix (η1) and two α-helices (α1-2) and is in direct contact with CPY. The major domain is made up of three segments: the strand-rich region 1 (SR1), strand-rich region 2 (SR2), and helical region 2 (HR2). IC is also revealed to have the ligand-binding site, which is conserved among PEBPs and the putative binding site of the polar head group of phospholipid.

![Figure 1](image1.png)  
*Figure 1.* Ribbon drawing of the IC–CPY complex at 2.7 Å resolution. IC and CPY are shown in blue and green, respectively. Sulfate ions are represented by red spheres.

![Figure 2](image2.png)  
*Figure 2.* A topology diagram of the secondary-structure elements in IC. β-Strands (β1-9) are represented by arrows, and α-helices (α1-3) and 310-helices (η1-4) by rectangles.

To further clarify the proteinase-inhibition mode of IC indicated by the three-dimensional structure of the complex, we analysed the biochemical properties of various IC mutants: the N-terminal unacetylated form (unaIC), an N-terminal modified form with the substitution of Gly for the acetyl group (gIC), the N-terminal seven-residues deleted form (d1-7IC), and the unacetylated and C-terminal deleted form (d217-219IC). The inhibition of anilidase activity of CPY by the native and mutant forms of IC showed that the inhibitor constants, Kᵢ, for unaIC, gIC, and d217-219IC were increased by 590-, 180-, and 550-fold, respectively, over that of the native and that the N-terminal deleted mutant (d1-7IC) had no detectable inhibitory activities against CPY (Fig 3). These results indicate that the N-terminal acetyl group has optimal properties for the efficient inactivation of CPY and that the N-terminal seven residues of IC, designated as the N-terminal inhibitory reactive site, are absolutely essential for the inhibitory activity of IC in solution.

![Figure 3](image3.png)  
*Figure 3.* Stoichiometry of CPY inhibition by IC and its mutants.