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 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.

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.

Crystallographic Studies of Binding Mode of Protein $\Gamma^C$ toward Carboxypeptidase Y

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

The structure of the $\Gamma^C$-CPY complex reveals that $\Gamma^C$ binds to CPY with multiple-binding sites and that the N-terminal portion (Ac-Met1–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.

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 $\Gamma^C$ 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 $\Gamma^C$ 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 $\Gamma^C$ 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.

To further clarify the proteinase-inhibition mode of $\Gamma^C$ indicated by the three-dimensional structure of the complex with CPY, we analyzed the biochemical properties of various $\Gamma^C$ 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 (gfI$^C$), 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 $\Gamma^C$, unaI$^C$, gfI$^C$, and d217-219I$^C$ produced single peaks in the position corresponding to the $\Gamma^C$-CPY complex but that the N-terminal deleted mutant (d1-7I$^C$) formed no complex with CPY (Figure 3).