Theoretical Studies on Redox Potential of Metalloproteins

Dissertation Abstract

Theoretical Studies on Redox Potential of Metalloproteins

金属タンパク質の酸化還元電位に関する理論的研究

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Abstract

We presented a theoretical study on electronic properties, in particular redox potential, of two metalloproteins, i.e. copper protein and iron-sulfur protein. As for copper protein, we investigated the cluster of type 1 copper center (T1CC) in relation to the electronic structure and several properties of T1CC. Then we extended our study by investigating the contribution of long-range interaction between copper and methionine residue as axial ligand to several properties of T1CC. The model cluster of T1CC in both studies comprised by a copper atom, a cysteine, two histidines and a methionine residue. We presented a SOMO orbital and spin density surface of T1CC in oxidized state. A significant contribution of M06 is found in bond constant calculation, especially in Cu-Met bond. As for iron-sulfur protein, we investigated redox potential and pK_a of iron-sulfur clusters with respect to spin structures. The model of iron-sulfur cluster consists of two irons, two bridging sulfur atom, and four methylthiolates. We found the most significance improvement with respect to the diffuse function was obtained from the calculation by AP UM06, which the absolute error decrease by 0.30 V. With the absolute error of 0.01 V, the best result presented by AP UM06/6-31++G(d,p) calculation.

1 Introduction

Copper proteins are metalloprotein that play a fundamental rote in a wide range of biomolecular processes such as electron transfer, oxygen transportation, and so on. One or more coper ions can be found in the active site of this protein. The copper protein containing one copper ion is called type 1 copper (T1Cu) proteins. There are several copper proteins that classified as T1Cu protein such as Azurin, Plastocyanin, Stellacyanin, etc. Meanwhile, copper proteins can also contain more than one copper ions like found in Multicopper Oxidases (MCOs). MCOs contain two copper centers, i.e. type I copper (T1Cu) and tri-nuclear copper (TNC), that located at different sites. T1Cu is composed by a copper ion coordinated to two histidines and a cysteine in a trigonal planar structure, and an axial ligand such as methionine and so on.[1] During the reaction, T1Cu accepts an electron from other substrates with higher redox potential and transfer the electron to TNC.[2] In the end of one cycle reaction, dioxygen molecules are reduced to yield two water molecules in TNC.[3]

There are several properties of T1Cu related to electronic structure and reaction that have been investigated experimentally by many groups. The structure of T1Cu has been identified from a strong absorption at around 600 nm and narrow hyperfine splittings in the electron paramagnetic resonance (EPR) spectroscopy.[4] Solomon and co-workers have also studied the characteristic of room temperature circular dichroism and magnetic circular dichroism spectra of several T1Cu proteins, such as Stellacyanin, Plastocyanin, and Azurin.[4] From the viewpoint of theoretical study, T1Cu has attracted many interest in relation to the electronic structure. Solomon and co-workers have investigated the interaction of copper ion and ligands by using quantum method.[2]

Generally, copper ion (Cu) in T1Cu bound to a cysteine (Cys) and two histidines (His) in equatorial position, and also bound to methionine (Met) in axial position. A short bond distance between Cu and Cys is known to be highly covalent and thus arise an intense of charge-transfer band.[5] On the contrary, Met bound to Cu with the bond distance more than 3 $\mathring{A}$ and interact with a long-range interaction.[5] Although the interaction between Cu and Met is relatively weak, several groups has reported that this interaction contribute

to protein function. Li and coworkers have found that the lack of the ligand give a contribution to redox potential of T1Cu protein.[6] Furthermore, several studies have revealed other roles of the axial ligand, such as steric protection of Cu ion and geometric control.

The other metalloprotein that commonly involved in many processes in biological system is iron-sulfur protein. This proteins present a broad range of redox potential and thus allow them to interact with many types of redox substrates by acting as electron carriers.[7] Iron-sulfur proteins contain one or more iron-sulfur clusters that comprised by one to eight iron atoms, cysteine's sulfur atoms, and inorganic acid labile sulfurs that connect the cluster with peptides. In supporting biological processes, this clusters present an important role such as radical chain stabilization, redox reaction, and Lewis acids. The classification of iron-sulfur clusters are suggested in the basis of the number of iron and sulfur atoms, structural motif, and its electrochemical and spectroscopic properties.

Iron-sulfur proteins can be classified into several classes. Ferredoxins is one of the classes that present low molar mass and also support in several biological pathways of electron transfer by acting as electron carriers. This proteins are recognized from the feature of EPR signal as that from a non-heme iron. Furthermore, ferredoxins are again classified into several classes. [2Fe-2S] ferredoxin is one of ferredoxins classes that contain a complex cluster of two iron atoms, two inorganic sulfur atoms, and four cysteine thiolates. [2Fe-2S] Ferredoxins have attracted much interest for their function in supporting many biological processes, and thus the number of studies to explore structure-function relationships of this protein have also been increased.

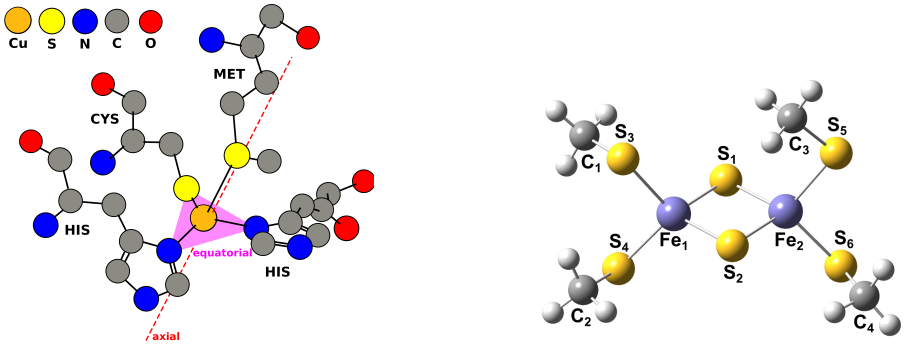
In this study we aim to investigate the electronic properties, in particular redox potential, of two metalloproteins, i.e. copper protein and iron-sulfur protein. As for copper protein, we prepared T1Cu cluster from a series of copper protein and calculate the properties of the cluster. In particular, we discussed the dependency of redox potential on DFT functional in relation to axial ligand contribution. As for iron-sulfur protein, we prepared [2Fe-2S] iron-sulfur cluster from ferredoxin and adrenodoxin. We calculated the redox potential of those proteins and discussed the contribution of spin structure to the accuracy of calculation.

2 Computational Methods

2.1 Electronic Structure and Properties of Copper Protein

T1Cu clusters were extracted from the series of X-ray crystal structure of copper proteins. In the case of oxidized state, the cluster of Azurin, Plastocyanin, Stellacyanin, and MCOs were extracted from X-ray crystal structure with PDB ID are 4AZU, 1PLC, 1X9R and 4NER, respectively. Meanwhile, the cluster of Azurin, Plastocyanin, Stellacyanin, and MCOs in reduced state were extracted from X-ray crystal structure with PDB ID are 1E5Y, 5PCY, 1X9YUand 4E9T, respectively. In this model, all T1Cu clusters are composed by a copper ion coordinated to two histidines and a cysteine in trigonal plane, and a methionine as axial ligand, except for Stellacyanin. The schematic diagram of T1Cu model cluster is presented in Figure 1(a). To calculate the contribution of axial ligand, we prepared a series of T1Cu cluster derived from Azurin protein and varied Cu-Met bond distances as 3.18, 3.28, 3.38, 3.48, and 3.58 $\AA$. The calculations are conducted by utilizing eight density functional theory (DFT) functionals upon to several classes of DFT functionals, i.e. (i) pure generalized gradient approximation (GGA) (PBE and BLYP), (ii) hybrid (B3LYP and M06), (iii) long-range corrected (LC)-GGA (LC-BLYP and LC-PBE), and (iv) LC hybrid (B97X and CAM-B3LYP).

We calculated several properties consist of molecular orbital, atomic par-



(a) (b)

Figure 2.1: (a) Schematic diagram of T1CC model cluster and (b) structure of iron-sulfur cluster.

tial charges and partial spin, ionization potential (IP) of reduced T1Cu and electron affinity (EA) of oxidized T1Cu. Also, we investigated the long-range interaction of axial Met ligand by calculating equilibrium bond distance and bond constant of Cu-Met bond, redox potential, and maximum absorption wavelength (MAW). The equlibrium bond distances and bond constants were calculated by approximating potential energy surface (PES) along with Cu-Met bond distance that formulated as[8]

$$V(r, K_r) = K_r(r - r_c)^2 \tag{2.1}$$

which K_r and r_c means bond constant and equilibrium bond constant, respectively. Meanwhile, redox potential (E_{redox}) of T1CC is calculated by using Nernst's equation

$$E_{\text{redox}} = \frac{\Delta G_{\text{redox}}}{nF} - E_{\text{NHE}} \tag{2.2}$$

which ΔG_{redox} represents the Gibbs free energy for redox reaction, E_{NHE} represents reference potential (4.44 V),, n and F are the number of electron involve in reaction, and Faraday constant (96.485 kJ/mol.V), respectively. In the meantime, MAW is approximated by using time-dependent DFT method with polarizable continuum solvation model ($\varepsilon = 10$). The accuracy of calculation is estimated by calculating relative error from the comparison between calculated results and experimental data, which the relative error given by

$$\text{Error}_{\text{relative}} = \frac{|P_{\text{calc}} - P_{\text{exp}}|}{P_{\text{exp}}}. \tag{2.3}$$

P_{calc} and P_{exp} mean the value of the properties obtained from calculation and experiment, respectively. All of the calculations are conducted by using Gaussian 09 package.[9]

2.2 Electronic Structure and Properties of Iron-Sulfur Protein

We investigated two kinds of iron-sulfur protein, i.e. ferredoxin and adrenodoxin, for typical example. The calculations were performed by using two DFT functionals, i.e. B3LYP and M06, combined with two basis sets, i.e. 6-31G(d)

and 6-31G(d,p), w/wo diffuse functions. The models of iron-sulfur cluster were prepared from X-ray crystal structures of ferredoxin (PDBID 1A70) and adrenodoxin (PDBID 2MJD and 2MJE). The clusters consist of two irons, two bridging sulfur atom, and four methylthiolates as a replacement of cysteine residues, as shown in Figure 1(b).

We approximated a various spin states of the clusters, raised by coupled iron, by using several spin approximations. Firstly, we calculate the energy of the cluster with the spin state that defined as high spin (HS) and low spin (LS), in which the energy in LS state was approximated by using broken symmetry (BS) technique. As for the LS states, other spin approximation, the so-called approximate spin projection (AP), was utilized that is formulated as[10]

$$E^{\mathrm{LS}}_{\mathrm{APBS}} = \alpha E^{\mathrm{LS}}_{\mathrm{BS}} - \beta E^{\mathrm{HS}}. \tag{2.4}$$

Meanwhile, we also used another spin approximation to estimated the energy in LS state. In this approximation, the effect of J spin coupling (JC) in Heisenberg Hamiltonian with an isotropic correction is considered, in which the ground state energy is formulated as[11]

$$E_0(\mathrm{GS})_{\mathrm{ox}} = E_0(\mathrm{BS}) - 5/2J_{\mathrm{ox}} \tag{2.5}$$

$$E_0(\mathrm{GS})_{\mathrm{red}} = E_0(\mathrm{BS}) - 2J_{\mathrm{red}} \tag{2.6}$$

where J means the spin coupling parameter.

Regarding solvent contribution around the vicinity of the cluster, we used conductor-like polarizable continuum model (CPCM), with $\varepsilon = 10$. Also, we used pseudo-counter ion scheme (PCIS) method as an alternative solvation model by adding a charge-dependent correction term that is derived from a Generalized Born theory.[12] The implementation of this correction scheme can reduce error in redox potential calculation of highly charged metal complexes.[12] We calculated standard redox potential (E_{redox}) by using a relationship of redox potential and the Gibbs free energy difference of oxidized and reduced states ($\Delta G_{\mathrm{redox}}$) that is written as

$$E_{\mathrm{redox}} = \frac{\Delta G_{\mathrm{redox}}}{nF} - E_{\mathrm{SHE}} \tag{2.7}$$

where n and F represent the number of electrons, and Faraday constant, respectively, while E_{SHE} represents absolute of standard hydrogen electrode, taken as 4.43 V. In this calculation, the free energy difference was directly calculated, instead of using a thermodynamic cycle. We defined the free energy difference as the difference between the free energy of the cluster in oxidized and reduced states, the so-called vertical ionization potential (VIP), as given by

$$\Delta G_{\mathrm{redox}} \approx E^{\mathrm{ox}}_{(\mathrm{aq})} - E^{\mathrm{red}}_{(\mathrm{aq})}. \tag{2.8}$$

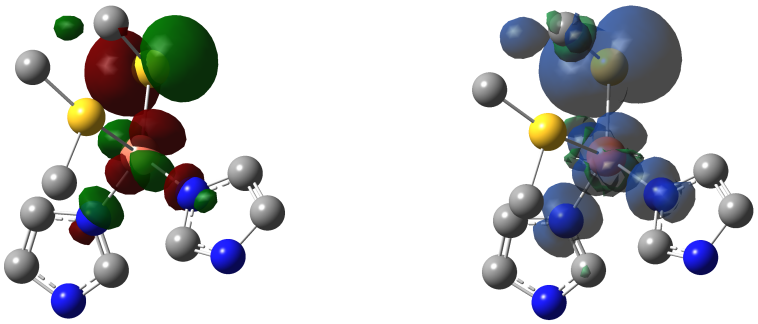
3 Results and Discussions

3.1 Type 1 Copper Cluster of Copper Protein

We presented a SOMO calculated by using M06, as shown in Figure 1(a). The shape of SOMO correspond to $d_{x^2-y^2}$ orbital of the copper ion, p orbitals of the sulfur and σ orbital of two nitrogen atoms. Regarding the interaction between copper ion and ligands, we found an antibonding orbital between copper ion and a cysteine and two histidines in trigonal plane. The orbital of methionine's sulfur did not present in SOMO indicate that the interaction between copper and methionine is significantly weak. Our calculation produce SOMO that the shape is in agreement with the reference.[2] We also present the surface of spin density calculated by using M06 in Fig. 1(b). The shape of the surface is found to be similar to SOMO orbital shown in Fig. 1(a).

We approximate the bond constant around T1Cu of Azurin, Plastocyanin, Stellacyanin, and MCOs from a series of PES obtained from M06 calculation. The value of the bond constants of T1Cu in Azurin are summarized in Table 3.1. From the results, we found the dependency of bond constant on DFT functional. Bond constant obtained from M06 calculation is consistently larger than those obtained from B3LYP calculation for all T1Cu cluster. Particularly, we found that the most significant dependency of bond constant is found in the interaction of Cu and axial ligand. The larger bond constant obtained from M06 calculation indicate that M06 calculation represent the bond interaction between copper and ligand in a stronger way than B3LYP does.

The plot of relative redox potential as a function of Cu-Met bond distance



(a) (b)

Figure 3.1: (a) SOMO orbital of T1Cu calculated by using M06 and (b) Spin density surface of T1Cu calculated by using M06.

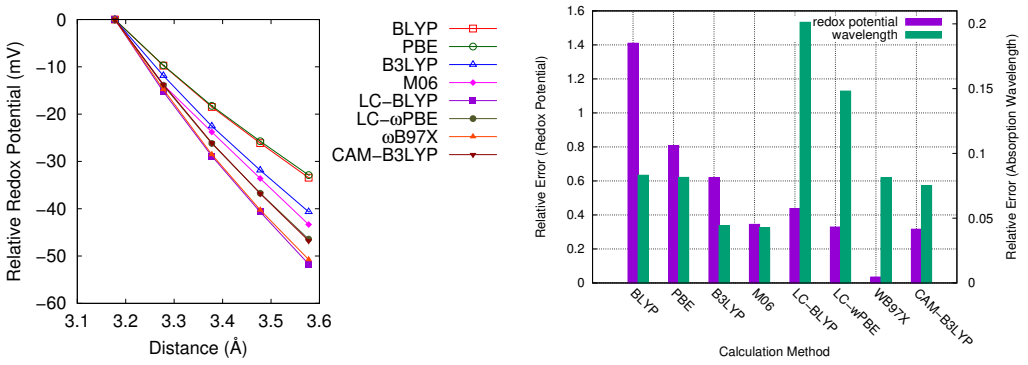
Table 3.1: Fitting parameter for bond distance around T1Cu of Azurin calculated by B3LYP (M06).

Bond	Reduced State			Oxidized State		
	r_{PDB}	r_c	K_r	r_{PDB}	r_c	K_r
Cu-N(His46)	2.00	1.97 (1.94)	109.39 (136.66)	2.06	1.96 (1.93)	107.82 (131.11)
Cu-N(His117)	2.11	2.00 (1.95)	106.04 (127.09)	2.19	2.05 (1.99)	72.16 (99.98)
Cu-S(Cys112)	2.27	2.14 (2.10)	135.11 (154.38)	2.28	2.20 (2.16)	96.67 (123.98)
Cu-S(Met121)	3.18	3.56 (3.28)	9.69 (25.80)	3.33	3.79 (3.39)	7.02 (27.93)

The unit of distance (r) and force constant (K_r) are Å and kcal mol^{-1}Å^{-2}, respectively.

is presented in Figure 2(a). From the Figure, we confirm the contribution of the interaction between Cu and axial Met ligand to redox potential. The value of redox potential is found to be decreased along with the increasing of the bond distance. In this calculation, we found that the series of LC-DFT is more sensitive to the change of the bond distance than non-LC-DFT. Amongst LC-DFT, LC-BLYP present the most sensitive functional with the tendency is close to ωB97X functional. Meanwhile, PBE and BLYP functional is much less sensitive to the change of the bond distance, which correspond to similar LC scheme deficiency. This results indicate the effect of LC scheme on the bond distance sensitivity of DFT functional for redox potential calculation.

Bearing on accuracy, the relative error of redox potential is calculated and shown in Figure 2(b) We found poor results produced from pure GGA calculation that is indicated by high value of the error. We also found that the accuracy of LC-DFT calculation is better than non-LC-DFT calculation,



(a) (b)

Figure 3.2: (a) Relative redox potential as a function of Cu-Met bond distance and (b) The relative error in the calculation of redox potential and absorption wavelength.

except for M06. The accuracy of M06 is found to be relatively similar to LC-DFT calculation. In this case, the performance of mixing scheme of exchange functional in M06 is quite similar to the contribution of LC scheme in LC-DFT. The ωB97X functional produce the best result indicating the LC scheme implemented in this functional is better than others. We also presented the contribution of LC scheme explicitly by comparing the accuracy of BLYP with LC-BLYP, and B3LYP and CAM-B3LYP. The utilization of LC scheme in BLYP and B3LYP reduce the error by 0.97 (from 1.40 to 0.43) and 0.30 (from 0.61 to 0.31), respectively. This results suggest the contribution of the LC scheme to improve the accuracy of redox potential.

3.2 [2Fe-2S] Cluster of Iron-Sulfur Protein

We summarize the calculation results of $\Delta\Delta E$ in Table 3.2. In the case of VIP with PCIS scheme, we found a little dependency of basis set on the calculation with B3LYP. Nevertheless, the implementation of diffusion function in the calculation with BS and JC UM06 give an improvement of $\Delta\Delta E$ value. The most significant improvement with respect to the diffuse function was obtained from the calculation with AP UM06, in which the absolute error decreases by 0.30 V (from 0.31 to 0.01 V). This findings indicate that the diffuse function give a contribution to improve the calculation of redox potential with M06

Table 3.2: The absolute error of redox potential difference.

Method	Spin Approximation			
	HS	BS	AP	JC
VIP scheme				
1	0.23	0.21	0.20	0.26
2	0.23	0.21	0.20	0.21
3	0.23	0.28	0.35	0.18
4	0.22	0.13	0.03	0.12
VIP with PCIS scheme				
1	0.19	0.17	0.16	0.22
2	0.19	0.17	0.16	0.17
3	0.19	0.24	0.31	0.22
4	0.18	0.09	0.01	0.08

HS: High spin, BS: Broken symmetry, AP: Approximated spin projected, JC: J spin coupling
1: B3LYP/6-31G(d), 2: B3LYP/6-31++G(d), 3: M06/6-31G(d), 4: M06/6-31++G(d)

functional. Bearing on DFT exchange correlation functional, the calculation by M06 produce the results that are more accurate than those by B3LYP, except for HS and AP approximations without diffuse function. For example, in VIP with PCIS scheme and BS approximation, the value of $\Delta\Delta E$ by B3LYP and M06 with diffuse function are found as 0.17 and 0.09 V, respectively. Overall, the best result was obtained from AP UM06/6-31++G(d,p) calculation with the absolute error of 0.01 V.

4 Conclusion

In this thesis, we presented a theoretical study on electronic structure and properties of two metalloproteins, i.e. copper protein and iron-sulfur protein. We presented a SOMO orbital of T1CC in oxidized state that is mainly raised from anti-bonding interaction between $d_{x^2-y^2}$ orbital of copper and π orbital of cysteine's sulfur. We also found that the shape of spin density surface is similar to the shape of SOMO. A significant contribution of M06 is found in bond constant calculation, especially in the case of the bond between copper (Cu) and methionine (Met). This indicate that M06 gives a significant contribution in bond constant calculation. We also found that M06 produce the

equilibrium bond distance that is closer to the distance obtained from X-ray crystal structure. In the case of redox potential, we found that the series of LC-DFT is more sensitive to bond distance than those of non-LC-DFT, in which the most sensitive one is presented by LC-BLYP. The series of LC-DFT were also found to be more accurate than those of non-LC-DFT, except M06.

As for iron-sulfur protein, we investigated redox potential and pK_a of iron-sulfur clusters with respect to spin structures. We did not found any contribution of diffuse function in improving the accuracy of all B3LYP calculation, except JC approximation. In contrary, the diffuse function present a significant improvement in the calculation by M06. The most significance improvement with respect to the diffuse function was obtained from the calculation by AP UM06, which the absolute error decrease by 0.30 V. This finding confirm the contribution of diffuse function in improving the accuracy of redox potential calculation by M06. Bearing on DFT functional, we found that M06 produce a results that is more accurate than B3LYP, except for HS and AP approximation without diffuse function. This results correspond to the kind of exchange functional used in both functionals. With the absolute error of 0.01 V, we obtained the best result from the calculation by AP UM06/6-31++G(d,p).

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学位論文審査報告書（甲）

１．学位論文題目（外国語の場合は和訳を付けること。）

Theoretical Studies on Redox Potential of Metalloproteins

（金属タンパク質の酸化還元電位に関する理論的研究）

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３．審査結果の要旨（600～650字）

当該学位論文に関して、各審査員が個別に検討した後、平成３０年７月２５日に予備審査を行い論文内容を詳細に検討した。その後平成３０年８月６日に行われた学位論文公聴会の後に審査委員会を開き、協議の結果以下のように判定した。

Isman Kurniawan氏は、タンパク質活性部位に金属イオンを含む金属タンパク質活性部位付近の電子構造を解析し、金属イオン周りの静的及び動的構造特性を解析した。特に、銅イオンを含むタイプＩ銅タンパク質活性部位に於いて、銅イオンとメチオニン中の硫黄イオンとの配位結合を理論的に解析した結果、活性部位付近の正しいダイナミックスを再現するためには分子間力を考慮した密度汎関数法を用いて評価することが重要であることを初めて指摘した。この結果は他の金属タンパク質のシミュレーション研究を実施する場合でも使用するパラメータを見直す必要性を示唆する。またIsman Kurniawan氏は、鉄硫黄タンパク質フェレドキシン、アドレノドキシンの酸化還元電位を理論的に求めた。酸化還元電位と酸解離定数の考察を行い、高スピン状態と低スピン状態の各状態のスピンカップリング項等の補正項を検討した。この結果を用いて、酸化還元電位はタンパク質環境依存性が高いことを明らかにした。この一連の研究結果は、金属タンパク質の酸化還元電位の計算の有効性を示すと共に、タンパク質間電子移動に関わる今後の理論や実験研究にも多くの寄与をもたらすものである。以上により、この論文は博士（理学）に値するものと判断した。

４．審査結果　(1) 判　　定（いずれかに○印）（合　格）・　不合格

(2) 授与学位　　博　士（　理学　）