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# Binding site selectivity of copper(II) ions transferred from azobenzene-amino acid Schiff base copper(II) complexes to lysozyme

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The objective of this study was to clarify the binding site and coordination environment, and to experimentally analyze the incorporation of copper(II) ions into egg white lysozyme crystals and their binding site selectivity. The original purpose of this research was to analyze the structure of the copper(II) complex bound to the protein crystal, but since the dissociation reaction of the copper(II) complex could not be ignored, we report here derivative findings on ionic bonding.

## 1 Introduction

In recent years, metal ion incorporation into biopolymers has become important in hybrid catalysis and drug discovery research [1,2]. To investigate how to control metal ion-induced protein conformational changes and aggregation, which are thought to be a contributing factor to Alzheimer's disease, we introduced copper(II) ions released from Schiff base copper (II) complexes [3] of amino acid derivatives (Ser, Thr, Tyr, and Val) containing hydrophobic azobenzene moieties into egg white lysozyme crystals by the immersion method and performed X-ray crystallography.

## 2 Experiment

Electron density analysis of the copper(II) ions and other ligands [3, 4] was performed using the analysis packages CCP4i and Coot, and 3D images were created using PyMOL. The binding residues and coordination numbers of the copper(II) ions and ligands were identified. Furthermore, docking simulations using Autodock and MIB2 were performed to compare the predicted binding sites . X- ray crystal structure analysis revealed that when azoamino acid Schiff base copper (II) complexes were introduced into egg white lysozyme crystals, the side chain structure and hydrophobicity of each copper (II) complex significantly affected the binding site selectivity and coordination number of the copper (II) ion.

## 3 Results and Discussion

For example, the tyrosine derivative copper (II) complex is highly hydrophobic, and the free copper(II) ion was confirmed to bind tightly to the lysozyme side chains , such as Arg14 , Arg21 , His15 , Thr89 , Leu129 , and Glu35 , with up to four-coordination as shown in Fig. 1. This is thought to be because it has a lower penetration into the protein than the amino acid derivative Schiff base copper(II) complex, which does not have an azobenzene moiety, and preferentially binds near the surface [2]. Furthermore, the binding of copper(II) ions to lysozyme exhibited different results from that of the Irving-Williams series, suggesting that the protein structure and coordination geometry may affect the copper(II) ion stability [3][4].

Future challenges include whether it is possible to predict the binding state of metal ions or metal complexes to proteins, and whether coordination bonds can be intentionally controlled based on predictions. The basic principle of metal selectivity (ease of binding of metal ions) generally follows the Irving–Williams series (Mn < Fe < Co < Ni < Cu > Zn). This is an empirical rule that indicates the hierarchy of stability when divalent first transition period metal ions form complexes with specific ligands (amino acid residues, etc.). However, the problem of "mismetalation" has also become a problem recently.

## References

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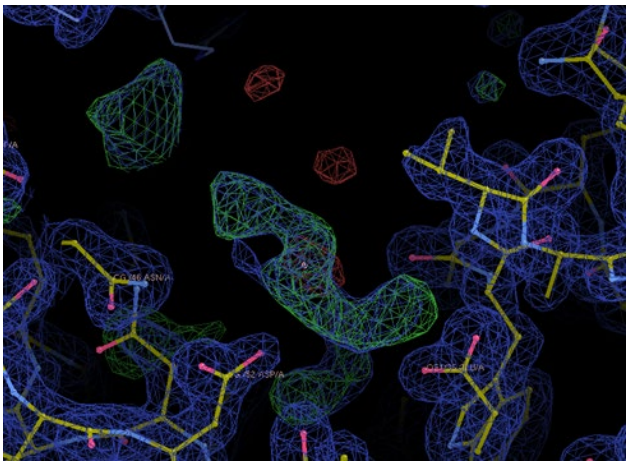

Fig. 1: Binding site of lysozyme and Ser-complex.

## Research Achievements

1. Binding sites of copper(II) ions or ligands from copper(II) complexes in lysozyme were confirmed.

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