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Enantioselective and Photochemical Reduction of $Co(acac)_3$ Catalyzed by Protein-hybrid Ruthenium Porphyrin

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A protein-hybrid photocatalyst was prepared by binding Ru^{II}(TCPP)(CO)(py) (TCPP = *meso*-tetrakis(4-carboxyphenyl)-porphyrin; py = pyridine) with Bovine Serum Albumin (BSA) covalently, and it reduced Λ-$Co(acac)_3$ ($acac^-$ = acetylacetonato) enantioselectively under photoirradiation.

Control of electron transfer is realized in biological systems, e.g. the photosynthetic system, by amino acid residues arranged in proteins. To clarify functions of amino acid residues for electron transfer and to construct artificial electron transfer systems are an important target for chemists. There are many and important reports for electron transfer in transition metal complexes,[1] organic molecules, and proteins.[2] However, enantioselective control is generally difficult in photo-induced electron transfer,[3] because a distance between chiral reactive centers in the transition state is relatively long. Especially, although there are a few reports on catalytic and enantioselective photo-chemical reactions,[4] there is no report regarding protein systems. We report here the preparation of protein hybrid ruthenium(II) porphyrin and the catalytic and enantioselective photoreduction of a hydrophobic substrate, $Co(acac)_3$ ($acac^-$ = acetylacetonato), by the protein hybrid catalyst. We chose Bovine Serum Albumin (BSA) as a carrier protein, because it is capable of including several hydrophobic substances.[5]

The BSA hybrid ruthenium porphyrin, Ru(TCPP)(CO)(py)-BSA, was prepared by the covalent amide-bond formation through the reaction between the succinimidyl ester[6] (23.8 mg) of the TCPP portion in the ruthenium(II) complex and BSA (349 mg) in a borate buffer (10 cm^3) at 25 °C for 4 days. Ru(TCPP)(CO)(py)-BSA was purified by Sephadex G-50 column chromatography and an ultra filter dialysis to remove the unbound species; the molar ratio of ruthenium porphyrin and BSA in the BSA hybrid system was spectrophotometrically confirmed to be 1:1 from the molar-ratio calculation with their absorption coefficients at 416.0 and 274.8 nm.

Figure 1 shows the electronic spectra of Ru(TCPP)(CO)(py), hybrid Ru(TCPP)(CO)(py)-BSA, and *in situ* prepared Ru(TCPP)(CO)(py)/BSA[7] (molar ratio = 1:1) in aqueous solutions (pH = 9.3). The Soret (410 nm) and Q bands (530 nm) of Ru(TCPP)(CO)(py) *per se* shifted to a longer wavelength region (416 and 535 nm, respectively) in both the hybrid and *in situ* prepared systems of ruthenium porphyrin and BSA. In order to determine the positions of ruthenium porphyrin in BSA, the average distances (R) between tryptophan-134 (or 212) of BSA and the ruthenium complexes were calculated from the tryptophan-134 (or 212) emission intensity.[8] The distances were 2.62 and 2.99 nm for the hybrid and *in situ* prepared system, respectively. This implies that the ruthenium porphyrins were located at the almost same positions of BSA in both systems. It is also noteworthy that the hydrophobic circumstances of BSA changed the lifetimes of their excited states drastically through the depression of the vibration relaxation of the photo-activated Ru(TCPP)(CO)(py); the relative lifetimes obtained from the emission intensities of Ru(TCPP)(CO)(py)/BSA and Ru(TCPP)(CO)(py)-BSA were 1.73 and 2.23 times longer than that of Ru(TCPP)(CO)(py), respectively.

The photoreduction of $Co(acac)_3$ catalyzed by the ruthenium porphyrins, the *in situ* prepared and BSA hybrid systems was then performed as follows; after thorough degassing of the 10% (v/v) 2-PrOH-borate buffer (pH = 9.3) containing 2.0 x 10^{-5} mol dm^{-3} of Ru(TCPP)(CO)(py)-BSA (or

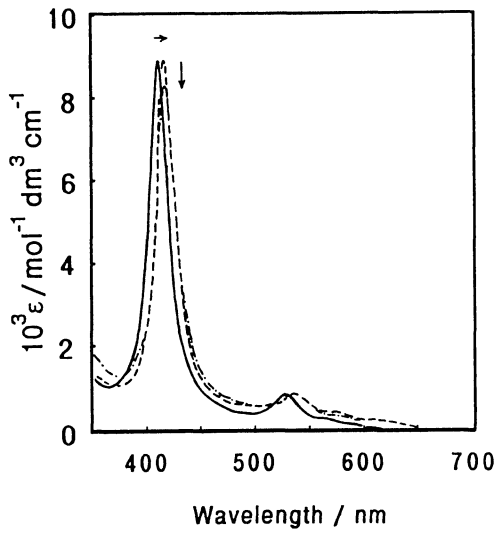

Fig. 1. The UV-vis spectra change of Ru(TCPP)(CO)(py) (1.0 x 10^{-5} mol dm^{-3}) in the absence of BSA (——), in the presence of BSA (---), and bound to BSA (-•-•-) at 25 °C.

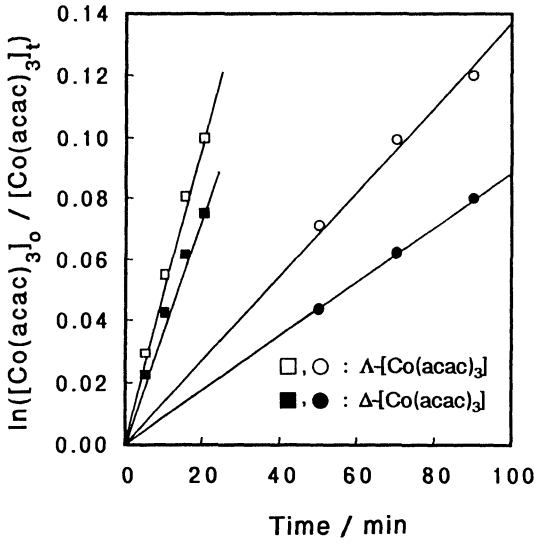

Fig. 2. Enantioselective $Co(acac)_3$ (1.2 x 10^{-3}mol dm^{-3}) reductions catalyzed by Ru(TCPP)(CO)(py) (2.0 x 10^{-5} mol dm^{-3}) in BSA (□,■) and bound to BSA (○,●) under photo-irradiation (λ > 400 nm) at 25 °C.

Table 1. Enantioselective $Co(acac)_3$ photo-reduction catalyzed by BSA hybrid ruthenium complexes[a)]

Catalysts	R[b)]/nm	k_{obsd}[c)]/$10^{-5}s^{-1}$		k^{Λ}/k^{Δ}	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$
		Λ	Δ		kJ mol^{-1}	J K^{-1} mol^{-1}
Ru(TCPP)(CO)(Py)/BSA	2.99	7.85	5.88	1.33	55.1	-145.1
Ru(TCPP)(CO)(Py)-BSA	2.62	2.37	1.50	1.58	91.5	-25.8

a) In 10% (v/v)-2-PrOH-$Na_2B_4O_7$ solutions under anaerobic conditions. b) Average distance between tryptophan-134 (or 212) and Ru(TCPP)(CO)(py) in BSA. c) At 25 °C.

Ru(TCPP)(CO)(py)/BSA) and 1.2 x 10^{-3} mol dm^{-3} of Λ(or Δ)-$Co(acac)_3$ under vacuum by five successive freeze-pump-thaw cycles, the reactants were transferred to a quartz cuvette, which was then irradiated by visible light of a 500-W xenon lamp with a Toshiba L-42 glass filter transmitting light of λ>400 nm. The photochemical reaction was monitored from the decay of the absorption band of $Co(acac)_3$ at λ_{max} = 595 nm. The results of photoreaction are depicted in Fig.2. The reactions obeyed pseudo-first-order kinetics and proceeded catalytically; turnover numbers (converted mole of Co(III) / mole of catalyst) were achieved to be 7 (or 6) in the $Co(acac)_3$ reduction with Ru(TCPP)(CO)(py)-BSA (or Ru(TCPP)(CO)(py)/BSA) for 90 (or 20) min, and *in situ* prepared Ru(TCPP)(CO)(py)/BSA, which has the flexible mobility of the Ru complex for the reaction with $Co(acac)_3$ in BSA, showed higher catalytic activity. Moreover, the reaction in the chiral field of BSA proceeded enantioselectively with the predominant reduction of Λ-$Co(acac)_3$ rather Δ-$Co(acac)_3$. The enantiomer rate ratios (k^{Λ}/k^{Δ}) of k^{Λ} to k^{Δ}, which were obtained individually, were 1.33 and 1.58 in the Ru(TCPP)(CO)(py)/BSA and Ru(TCPP)(CO)(py)-BSA systems respectively (Table 1). The CD spectrum of Ru(TCPP)(CO)(py)-BSA was shown at 208 nm (peak) and 220 nm (shoulder), however no conformational changes were observed in the photoreaction. There were also no differences in the CD spectra between the hybrid system and *in situ* prepared system. Therefore, the slightly higher enantioselectivity of Ru(TCPP)(CO)(py)-BSA compared with that of Ru(TCPP)(CO)(py)/BSA, probably resulted from the restricted motion of the Ru(TCPP)(CO)(py) moiety bound to BSA in the reaction with $Co(acac)_3$.

Activation parameters were then elucidated for the present photoreduction of racemic $Co(acac)_3$ in the temperature range of 20 - 40 °C (Table 1); in the Ru(TCPP)(CO)(py)-BSA (or Ru(TCPP)(CO)(py)/BSA) system, the activation enthalpy $\Delta H^{\ddagger}$ was 91.5 (or 55.1) kJ mol^{-1} and the activation entropy $\Delta S^{\ddagger}$ was -25.8 (or -145.1) J K^{-1} mol^{-1}. The larger $\Delta H^{\ddagger}$ (or $\Delta S^{\ddagger}$) value of the BSA hybrid system than that of the *in situ*

prepared one supported the above-mentioned restricted motion and orientation of the ruthenium porphyrin in the hybrid system, especially at the transition state of the electron-transfer reaction between Ru(TCPP)(CO)(py)-BSA and $Co(acac)_3$.

In conclusion, the newly synthesized protein-hybrid ruthenium-complex catalyst, Ru(TCPP)(CO)(py)-BSA, reduced a hydrophobic substrate, $Co(acac)_3$, catalytically and enantioselectively through the efficient photo-induced electron transfer from the former to the latter in the hydrophobic and chiral field of BSA.

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6) Ru(TCPP)(CO)(py) (998 mg) was esterificated with N-hydroxysuccinimide (230 mg) by dicyclohexylcarbodiimide (413 mg) in pyridine at 0 °C for 1 hour. After stirring at 0 °C for another 3 hours, the solution was stored in a refrigerator over night. Precipitated dicyclohexylurea was removed by filtration, and the solvent was evaporated to yield crystals. Anal. Found: C, 57.78; H, 5.10; N, 7.28%. Calcd for $RuC_{62}H_{51}N_7O_{19}$; C, 57.32; H, 3.96; N, 7.55%.

7) Ruthenium porphyrin dissolved in 2-PrOH (1.0×10^{-4} mol dm^{-3}, 2.0 cm^3) was added to the borate buffer (pH = 9.3, 20 cm^3) containing BSA (13.6 mg).

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