Efficient Photogeneration of Oxidizing (Compound II-like) Species of Semisynthetic Myoglobin Appending Ruthenium Tris(2,2'-bipyridine)

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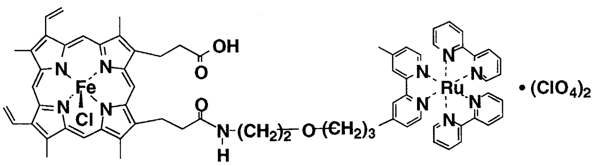
An efficient photogeneration method for an oxidizing active species of semisynthetic myoglobin bearing ruthenium tris(2,2'-bipyridine) (Ru(bpy)$_3$) through intramolecular electron abstraction is described.

Photoregulation of enzymes / proteins functions is considered to be an important concept which affords a novel biocatalysts, so-called photoenzymes, in protein molecules engineering.[1] Incorporation of photochromic molecules into some enzymes successfully induced a photo-reversible conformational change in the polypeptide backbone so as to switch their activities.[2] As the other promising approach, one can raise a concept that photosensitizers have been covalently attached to redox active proteins in order to control the redox states of their active centers by photo-induced electron transfer.[3,4] The photo-redox proteins have been mainly applied as reductive reagents so far, because they can only be yielded by photo-reduction, but not by photo-oxidation. For example, we recently demonstrated the light-driven activation of myoglobin by the photo-initiated electron injection from the excited state of covalently-attached Ru(bpy)$_3$ to the heme active site.[4] In contrast to these approaches, Gray and coworkers proposed that an electron abstraction by the photo-generated Ru (III) complex may produce an oxidizing iron (IV) heme even in the simple model system.[5] With the aim at the development of a new oxidative photoenzyme, we attempted to synthesize a powerful oxidative heme center by visible light in our Ru(bpy)$_3$-pendant myoglobin derivatives.

We used a protohemes (**1**) connecting Ru(bpy)$_3$ with a ether-spacer which was conveniently reconstituted with apo-myoglobin (apo-Mb, horse heart) in a same manner as that previously reported by us.[4,6]



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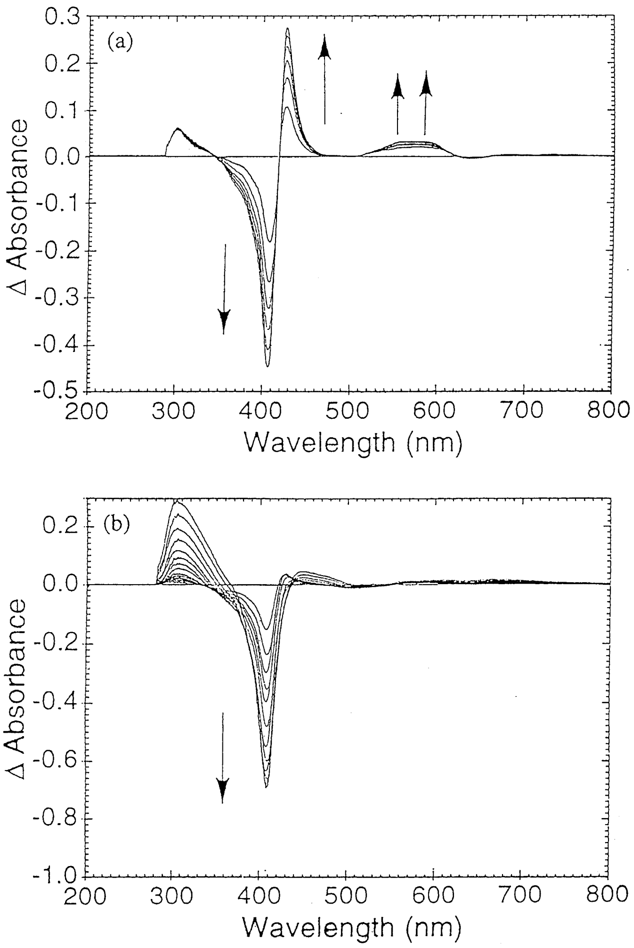


Figure 1. Time courses of UV-visible difference spectral changes by visible light irradiation (wavelength cutoff below 450 nm) under unaerobic conditions at 25 °C : (a) Ru(bpy)$_3$-Mb (**1**), (b) the intermolecular system

The visible-light irradiation (wavelength cut off below 450 nm) to the aqueous solution (50 mM phosphate buffer, pH 7.5) containing Ru(bpy)$_3$-Mb (**1**) (Fe (III) state, 10 μM) in the presence of [Co(NH$_3$)$_5$·Cl]Cl$_2$ (a sacrificial acceptor, 3 mM) under Ar atmosphere,[8] gave a clear absorbance shift from 408 nm to about 422 nm. Figure 1a shows UV-visible difference spectra of Ru(bpy)$_3$-Mb (**1**) during photo-illumination. The absorption maxima at 407 and 630 nm due to met-form of Mb (i. e., iron (III) state of heme) decrease with the simultaneous increase in the absorptions at 426, 560, and 580 nm, which are closely similar to oxoferryl Mb prepared by met-Mb with H$_2$O$_2$ (Fe (IV) = O, so-called compound II).[7] Electron paramagnetic resonance (EPR) signals at 5K due to the ferric high spin (g=6.0, 2.0) almost disappeared after photoirradiation, indicating that the ferric (Fe (III)) Mb changed to a high valent iron species during this reaction. Addition of *o*-methoxyphenol (*o*-MP), a typical substrate in a peroxidation reaction assay, to the formed compound II-like Ru(bpy)$_3$-Mb (**1**), regenerates the met-state of Mb and consequently an oxidized product, tetraguaiacol, was explicitly detected by UV-visible spectra at 475 nm (data not shown). It is clear, therefore, that the photogenerated species certainly has an oxidizing activity to some organic chemicals. No reaction occurred in the dark.

An important control experiment, where the photoirradiation to the simple mixture of Ru(II) $(bpy)_3$ and native Mb in the presence of $[Co(NH_3)_5\cdot Cl]Cl_2$ (i.e., the intermolecular system : [native Mb] = [Ru(II) $(bpy)_3$] = 10 µM, $[Co(NH_3)_5\cdot Cl]Cl_2$ = 3 mM, pH 7.5 with 50 mM phosphate buffer) was conducted, results in no appearance of compound II, instead, a decrease of Soret band mainly took place (See Figure 1b). The circular dichroism (CD) spectrum of the intermolecular system shows that α-helix content considerably diminished by photoreaction, relative to $Ru(bpy)_3$-Mb (**1**). These results clearly indicate that Ru(III) $(bpy)_3$, a strong oxidant generated in a bulk solution, randomly attacks Mb molecule to decompose it. On the contrary, the active-site-specific placement of Ru(III) $(bpy)_3$ in $Ru(bpy)_3$-Mb (**1**) can selectively abstract electron from the iron (III) heme center to promote the formation of the oxidative active center without serious side reactions. No photoreaction takes place without apo-Mb (i.e., the heme **1** only).

The initial rate of the generation of compound II is found to depend on the concentration of the sacrificial acceptor, $[Co(NH_3)_5\cdot Cl]Cl_2$ (pH 7.5). The reaction never proceeds in the absence of $[Co(NH_3)_5\cdot Cl]Cl_2$. The linear dependence of the rate on the Co (III) ammine complex in its lower concentration (0 ~ 5 mM) indicates that the rate-determining step of the net reaction is the oxidation step of the excited Ru (II) complex by Co (III) in the low Co (III) concentration.

The rate also shows a bell-shaped dependence on pH of the reaction solution (Figure 2). At pH 6, the photo-oxidation does not take place at all. When pH is shifted to basic region (pH 7 ~ 8), the photoreaction is commenced and remarkably accelerated. This is consistent with Gray's proposal in which electron can be more easily abstracted from the hydroxide (OH^-)-coordinating iron (III) heme, rather than from the aquo (H_2O)-coordinating one.[8] In more basic pH region, however, the formation of compound II is proven to be suppressed again. This deceleration may be due to the fact that $[Co(NH_3)_5\cdot Cl]Cl_2$ loses its ability as a sacrificial acceptor in too basic solution.

In conclution, our engineered myoglobins with active-site-directed modification can be oxidatively photo-excited to generate compound II through the spatially controlled electron transfer.[9] This also demonstrates that the outer sphere reaction can yield a high valent iron center in hemoproteins, as well as the conventional inner sphere mechanism using H_2O_2. We believe that the present system is suitable for investigating dynamic changes of protein structure from a resting state to an activated one in details.

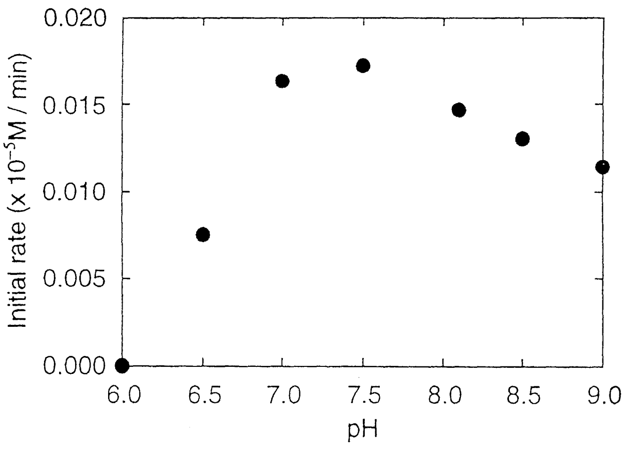
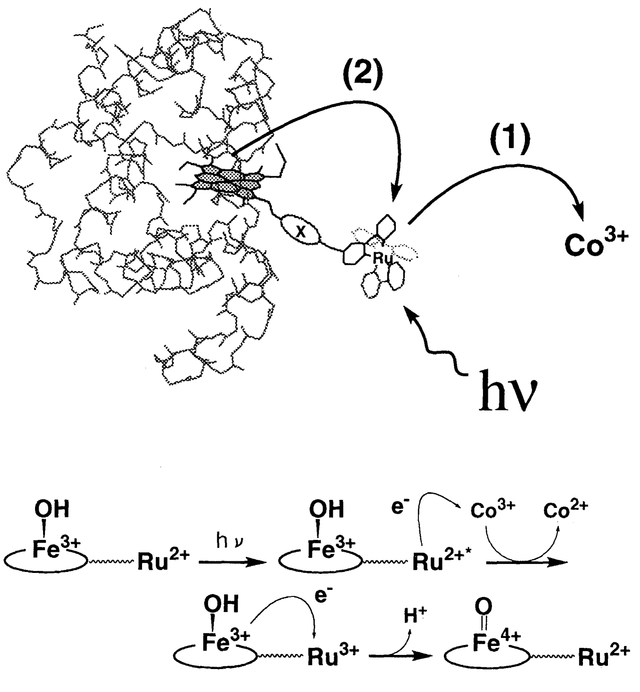


Figure 2. pH dependence of initial rates of compound II generation of $Ru(bpy)_3$-Mb (**1**).



Scheme 1.

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