Ab initio study of ion replacement in Spinach plastocyanin protein

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Ion replacement in protein active site is attracting considerable interest due to its application in some spectroscopic methods like perturbed angular correlation. Theoretical consideration of ion replacement in plastocyanin was done using the two layer ONIOM method. The copper (I) and copper (II) ions in the plastocyanin active site were replaced by silver (I) and cadmium (II), respectively. Optimization of the four proteins was done with LANL2DZ and SDD basis sets. Molecular orbital, natural bond orbital and atom in molecule analysis were used to determine the electronic structures of the four active sites. The total energy of copper (I) plastocyanin is lower than that of silver (I) plastocyanin. Copper (II) plastocyanin showed a lower energy than cadimum(II) plastocyanin. HOMO and LUMO orbital energies of the copper (I) and cadmium (II) active sites are lower than those of the silver (I) and copper (II) active sites, respectively. The four active sites displayed different charge distribution. Atom in molecule theory demonstrated different values for electronic density and bond ellipticity for the four active sites.

Key words: Electronic structure; Protein active site; *ab initio*; DFT; AIM; NBO

1. INTRODUCTION

Large molecular systems such as catalysts, nanotubes and bimolecular systems are not simple enough to be investigated by quantum methods alone. Therefore, the ONIOM method has been developed to overcome this problem. ONIOM method has been used to study the mechanism of enzyme catalyzed reactions, electronic structure of protein active site and ion replacement in protein structure [1–8].

Plastocyanin (PC), also known as blue copper (BC) protein, is a small soluble copper protein with a barrel-like structure. Multiconfigurational approach has been used for consideration of the electronic structure and charge transfer of model complex systems of plastocyanin. Excellent agreement has been found between the experimental and the calculated spectra [9]. Smbhu *et al.* used a theoretical method for poplar plastocyanin to calculate the standard reduction potentials *in vitro*. They showed that calculated potentials are in excellent agreement with the experimental one [10]. Density functional theory (DFT) calculation of the active site of plastocyanin was performed for model complexes. These model complexes were constructed by exchanging His,

Cys and Met residues by imidazol, methyl-thiolate and dimethyl-sulfide, respectively [11]. Perturbed angular correlation spectroscopy (PAC), which is based on gamma ray emission from radioactive nuclei, has been used to study the binding of plastocyanin to reduced photosystem 1 of spinach by substituting copper (I) and copper (II) by silver (I) and cadmium (II), respectively [12,13]. A question that needs to be answered is whether the electronic structure of silver(I) and cadmium(II) active sites is similar to that of copper(I) and copper(II) active sites or not. The present work is an attempt to show the main difference between the electronic structures of the active sites of copper(I) and silver(I) protein and also between copper(II) and cadmium(II) protein using ONIOM approach, molecular orbital (MO), natural bond orbital (NBO) and atom in molecule(AIM) theory analysis.

2. COMPUTATIONAL DETAILS

The experimental X-ray structure of plastocyanin was retrieved from the Brookhaven Protein Databank (PDB ID: 1ylb and 1plc). PDB code of reduced and oxidized form of plastocyanin is 1ylb and 1plc, respectively. The two layer ONIOM hybrid method was used for calculations. The active site of plastocyanin consisting of 38 atoms including metal ion and side chain of His37, His87, Cys84 and Met92 was used as QM region.

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Beckes three-parameter hybrid exchange functional and the Lee-Yang-Parr correlation functional DFT hybrid B3LYP method [14,15] by employing the effective core potentials of Hay and Wadt with double-e valence (LanL2DZ) basis set [16] and the Stuttgart Dresden Dunning pseudopotentials (SDD) basis set [17,18] were used for the QM region. For the remaining part of the protein structure UFF force field was used. All calculations were performed using the GAUSSIAN 03 package [19]. NOC software, downloaded from *http://noch.sourceforge.net/*, was used for representation of protein structure. The active site complex of the proteins was extracted from the optimized protein structure and was used for further analysis. NBO analysis was used for evaluating atomic charge and second-order interaction energies [20]. The topological properties of electron density were analyzed for the four active site complexes using the AIM2000 program [21 Correspondence 24].

3. RESULTS AND DISCUSSION

3.1 Spinach plastocyanin protein

Fig. 1 shows the PDB structure of the spinach plastocyanin. As clearly seen, there is one active site in this protein. The active site is consisting of one copper ion, two histidines, one cysteine and one methionin residue. For the present study, we used the copper ion and the side chain of the mentioned residues as high layer and the other parts of the protein as low layer.
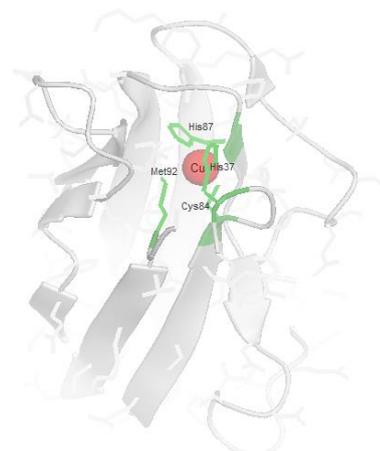
Fig. 1. Spinach plastocyanin protein structure along with active sites

3.2 Energy analysis

Table 1 shows the high and low layer energy of ONIOM calculation for all basis sets. The main difference among the four active site energies is seen in the energy of the model system with high level of the calculation. Copper (I) active site shows the lowest energy among other active sites. The order of decrease in total energy is as follows:

Copper (I) < copper (II) < silver (I) < cadmium (II) active site

Data in Table 1 show that energy values are not sensitive to the selection of basis set.

3.3 Structure parameters analysis

Table 2 illustrates the bond distance of the metal ion of the protein active site from His to the sulfur atom of the Cys and Met ligand. Bond distances were derived from two PDB entries that were

Table 1. Energy analysis of the four plastocyanin active sites

Basis set	Cu^+-PC		Ag^+-PC		Cu^{2+}-PC		Cd^{2+}-PC	
	SDD	LANL2DZ	SDD	LANL2DZ	SDD	LANL2DZ	SDD	LANL2DZ
E_{low}(model)	0.3	0.3	0.26	0.26	0.34	0.35	0.31	0.31
E_{high}(model)	−1683.6	−906.3	−1633.2	−855.8	−1683.2	−905.9	−1654	−757.9
E_{low}(real)	2.32	2.27	2.26	2.43	2.29	2.3	2.24	2.25
E_{total}	−1681.6	−904.3	−1631.2	−853.9	−1681.3	−903.9	−1652	−755.9

Table 2. Bond distances of the four active sites along with experimental data

X^*-PC	Cu^+-PC		Ag^+-PC			Cu^{2+}-PC		Cd^{2+}-PC		
Basis set	*SDD*	*LANL2DZ*	*SDD*	*LANL2DZ*	*Exp*	*SDD*	*LANL2DZ*	*SDD*	*LANL2DZ*	*Exp*
His87										
X-N	1.99	2.02	2.23	2.26	2.09	1.97	1.99	2.21	2.26	2.06
His37										
X-N	1.99	2.02	2.23	2.25	2.07	1.97	1.96	2.22	2.25	1.91
Met92										
X-S	3.09	3.11	3.28	3.52	3.10	2.60	2.59	2.71	2.77	2.82
Cys84										
X-S	2.21	2.28	2.44	2.57	2.12	2.42	2.27	2.72	2.74	2.07

*X stands for metal ion

Table 3. Bond angles of the four active sites along with experimental data

X*-PC	Cu+-PC		Ag+-PC			Cu2+-PC		Cd2+-PC		
		LANL2D		*LANL2D*			*LANL*		*LANL2D*	
Basis set	*SDD*	*Z*	SDD	*Z*	Exp	*SDD*	*2DZ*	*SDD*	*Z*	Exp
His87										
X-N-C20	125.32	125.38	125.31	124.62	126.46	124.89	122.12	121.07	124.41	125.99
X-N-C17	126.78	127.01	126.76	127.46	127.89	127.71	129.86	131.45	128.38	123.93
His37										
X-N-C5	123.14	122.26	121.28	122.47	125.98	121.71	124.10	118.90	115.54	128.97
X-N-C2	129.12	130.10	130.70	130.22	128.63	129.86	127.46	133.58	137.27	123.10
Met92										
X-S-C30	98.57	97.98	107.41	89.27	117.26	116.61	111.66	107.47	110.44	96.48
X-S-C28	129.67	130.65	138.29	138.95	122.51	135.80	130.51	127.81	123.03	126.86
Cys84										
X-S-C	109.90	109.38	111.03	109.78	113.63	105.12	108.65	99.88	104.85	110.42

*X stands for metal ion

mentioned above. Except the distance between sulfur and copper (I) ion, LAnl2dz basis set can reproduce experimental bond distance lengths more accurately than SDD basis set in the copper (I) active site. ONIOM calculation with SDD basis set can predict all bond lengths more accurately than Lanl2dz one. Besides bond distances between sulfur and metal ion in the Met ligand, ONIOM calculation with the Lanl2dz basis set shows the accurate bond lengths for other distances of the copper (II) active site. For cadmium (II) active site Lanl2dz basis set shows strong prediction with respect to SDD basis set for metal-sulfur distance in Met92 ligand, but weak prediction is seen for other bond distances.

Table 3 shows selected bond angles of the four active sites. For copper (I) active site, Lanl2dz basis set can reproduce X-N- $C\delta$ and X-N- $C\gamma$ bond angles of the His 87 and also X-N- $C\beta$ of the His 37 more accurately than SDD basis set. For silver (I) active site, Lanl2dz basis set gives better prediction than SDD basis set for X-N- $C\gamma$ bond angle of the His87 and X-N- $C\delta$, X-N- $C\beta$ of the His 37. Except X-N- $C\gamma$ bond angle of the His 87, LanL2dz shows better prediction than SDD basis set. Finally, except X-N- $C\delta$, X-N- $C\beta$ and X-S- $C\varepsilon$ bond angle, the prediction of bond angles by Lanl2dz is worse than by SDD basis set.

3.4 MO analysis

Fig. 2 shows the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the four active sites. The HOMO and LUMO energies varied as follows:

$E_{Homo-Cd(II)} < E_{Homo-Cu(II)} < E_{Homo-Cu(I)} < E_{Homo-Ag(I)}$

$E_{Lumo-Cd(II)} < E_{Lumo-Cu(II)} < E_{Lumo-Cu(I)} < E_{Lumo-Ag(I)}$

Based on these results, one can conclude that Ag^{1+} active site in plastocyanin can donate an electron to a partner more feasibly than Cu^{1+} active site. Also, comparision of LUMO orbitals energies shows that

Cd^{2+} active site can receive an electron more comfortably than Cu^{2+} active site.

3.5 NBO analysis

Table 4 shows the natural charge of atoms in the four active sites. As clearly seen, insertion of silver ion into the active site increases some atom charges such as $C\varepsilon$ of the His37; $C\beta$ and $C\delta$ of the His87 and reduces some atom charges such as $N\delta$ of the His37; $C\gamma$ of the His87; $C\beta$ of the Met92 and $S\gamma$ of the Cys84. Insertion of cadmium (II) into the copper (II) active site caused decreasing of charge density on $C\beta$ of the His37 and $C\beta$ of the His87.

Table 5 shows the stabilization energy of the donor-acceptor interaction of the four active sites. For all active sites, the strongest interaction is between $N\varepsilon$ lone pair and $\sigma^*_{N\delta-C\varepsilon}$ bond.

Table 4: Atomic charges of plastocyanin active sites

	Cu+ *active site*	Ag+ *active site*	Cu2+ *active site*	Cd2+ *active site*
	Charge			
His37				
$C\beta$	0.557	0.559	–0.009	0.033
$C\gamma$	–0.171	–0.254	0.099	0.063
$C\delta$	0.261	0.061	0.075	0.069
$N\delta$	–0.178	0.026	–0.545	–0.443
$C\varepsilon$	0.094	–0.093	0.26	0.21
$N\varepsilon$	–0.156	–0.161	–0.234	–0.267
His87				
$C\beta$	0.564	–0.528	–0.009	0.056
$C\gamma$	–0.18	0.319	0.085	0.058
$C\delta$	0.269	–0.267	0.085	0.077
$N\delta$	–0.159	–0.402	–0.541	–0.441
$C\varepsilon$	0.074	0.312	0.268	0.227
$N\varepsilon$	–0.159	–0.125	–0.235	–0.265
Met92				
$C\beta$	–0.128	0.58	0.121	0.076
$C\gamma$	–0.027	–0.123	–0.086	–0.092
$S\delta$	0.032	0.075	0.048	0.003
$C\varepsilon$	–0.087	–0.086	–0.152	–0.142
Cys84				
$C\beta$	–0.547	–0.075	0.09	0.156
$S\gamma$	–0.082	0.0104	–0.217	–0.079
Metal				
Cu+	0.557	-	-	-
Ag+	-	0.387	-	-
Cu2+	-	-	1.404	-
Cd2+	-	-	-	1.329
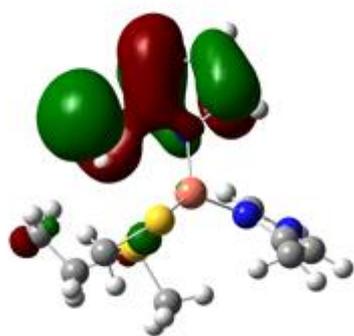
HOMO-Cu(I)
E= -0.33419 hartree
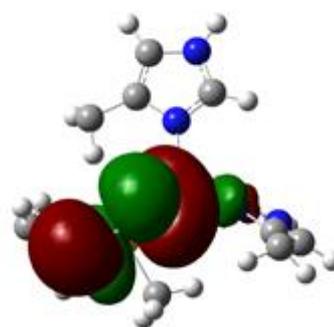
LUMO-Cu (I)
E= -0.28245 hartree
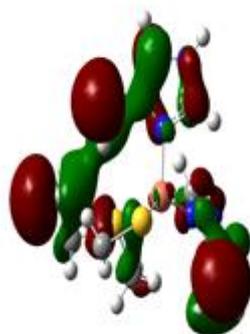
HOMO-Cu (II)
E= -0.42924 hartree

LUMO-Cu (II)
E= -0.41315 hartree
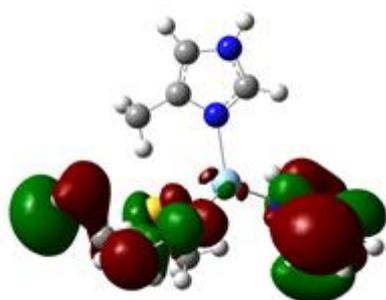
HOMO-Ag (I)
E= -0.28778 hartree
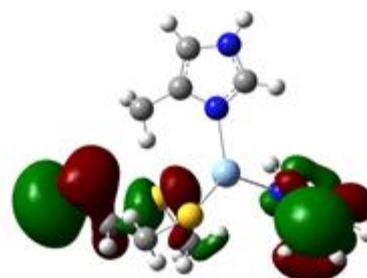
LUMO-Ag (I)
E= -0.27423 hartree
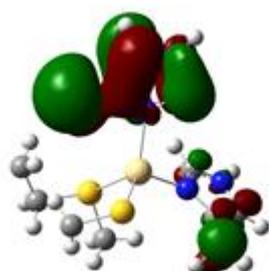
HOMO-Cd (II)
E= -0.47696 hartree
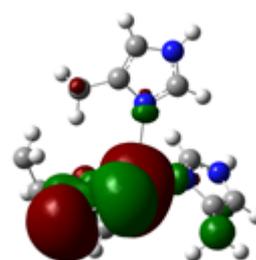
LUMO-Cd (II)
E= -0.41156 hartree

Fig. 2.HOMO and LUMO orbitals of plastocyanin active sites

Table 5: Stabilization energies of donor-acceptor interaction

	Cu^+ *active site*	Ag^+ *active site*	Cu^{2+} *active site*	Cd^{2+} *active site*
	Charge transfer E(2), E(j)-E(i),F(i,j)			
BD(Cu- Cβ)(Met92)→ BD*(Cγ-β)(Met92)	30.24–0.41–0.143	–	–	–
LP(Nε)(His37) →BD*(Cγ – Cδ)(His37)	34.43–0.59–0.188	–	–	–
LP(Nε)(His37) →BD*(Nδ– Cε)(His37)	49.10–0.47–0.193	–	–	–
LP(Nε)(His87) →BD*(Cγ – Cδ)(His87)	34.88–0.59–0.189	–	–	–
LP(Nε)(His87) →BD*(Nδ– Cε)(His87)	47.67–0.46–0.188	–	–	–
LP(Nδ)(His37) →BD*(Sg-Cu)(Cys)	–	–	48.45–0.83–0.184	–
LP(Nε)(His37) →BD*(Cγ – Cδ)(His37)	–	–	50.62–0.55–0.158	–
LP(Nε)(His37) →BD*(Nδ– Cε)(His37)	–	–	147.00–0.41–0.219	–
BD*(Cβ –Cu)(Met92) →LP*(Cu38)	–	–	213.86–0.02–0.109	–
BD*(Cβ-Cu)(Met92) →Ry*(Cu38)	–	–	43.29–0.13–0.126	–
LP(Nδ)(His37) →LP*(Ag)	–	28.51–0.72–0.190	–	–
LP(Nε)(His37)→BD*(Nδ– Cε)(His37)	–	75.04–0.47–0.239	–	–
LP(Nδ)(His37) →LP*(Ag)	–	29.53–0.78–0.200	–	–
LP(Nε)(His87)→ BD*(Nδ– Cε)(His87)	–	–	–	35.79–0.48–0.168
BD (Cβ -Sγ)(His87)→ LP*(Cd)	–	–	–	18.82–0.33–0.108

3.6 AIM analysis

Table 6 shows the results of AIM analysis for all active site complexes. In the case of copper (I) active site, the data show that high electron density exists for the Cu-N bond of the His37 and His87 ligands. Because of $\nabla^2\rho<0$, no charge depletion is registered for all bonds. Bond ellipticity(ε), which can be used to detect conjugation and hyperconjugation, is large only for Cu-S bond of Met92 and Cys84 ligands. For Ag (I) active site, the same trend as for copper (I) active site is seen. The charge density behavior of copper (II) active site is similar to that of copper (I) and silver (I) active sites, but high bond ellipticity is found only for Cu-S of the Cys84 ligand. For the cadmium active site, the behavior of charge density is similar to that for other active sites, but bond ellipticity of Cd-S of Met92 ligand shows a high value.

Table 6: Atom in molecule properties of metal-ligand bonds

X^*	X–N(His87)	X–N(His37)	X–S(Met92)	X-S(Cys84)
Cu^+				
ρ	0.077	0.077	0.015	0.062
$\nabla^2\rho$	–0.122	–0.117	–0.007	–0.060
ε	0.050	0.064	0.101	0.110
Ag^+				
ρ	0.065	0.065	0.009	0.047
$\nabla^2\rho$	–0.074	–0.073	–0.005	–0.039
ε	0.059	0.069	0.093	0.092
Cu^{2+}				
ρ	0.080	0.086	0.019	0.064
$\nabla^2\rho$	–0.124	–0.136	–0.010	–0.057
ε	0.064	0.075	0.077	0.101
Cd^{2+}				
ρ	0.061	0.062	0.032	0.028
$\nabla^2\rho$	–0.061	–0.063	–0.023	–0.021
ε	0.070	0.074	0.081	0.079

*X stands for metal ion

4. CONCLUSION

The ion replacement in plastocyanin was studied by the ONIOM calculation method. The results revealed the main difference between the four proteins. Total protein energy of copper (I) and copper (II) was found to be lower than that of silver (I) and cadmium (II) proteins, respectively. Also, HOMO and LUMO orbital energies of copper (I) and cadimum (II) active sites are lower than those of silver (I) and copper (II) active sites. Natural charges of atoms in the four active sites illustrated that substitution of ion in plastocyanin can cause different charge distribution in the plastocyanin active site. Also, atom in molecule theory showed different electronic density and bond ellipticity for bonds of the central metal ion with atoms of surrounding ligands. Although the substitution of silver (I) with copper (I) in PAC spectroscopy can help to derive useful information about binding of plastocyanin to photosytem I, ONIOM results show that the electronic structures of the substituted active sites are different.

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АВ INITIO-ИЗСЛЕДВАНЕ НА ЗАМЕСТВАНЕТО НА ЙОНИ В ПРОТЕИНА ПЛАСТОЦИАНИН В СПАНАКА

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(Резюме)

Замяната на йони в активните центрове на протеини предизвиква значителен интерес поради нейното приложение при някои спектроскопски методи, например при PACS (Perturbed Angular Correlation Spectroscopy). Теоретичното разглеждане на замяната на йони в пластоцианина е извършено с помощта двуслойния ONIOM-метод. Медните йони (едно и двувалентни) в активния център на пластоцианина се заместват съответно със сребърни (I) и кадмий (II). Извършена е оптимизация на четирите протеина с LANL2DZ и SDD базисни мрежи. Молекулните орбитали, естествените орбитали на връзките и атомите са използвани за определяне на електронните структури на четирите активни центрове. Общата енергия на пластоцианина с мед (I) е по-ниска от тази на пластоцианин със сребро (I). Пластоцианинът с мед (II) показва по-ниска енергия от този с кадмий (II). HOMO и LUMO-орбиталните енергии на активните центрове с мед (I) и кадмий (II) са по-ниски от тези при сребро (I) и мед (II). Четирите активни центрове показват различно разпределение на заряда. Установени са различни стойности на електронната плътност и елиптичността на връзките за четирите активни центрове.