VII-G Artificial Photoreaction systems on a Protein Surface

New methodologies are developed which one can construct an artificial photoreaction systems on a protein matrix. Using the protein-based photosystems, we aim to investigate characteristics for electron transfer phenomena in a protein matrix. Furthermore, based on these results, we would like to design and semisynthesize an efficient photoreaction system such as an artificial photoreaction center.

VII-G-1 Direct Observation of the Ferric-Porphyrin Cation Radical as an Intermediate in the Photo-Triggered Oxidation of Ferric- to Ferryl-Heme Tethered to $Ru(bpy)_3$ in Reconstituted Myoglobin

HAMACHI, Itaru[1,2]; TSUKIJI, Shinya[1]; SHINKAI, Seiji[1]; OISHI, Shigero[3]
([1]Kyushu Univ.; [2]IMS; [3]Kitasato Univ.)

[*J. Am. Chem. Soc.* **121**, 5500 (1999)]

Using semisynthetic myoglobins ($Ru(bpy)_3$–Mbs) with covalently-appended $Ru(bpy)_3$ (bpy = 2,2'-bipyridine), an oxidized-Mb is photo-produced through intramolecular electron abstraction reaction as a key step. UV-visible spectra, electron paramagnetic resonance measurements and reactivity tests identify the photo-oxidized Mb as a ferryl-species (*i.e.* Fe^{4+}–heme). By circular dichroism (CD) spectroscopy, high performance liquid chromatography (HPLC) and SDS polyacrylamide gel electrophoresis (SDS-PAGE), the photo-oxidation proceeds without the damage of the protein structure. Significantly, we report the first direct observation of ferryl-Mb photogeneration via the intermediate porphyrin cation radical. As a consequence of this observation and proposed mechanism, the rate constants for each step can be clearly determined. The photo-excited $Ru^{2+}(bpy)_3$ is oxidatively quenched by $[Co(NH_3)_5Cl]^{2+}$, a sacrificial acceptor, to produce $Ru^{3+}(bpy)_3$ which then proceeds to abstract an electron from the porphyrin ring with a first order rate constant of $7.1 \times 10^5\ s^{-1}$, in the first step. The electron transfer is followed by iron(III) oxidation by the porphyrin radical with concurrent deprotonation (a first order rate constant of $4.0 \times 10^4\ s^{-1}$ at pH 7.5, and $2.0 \times 10^5\ s^{-1}$ at pH 9.0) in the second step. Consistent with this mechanism, it is demonstrated that the rate of the fast step of the porphyrin radical generation is independent of pH, whereas the slower step of ferryl-heme formation is dependent on pH. Simulation of the detailed pH dependence of the kinetics clearly shows that the deprotonation-protonation equilibrium of the protein matrix can control the ferryl-heme generation in a heme pocket of Mb.

VII-G-2 Construction of Artificial Photosynthetic Reaction Centers on a Protein Surface: Vectorial, Multistep, and Proton-Coupled Electron Transfer for Long-Lived Charge Separation

HU, Yi-Zhen[1]; TSUKIJI, Shinya[1]; SHINKAI, Seiji[1]; OISHI, Shigero[3]; HAMACHI, Itaru[1,2]
([1]Kyushu Univ.; [2]IMS; [3]Kitasato Univ.)

[*J. Am. Chem. Soc.* **122**, 241 (2000)]

Artificial photosynthetic reaction centers have been constructed on a protein surface by cofactor reconstitution, which mimic the function of photosynthetic organisms to convert light energy to chemical potential in the form of long-lived charge-separated states. They feature a ruthenium tris(2,2'-bipyridine) moiety as the sensitizer, which is mechanically linked (*i.e.* in catenane-type) with a cyclobis(paraquat-*p*-phenylene) unit (BXV^{4+}, acceptor) and covalently linked with a protoheme or Zn-protoporphyrin (donor) located in the myoglobin pocket. Reconstitution of apo-myoglobin (Mb) with **1** and **2** affords the two Mb-based artificial triads, Mb-$(Fe^{III}OH_2)$–Ru^{2+}–BXV^{4+} and Mb(Zn)–Ru^{2+}–BXV^{4+}. Laser flash photolysis of the $Ru(bpy)_3$ moiety of Mb$(Fe^{III}OH_2)$–Ru^{2+}–BXV^{4+} in an aqueous solution yields an initial charge-separated state, Mb$(Fe^{III}$-$OH_2)$–Ru^{3+}–$BXV^{3+\bullet}$, via noncovalent electron transfer, followed by dark electron transfer to generate an intermediate consisting of porphyrin cation radical, Mb-$(Fe^{III\bullet}(OH_2)$–Ru^{2+}–$BXV^{3+\bullet}$. Mb$(Fe^{III\bullet}(OH_2)$–Ru^{2+}–$BXV^{3+\bullet}$ thus generated is subsequently converted, via a proton-coupled process and with a quantum yield of 0.005, into the final charge-separated state, Mb$(Fe^{IV}$=O)–Ru^{2+}–$BXV^{3+\bullet}$, which bears an energy more than 1 eV above the ground state and a lifetime (τ > 2 ms) comparable to that of natural photosynthetic reaction center. By analogy with a related system reported previously, it was considered that back ET from $BXV^{3+\bullet}$ to Mb$(Fe^{IV}$=O) might be coupled to the protonation of Mb$(Fe^{IV}$=O) and governed by the slow interconversion between the metal-oxo form and the proton-activated species, rendering the CS state Mb$(Fe^{IV}$=O)–Ru^{2+}–$BXV^{3+\bullet}$ specially long-lived. Control experiments clearly demonstrated that partial incorporation of the triads into the protein matrix plays a crucial role in regulating the electron transfer pathway and stabilizing the charge separation state.

VII-G-3 Direct Comparison of Electron Transfer Properties of Two Distinct Semisynthetic Triads with Non-Protein Based Triad: Unambiguous Experimental Evidences on Protein Matrix Effects

HU, Yi-Zhen[1]; TAKASHIMA, Hiroshi[1]; TSUKIJI, Shinya[1]; SHINKAI, Seiji[1]; NAGAMUNE, Teruyuki[3]; OISHI, Shigero[4]; HAMACHI, Itaru[1,2]
([1]Kyushu Univ.; [2]IMS; [3]Univ. Tokyo; [4]Kitasato Univ.)

[*Chem. Eur. J.* **6**, 1907(2000)]

In order to understand the roles of protein matrix in electron transfer (ET) within biological systems, a heme-based donor (Zn-heme: ZnPP)-sensitizer ($Ru^{2+}(bpy)_3$)-acceptor (cyclic viologen: BXV^{4+}) triad **1**

was used as a probe molecule. Two semisynthetic systems, Cyt-b_{562}(**1**) and Mb(**1**), in which the triad is incorporated into cytochrome b_{562} (Cyt-b_{562}) or into myoglobin (Mb), were constructed by cofactor reconstitution. These two semisynthetic proteins were compared with the triad itself (*i.e.* without protein matrix) using absorption spectroscopy, steady state emission and excitation studies, laser flash photolysis experiments, and molecular modelling. Photoexcitation of the ZnPP moiety of Cyt-b_{562}(**1**) or Mb(**1**) leads to a direct ET from the triplet state of ZnPP state (^{3}ZnPP) to BXV^{4+} to generate a final charge separated (CS) state, Cyt-b_{562}(Zn^+)–Ru^{2+}–$BXV^{3+\bullet}$ or Mb(Zn^+)–Ru^{2+}–$BXV^{3+\bullet}$. On the other hand, direct ET from the excited ZnPP moiety to the BXV^{4+} moiety is also involved in **1** in the absence of the protein matrix, but the excited state of ZnPP involved is not ^{3}ZnPP, but the singlet excited state (^{1}ZnPP) in this pathway. When the $Ru^{2+}(bpy)_3$ moiety of Cyt-b_{562}(**1**) or Mb(**1**) is excited, a stepwise ET relay occurs with the ion-pair, Cyt-b_{562}(Zn)–Ru^{3+}–$BXV^{3+\bullet}$ or Mb(Zn)–Ru^{3+}–$BXV^{3+\bullet}$, as an intermediate, leading to the same final CS state as that generated in the direct ET pathway. The lifetimes of the corresponding final CS states were determined to be 300 ns for **1** in the absence of the protein matrix, 600–900 ns for Cyt-b_{562}(**1**) and 1.1–18 μs for Mb(**1**), the values of which are greatly affected by the protein matrix. Molecular modeling study of the three systems consistently explained the differences of their photophysical behavior.

VII-G-4 Cyclodextrin-Appended Myoglobin as a Tool for Construction of a Donor-Sensitizer-Acceptor Triad on a Protein Surface

HAMACHI, Itaru[1,2]; TAKASHIMA, Hiroshi[1]; HU, Yi-Zhen[1]; OISHI, Shigero[3]; SHINKAI, Seiji[1]
([1]Kyushu Univ.; [2]IMS; [3]Kitasato Univ.)

[*Chem. Commun.* 1127 (2000)]

A protein-based and noncovalently-linked donor-sensitizer-acceptor triad has been prepared by self-assembly via mechanical linkage and hydrophobic interaction, and its photoinduced electron transfer properties has been studied. Cyclodextrin(CD)-appended hemes are successfully reconstituted with apo-myoglobin to yield CD-appended myoglobins. Upon addition of viologen-connected ruthenium tris(bipyridine) bearing adamantane unit, a donor-sensitizer-acceptor triad is formed on a protein surface, which shows a stepwise, vectrial electron transfer reaction by visible light irradiation. Clearly, this is a novel type of supramolecular photoreaction system.