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Preliminary Investigation of Copper(II) Ion Binding or Complex Coordination in Lysozeme Molecules

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Abstract

Hydrophobic Val derivative Schiff base copper(II) complexes and dipeptide (AlaAla, GlyGly) derivative Schiff base copper(II) complexes were introduced into egg white lysozyme. X-ray crystal structure analysis revealed amino acid derivative Schiff base copper(II) complexes were obtained. Herein we discuss primarily on the binding mode of copper(II) of the complexes obtained with egg white lysozyme. The electron density of copper(II) ions was confirmed by X-ray crystal structure analysis. The Val derivative Schiff base copper(II) complex was weakly bound at Arg114 of egg white lysozyme. In other copper(II) complexes, binding of copper(II) ions with dissociated ligands to various residues was observed. The binding sites of copper(II) ions were compared with computational scientific predictions.

Keywords

Copper, Schiff Base, Lysozyme, Metal-Protein Binding, Computational Methods

1. Introduction

1.1. Metal Binding and Theoretical Approach

Metal ions are abundant on earth, and in excess amounts they are toxic within biopolymers, but in stoichiometric amounts they play an important role as catalysts by binding with proteins. Artificial metalloproteins have two properties (homogeneous metal catalysis and enzyme catalysis), and they have the ability to impart new catalytic functions to polymers by incorporating metal-containing moieties into the protein scaffold [1] [2] [3] [4].

In addition, unlike metal complexes that use simple amino acids or peptides as ligands, there are four main synthesis strategies (covalent bonding, supramolecular bonding, coordination bonding, and metal substitution) [5]. However, computational scientific design of metal ion-protein interactions is not always as well established as for drug-organic compound ligand-receptor proteins due to the complex electronic structure of metals. Formation of coordination bonds is generally not simple by considering steric situations as well as thermodynamic conditions of protein molecules.

Recently, using deep learning, methods for predicting the position of metal ions in protein structures (using Protein Data Bank (PDB)) have been developed to accurately predict the positions of metal ions within proteins [6]. For example, an experimental dataset of high-resolution crystal structures containing zinc sites was used to train a geometric predictor and a deep learning predictor [7]. These trainings are based on experimental zinc(II) ion sites. The coordination environment is extracted and the metal is extracted from the protein environment which has been voxelized. The process of visualizing a three-dimensional object by combining two-dimensional image pixels with the smallest unit of a small cube may be used in three-dimensional graphics. It is a fully convolutional two-dimensional Convolutional Neural Network (CNN) trained to predict density. The metal is placed at the geometric center of the high scoring residue according to the probability map. The final ranking of the sites is obtained using a probability map.

1.2. Experimental Methods in Conventional Crystallography

By the way, the heavy atom isomorphic replacement method is a phase determination method that is widely used for protein structural analysis for a long time [8] [9]. In this method, in addition to native crystals, crystals with heavy atoms (metal ions, metal complexes, polynuclear metal complex clusters, etc.) bonded to specific sites of the protein are prepared. This method determines the phase from the difference in intensity of diffraction data, which sometimes resulted in forming artificial metalloproteins consequently [10]. It is also confirmed by means of not only X-ray crystallography but also X-ray fluorescence [11] when a heavy atom compound permeates through a single crystal from a solution, it usually binds specifically and gradually to proteins [12].

2. Results and Discussion

2.1. Introducing Complexes into Crystals

Comparing the co-crystallization method (adding a heavy atom solution before crystallization) and the soaking method (heavy atom replacement method), it was found that the electron density of heavy atoms was not observed in the co-crystallization method, and that in the soaking method. Although the electron density of heavy atoms was confirmed, it was often not introduced into proteins. In other words, we have experienced that it is difficult to efficiently introduce metal ions into protein molecules without complex transport and dissociation processes.

2.2. Binding Sites and Features

Docking calculations of hen egg lysozyme and copper(II) ions Schiff base complexes were performed using computational chemistry simulations (**Figure 1**). Previous reports have indicated that ALA and HIS scores were relatively high for amino acid side chains in lysozyme. Furthermore, binding simulation predictions [13] have revealed that copper(II) ions are likely to be incorporated into ALA and HIS of lysozyme. However, results obtained from our tentative experimental study did not show this tendency. The prediction program has been improved to utilize AlphaFold2 and Protein Structure Database to acquire predicted structures to perform metal ion docking and predict binding residues [14]. The results were compared with experimental results based on the score values of amino acid side chains (**Table 1**). The result obtained indicated that in some cases the copper(II) ions dissociated from the ligands and were incorporated into hen egg lysozyme (**Figure 2(a)**). Additionally, copper(II) complexes were observed near amino acids with potentially coordinating side chains (**Figure 2(b)** and **Figure 2(c)**) [15] [16] [17]. Interestingly, ion dissociation from the ligand was more facilitated into the protein crystal than the "bare" ion from dissolution of some copper(II) salts.

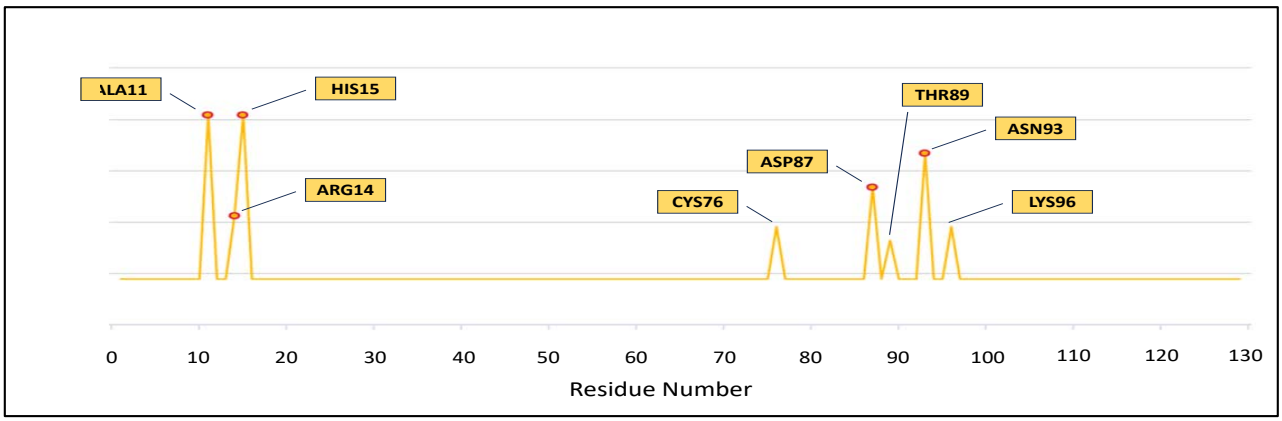
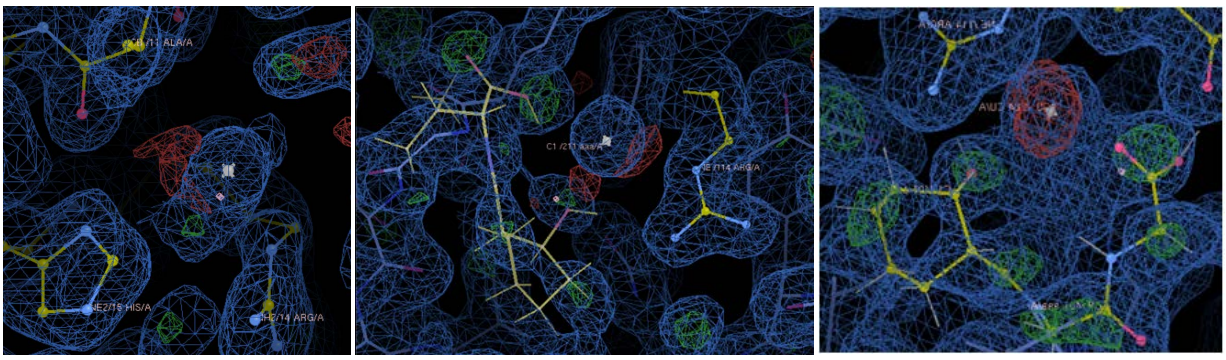


Figure 1. Copper(II) binding residues of lysozyme by MIB simulation [13].



(a) (b) (c)

Figure 2. Copper(II) ion from (a) ALA-ALA (b) GLY-GLY and (c) VAL derivative Schiff base copper(II) complexes binding sites with lysozyme based on tentative X-ray crystallography.

Table 1. Copper(II) binding residues of lysozyme after soaking copper(II) complexes.

Entry	Copper(II) binding residues
1	TRP108, VAL109
2	GLU35
3	ALA42, THR43, GLN41
4	ARG73, ASN74, CYS64, ASN65, ARG61, SER60, SER72
5	THR69, PRO70
6	ARG21, GLY22
7	GLY126, CYS127, ARG128
8	ASN59
9	LYS13, LEU129
10	GLN121, ALA122
11	ASP18, LEU17, ASN19
12	ASP87, ILE88
13	THR69, PRO70, ARG68
14	ILE58, ASN59
15	ILE78 PRO79, ASN74
16	THR69, PRO70, ARG68, GLY67, SER72
17	ASP119, ARG125
18	ILE58, ASN59
19	ASN113
20	LEU56, GLN57, ILE55, GLY54, TYR53
21	ASN65
22	PRO70, GLY71

3. Conclusion

As far as we have investigated with this method so far, in this way, we have not observed the coordination mode of copper(II) ions binding to neighboring three or more amino acid residues that exhibits the blue-purple color of the so-called biuret reaction.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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