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Structural and physical properties of salen-type copper(II) complexes in lysozyme for oxygen reduction

Kumpei Kashiwagi[a], Sayantan Pradhan*[b], Tomoyuki Haraguchi[a] and Takashiro Akitsu*[a]

[a]Department of Chemistry, Tokyo University of Science, Tokyo, Japan

E-mail: akitsu2@rs.tus.ac.jp

[b]Chemical Sciences Division, Saha Institute of Nuclear Physics, Kolkata-700 064, India

E-mail: sayan23us@gmail.com

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Artificial metalloenzymes (ArM) have emerged as a promising strategy to emulate the catalytic efficiency of natural metalloenzymes in new to nature reactions. ArM consisting of lysozyme derived from hen egg white lysozyme (HEWL) and several chiral salen-type Cu(II) complexes was prepared in this study. Twenty types of salen-type metal complexes were obtained from crystal database and then were virtually screened with help of molecular docking procedure. Docking results verified that the Cu(II) complexes interacts with lysozyme by hydrogen and hydrophobic bonds. Four Cu(II) complexes with best docking score were synthesized. It was confirmed by the CV curve that there is an electrochemical difference between lysozyme simple substance and complex to attempt to reduce oxygen.

Keywords: Artificial metalloprotein, salen-type Cu(II) complexes, DFT, docking.

Introduction

In recent years, by introducing a non-natural metal ion or a metal-containing prosthetic group into a protein, it is possible to dramatically expand the diversity of the function of the protein and its application range, and the reported artificial metal protein[1] has been implemented in a wide range of fields including tumor markers and fuel cell electrodes. The inventors of the present invention we have previously shown that have found that in an artificial metal protein complex system comprising human serum albumin (HSA) and a salen-type zinc complex, a change in current amount due to molecular orientation of a metal complex, crystallization by a complex system of laccase and a salen-type zinc complex reported[2,3].

Therefore, in this study, we focused on relatively inexpensive HEWL and observed the behaviour by creation of complex system of salen-type copper(II) complex and HEWL. Since laccase which is an oxygen reductase reacts at the active center of copper, copper was used as the central metal of the metal complex[4].

About 20 types of salen-type metal complexes in CSD (The Cambridge Structural Database) were virtually screened with help of molecular docking procedure. We also examined the optimal structure and affinity of the complex and lysozyme using DFT (Density Functional Theory) calculations and considered the electron transfer pathway within the complex. In order to easily synthesize and compare the effects of docking score and substituent effects, the four complexes PIFKIY, MAGSOD, PAYDEY, DAHBET were selected. By performing CV (Cyclic Voltammetry) measurement there from, the oxygen reducing ability of the complex containing lysozyme and the complex itself was compared. CV validated the docking results.

It was confirmed that affinity with lysozyme was increased by attachment of a hydrophobic group to the aldehyde side. An oxygen reduction peak was observed in a complex system using lysozyme of all four complexes. From these four measurement results, we discuss the influence of salen-type metal complexes introduced into lysozyme together with DFT calculation results.

Experimental

First virtual screening of salen-type metal complexes:

The three-dimensional (3D) structure of lysozyme (PDB id: 1DPX) was obtained from PDB. The structure of the ligand (metal complex) was obtained from CSD. 3D optimization was carried out using density functional theory (DFT) with the help of Gaussian 09 and Gauss View 5.0.9 packages[5,6]. DFT was performed by B3LYP method and 6-31G base set[7,8]. The 3D optimized structure was acquired for docking by adding Gasteiger charge, detecting routes and selecting torsion from the torsion tree of the AutoDock Tools panel[9,10].

Water from lysozyme protein file has been removed, bound ligand to the protein structure has been deleted and hydrogens added to the protein structure in order to make lysozyme structure compatible for docking with help of Autodock Tools. Center Grid box x: –1.388, y: 19.456, z: 19.336 and number of points in x,y,z dimensions are measured as 30×30×30 $Å^3$ respectively and grid spacing has been taken as 0.3750 Å. Docking process has been executed by using Lamarckian genetic algorithm[11].

Preparation of electrode:

Chiral Schiff base complexes (Fig. 1) have been reported elsewhere[12–15]. The yields were 71.1, 77.6, 68.7, and 49.2% for **1**-**4**, respectively. To confirm preparation of these known complexes, only IR, UV-Vis, and CD (if possible) (Fig. 2) spectra were measured. IR (KBr) C=N: 1648, 1629, 1631, and 1620 cm^{-1} for **1**-**4**, respectively. To a solution of salicylaldehyde (1.00 mmol : **1** = 0.1598 g, **2** = 3603 g, **3** = 0.1243 g, **4**

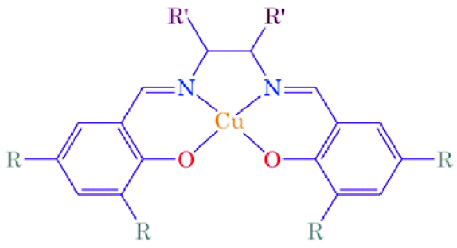


Fig. 1. Molecular structures of chiral salen-type Cu(II) complexes. R and R′ = H and H for MT1, t-Bu and H for MT2, H and cyclohexane for MT3, and t-Bu and cyclohexane for MT4, respectively.

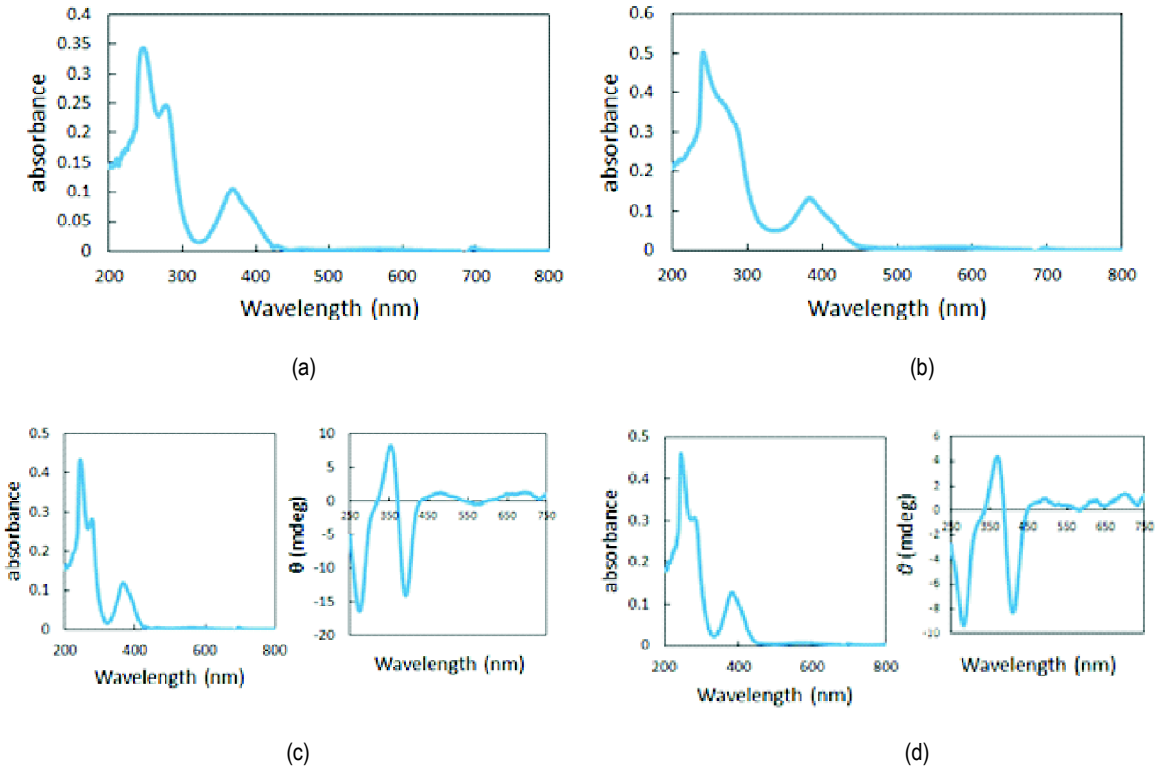


Fig. 2. UV-Vis (and CD) spectra for **1** (a), **2** (b), **3** (c) and **4** (d) in 0.01 mM $CHCl_3$ solutions.

= 0.2342 g) dissolved in methanol (50 mL) was added diamine (0.500 mmol: **1** = 0.03897 g, **2** = 0.04634 g, **3** = 0.05816 g, **4** = 0.05786 g) was added dropwise and stirred at 313 K for an hour to obtain a yellow solution of the ligand. Cu(II) acetate monohydrate (0.500 mmol, **1** = 0.1285 g, **2** = 0.1535 g, **3** = 0.0998 g, **4** = 0.100 g) was added to the obtained solution, and the mixture was stirred for 1.5 h, and the solution was filtered to obtain a precipitate. Yield **1** = 0.1518 g (71.67%), **2** = 0.3302 g (77.62%), **3** = 0.1315 g (68.65%), **4** = 0.1495 g (49.23%). The complex to be measured for electrochemical measurements was mixed with CNT (to increase the amount of current) and fixed on the electrode by glutaraldehyde. Commercial lysozyme was added to it and film formation was carried out on the RRDE electrode by Nafion®.

Electrochemical evaluation of direct bioelectrolysis:

Electrochemical measurements were carried out using a potentiostat (ALS, DY 2323). A platinum spiral wire and an Ag/AgCl electrode (saturated with NaCl) were used as counter and reference electrodes, respectively. It was investigated by performing cyclic voltammetry in a range of –0.8 V to +0.6 V vs Ag/AgCl using a sodium acetate buffer solution (0.20 *M*, pH 5.0) as a supporting electrolyte. The potential sweep rate was 50 mV/s.

Physical measurements:

Infrared (IR) spectra were recorded as KBr pellets on a JASCO FT-IR 4200 plus spectrophotometer in the range 4000–400 cm^{-1} at 298 K. Electronic (UV-Vis) spectra were obtained on a JASCO V-570 UV-Vis-NIR spectrophotometer in the range 1500–200 nm at 298 K. Circular dichroism (CD) spectra were obtained on a JASCO J-820 spectropolarimeter in the range 900–250 nm at 298 K.

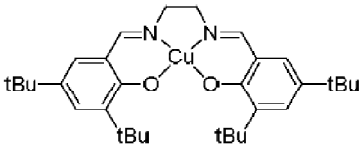
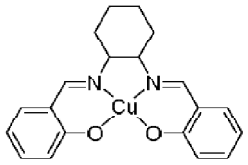
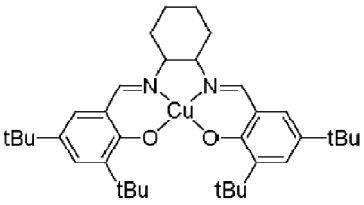
Results and discussion

Virtual screening:

Crystal structure database (CSD) survey and preliminary docking simulation with GOLD (CCDC) resulted in the best four complexes (**1**-**4**) employed have been carried out prior to this study[16]. Crystal structures of CCDC codes PIFKIY, MAGSOD, PAYDEY, and DAHBET(with characterization data) of **1**-**4** were used as 3D structures of complexes for AutoDock treatments[12–15].

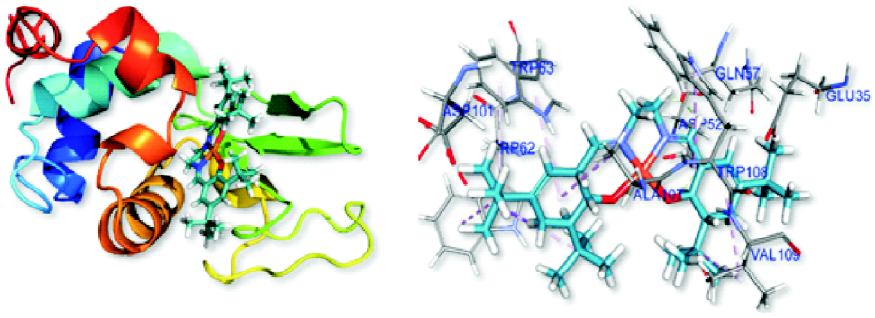
Docking of metal complexes has been performed out with AutoDock 4.2.6 on windows platform with 8 GB RAM and Intel I 5 processor. Metal complex **2** (MT2) showed the highest docking score i.e. –8.6 kcal/mol (Table 1). More precise docking with MT2 was performed. From Fig. 3, MT2 formed several bonds with lysozyme.

Table 1. Metal complex and their docking scores

Complexes structure and name	Docking score (kcal/mol)
 MT1(PIFKIY)	–6.4
 MT2 (MAGSOD)	–8.6
 MT3 (PAYDEY)	–7.8
 MT4 (DAHBET)	–7.0

Electrochemical effect:

In CV measurement (Fig. 4), there was hardly any difference in the results of N_2 filling and O_2 filling with lysozyme alone. In the complex system of the selected four metal complexes and lysozyme, an increase in the reduction peak under O_2 loading was observed, and the synergistic effect be-



(a) (b)

Fig. 3. Comprehensive perception of lysozyme receptor and MT2 interaction after docking. (a) MT2 is docked in active site of lysozyme receptor. Secondary structure of lysozyme receptor represented by ribbon model, and MT2 is represented by ball and stick model and (b) interactions of ligand with lysozyme receptor amino acids omitting lysozyme receptor, ligand surrounding amino acids are in three letters code represented in dark blue.

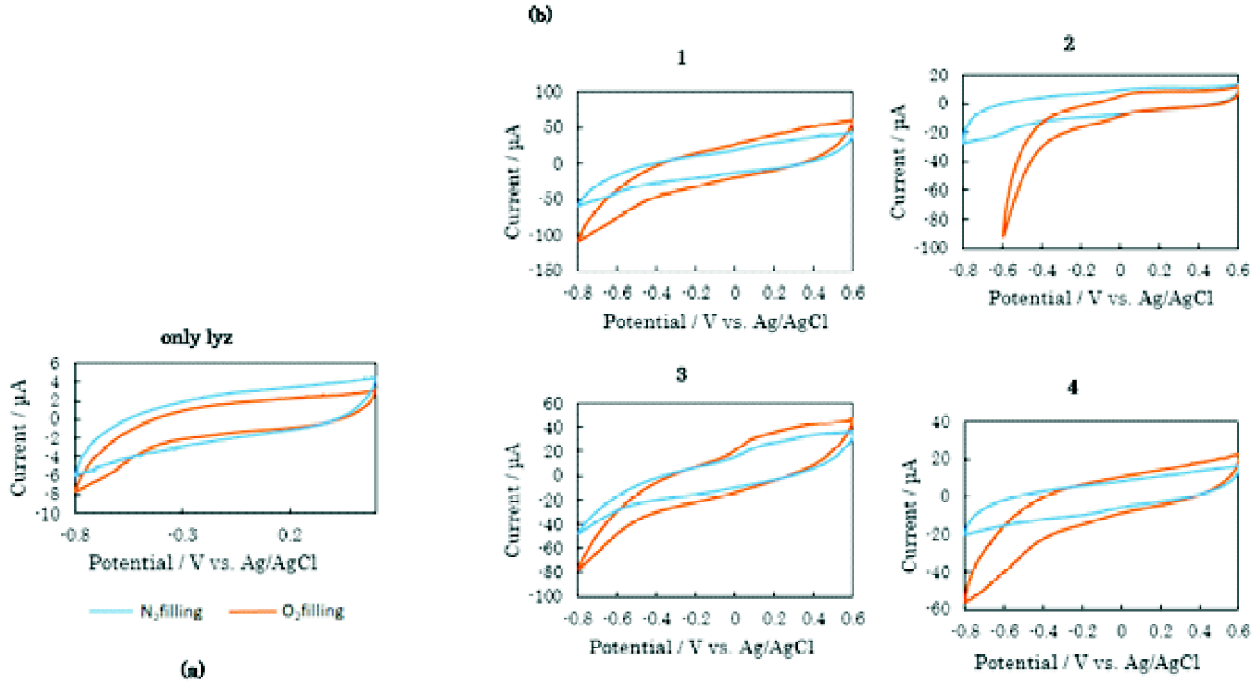


Fig. 4. CV measurement result under N2 or O2 filling, (a) lysozyme simple substance and (b) complex system of salen-type copper complex and lysozyme.

tween the metal complex and lysozyme was clarified. In the MT2 complex with the highest docking score (Table 2), the increase in CV measurement was also the largest among the four complexes. As the affinity with lysozyme increased, it is considered that the electron transfer pathway was established. Thereby smoother electron transfer takes place.

Table 2. Bond distances and type between lysozyme receptor and MT2

Bond name	Distance (Å)	Bond category	Bond type
P:MT2:H13 - A:GLN57:O	2.01	Hydrogen bond	Carbon hydrogen bond
P:MT2:H54 - A:TRP62	2.65	Hydrophobic	Pi-Sigma
P:MT2:H49 - A:ASP52:OD2	2.71	Hydrogen bond	Carbon hydrogen bond
P:MT2:H15 - A:GLN57:O	2.84	Hydrogen bond	Carbon hydrogen bond
A:TRP62 - P:MT2:C2	4.11	Hydrophobic	Pi-Alkyl
P:MT2:C23 - A:VAL109	4.19	Hydrophobic	Alkyl
A:TRP62 - P:MT2:C29	4.74	Hydrophobic	Pi-Alkyl
A:TRP62 - P:MT2:C2	4.90	Hydrophobic	Pi-Alkyl
A:ALA107 - P:MT2	4.93	Hydrophobic	Alkyl
A:TRP63 - P:MT2	5.03	Hydrophobic	Pi-Alkyl
A:TRP63 - P:MT2:C2	5.18	Hydrophobic	Pi-Alkyl
A:VAL109 - P:MT2	5.32	Hydrophobic	Alkyl
A:TRP108 - P:MT2	5.46	Hydrophobic	Pi-Alkyl

Conclusions

In conclusion, the present inventors prepared a supramolecular system as an artificial metal protein composed of several chiral salen-type Cu(II) complexes in lysozyme and experimentally confirmed its docking characteristics. MT2, which showed the highest docking score among the four metal salen complexes selected, has the greatest increase in the reduction peak of the CV measurement, so it is related to the docking score. This concept may be applied to artificial metal proteins.

Acknowledgements

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Supporting Information

Docking score and tables are given (SI-1).

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