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$Ru(bpy)_3$-based artificial receptors toward a protein surface: selective binding and efficient photoreduction of cytochrome c

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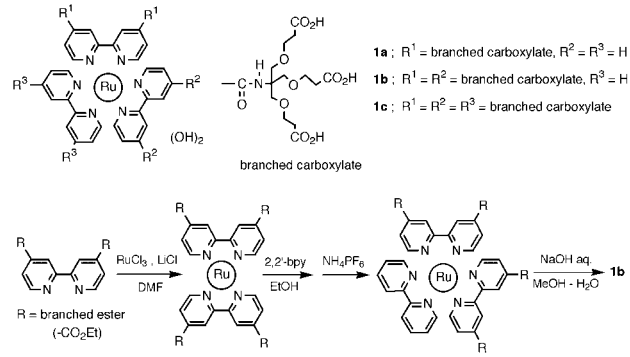
The binding properties of a series of $Ru(bpy)_3$ complexes to cytochrome c are described; these compounds act as selective cytochrome c receptors and one derivatie is shown to be a photodriven modulator of the cytochrome c redox state.

Site-specific recognition on a protein surface, including a definite α-helix or β-sheet structure, with an artificial molecule is one of the major goals of recent bioorganic chemistry. A few ligands and receptors have been synthesized to bind proteins by using non-covalent and multi-point forces *via* hydrogen bonding, electrostatic or hydrophobic interactions.[1] However, design of such receptors is generally difficult because protein surfaces are relatively larger than conventional target molecules in host–guest chemistry. We recently demonstrated that lipid bilayer surfaces are promising for domain selective binding of a protein *via* electrostatic interaction.[2] However, more sophisticated interactions are not expected in such a molecular assembly since the spatial juxtaposition of interaction points is not elaborately carried out. Instead we made an attempt to utilize the coordination sphere of ruthenium tris-bipyridine complexes [$Ru(bpy)_3$], asymmetric functionalization of which is effected by simple coordination chemistry.[3] In this communication, we describe the binding properties of a series of $Ru(bpy)_3$ complexes to cytochrome c (Cyt-c) and the efficient electron injection which occurs through photoinduced electron transfer (ET) in their non-covalently linked systems.

The poly-anionic $Ru(bpy)_3$ complexes **1a**–**c** (Scheme 1) were constructed *via* the corresponding Ru bis-bpy complexes as intermediates. The bpy ligand bearing dendritic carboxylate moieties was synthesized according to the modified method in the literature.[4] All compounds were satisfactorily identified by NMR, IR, MALDI-Tof-mass spectroscopy and elemental analysis.‡ The photophysical properties of **1a**–**c** were investigated by absorption and emission spectroscopies, and emission lifetime experiments in a phosphate buffer (pH 7.0). The peak shapes and intensities (ε value at absorption maximum) in the UV-visible spectra of **1a**–**c** are similar to those in the spectrum of the original $Ru(bpy)_3$ complex, except for the 10 nm red-shifted λ_{max} of the MLCT band. This is due to the electron-withdrawing groups at the 4,4′-positions of the bpy ligand. The emission spectra are almost identical to that of the original $Ru(bpy)_3$ complex (λ_{em} 610 nm) upon excitation at the corresponding MLCT band (λ_{em} 615 nm for **1a**, 630 nm for **1b, 1c**). The emission lifetimes of $Ru(bpy)_3$, **1a** and **1b** were determined to be in the range 600–800 ns under anaerobic conditions (ex. 480 nm, em. 610 nm), whereas the lifetime of **1c** is elongated (1500 ns) by the cage effect due to the introduced branching on the three bpy ligands.[5]

The affinity of **1a**–**c** for various proteins was estimated by ultrafiltration binding assays. After incubation of the mixture containing a $Ru(bpy)_3$ derivative (3 μM) and a protein (9 μM) in buffer solution at pH 7.0 (10 mM phosphate) for 1 h, the solution was subjected to ultrafiltration [centricon-10 (Amicon), centrifuge at 7000 rpm, 25 °C]. The protein-bound



Scheme 1 Structures of $Ru(bpy)_3$-based poly-anionic complexes, **1a**–**c**.

$Ru(bpy)_3$ was not filtered and the fraction of the non-bound $Ru(bpy)_3$ component was determined by monitoring the filtrate spectrophotometrically. The results are summarized in Fig. 1. Obviously, the fraction of protein-bound $Ru(bpy)_3$ increases with an increase in the number of introduced carboxylate groups. Poly-anionic **1c**, bearing 18 carboxylates can bind to the Cyt-c surface 10 times more tightly than the original $Ru(bpy)_3$ complex. The surface of Cyt-c is positively charged at neutral pH, since the isoelectric point (pI) is 10.0.[6a] It is reasonable to assume that the poly-carboxylate clusters of the $Ru(bpy)_3$ complexes can bind to a cationic domain of the Cyt-c surface. Myoglobin (Mb, pI = 7.0), horseradish peroxidase (HRP, pI = 8.0), and cytochrome b_{562} (Cyt-b_{562}, pI = 5.0) were also used as target proteins under the same conditions.[6] The $Ru(bpy)_3$ derivatives pass through the ultrafiltration membrane in these cases, indicating that all Ru complexes examined here exhibit much lower affinities for these proteins, than for Cyt-c. It is clear that the poly-anionic $Ru(bpy)_3$-based complexes **1a**–**c** selectively bind to the Cyt-c surface.

Based on the redox potentials of Cyt-c [0.26 V (Fe^{3+}/Fe^{2+}) *vs.* NHE] and $Ru(bpy)_3$ [−0.86 V ($Ru^{3+}/{}^*Ru^{2+}$) *vs.* NHE], ET from the excited state of $*Ru^{2+}(bpy)_3$ to oxidized Cyt-c is energetically favorable.[3b,7,8] Thus, we studied the photoreduction of Cyt-c by visible light. Upon steady-state photoirradiation [high

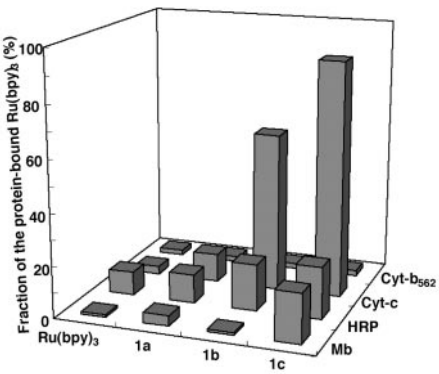


Fig. 1 Summary of the fraction (%) of protein-bound $Ru(bpy)_3$ complexes in solutions of each complex with a protein.

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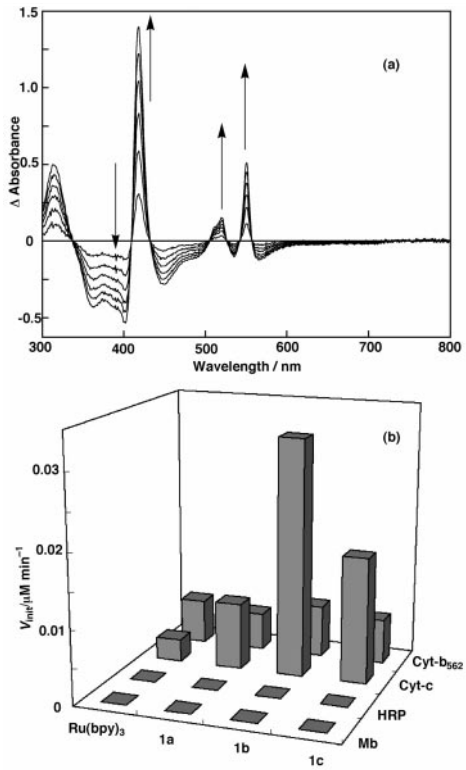

Fig. 2 (a) Time-course changes of the difference absorption spectra upon steady-state photoirradiation. (b) Summary of the initial rates (V_{init}) of photoreduction of various hemoproteins catalyzed by Ru(bpy)$_3$ derivatives.

pressure Hg-lamp (λ > 450 nm)] of a degassed solution consisting of Cyt-c (9 μM), a Ru complex (3 μM) and EDTA (10 mM),[3a,9] absorption bands at 520 and 550 nm attributable to the Q-bands of reduced Cyt-c(II) appeared [Fig. 2(a)]. The initial rates (V_{init}) are summarized in Fig. 2(b). Compared with the original Ru(bpy)$_3$ complex, it is apparent that **1a**–**c** accelerate the photoreduction of Cyt-c. Interestingly, the most effective ET relay was achieved by **1b**, having 12-carboxylates, not by **1c**, having 18-carboxylates. This suggests that not only the number of carboxylate groups, but also the asymmetric spatial orientation around the Ru center are crucial in the net photoreduction efficiency. On the other hand, no acceleration was observed for Mb, HRP and Cyt-b$_{562}$ as substrates of photoreduction, showing good agreement with the results of the binding experiments.

In the present photoreduction system, the Ru complexes should inject electrons into positively charged Cyt-c and simultaneously abstract electrons from negatively charged EDTA. In order to clarify the effect of asymmetric geometry, the ET quenching rates (k_{ET}) of the excited Ru complex by a cationic or anionic quencher were separately investigated by emission lifetime measurement. Since the sacrificial donor EDTA which is used in the photoreduction study has three negative charges at pH 7.0, the $Fe(CN)_6^{3-}$ anion was chosen as the negatively charged quencher.[10] The rate constant, k_{ET}, was calculated by the Stern–Volmer equation and decreases in the order Ru(bpy)$_3$ (k_{ET} = 1.5×10^{10} M^{-1} s^{-1}) > **1a** (4.2×10^9 M^{-1} s^{-1}) > **1b** (2.4×10^8 M^{-1} s^{-1}) > **1c** (8.9×10^7 M^{-1} s^{-1}), showing that ET becomes less efficient with the increasing number of carboxylate residues on the Ru(bpy)$_3$ derivatives.[11] On the other hand, when Cyt-c was used as a cationic quencher, the tight binding of **1c** and **1b** with Cyt-c prevented us from analyzing ET rates as a dynamic quenching mechanism.[12] Instead, pseudo intramolecular ET rates were determined: k_{ET} = 1.9×10^6 s^{-1} for **1c** and 1.8×10^6 s^{-1} for **1b**. In the case of **1a** and Ru(bpy)$_3$, ET rates were estimated from a typical dynamic quenching as follows: 8.3×10^9 M^{-1} s^{-1} for **1a** and 4.1×10^9 M^{-1} s^{-1} for Ru(bpy)$_3$. Thus, at a low concentration of Cyt-c (9 μM), the apparent rate constants are in the order **1c** = **1b** > **1a** > Ru(bpy)$_3$, which is almost opposite to the case with $Fe(CN)_6^{3-}$. These results suggest that the net photoreduction efficiency is controlled by a balance between the accessibility of the Ru complexes to Cyt-c and to sacrificial EDTA.

In conclusion, we have demonstrated that a Ru(bpy)$_3$ derivative **1b** which has an asymmetric charge distribution acts not only as a Cyt-c receptor, but also a photo-driven modulator of the redox state of Cyt-c. The present findings are anticipated to provide access to a combinatorial library of coordination chemistry to generate new Ru(bpy)$_3$-based function modulators which can selectively control protein activity.

Notes and references

‡ *Selected mass spectroscopic and elemental analysis data*: **1a**: MS(MALDI-Tof, CHCA) 1296.36 [M − $(OH)_2$]$^+$; Anal. Calc. for $C_{58}H_{66}N_8O_{22}RuNa_2$: C, 50.59; H, 4.79; N, 8.14. Found C, 50.48; H, 4.45; N, 8.11%. **1b**: MS(MALDI-Tof, CHCA) 2023.89 [M − $(OH)_2$]$^+$; Anal. Calc. for $C_{86}H_{105}N_{10}O_{42}RuNa_5$: C, 47.67; H, 4.67; N, 6.72. Found C, 47.60; H, 4.67; N, 6.72%. **1c**: MS(MALDI-Tof, CHCA) 2750.36 [M − $(OH)_2$]$^+$; Anal. Calc. for $C_{114}H_{152}N_{12}O_{62}RuNa_{17}$: C, 43.13; H, 4.82; N, 5.29. Found C, 43.33; H, 4.46; N, 5.56%.

1 For example, (*a*) H. S. Park, Q. Lin and A. D. Hamilton, *J. Am. Chem. Soc*., 1999, **121**, 8; (*b*) Y. Hamuro, M. C. Calama, H. S. Park and A. D. Hamilton, *Angew. Chem., Int. Ed. Engl*., 1997, **36**, 2680 and references cited therein.

2 I. Hamachi, A. Fujita and T. Kunitake, *J. Am. Chem. Soc.*, 1997, **119**, 9096.

3 (*a*) I. Hamachi, S. Tanaka and S. Shinkai, *J. Am. Chem. Soc.*, 1993, **115**, 10458; (*b*) S. Sakai and T. Sasaki, *J. Am. Chem. Soc*., 1994, **116**, 1587; (*c*) I. Hamachi, S. Tsukiji, S. Shinkai and S. Oishi, *J. Am. Chem. Soc*., 1999, **121**, 5500; (*d*) Y.-Z. Hu, S. Tsukiji, S. Shinkai and I. Hamachi, *Chem. Lett.*, 1999, 517.

4 (*a*) G. R. Newkome, X. Lin and J. K. Young, *Synlett*, 1992, 53; (*b*) J. Issberner, F. Vögtle, L. D. Cola and V. Balzani, *Chem. Eur. J*., 1997, **3**, 706; (*c*) G. R. Newkome, R. Güther, C. N. Moorefield, F. Cardullo, L. Echegoyen, E. Perez-Cordero and H. Luftmann, *Angew. Chem., Int. Ed. Engl*., 1995, **34**, 2023; (*d*) P. J. Dandliker, F. Diederich, M. Gross, C. B. Knober, A. Louati and E. M. Sanford, *Angew. Chem., Int. Ed. Engl*., 1994, **33**, 1739.

5 This value is almost comparable to that of similar compounds, such as dendritic Ru(bpy)$_3$ under anaerobic conditions (1940 ns); see ref. 4(*b*).

6 (*a*) P.-A. Albertsson, S. Sasakawa and H. Walter, *Nature*, 1970, **228**, 1329; (*b*) P. D. Barker, J. L. Butler, P. de Oliveria, H. Allen O. Hill and N. I. Hunt, *Inorg. Chim. Acta*, 1996, **252**, 71.

7 (*a*) P. L. Drake, R. T. Hartson, J. McGinnis and A. G. Sykes, *Inorg. Chem*., 1989, **28**, 1361; (*b*) G. J. Kavarnos and N. J. Turro, *Chem. Rev*., 1986, **86**, 401; (*c*) I. M. Kolthoff and W. J. Tomsicek, *J. Phys. Chem*., 1935, **39**, 945.

8 Although the redox potentials of **1a**–**c** have not been determined thus far, it was reported that the presence of amide groups at the 4 and 4′ positions of bpy positively shifts the redox potential of both $*Ru^{2+}/Ru^{3+}$ and Ru^{2+}/Ru^{3+}. Still, this ET reaction seems favorable, since the potential shift was reported to be within only 0.3–0.4 V; see V. Skarda, M. J. Cook, A. P. Lewis, G. S. G. McAuliffe, A. J. Thomson and D. J. Robbins, *J. Chem. Soc., Perkin Trans. 2*, 1984, 1309. Details are now under way in our laboratory.

9 No obvious effect on the photoreduction was seen in the control experiments; (i) in the absence of Ru(bpy)$_3$ derivatives and (ii) under dark conditions.

10 D. J. Pietrzyk and C. W. Frank, *Analytical Chemistry*, Academic Press, London, 1974, p. 435.

11 The k_{ET} between a Ru(bpy)$_3$, Ru(bpy)$_3$-based dendrimer and anthraquinone-2,6-disulfonate anion (AQ^{2-}) were recently obtained by similar titration experiments of lifetimes, *i.e.* k_{ET} = 1.2×10^{10} M^{-1} s^{-1} for Ru(bpy)$_3$ and k_{ET} = 2.7×10^9 M^{-1} s^{-1} for the Ru(bpy)$_3$-based dendrimer; see F. Vögtle, M. Plevoets, M. Nieger, G. C. Azzellini, A. Credi, L. D. Cola, V. D. Marchis, M. Venturi and V. Balzani, *J. Am. Chem. Soc*., 1999, **121**, 6290.

12 The emission lifetimes of the Ru complexes gradually become shortened by addition of Cyt-c, but the changes for **1b** and **1c** are saturated at low concentrations of Cyt-c.

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