Photocontrol of the redox state of ruthenium–tris(bipyridine)-appended cytochrome b_{562}

Itaru Hamachi,*[a] Shigeaki Tanaka,[a] Shinya Tsukiji,[a] Seiji Shinkai,[a] Masafumi Shimizu[b] and Teruyuki Nagamune[b]

[a] *Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Hakozaki Fukuoka 812-81 Japan*

[b] *Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Hongo, Bunkyo-ku 113 Japan*

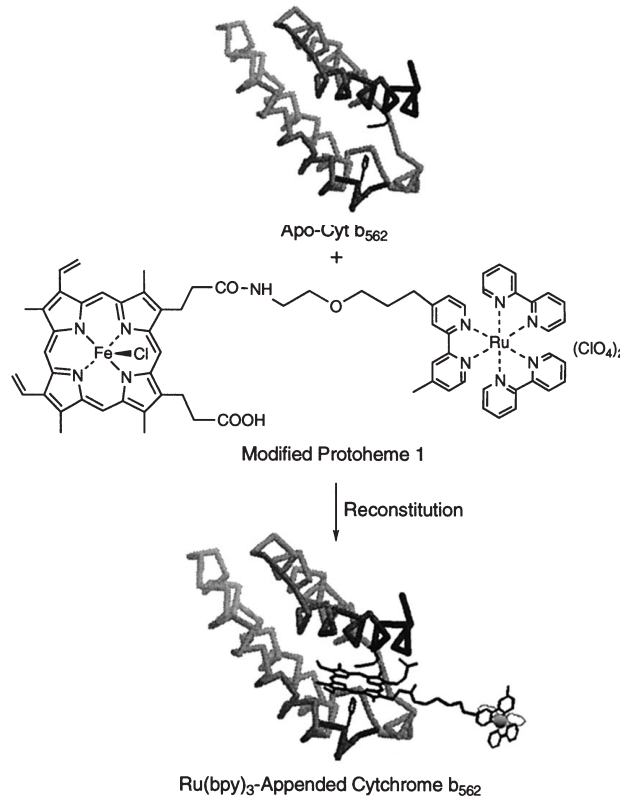
Ruthenium–tris(bipyridine)-appended heme is successfully reconstituted with apo-cytochrome b_{562} (Cyt-b_{562}); the redox state of the active site in the resultant semisynthetic Cyt-b_{562} can be switched by visible light irradiation.

Photocontrol of naturally occurring enzymes/proteins activity is one of the attractive targets in the recent protein engineering.[1] For the redox-active enzymes or proteins, it seems promising to modulate the redox state of the active site of the enzymes by photoelectron injection or abstraction.[2] We recently demonstrated that the dioxygen binding activity of myoglobin was successfully photoregulated by the covalent attachment of a ruthenium polypyridine complex to the heme site.[3] This approach is so convenient that it is desirable to establish the generality of our proposed method. In addition to myoglobin, we therefore applied the reconstitution method to another hemoprotein, cytochrome b_{562}, an electron-transporting hemoprotein, which possesses one protoheme in its active site.[4]

The soluble Cyt-b_{562} was obtained and purified from *Escherichia coli* (strain TB-1 harboring pNS207 plasmid)[5] except that Cyt-b_{562} was released from periplasmic space by treatment with chloroform.[6] The apoprotein was prepared at 4 °C using the butanone extraction method.[7] A synthetic heme **1** bearing ruthenium tris(2,2′-bipyridine) dissolved in dimethyl sulfoxide was added dropwise to the resultant apo-Cyt-b_{562} solution (50% aqueous Me_2SO). After incubation at 4 °C for 1 h, the mixture was purified by gel-column chromatography [Sephadex G-50, 50 mM phosphate buffer (pH 7.0) eluent] to obtain a red solution. The UV–VIS spectrum of this solution showed a sharp absorption at 419 nm due to the Soret band and a weak band at 530 nm due to the Q band of the heme cofactor. Upon reduction with $Na_2S_2O_4$, the absorption maxima shifted to 427, 531 and 561 nm. These absorption maxima are identical to those of the oxidized Fe^{III} and reduced Fe^{II} state of native Cyt-b_{562}.[4] These results indicate that the synthetic heme **1** was successfully incorporated into the heme crevice of apo-Cyt-b_{562} [Ru(bpy)$_3$–Cyt-b_{562}, Scheme 1].

Steady-state emission of Ru(bpy)$_3$ (emission at 610 nm, excitation at 450 nm) in an intermolecular mixture of Ru(bpy)$_3$ and native Cyt-b_{562} was only slightly lowered (0.8-fold intensity), compared to that of the Ru(bpy)$_3$ itself. On the other hand, the emission intensity of Ru(bpy)$_3$ in Ru(bpy)$_3$–Cyt-b_{562} was found to be markedly weaker than that of an intermolecular mixture (0.013-fold). This suggests that the intramolecular electron transfer quenching of the excited Ru^{II} state by the heme in Ru(bpy)$_3$–Cyt-b_{562} occurs more efficiently than in the intermolecular system under the present experimental conditions (protein concentration 0.3–10 μM).

Fig. 1 shows the absorption spectral change of Ru(bpy)$_3$–Cyt-b_{562} during visible light irradiation (using a high-pressure Hg lamp, cut-off at $\lambda < 450$ nm) in the presence of *N*,*N*-dimethylaminobenzoate (NNDB), a sacrificial donor.[8] The absorbances at 419 and 530 nm gradually decrease and those at 430, 538 and 580 nm grow with six isosbestic points at 420, 444, 518, 538, 550 and 569 nm. These spectral changes clearly reveal the photoreduction of the oxidized Cyt-b_{562} to the reduced form. The inset of Fig. 1 compares the photoreduction efficiency of Ru(bpy)$_3$–Cyt-b_{562} to that for the intermolecular reaction system [*i.e.* a simple mixture of Ru(bpy)$_3$, native Cyt-b_{562} (1 : 1 molar ratio) and NNDB]. Apparently, the intermolecular reaction proceeds very slowly. The initial reaction rate of the intramolecular Ru(bpy)$_3$–Cyt-b_{562} (0.05 μM min^{-1}) is 28 times greater than the value of the intermolecular control experiment (0.0018 μM min^{-1}). The quantum yield (Φ) of this photoreduction was determined to be 1.4–8.2 $\times$ 10^{-4} at protein concentrations of 0.7–7.6 μM. The activation efficiency is more than 10 times greater than that of the intermolecular system (Φ = 0.1–0.3 $\times$ 10^{-4}).† This difference is in good agreement with the above-mentioned emission quenching efficiency; photoreduction does not take place in the absence of NNDB. The visible light on–off experiment showed that the visible light is essential for the reduction of the active center of Ru(bpy)$_3$–Cyt-



Scheme 1 Reconstitution of ruthenium–tris(bipyridine)-appended heme to apo-cytochrome b_{562}

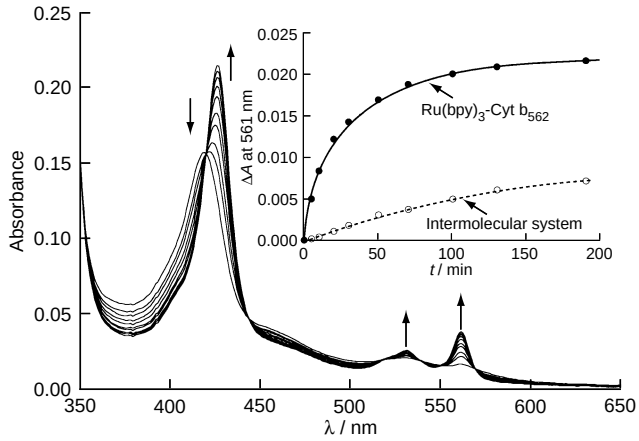


Fig. 1 Absorption spectral changes of $Ru(bpy)_3$–Cyt-b_{562} upon steady-state photoirradiation (wavelength cut-off below 450 nm, high-pressure Hg lamp) under anaerobic conditions: 1.5 μM $Ru(bpy)_3$–Cyt-b_{562} and 10 mM NNDB in phosphate buffer (50 mM, pH 7.0) at 25 °C. The inset shows the time course of the photogeneration of red-Cyt-b_{562} in the intramolecular [$Ru(bpy)_3$–Cyt-b_{562}] reaction system (——) and the intermolecular reaction system [$Ru(bpy)_3$ + native Cyt-b_{562}] (- - -).

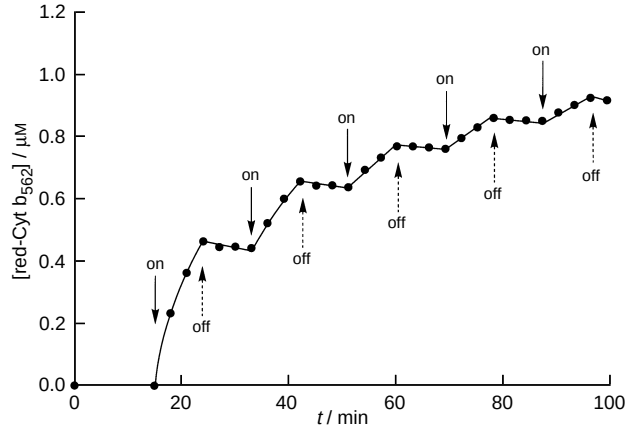


Fig. 2 The light on–off experiment for $Ru(bpy)_3$–Cyt-b_{562}. The solid and dashed arrows indicate switching on and off, respectively: 1.5 μM $Ru(bpy)_3$–Cyt-b_{562}, 10 mM NNDB in phosphate buffer (50 mM, pH 7.0) at 25 °C.

b_{562} in the present system. As shown in Fig. 2, the reduced form of $Ru(bpy)_3$–Cyt-b_{562} is not generated in the absence of visible light. The reduction smoothly started upon light irradiation and completely stopped when the light was turned off. This process can be repeated by turning the light on and off.

Next, we conducted the tandem photoreduction of cytochromec (Cyt-c) using the semisynthetic $Ru(bpy)_3$–Cyt-b_{562}. When a mixed solution of Cyt-c and $Ru(bpy)_3$–Cyt-b_{562} (5 : 1 mol : mol) was irradiated by visible light in the presence of NNDB, reduced Cyt-c was smoothly formed and $Ru(bpy)_3$-Cyt-b_{562} catalytically cycled (turnover frequency = 0.33 h^{-1}) this reaction. We found that the present system was 3.5 times more efficient than the conventional intermolecular reaction [*i.e.* Cyt-c : Cyt-b_{562} : $Ru(bpy)_3$ = 5 : 1 : 1].‡

In conclusion, with the aim of preparation of artificial photoenzymes, we successfully expanded the feasibility of the reconstitution method of the photosensitizer-appended cofactor to a hemoprotein other than myoglobin. It is suggested that the present semisynthetic $Ru(bpy)_3$–Cyt-b_{562} has an interesting potential to regulate the activity of other proteins by photocoupling reactions. The detailed photocatalytic properties of $Ru(bpy)_3$–Cyt-b_{562} are now being actively studied in our laboratory.

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Footnotes and References

* E-mail: itarutcm@mbox.nc.kyushu-u.ac.jp

† Quantum yields were determined using ferrioxalate actinometry under the following experimental conditions: protein concentration 0.7–7.6 μM, 10 mM NNDB, pH 7.0 at 25 °C under an Ar atmosphere.

‡ The reduction efficiency was estimated by subtracting the slightly overlapped autoreduction of Cyt-c from the apparent reduction rate.

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