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Binding of various azobenzene-amino acid Schiff base copper(II) complexes with egg white lysozyme and their X-ray crystallographic analysis

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Based on computational studies, herein we have introduced Schiff base copper(II) complexes of amino acid (SER, THR, TYR, VAL) derivatives with azobenzene moieties into lysozyme crystals by the immersion method and co-crystallization, and performed X-ray crystal structure analysis to discuss the binding mode and the three-dimensional structural changes associated with photoisomerization.

1 Introduction

In recent years, research into the conjugation of azo compounds to proteins to impart photoresponsiveness and photocontrol of chemical functions has been attracting attention. We have conjugated an amino acid derivative Schiff base copper(II) complex to egg white lysozyme and succeeded in imparting SOD (superoxide dismutase) activity [1,2]. Furthermore, we have clarified the interactions between the amino acid side chains in lysozyme and the metal ions and ligands of the THR derivative complex by X-ray crystal structure analysis.

2 Experiment

Using various amino acids with azobenzene moiety, experiments were carried out according to the literature [3]. Commercially available lysozyme (Wako-Fujifirm) was used. After adjusting phosphate buffer solutions of copper(II) complexes and lysozyme [1,4], we confirmed conventional spectra and grown the binding single crystals. Data collection of X-ray diffraction was carried out at KEK-PF BL-5A as automatic measurement. The electron density of copper(II) ions was confirmed using the analysis program packages CCP4i and Coot, and 3D images were created using the graphic software PyMOL. From the results, data in which the complexes were appropriately introduced were selected and refined.

3 Results and Discussion

When the amino acids VAL, TYR, and SER were used, copper ions were coordinated to GLU-35 and VAL-109, and interaction with an azobenzene-Schiff base copper(II) complex was confirmed. When THR was used, no binding or coordination of metal complexes was observed. When soaking was performed during photoisomerization by UV light irradiation, crystal structure analysis did not confirm large electron density in the ligands or complexes.

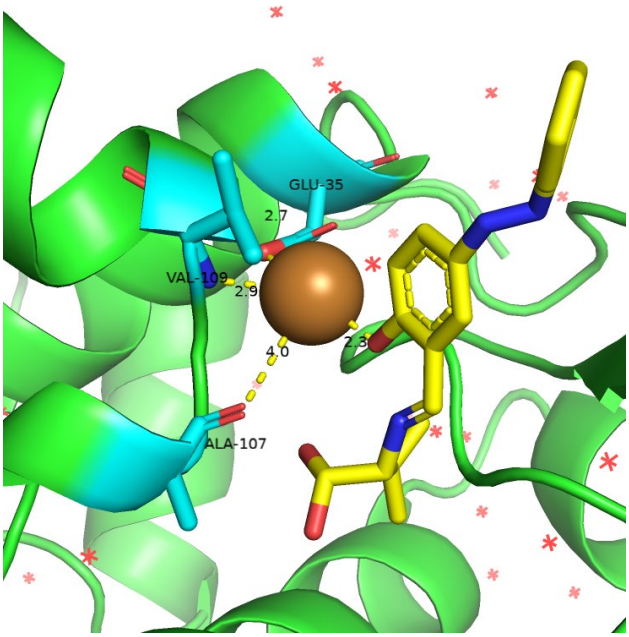

Fig. 1: Binding site of lysozyme and VAL-complex.

Research Achievements

1. In the complexation of lysozyme with Schiff base copper(II) complexes of amino acids containing azobenzene moieties (tyrosine, threonine, valine, serine), it was revealed that the amino acids tyrosine, valine, and serine interacted with GLU-35 and VAL-109.
2. When threonine was used as the amino acid, no binding of the complex or ligand to lysozyme was confirmed.
3. In the case of related complexes that did not contain azobenzene, the copper(II) ion almost always dissociated during soaking. In this study, crystals that maintained the metal complex and bound to lysozyme were obtained using several amino acid derivative ligands.

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References

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