A Convenient Synthesis of a Ru(bpy)$_3$-based Catenane-type Triad and Its Incorporation into a Protein Scaffold

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(Received March 1, 1999; CL-990132)

A catenated heme cofactor consisting of a donor (protoheme), a sensitizer (Ru(2,2'-bipyridine)$_3$) and an acceptor (cyclobis(paraquat-*p*-phenylene) was prepared by stepwise coordination, followed by iron-insersion. Reconstitution of apo-myoglobin with this cofactor afforded a protein-based, noncovalently-linked photosynthetic triad.

Photoinduced electron transfer in proteins has been the subject of extensive theoretical[1] and experimental[2] research efforts. The donor-acceptor distances, environmental and structural factors were found to control the electron transfer rates in the protein systems. The unique organization of the donor-acceptor units within the photosynthetic reaction center leads to vectorial electron transfer and effective charge separation.[3] Previous research on artificial photosynthesis were directed to the organization of molecular or supramolecular donor-acceptor diad, triad, etc. assemblies.[4] The conformational flexibility of many of these systems prevents charge separation due to the rapid recombination of the spatially intimate redox products. Incorporation of a molecular dyad or triad in protein system could structurally rigidify the donor-acceptor pair, thereby leading to efficient charge separation. We and other groups have reported that reconstitution of apo-hemoproteins, e.g. apo-myoglobin, with Fe(III)-, Co(II)- or Zn(II)-protoporphyrin IX appending chromophore, donor or acceptor, yields structurally-defined donor-acceptor systems.[5] Effective charge separation and photocatalytic functions were accomplished with these systems. As a further development of this method, here we report the construction of a myoglobin-based donor-sensitizer-acceptor triad (Mb(Fe^{III}OH$_2$)-Ru^{2+}-BXV^{4+}), in which the acceptor (cyclobis(paraquat-*p*-phenylene), BXV^{4+}) and the sensitizer (Ru(bpy)$_3$) are noncovalently linked in a catenane type, and the sensitizer is covalently linked with the donor (protoheme) located in the myoglobin surface (Figure 1).

We recently reported that photoexcitation of a semisynthetic myoglobin appending Ru(bpy)$_3$ in the presence of a sacrificial acceptor generates its oxoferryl state (Mb(Fe^{IV}=O), so-called compound II) via the coupling of inter- and intramolecular ET.[5c,d] This success prompted us to attempt to attach an electron acceptor to the system, which might thus be photo-activated via intramolecular ET. Recently, a novel [2]catenane incorporating Ru(bpy)$_3$ (sensitizer) and BXV^{4+} (acceptor) was reported by Duerr group.[6] The photoinduced forward electron transfer in this catenane is very fast and its charge-separated state is relatively long-lived. Taking the advantage of this catenane, a triad compound (**1**) was thus designed.

Compound **1** is a tris(2,2'-bipyridine) ruthenium complex with three different ligands, i.e. tris(heteroleptic) complex. Although

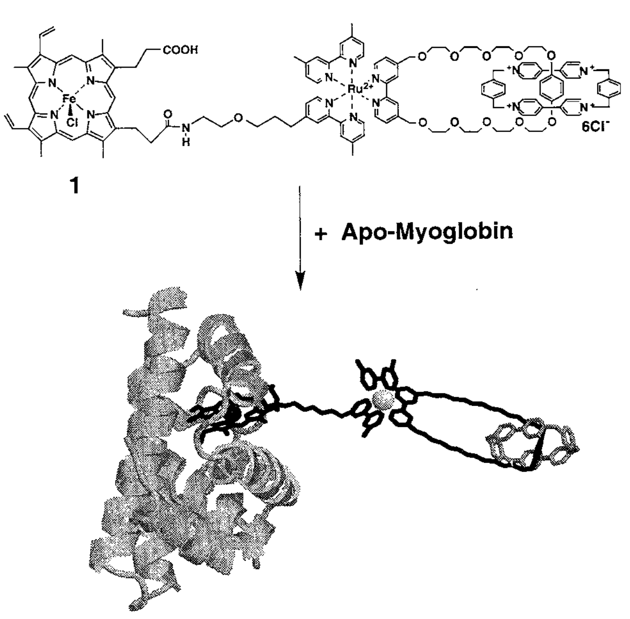
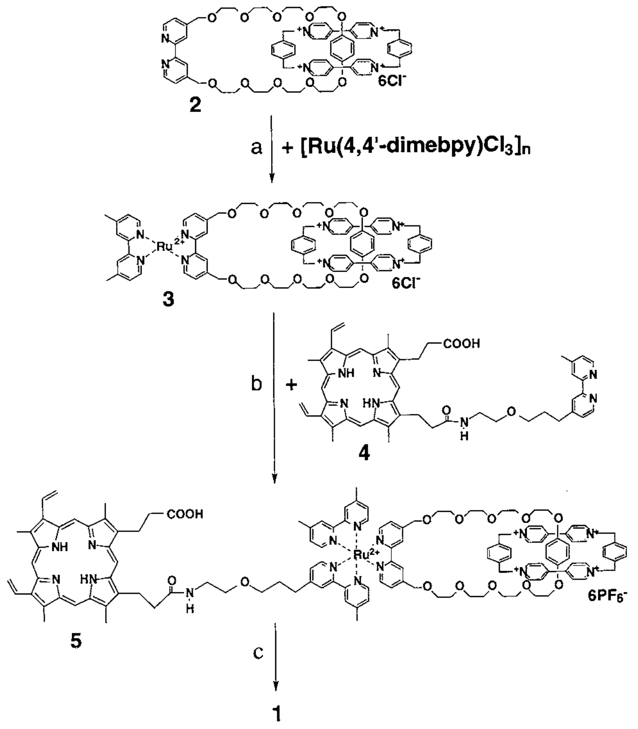


Figure 1. Schematic illustration of the cofactor reconstitution of apo-myoglobin with compound **1**.



Scheme 1. Reagents and conditions: (a) EtOH-H$_2$O (1:1), reflux under N$_2$ in the dark, 8 h. (b) EtOH, reflux under N$_2$ in the dark, 22 h, NH$_4$PF$_6$, 60% yield. (c) FeCl$_2$•4H$_2$O, DMF, 65 °C, 8h, Et$_4$NCl, 78% yield.

the syntheses of tris(bidentate) ruthenium complexes have been extensively reported, existing procedures are most effective for incorporation of at most two different ligands into the coordination sphere, and examples of tris(heteroleptic) complexes are very limited.[7] Recently, Keene and coworkers reported a synthetic method for heteroleptic tris(bidentate) ruthenium complex based on the sequential addition of the polypyridyl ligands to $[Ru(CO)_2Cl_2]_n$ (5 steps).[7a,b] In this method, however, a strong oxidant, trimethylamine *N*-oxide (TMNO), is needed for the decarbonylation, which limits the application of this method for the synthesis of oxidation-sensitive complex. Since both the porphyrin and the BXV^{4+} cyclophane units can be destroyed in the presence of TMNO, the existing method can not be used for the synthesis of compound **1**. Thus, a new synthetic strategy was employed, as depicted in scheme 1. We should note that a short communication reported a similar synthetic method for a Ru tris(diimine) heteroleptic complex but no detail was described.[7c]

Reaction of the catenane ligand (**2**)[6] with the precursor $[Ru(4,4\text{-dimethylbipyridine})Cl_3]_n$[8] affords, after column chromatography on silica gel (eluent MeOH/2M $NH_4Cl/MeNO_2$, 4:2:3) and anion exchange, the catenane complex $\mathbf{3(Cl)_6}$ in 70% yield.[9] Treatment of $\mathbf{3(Cl)_6}$ with the protoporphyrin-pendanted bipyridine **4**[5a,b] in ethanol under refluxing, followed by column chromatography on silica gel (eluent MeOH/2M $NH_4Cl/MeNO_2$, 3:2:3) and anion exchange, gives the tris(heteroleptic) complex $\mathbf{5(PF_6)_6}$ in 60% yield.[10] Compound $\mathbf{1(Cl)_6}$ was obtained by iron-insertion of $\mathbf{5(PF_6)_6}$ and subsequent anion exchange in 78% yield.[11] Compared with the method reported by Keene et al.,[7a,b] it is clear that the present one bears the advantages of milder reaction conditions, simpler procedures and higher yield. Thus, the synthetic technique described here provides an efficient route for the preparation of tris(heteroleptic) species of ruthenium (II) in general.

The reconstitution of **1** with apo-Mb was conducted according to the modified method of Hamachi *et al.*.[5a,b] To an apo-Mb solution in H_2O was added dropwise a 1.4-equivalent of $\mathbf{1(Cl)_6}$ dissolved in H_2O (3 mM) in ice-bath. The mixture was incubated at 4 °C for 6 hours and then dialyzed in 10 mM KH_2PO_4 buffer (pH 7.0) two times. Purification of the mixture by gel chromatography on sephadex G-25 afforded the triad $Mb(Fe^{III}OH_2)\text{-}Ru^{2+}\text{-}BXV^{4+}$ in 56% yield.

Spectrophotometric titration of **1** with apo-Mb clearly showed 1:1 complex formation (inset of Figure 2). The absorption spectrum of the met-form (ferric state, Fe(III)) of $Mb(Fe^{III}OH_2)\text{-}Ru^{2+}\text{-}BXV^{4+}$ shows a sharp soret band at 409 nm, a Q-band at 630 nm, a sharp band at 284 nm (LC band of $Ru(bpy)_3$) and a shoulder at 460 nm (MLCT band of $Ru(bpy)_3$) (Figure 2). This spectrum is nearly identical to the sum of the spectra of native Mb[12] and the BXV^{4+}-catenated $Ru(bpy)_3$ complex reported by Duerr group.[6] Reduction of the met-form of $Mb(Fe^{III}OH_2)\text{-}Ru^{2+}\text{-}BXV^{4+}$ with $Na_2S_2O_4$ and the subsequent air-oxidation gave absorption spectra corresponding to those of the deoxy- (434 nm) and oxy- (417 nm, 543 nm and 582 nm) forms, respectively. These redox properties are essentially identical to those of native Mb and the semisynthesized Mb appending $Ru(bpy)_3$,[5a,b,12] indicating that compound **1** was satisfactorily reconstituted with apo-Mb.

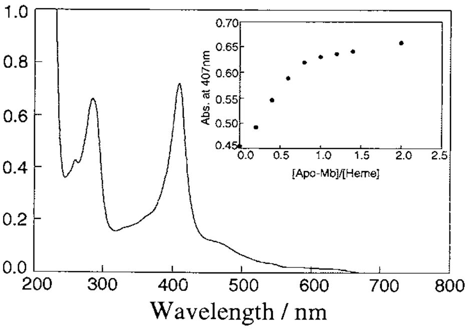


Figure 2. Absorption spectrum of $Mb(Fe^{III}OH_2)\text{-}Ru^{2+}\text{-}BXV^{4+}$ (7 μM) in 50 mM phosphate buffer (pH 7.5). Inset: spectrophotometric titration of **1** with apo-Mb in H_2O.

In conclusion, a donor-sensitizer-acceptor triad, linked in a catenane fashion, was constructed in myoglobin by cofactor reconstitution. To the best of our knowledge, this is the first protein-based noncovalently linked artificial triad. The Mb-based triad thus semisynthesized is expected to be useful for study on step-wise intramolecular electron transfer events in a protein matrix.

Y.Z.H is a postdoctoral fellow of the Japan Society for the Promotion of Science (JSPS) and S.T. is a JSPS fellow for Japanese Junior Scientists.

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9 $\mathbf{3(Cl)_6}$: ^{1}H-NMR (250 MHz, D_2O): δ (ppm) = 2.51 (d, 4.5H, $-CH_3$ groups of the cis-isomer), 2.84 (s, 1.5H, $-CH_3$ groups of the trans-isomer), 3.60-4.20 (m, 36H, ethylene groups and hydroquinone), 4.99 (s, methylene groups of the crown bpy, overlaped with the DOH peak), 5.89 (d, 8H, $-CH_2-$ of BXV^{4+} cyclophane), 7.00-9.90 (m, 36H, aromatic H from bpy and BXV^{4+}, overlaped with each other). FAB-MS (NBA) for $C_{82}H_{90}N_8O_{10}Cl_6Ru$ (M = 1661.4): m/z = 1658.4 $[M\ -3H]^+$, 1625.5 $[M - Cl]^+$, 1590.5 $[M- 2Cl]^+$, 1553.6 $[M - 3Cl]^+$, 1518.6 $[M - 4Cl]^+$, 998.3 $[M - 4Cl - BXV^{4+}]^+$, 963.5 $[M - 5Cl - BXV^{4+}]^+$.

10 $\mathbf{5(PF_6)_6}$: FAB-MS for $C_{132}H_{143}N_{15}O_{14}P_6F_{36}Ru$ (M = 3134.50): m/z = 2989.0 $[M - PF_6]^+$, 2844.1 $[M - 2PF_6]^+$, 2700.0 $[M - 3PF_6 + H]^+$, 1888.9 $[M - 5PF_6 - BXV^{4+}]^+$, 1100.5 $[BXV^{4+} + 4PF_6]^+$, 955.3 $[BXV^{4+} + 3PF_6]^+$, 810.3 $[BXV^{4+} + 2PF_6]^+$, 665.0 $[BXV^{4+} + PF_6]^+$. Elemental Anal. Found: C 49.87, H 4.83, N 6.68%. Calcd for $C_{132}H_{143}N_{15}O_{14}P_6F_{36}Ru{\bullet}2H_2O$ (3170.526): C 50.03, H 4.67, N 6.63%. Stereoisomers regarding of the Ru coordination sphere were not separated here.

11 $\mathbf{1(Cl)_6}$: ESI-TOF MS for $C_{132}H_{141}N_{15}O_{14}Cl_7FeRu$ (M = 2566.8): m/z = 837.0 (+3) $[M - Fe]^{3+}$, 802.3 (+3) $[M - Fe - 3Cl]^{3+}$, 790.3 (+3) $[M - Fe - 4Cl]^{3+}$, 619.4 (+4) $[M - Fe -Cl]^{4+}$, 597.2 (+4) $[M - 5Cl]^{4+}$, 588.2 (+4) $[M - 6Cl]^{4+}$. Elemental Anal. Found: C 58.85, H 5.21, N 7.79%. Calcd. for $C_{132}H_{141}N_{15}O_{14}Cl_7FeRu{\bullet}6H_2O$ (2674.8): C 59.22, H 5.72, N 7.85%.

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