Advanced Research Center for Beam Science
- Structural Molecular Biology -

http://www.scl.kyoto-u.ac.jp/~hata/indexE.html

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Dr SHERMAN, Evegeny	Department of Physics, University of Toronto, Canada, 15–26 October 2006
Dr JULIEN, Christian	Institut des Nanoscience de Paris, Universite Pierre et Marie Curie, France, 5–6 September 2006

Scope of Research

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 biomacromolecular 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 spectromator 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.

Research Activities (Year 2006)

Grants

Hata Y, Structural Analyses of Gene-products Involved in Protein Structure Formation, Protein 3000 Project, 1 April 2002–31 March 2006.

Sanjoh A (Protein Wave Corporation), Hata Y *et al.*, Studies and Developments on Practical Use of Devices for Growth of Protein Crystals Suitable for Ultra-high Resolution X-ray Analysis, Grant for Support of Studies and Developments by Cooperation of Industry, Academic and Public, Kyoto Sangyo 21 Foundation, 1 April 2004–31 March 2006.

Ito Y, Development of Basic Technologies for New Functional Particle Materials, Kyoto Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST, 2004–2008.

Crystallographic Studies of Binding Mode of Protein I^C toward Carboxypeptidase Y

The protein I^C from *Saccharomyces cerevisiae* inhibits carboxypeptidase Y (CPY) by forming the 1:1 complex. We determined the 2.7 Å crystal structure of the I^C-CPY complex by X-ray crystallography to reveal the inhibitory mode of I^C against CPY (Figure 1).

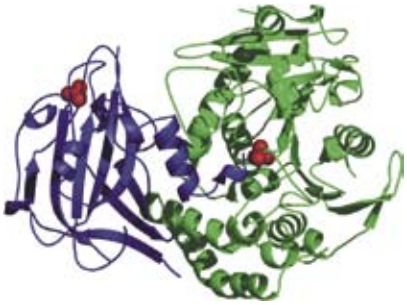

Figure 1. Ribbon drawing of the I^C-CPY complex at 2.7 Å resolution. I^C and CPY are shown in blue, green, respectively. Sulfate ions are represented by red spheres.

The structure of the I^C-CPY complex reveals that I^C binds to CPY with multiple-binding sites and that the N-terminal portion (Ac-Met1I–Lys73I) contains not only the N-terminal inhibitory reactive site (Figure 2a) but also a large portion of the secondary binding site (Figure 2b). In addition, the complex structure reveals that the specific binding of the N-terminal acetyl group to the active site of CPY is a novel proteinase–protein inhibitor interaction, and contributes to a tight interaction with CPY and to its complete inactivation.

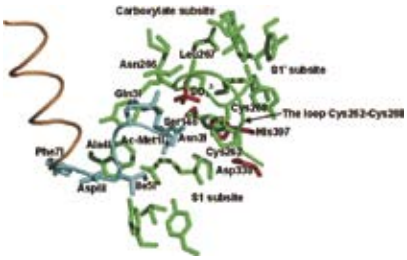

Figure 2a. Binding of the N-terminal inhibitory reactive site to the active site of CPY. The side-chains of residues that form the catalytic triad (red) and substrate-binding sites of CPY and the inhibitory reactive site of I^C are depicted as stick models.

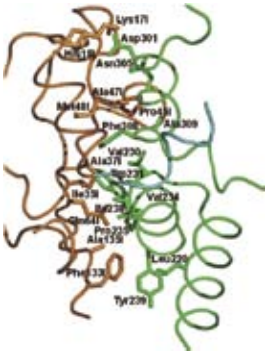

Figure 2b. The secondary binding site of I^C. The side-chains of residues involved in the interaction at the binding interface are depicted as stick models with labels.

To further clarify the proteinase-inhibition mode of I^C indicated by the three-dimensional structure of the complex with CPY, we analyzed the biochemical properties of various I^C mutants: the N-terminal unacetylated form (unaI^C), the N-terminal modified form in which Gly is added to the N-terminus to mimic the acetyl group (gI^C), the N-terminal deleted form (d1-7I^C), and the unacetylated and C-terminal deleted form (d217-219I^C). Gel filtration chromatography of mixtures of the native and mutant inhibitors with CPY showed that I^C, unaI^C, gI^C, and d217-219I^C produced single peaks in the position corresponding to the I^C-CPY complex but that the N-terminal deleted mutant (d1-7I^C) formed no complex with CPY (Figure 3).

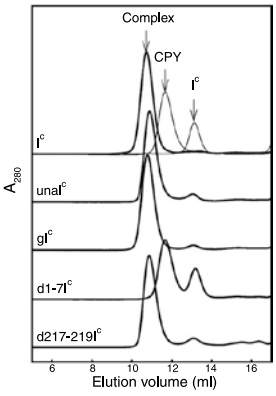

Figure 3. Gel filtration analysis of equimolecular mixtures of the native and mutant forms of I^C with CPY. Thick lines, the mixtures; thin lines, free CPY and I^C used as the reference.

The majority of endoproteinase inhibitors and carboxypeptidase inhibitors, the three-dimensional structures of which were previously elucidated at atomic resolution, were directed toward their target proteinases so that they interact with the active sites of the proteinases in a substrate-like manner through an inhibitory reactive site alone. In contrast, the inhibition and interaction modes of I^C toward CPY have the three features that are different from those of the canonical inhibitors described above: (1) the masking of the active site of CPY in a non-substrate-like manner, (2) the involvement of the N-terminal acetyl group introduced posttranslationally in the complete inhibition of the proteinase, and (3) multiple-site binding to the proteinase. Thus, the binding of I^C toward CPY is the prototype of a novel class of proteinase-protein inhibitor interactions. Furthermore, considering the loss of the binding affinity of the N-terminal deleted mutant (d1-7I^C) to CPY (Figure 3), it is reasonable to assume that I^C firstly binds to CPY via the inhibitory reactive site alone, and then the secondary CPY-binding site come into contact with the enzyme to form the stable complex between the proteins.