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Cyclodextrin-appended myoglobin as a tool for construction of a donor–sensitizer–acceptor triad on a protein surface

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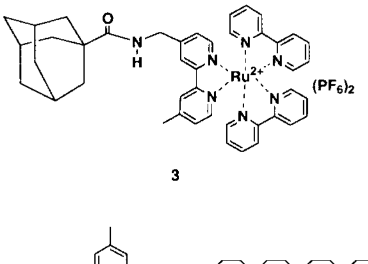
A protein-based and noncovalently-linked donor–sensitizer–acceptor triad has been prepared by self-assembly *via* mechanical linkages and hydrophobic interactions, and its photoinduced electron transfer properties have been studied.

Multi-step electron transfer (ET) in protein matrices is the key process of biological energy transduction in photosynthetic and mitochondrial respiratory systems.[1,2] A number of efforts have shown that model proteins suitably modified with an electron donor or acceptor are valuable for understanding such complicated ET mechanisms.[3] Most of the models reported so far, however, are simple donor–acceptor diads because of serious limitations for modifying native protein matrices. We recently reported a donor–sensitizer–acceptor triad attached to protein matrices using cofactor reconstitution methods.[4] Here, we describe a novel strategy for the construction of a triad by self-assembly. The protein-based triad, which consists of cyclodextrin(CD)–appended Zn–myoglobin (Zn-Mb-CD), an adamantane-modified Ru(bpy)$_3$ and a bis(viologen)cyclophane (BXV^{4+}), is noncovalently connected by elaborately employing mechanical linkages[5] and hydrophobic interactions.

The β-CD-modified protoporphyrins IX (**1a** and **1b**) were synthesized according to Scheme 1. Protoporphyrin IX (PP) mono-ethylester was condensed with 6-monoamino-peracetylated-β-CD in the presence of BOP to yield a CD-appended heme **2**. Subsequent metal insertion into the heme center followed by hydrolysis of the ester groups gave the target compound **1**. Tris-heteroleptic Ru(bpy)$_3$ derivatives (**3**, **4**) used herein were synthesized according to the synthetic route reported previously by us.[4,5] All of the compounds were characterized by FT-IR, NMR, MALDI-TOF mass spectrometry, and elemental analysis.



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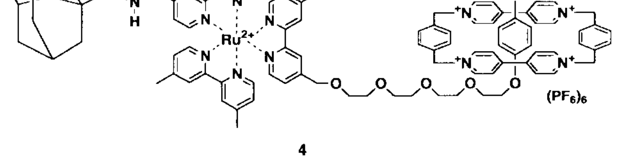

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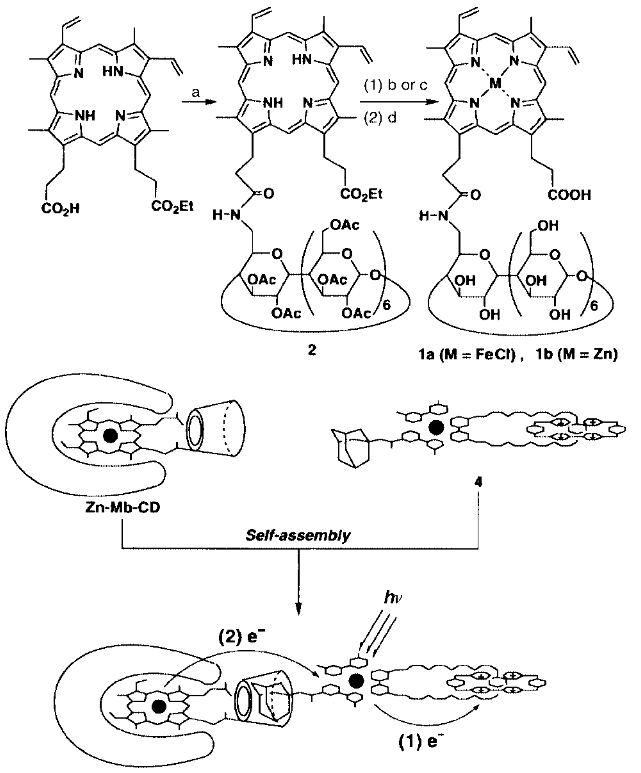
Reconstitution of **1a** and **1b** with apo-Mb were carried out according to the literature reported by us.[4] The yield of Zn-Mb-CD and Fe(III)-Mb (Fe-Mb-CD) were 36% and 40%, respectively. The absorption and/or fluorescence spectra of Zn-Mb-CD and Fe-Mb-CD are very similar to those of their corresponding native forms, Zn-Mb and Fe-Mb.[6] Spectroscopic titration experiments clearly show that 1 equiv. of **1** is quantitatively complexed with apo-Mb (data not shown). In Fe-Mb-CD, the spectroscopic changes due to the ligand exchange and redox and oxygen-binding reactions were almost identical to native Mb.[6] These results undoubtedly imply that the heme unit of **1** is located in the natural heme crevice of Mb.

Binding of Ru(bpy)$_3$ derivatives bearing adamantane units to the CD-appended Mb's was monitored by emission quenching of Ru(bpy)$_3$ of **3** by Fe-Mb-CD. The emission lifetime of **3** was determined to be *ca.* 500 ns in the absence of Fe-Mb-CD. By addition of Fe-Mb-CD (10 μM) to a solution of **3** (1 : 1 ratio), we observed a faster decay (64%, the lifetime of 100 ns) of the emission at 600 nm, as well as the normal lifetime of 500 ns (36%). The shorter lifetime is attributable to the fraction of **3** bound to Fe-Mb-CD.[7] Based on these fractions, the binding constant of the adamantane unit of **3** with the CD unit of Fe-Mb-CD was estimated to be *ca.* 5×10^5 M^{-1}, suggesting that the



Scheme 1 *Reagents and conditions*: a, 6-monoamino-peracetylated-β-CD, BOP (1 equiv.), DIEA (1 equiv.), dry DMF, N$_2$, r.t., 9 h, 35%; b, Zn(OAc)$_2$ (10 equiv.), CHCl$_3$–MeOH (1 : 1, v : v), r.t., 5 h, 95%; c, FeCl$_2$ (12 equiv.), dry DMF, N$_2$, 65 °C, 5 h, 48%; d, 1 N NaOH (42 equiv.), MeOH–THF (1 : 1, v : v), r.t., 17 h, 89% (**1a**) and 76% (**1b**).

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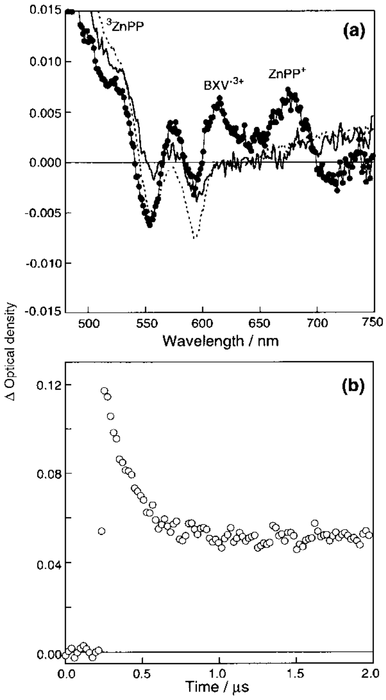

Fig. 1 (a) Transient absorption spectra observed after laser excitation of Zn-Mb-CD (10 µM)/**4** (20 µM) and Zn-Mb (10 µM)/**4** (20 µM) complexes in deaerated phosphate buffer (pH 7.0, 50 mM) solution at the delay time of 50 ns. (—●—) Zn-Mb-CD/**4**, λ_{ex} = 460 nm; (·····) Zn-Mb-CD/**4**, λ_{ex} = 596 nm; (——) Zn-Mb/**4**, λ_{ex} = 460 nm. (b) Time profile monitored at 670 nm in Zn-Mb-CD/**4** after laser excitation at 460 nm.

$Ru(bpy)_3$ derivatives **3** and **4** can form tight complexes with Zn-Mb-CD or Fe-Mb-CD.

Next, a donor–sensitizer–acceptor triad system was prepared by spontaneous assembly of Zn-Mb-CD with the $Ru(bpy)_3$-based diad **4**. Fig. 1a shows the transient absorption spectrum obtained by photoexcitation of an aqueous solution containing Zn-Mb-CD (10 µM)/**4** (20 µM) at 460 nm (MLCT band of $Ru(bpy)_3$; —●—) and 596 nm (Q-band of ZnPP; ······): Photolysis at 460 nm led to the immediate appearance of absorption bands due to the viologen radical and the ZnPP radical at 610 nm and 670 nm, respectively, in addition to the ZnPP triplet absorption at 470 nm.[4,5,8] In contrast, the viologen and ZnPP radicals are not photo-produced in a mixture of native Zn-Mb and **4** (*i.e.* without CD; ——) under the same conditions. This is direct evidence for the formation of a pseudo intramolecular Zn-Mb-CD/**4** complex, in which a charge-separated (CS) state between ZnPP and cyclic viologen is generated by photolysis of the MLCT band of $Ru(bpy)_3$ (460 nm). Interestingly, the CS state was not observed by photoexcitation of Zn-Mb-CD/**4** at 596 nm excitation, indicating that a direct electron transfer from the excited ZnPP to cyclic viologen does not occur. Moreover, both the excited singlet and triplet states of the ZnPP unit of Zn-Mb-CD were not considerably quenched by **4**. This implies that an energy transfer from excited ZnPP to $Ru(bpy)_3$ does not take place in the Zn-Mb-CD/**4** complex. Conceivably, the CS is photo-generated by a stepwise electron transfer as shown in Fig. 2. Initial electron transfer occurs from the photo-excited $Ru^{II}(bpy)_3$ to the cyclic viologen,[5] followed by reduction of the produced $Ru^{III}(bpy)_3$ by ZnPP to yield the final CS state.[4] It is noteworthy that both ET steps occur in noncovalently linked donor–acceptor pairs.

The kinetic behavior of the CS state of Zn-Mb-CD/**4** was monitored at 670 nm. As shown in Fig. 1b, the absorbance at 670 nm appears within 50 ns, indicating that steps 2 and 3 of Fig. 2 proceed faster than 50 ns. Unfortunately, the precise rate constants for steps 2 and 3 have not been determined due to instrumental limitations. The lifetime of the CS state was determined to be *ca.* 640 ns from the decay process shown in Fig. 1b.[9] The quantum yield of the CS state was estimated to be 0.03 by using 5,10,15,20-tetraphenylporphyrin as a reference.[10]

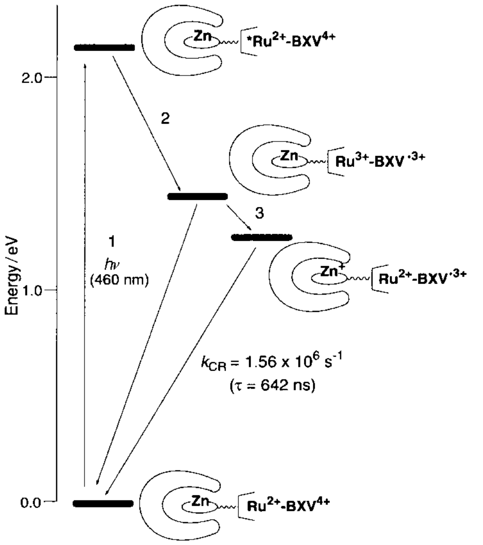

Fig. 2 Schematic energy diagram for the photoinduced reactions in the Zn-Mb-CD/**4** complex.

In conclusion, we have successfully prepared a noncovalently linked donor–sensitizer–acceptor triad on a myoglobin surface using a rational combination of mechanical bonding and hydrophobic interactions. The present approach may extend to the utilization of various kinds of donors and/or acceptors in semisynthetic systems, by which one can obtain valuable insight into ET events occurring in a protein matrix.

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8 The appearance of the ZnPP triplet absorption is due to the weak light absorption by the ZnPP moiety at 460 nm (*ca.* 10%) which decays with a lifetime of 2 ms. Since the emission of **3** was not quenched in the presence of Zn-Mb-CD, energy transfer from excited $Ru(bpy)_3$ to ZnPP can be ruled out.

9 On the time scale detected (2 µs), the decay curve in Fig. 1b does not reach zero because the ZnPP triplet state exhibits an absorption residue at 670 nm. The remaining fraction decays with a lifetime similar to that of the ZnPP triplet state of Zn-Mb-CD (*ca.* 2 ms).

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