Proteins as Semiconductor devices

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1 Introduction

Bioelectronics is one of the most promising technologies for the next generation, because bio-materials have excellent characters which cannot be realized by the silicon devices. Actually, biosensors have been already commercialized by enzymes. Recently, bio battery (biofuel cell) evolving from the biosensor has been actively developed by various groups with us [1]. It is however difficult to truly open up the bioelectronics world, without understanding the ET behaviour in bio-materials, especially in protein. Thus we tried to investigate the ET in protein by using a precise wave function calculated from a whole structure of protein (zinc-substituted cytochromes c and b_{562}: Zn-cyts c and b_{562}) [2, 3].

Attempting to understand the photoinduced ET mechanism, we have calculated an all-electron wave function for the ground-state of Zn-cyts c and b_{562} on the basis of DFT. Gouterman's 4-orbitals are assigned on the basis of the excited states of chromophore model for Zn-porphine complex calculated with the time-dependent DFT method. ET rates between each 4-orbitals and other MOs were estimated using Fermi's golden rule.

$$k_{i \to f} = 2\pi/\hbar \times |H_{fi}|^2 \delta(E_{fi}) \quad (1)$$

where H_{fi} is the electronic coupling matrix elements, E_{fi} is the MO energy difference between final and initial states, and δ is the delta-function. In Zn-cyt c, it appeared that the two occupied MOs of the 4-orbitals show exclusively higher ET rate from/to particular MOs that localize on outermost amino acid residues, whereas ET rates involving the two unoccupied MOs of the 4-orbitals are much slower. On the other hand the two unoccupied MOs of the 4-orbitals show much higher ET rate from/to particular MOs in Zn-cyt b_{562}. These results imply that the intramolecular ETs in photoexcited Zn-cyt c or b_{562} are governed by the hole transfer through occupied or unoccupied MOs, respectively. This description may also comprehend both Dutton's model and Gray's model. According to the H_{fi} analyses, the H_{fi} values around the 4-orbitals region are almost homogeneous and their influences on ET rate constants should be relatively small. Hence, if the $\delta(E_{fi})$ effect is small, the ET rate will be well-described with Dutton's model where a mass of peptides can be treated as a continuous medium. On the other hand, the Gray model is rather preferable when the effect of $\delta(E_{fi})$ is not negligible.

The couplings of MOs between zinc porphyrin core and specific amino acid residues on the protein surface have been demonstrated in Zn-cyts c and b_{562} immobilized on an Au electrode via SAM. The voltage dependence of the photocurrent appeared to be ohmic behavior like a photoconductor in Zn-cyt c and to be rectifying behavior like a photodiode in Zn-cyt b_{562}, which strongly support the intramolecular ET mechanism in Zn-cyts c and b_{562} proposed on the basis of the theoretical calculations.

2 Calculations

we computed the all-electron wavefunction of Zn-cyts c and b_{562} by using Protein DF. For the excited states, we performed a time-dependent DFT (TDDFT) calculation of the chromophore model (CM) consisting of Zn-porphine with the two axial ligands and we have assigned molecular orbitals (MOs) responsible for the excited states of Zn-cyts c and b_{562}.

2.1 Zn-cyt c

The horse heart Zn-cyt c contains 104 amino acid residues and one c-type Zn-protoporphyrin. The protoporphyrin is bound covalently to polypeptide (apo-protein) of Zn-cyt c via thioether bonds between two vinyl side chains and Cys14 and Cys17. Residues His18 and Met80 form the fifth and sixth axial ligands of zinc- protoporphyrin, respectively. The numbers of atoms and electrons are 1764 and 6590, respectively.

The coordinate of Zn-cyt c (horse heart) was obtained from the protein data bank as 1m60. A molecular mechanics calculation was performed using the Generalized Born method (dielectric constant is 80) with Discovery Studio Modeling 1.5 (force field: CHARMm) to relax local structure distortions, in the state that the structure of Zn-protoporphyrin was fixed. At the neutralization, Na$^+$ and Cl$^-$ ions were placed around the anionic and cationic residues, respectively. For an anionic residue where a Na$^+$ ion could not be placed for steric reason, a hydrogen atom was added to the carbonyl oxygen with 0.96Å fixed bond length in the direction of the nearest hydrogen of water molecule. Whereas for a cationic residue where Cl$^-$ ion could not be placed for steric reason, the hydrogen nearest to an oxygen of water molecules was removed. 5258 water molecules (TIP3) were placed around the protein. In order to relax the Na$^+$ and Cl$^-$ ions, all water molecules and Na$^+$ and Cl$^-$ ions were subjected to a molecular mechanics energy minimum calculation under a fixed coordinate of the protein structure. In the DFT calculation, the TIP3 water molecules were removed and Na$^+$ and Cl$^-$ ions were treated as point charges +1 and -1, respectively.

The all-electron calculation of Zn-cyt c was carried out by using the Gaussian-based DFT program, proteinDF with VWN functional and sg-1 grid, following the method for native cytochrome c reported by Sato et al [4]. The basis and auxiliary function sets used are listed in Table 1. The effective initial guess was constructed using quasi-canonical localized orbitals (QCLOs). Firstly, we decomposed Zn-cyt c into Zn-protoporphyrin and apo-protein. The apo-protein was further decomposed into single amino acid fragments, 1, 2 …104 residues. The ordinary all-electron calculations were performed for the 104 molecules. Then, all-electron calculations of the 102 tri-peptide fragments, 1-3, 2-4, 3-5…102-104 were carried out. In this step, the initial electron density was estimated from the corresponding electron density expanded by using the auxiliary functions for each single residue in the results of the previous step. The calculated canonical MOs of the tri-peptides were then localized on each atom and the QCLOs for the fragment defined by main chain or side chain regions were then obtained. Iterating this type of elongation procedure, we prepared fifteen peptides of the apo-protein: 1-7, 6-14, 13-19, 18-24, 22-30, 29-37, 36-44, 43-51, 50-58, 57-65, 64-72, 71-80, 80-86, 85-95, and 94-104.

The Zn-protoporphyrin part was built up through another elongation pathway. We computed a chromophore model (CM)

consisting of Zn-porphine, 5-ethylimidazole for His18 and methyl propyl sulfide for Met80. The geometric data of these elements were derived from the computational structure of Zn-cyt c constructed above. We replaced all side chains of protoporphyrin with hydrogen atoms to make Zn-porphine. 5-ethylimidazole, methyl propyl sulfide and Zn-porphine were calculated separately, then the model was expanded to the Zn-protoporphyrin complex containing Zn-cyt c and its surrounding small peptides, 13-19 and 79-81. All side chains of protoporphyrin were introduced, and Cys14, Cys17 and two vinyl side chains were connected via thioether bonds. Finally we combined the results of the Zn-protoporphyrin complex and the other parts of apo-protein, and carried out the all-electron calculation of Zn-cyt c. This calculation includes 1764 atoms, 6590 electrons and 8786 MOs.

The excited states calculation of chromophore in Zn-cyt c was performed by using the TDDFT method with the Gaussian 03 (Rev.B03) program package. The basis sets and the functional are the same as those of the all-electron calculation of Zn-cyt c. The model structure was adopted to the CM described above. The symmetry is C_1 and ten excited states of CM were calculated.

2.2 Zn-cyt b_{562}

Calculation of Zn-cyt b_{562} was performed according to above method. The Zn-cyt b_{562} contains 106 amino acid residues and one b-type Zn-protoporphyrin. Residues His102 and Met7 form the fifth and sixth axial ligands of Zn-protoporphyrin, respectively. The coordinate of cyt b_{562} was obtained from the protein data bank as 256B and Zn-cyt b_{562} structure was made by substituting Zn for Fe. This calculation includes 1722 atoms, 6628 electrons and 8758 MOs.

3 Conclusion

The intramolecular ET rate constants of photoexcited Zn-cyt b_{562} were estimated from [Eq. (1)] by using each and related to the Gouterman's 4-orbitals. As is seen in the case with Zn-cyt c, distinct ET rates for specific couples of MOs in Zn-cyt b_{562} are observed. Dominant intramolecular ET rate constants for each Gouterman's 4-orbital of Zn-cyt b_{562} and Zn-cyt c are summarized in Table 1. In Zn-cyt b_{562}, the rate constants between unoccupied MOs are one order of magnitude larger than those between the occupied MOs. The two unoccupied MOs are localized on the outermost amino acid residues around Gly70 (MO3331) and Pro56 (MO3332), respectively, which are located on the opposite side of the protein-SAM interface and exposed to the solution (Scheme 1, Figure 2a, Figure S2, Table S2). On the other hand, the two occupied MOs are localized on the Glu18 (MO3300) and Asp50 (MO3307), respectively, and both reside on the electrode-contact side. Hence the major mechanism of ET in the photoexcited Zn-cyt b_{562} is "electron transfer (ET) in the conduction band" through two-states coupling between unoccupied MOs, showing that the photoexcited Zn-cyt b_{562} is an n-type semiconductor. This is quite different from Zn-cyt c where "hole-transfer (HT) in the valence band" between occupied MOs dominates. This protein is a p-type semiconductor, even though the "dopant" is the same as in Zn-cyt b_{562}.

Why is one protein n-type and the other p-type semiconductor? We analyzed the ground state electronic structures of both proteins. MO energies of Zn-cyt b_{562} are generally higher, compared to those of Zn-cyt c, which reflects difference in charge of the two proteins: Zn-cyt b_{562} and Zn-cyt c have negative (isoelectric point, pI = 5.5) and positive (pI = 9.6) charges in pH 7.0, respectively. Importantly, the effect of the negative charge of Zn-cyt b_{562} to its 4-orbitals energies are more prominent than the other porphyrin MOs assigned to porphyrin (σ) orbitals and the MOs localized on amino acid

residues. The difference in MO energy levels of each corresponding 4-orbitals between the two systems are almost in the same extent, +2.34 eV, though the average energy shift from Zn-cyt c to Zn-cyt b_{562} is +1.38 eV. Hence the relative energy level of 4-orbitals against protein bands consisting of MOs localized on amino acid residues is very different between the two proteins. In Zn-cyt b_{562}, the unoccupied 4-orbitals (MOs 3326 and 3329) locate in the lower region of the protein's conduction band, whereas such an overlap is not found in Zn-cyt c. On the other hand, the occupied 4-orbitals (MOs 3302 and 3304) are placed at a shallow position in the higher valence band of Zn-cyt b_{562}. In comparison to Zn-cyt c, the MOs with holes and the valence band with amino acid character are more weakly coupled in Zn-cyt b_{562}. These electronic structure differences between the two proteins dictate the molecular-semiconductor nature of the proteins.

Table 1. Calculated ET parameters for Zn-cyt b_{562} and Zn-cyt c

Proteins	MO Pairs	H_{fi}(eV)	E_{fi} (eV)	$k_{i\rightarrow f}$ (sec^{-1})
Zn-cyt b_{562}	3302_3300	-8.52×10^{-1}	-4.41×10^{-2}	1.57×10^{17}
	3304_3307	-6.94×10^{-1}	$+1.11\times10^{-1}$	4.15×10^{16}
	3326_3325	-5.71×10^{-1}	-5.50×10^{-2}	5.66×10^{16}
	3329_3331	$+1.15\times10^{0}$	$+9.11\times10^{-3}$	1.39×10^{18}
	3329_3332	-7.33×10^{-1}	$+1.48\times10^{-2}$	3.46×10^{17}
Zn-cyt c	3268_3270	-3.51×10^{-1}	$+2.27\times10^{-2}$	5.17×10^{16}
	3272_3271	-6.15×10^{-2}	-9.24×10^{-5}	3.91×10^{17}
	3297_3296	-4.91×10^{-2}	-1.59×10^{-2}	1.45×10^{15}
	3299_3298	$+9.40\times10^{-2}$	-1.14×10^{-1}	7.42×10^{14}

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