Calatytic Mechanism and Activity of Bis(2,2′ : 6′,2″-terpyridine) rhodium(III) for the Reduction of NAD$^+$ into NADH in a Photosensitized Reaction System

Keisuke UMEDA, Asao NAKAMURA, and Fujio TODA*
Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology,
4259 Nagatsuta, Midori-ku, Yokohama 227

(Received October 8, 1991)

Rh(terpy)$_2^{3+}$ was used as a reduction catalyst for NAD$^+$ into NADH in a photosensitized reaction system containing Ru(bpy)$_3^{2+}$ (as a photosensitizer) and TEOA (triethanolamine) (as an electron donor). At pH 7.7, an effective NADH-formation (1.04 mM (1 M=1 mol dm^{-3})) was observed after 90 min of light-irradiation, whereas a low yield of NADH at pH 6.5 or pH 10.0 (0.1 and 0.21 mM respectively) was obtained after 90 min of irradiation. Although the electron donor activity of TEOA is likely to be dependent on the pH conditions, the photosensitized reaction in the TEOA–Ru(bpy)$_3^{2+}$–Rh(terpy)$_2^{3+}$ system showed little pH influence in that growth in the absorbance at 440 nm, since the reduction of Rh(terpy)$_2^{3+}$ at pH 7.7 is ca. 1.5-fold as large as at pH 6.3. The main reason for the NADH-formation dependence on the pH was found to be caused by the dependence of the catalytic activity of the reduced form of Rh(terpy)$_2^{3+}$ on the pH conditions. In addition, the IR spectrum of Rh(terpy)$_2^{3+}$ reduced at pH 8.0 shows a peak due to rhodium-hydride stretching (2100 cm^{-1}), whereas that Rh(terpy)$_2^{3+}$ reduced at pH 10.0 and/or pH 6.5 shows no peak in that region of wavenumbers. These results indicate that the reduction of NAD$^+$ into NADH in the photosensitized reaction system is catalyzed by Rh(terpy)$_2^{3+}$ according to hydride-transfer route.

Photosynthesis performs a conversion from solar energy, which is clean and limitless, to chemical free energy that is accumulated as high-energy compounds. During this decade, the mechanism of the photosensitized reaction has been given much attention, and many systems for the model reaction of photosynthesis have been reported, such as photosensitized electron transfer,[1] the photoreduction of water to generate hydrogen,[2—4] the photoregeneration of NAD(P)H followed by the formation of alcohol and/or amino acid with combination of appropriate enzyme,[5] and so on.[6] NAD(P)H, which is reduced form of NAD(P)$^+$, plays an important role *in vivo* as a coenzyme, and has also been studied in systems containing a substrate and an enzyme to produce asymmetrical compounds *in vitro*.[7] The reduction of NAD$^+$ into NADH generally requires an enzyme, such as a dehydrogenase; enzymes are often highly selective catalyst to give 1,4-NADH, which shows enzymatic activity, but undergo degradation when the system is run for an extended period of time. Enzyme-free systems are thus desirable alternatively. Unfortunately, the reduction of NAD$^+$ by a high-potential photosensitizer,[8] or by electrochemical means,[9,10] produces a mixture of dimers (NAD$_2$) and NADH isomers (1,2-NADH and 1,6-NADH), which are not enzymatically inactive.

Wienkamp and Steckhan recently proposed that tris(2,2′-bipyridine) rhodium(III), Rh(bpy)$_3^{3+}$ is an effective catalyst for the photochemical[11] or electrochemical[12] reduction of NAD$^+$; some articles[13] have proposed rhodium complexes to be candidates for a catalyst for the reduction of NAD$^+$. We have also carried out studies in this field, and have reported[14] on the catalytic activity of bis (2,2′ : 6′,2″-terpyridine)rhodium(III), Rh(terpy)$_2^{3+}$, as well as Rh(bpy)$_3^{3+}$, both of which show regioselectivity for the reduction at the 4-position of NAD$^+$. In addition, it was proved that Rh(terpy)$_2^{3+}$ retains its catalytic activity for a long term in a photochemical reaction, due to the stability of the bis(tridentate)ligand.

There is now a question concerning the route for the reduction of NAD$^+$: Whether two electrons and a proton are transferred (Eq. 1), or a hydride ion is transferred (Eq. 2),

$$\mathrm{NAD^+ + 2e^- + H^+ \rightarrow NADH} \qquad (1)$$

or

$$\mathrm{NAD^+ + H^- \rightarrow NADH.} \qquad (2)$$

Although NAD$^+$ *in vivo* accepts an H$^-$ to produce NADH, an H$^-$ transfer is not always the route *in vitro*, as can be seen when dithionite is the reductant.[15]

The elucidation of the NAD$^+$-reduction mechanism in a photochemical system with a catalyst is of interest, and would lead to the development of a high-power catalyst for NAD$^+$-reduction.

In this paper, we discuss the photochemical reduction of NAD$^+$ with Rh(terpy)$_2^{3+}$ as the catalyst, and elucidate its reducing mechanism.

Results and Discussion

Dependence of NAD$^+$-Reduction on the pH. As mentioned above, the reduction of NAD$^+$ into NADH requires either two electrons and one proton or hydride ion. It is simply considered that the pH in the reaction system affects the NADH-formation in both reduction routes. As a starting point, examinations of the photoreduction of NAD$^+$ were performed under several pH conditions in a TEOA (250 mM (1 M=

1 mol dm^{-3}))–Ru(bpy)$_3^{2+}$ (50 μM)–Rh(terpy)$_2^{3+}$ (250 μM)–NAD$^+$ (4 mM) system; NADH-formation was investigated under different pH conditions. Figure 1 shows the growth in the NADH concentration when the reaction mixture was irradiated with visible light. It is clear from Fig. 1 that the rate of NADH formation shows great efficiency at pH 7.7, compared to that of at pH 6.5 or pH 10.0; 1.04 mM of NADH was produced at pH 7.7 after 90 min irradiation, whereas only 0.1 mM or 0.21 mM was produced at pH 6.5 or pH 10.0, respectively. There are some possible explanations about the result given in Fig. 1. One concerns the stability of NAD$^+$ or NADH, in which NAD$^+$ is degradable under alkaline conditions, and the NADH under acid conditions undergoes hydration.[16] In order to eliminate such additional phenomena within the time range of the irradiation and preparation of the reaction mixture, NAD$^+$ in a solution buffered at pH 10.0 and NADH at 6.5 were incubated at 25 °C; those remaining in the solution were analyzed by HPLC. It is clear from Fig. 2 that very slow degradation was observed for NAD$^+$ in a pH 10.0 solution and for NADH in a pH 6.5 solution, and that a half period of 11.6 h for NAD$^+$ and 9.5 h for NADH was calculated by fitting according to first-order kinetics. These results, however, demonstrate that the stability of NAD$^+$ and NADH in the pH range from 6.5 to 10.0 is little able to damage the photochemical reduction systems.

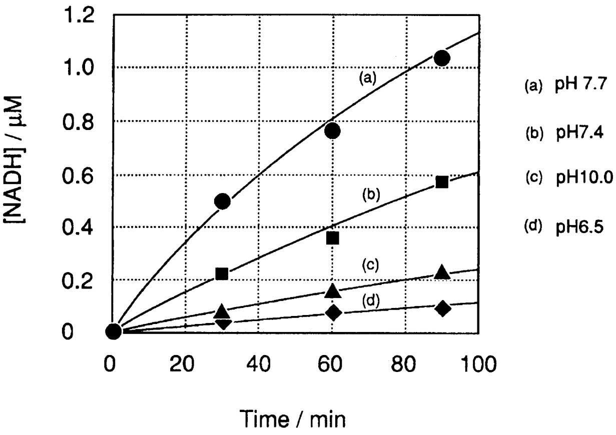


Fig. 1. Variation of [NADH] during irradiation at 25 °C to the reaction mixture at (a) pH 7.7, (b) pH 7.4, (c) pH 10.0, and (d) pH 6.5. The reaction mixture was composed of 250 mM TEOA, 50 μM Ru(bpy)$_2^{3+}$, 250 μM Rh(terpy)$_2^{3+}$, and 4 mM NAD$^+$ with 2 M NaCl. Only in a pH 10.0 solution was EDTA used instead of TEOA, due to insoubility of TEOA in this condition.

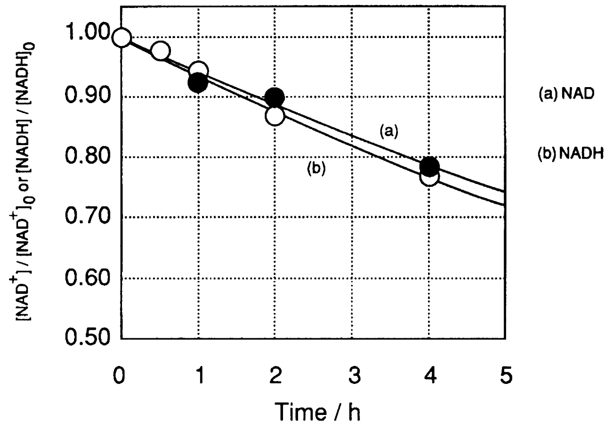


Fig. 2. Ratio of the concentration of retaining NAD$^+$ at pH 10.0 (a) or NADH at pH 6.5 (b) to that of the early one, respectively. Curve fitting was performed according to first-order kinetics.

As has been previously reported, it is known that NAD(P)H acts as an electron donor in photochemical systems to regenerate a photo-oxidized sensitizer,[17] or to quench a reductively excited sensitizer.[18] The behavior of NADH as an electron donor in our system, which seems to diminish the accumulation of NADH, should be ruled out, since the concentration of TEOA is much higher than NADH, especially during the early stage of the reaction. The TEOA-reaction therefore dominates over the NADH reaction in competition. Moreover, the oxidative degradation of NADH presumably proceeds according to Eq. 3, in which deprotonation at the second step occurs preferably in

$$\text{NADH} \xrightarrow{e^-} \text{NADH}^{+\bullet} \xrightarrow{H^+} \text{NAD}^{\bullet} \xrightarrow{e^-} \text{NAD}^+ \tag{3}$$

alkaline to acidic conditions. In addition, when irradiation was carried out on the NADH (1 mM)–Ru(bpy)$_3^{2+}$ (50 μM)–Rh(terpy)$_2^{3+}$ system, the comsumption of NADH proceeded slowly, indicating that the action of NADH as an electron donor has no relation to the result given in Fig. 1. For these reasons, the difference in NADH yielding by a photoreduction between pH conditions originates due to other factors, one of which is that a photosensitized reaction in the TEOA–Ru(bpy)$_3^{2+}$–Rh(terpy)$_2^{3+}$ system would be influenced by the pH conditions; another is that the catalytic activity of the reduced form of Rh(terpy)$_2^{3+}$ would be dependent on the pH conditions. These two questions are examined below.

Influence of the pH in the Photochemical Reaction in the TEOA–Ru(bpy)$_3^{2+}$–Rh(terpy)$_2^{3+}$ System. The quenching reaction of the excited state of Ru(bpy)$_3^{2+}$ under several pH conditions was evaluated according to Stern–Volmer kinetics (Fig. 3); the quenching constants are summarized in Table 1. Rh(terpy)$_2^{3+}$ is evidently an effective quencher; on the contrary, TEOA quenched with a small rate constant. These facts support the idea that the oxidative quench of Ru(bpy)$_3^{2+*}$ by Rh(terpy)$_2^{3+}$ is the first stage in the photosensitized reaction in the TEOA–Ru(bpy)$_3^{2+}$–Rh(terpy)$_2^{3+}$ system, followed by the reduction of Ru(bpy)$_3^{3+}$ by TEOA into the regeneration of Ru(bpy)$_3^{2+}$:

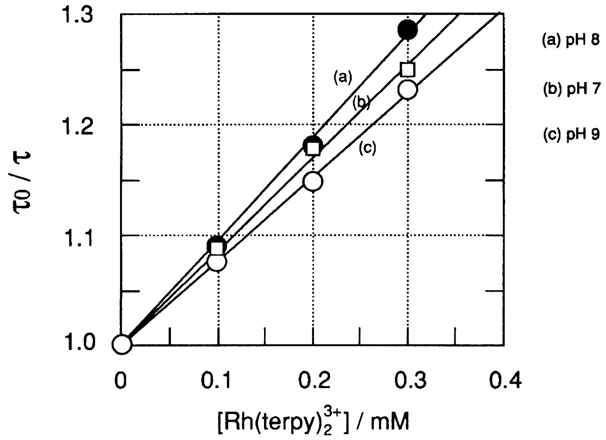


Fig. 3. Stern–Volmer plot for quenching excited Ru(bpy)$_2^{3+}$ by Ru(terpy)$_2^{3+}$ at (a) pH 8, (b) pH 7, and (c) pH 9 at 25 °C. All solutions were with 2 M NaCl and buffered by 0.1 M tris–HCl.

Table 1.

pH	NaCl/M	Quencher	$k_q/10^9$ M^{-1} s^{-1}
9	2	Rh(terpy)$_2^{3+}$	1.7
9	0	Rh(terpy)$_2^{3+}$	0.34
9	2	TEOA	0.0015
8	2	Rh(terpy)$_2^{3+}$	2.1
8	0	Rh(terpy)$_2^{3+}$	0.61
7	2	Rh(terpy)$_2^{3+}$	1.9
7	0	Rh(terpy)$_2^{3+}$	0.68
7	2	TEOA	0.00037

$$\mathrm{Ru(bpy)_3^{2+*} + Rh(terpy)_2^{3} \rightarrow Ru(bpy)_3^{3+} + Rh(terpy)_2^{2+}} \quad (4)$$

and

$$\mathrm{Ru(bpy)_3^{3+} + TEOA \rightarrow Ru(bpy)_3^{2+} + TEOA_{OX}}. \quad (5)$$

Regarding the quenching rate constants (k_q) in case of using Rh(terpy)$_3^{3+}$ as a quencher, the k_q under 2 M NaCl conditions is grater than the k_q under no NaCl conditions (Table 1). The influence of the ionic strength on k_q was reported previously.[19,20] Chioboli and co-workers clarified the salt effect on k_q by means of Debye–Hückel model. On the basis of their report, it is understood that the chloride anion weakens the repulsion between positively charged Ru(bpy)$_3^{2+}$ and Rh(terpy)$_2^{3+}$, resulting in high quenching constants. In this respect, the same interpretation can be applied to k_q in the case of no NaCl conditions. The quenching-rate constants under no NaCl conditions was dependent on the pH, because too much HCl was provided under acidic conditions than under basic conditions. Thus, too much Cl^- was present in an acidic solution and the k_q value was high under acidic conditions.

The difference between the quenching-rate constants by TEOA at pH 7 and 9 are caused by its protonation. It is known that TEOA, which is a tertiary amine, exists in equilibrium between the protonated form and the molecular form in an aqueous solution with $pK_a=8.1$ at 25 °C.[4] Accordingly, TEOAH$^+$ is dominated at pH 7.0 and cannot quench Ru(bpy)$_3^{2+*}$ reductively.[21]

Based on the facts above mentioned, if the run of the photoreduction in TEOA–Ru(bpy)$_3^{2+}$–Rh(terpy)$_3^{2+}$ depends on the pH, it would be the stage that TEOA reacts with Ru(bpy)$_3^{3+}$ to regenerate Ru(bpy)$_3^{2+}$.

TEOA is in equilibrium between the protonated form and the deprotonated form as mentioned above; only the deprotonated form can act as an electron donor. When the concentration of the total TEOA is 25 mM, it is calculated using pK_a that the concentration of the deprotonated TEOA is 71 mM in a pH 7.7 solution and 6 mM in a pH 6.5 solution. For this reason, TEOA at pH 6.5 seems to act inefficiently as an electron donor. It is therefore likely that the low yield of NADH due to a photoreduction at pH 6.5 is caused by the donor-activity of TEOA. In order to evaluate this possibility, irradiation was carried out on the TEOA–Ru(bpy)$_3^{2+}$–Rh(terpy)$_2^{3+}$ system at pH 6.5 and 7.7. Figure 4 presents the absorption spectra of the photo-reduction products after 50 min of irradiation at pH 6.5 and 7.7, respectively; Figure 5 shows the growth in absorption at 440 nm as a function of the irradiation time. As can be seen in Figs. 4 and 5, the growth in absorbance due to the reduction of Rh(terpy)$_2^{3+}$ at pH 7.7 is about 1.5-fold as great (7.7) as in a pH 6.5 solution. Although these results coerce presumably caused by the donor-activity of TEOA, the significant difference in the concentration of basic TEOA did not directly reflect the photoreduction of Rh(terpy)$_2^{3+}$. On the bases of these results, it is summarized that this photochemical reaction stage is not significantly dependent on the pH.

Incidentally, regarding the photoreduction product of Rh(terpy)$_2^{3+}$ observed in the absorption spectra (Fig. 4), the following interpretations are mentioned.

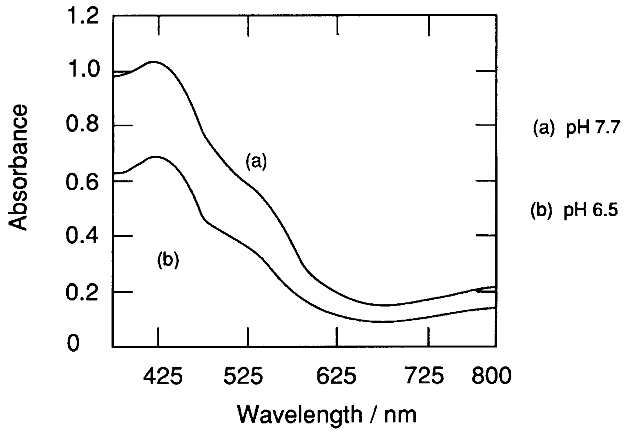


Fig. 4. Absorption spectra of Rh(terpy)$_2^{3+}$ reduced photochemically at 25 °C by 50 min of irradiation to a reaction mixture comprising TEOA (250 mM), Ru(bpy)$_3^{2+}$ (50 μM), and Rh(terpy)$_2^{3+}$ (250 μM) at (a) pH 7.7 and (b) pH 6.5. The absorption band of Ru(bpy)$_3^{2+}$ was removed in both spectra.

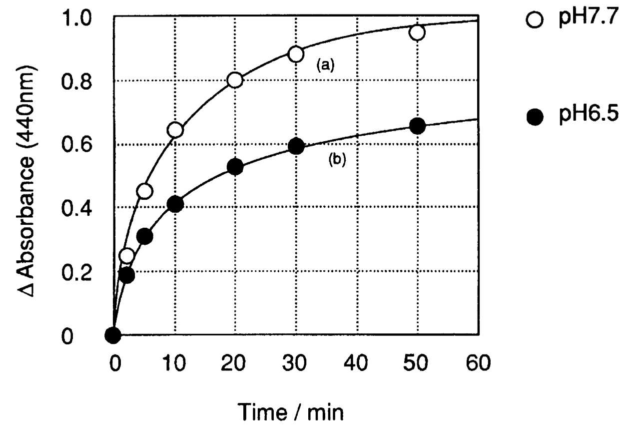


Fig. 5. Growths in the absorbance at 440 nm during irradiation of a reaction mixture at 25 °C at (a) pH 7.7 and (b) pH 6.5.

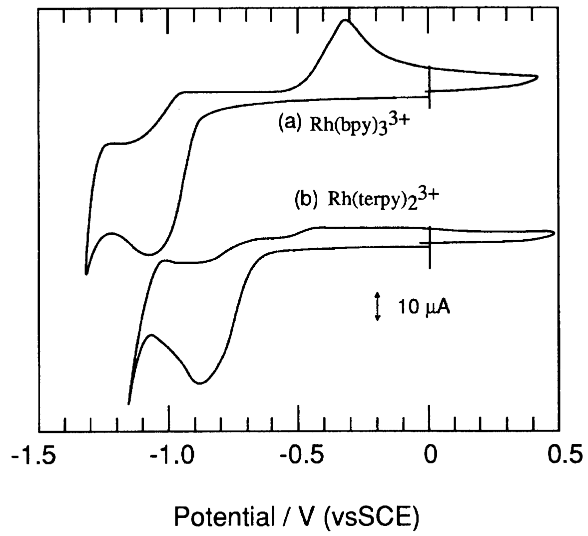


Fig. 6. Cyclic voltammogram of (a) Rh(bpy)$_3^{3+}$ and (b) Rh(terpy)$_2^{3+}$ in a 0.05 M NaOH aqueous solution with a sweep rate of 0.3 V s^{-1}. [Rh(bpy)$_3^{3+}$]=2 mM, [Rh(terpy)$_2^{3+}$]=1.5 mM.

First, the same spectral features were observed whether the photosensitized reduction was performed at pH 7.7 or pH 6.5, and the photoreduced species of Rh(terpy)$_2^{3+}$ detected by the absorption spectra (Fig. 4) seem to be identical. Nevertheless, a remarkable difference in NADH-generation under the pH conditions (Fig. 1) was observed. Accordingly, the reduced species of Rh(terpy)$_2^{3+}$ detected by the absorption spectra is not presumably that which catalyze the NAD$^+$-reduction.

Second, the photoreduction product of Rh(terpy)$_2^{3+}$ is presumably not the Rh$^{\mathrm{II}}$ species, but the Rh$^{\mathrm{I}}$ species which is presumably generated by the disproportionation of Rh$^{\mathrm{II}}$(terpy)$_2^{2+}$, since the monometal complex of Rh$^{\mathrm{II}}$ is unstable.[22] The disproportionation of Rh(terpy)$_2^{2+}$ is supported by the feature of a cyclic voltammogram obtained for Rh(terpy)$_2^{3+}$ and for Rh(bpy)$_3^{3+}$ (Fig. 6). Both of these show the characteristic broad cathodic wave with E_{p} at −0.88 V (vs. SCE) for Rh(terpy)$_2^{3+}$ and at −1.07 V (vs. SCE) for Rh(bpy)$_3^{3+}$. Sutin et al.[4] proposed for Rh(bpy)$_3^{3+}$ that after Rh(bpy)$_3^{3+}$ is reduced at an electrode, a rapid disproportionation of Rh(bpy)$_3^{2+}$ occurs, and that the broad wave can be ascribed to that reaction; the disproportionation of Rh(bpy)$_3^{2+}$ was ascertained by Brown et al.[23] With the interpretation by Sutin et al.,[4] the broad wave for Rh(terpy)$_2^{3+}$ as for Rh(bpy)$_3^{3+}$ demonstrates the rapid disproportionation of Rh(terpy)$_2^{2+}$ into Rh(terpy)$_2^{+}$ and Rh(terpy)$_2^{3+}$; presumably, Rh(terpy)$_2^{+}$ contributes the reduction of NAD$^+$, such as the Rh(bpy)$_3^{3+}$ catalyzing system,[12] and the catalyzing species of Rh(terpy)$_2^{+}$ cannot be detected by the visible absorption spectrum.

Influence of the pH Conditions on the Catalytic Activity of the Reduced Form of Rh(terpy)$_2^{3+}$. In respect to NADH generation, which showed a different tendency under pH conditions, another possibility is that the catalytic activity of Rh(terpy)$_2^{3+}$ depends on the pH conditions of the reaction system. This subject was examined by measuring the reactivity of reduced Rh(terpy)$_2^{3+}$, which was produced by NaBH$_4$ with NAD$^+$ at several pH. The reaction mixtures were analyzed by HPLC. Figure 7 presents the absorption spectra of Rh(terpy)$_2^{3+}$ reduced by NaBH$_4$. Similar spectral features, compared to the photoreduction product (Fig. 4), were also obtained in this case. It is expected that the reductive condition by NaBH$_4$ is similar to that of photoreduction. Figure 8 present the results of a HPLC analysis monitored at 340 nm. In a reaction mixture of pH 8.2, a significant peak assigned to 1,4-NADH after 45 min of incubation at 25 °C was observed after 9.5 min of retention time. On the other hand, the peak observed at that time is very small in pH 10.0, and especially a pH 6.5 mixture. (Note that the large peak at 12.5 min was assigned to NAD$^+$ [25] and the peak at 6.5 min is unidentified). It is unlikely that NaBH$_4$ reduce NAD$^+$ directly, since the amount of NaBH$_4$ added to the Rh(terpy)$_2^{3+}$ solution was 80% equivalent to Rh(terpy)$_2^{3+}$, and because about a 5 min interval (which seemed to be long enough to consume NaBH$_4$) was spaced before adding NAD$^+$. Moreover, when NAD$^+$ is reduced by NaBH$_4$, 1,6-NADH and 1,2-NADH must be produced (Fig. 8(b)).[9] Taking these aspects into account, it is obvious that Rh(terpy)$_2^{3+}$ reduced by NaBH$_4$ catalyzed the NAD$^+$-reduction into (1,4-)NADH. These results are in this case in good agreement with those of NADH-generation in the photoreduction, as shown in Fig. 1. It was found that the reduction of NAD$^+$ catalyzed by reduced Rh(terpy)$_2^{3+}$ is dependent on the pH, and that catalytic activity appears in a narrow pH range near to 8.0.

Reduction Mechanism of NAD$^+$ by Rh(terpy)$_2^{3+}$. The results obtained in this study are that Rh(terpy)$_2^{3+}$ catalyzing the reduction of NAD$^+$ was observed near pH 8.0 in both photochenical and chemical

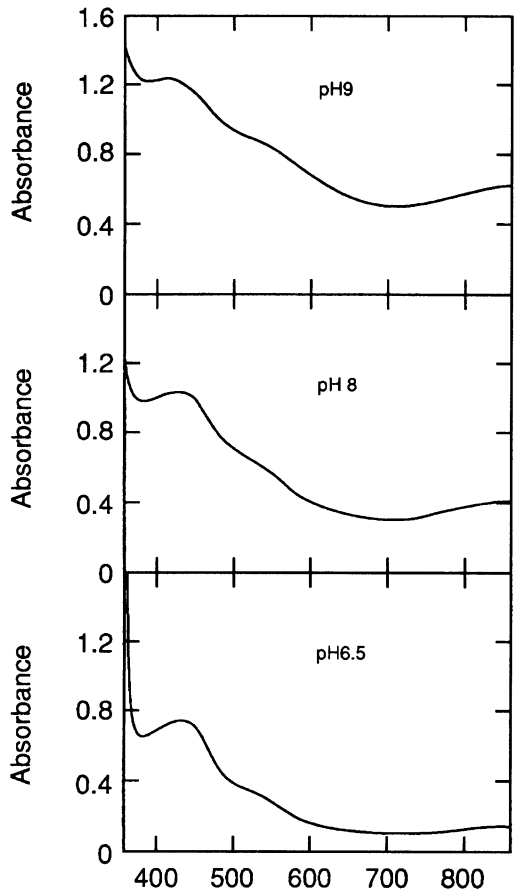


Fig. 7. Absorption spectra of $\mathrm{Rh(terpy)_2^{3+}}$ reduced by $\mathrm{NaBH_4}$ in a deareated buffer solution at (a) pH 9, (b) pH 8, pH 6.5.

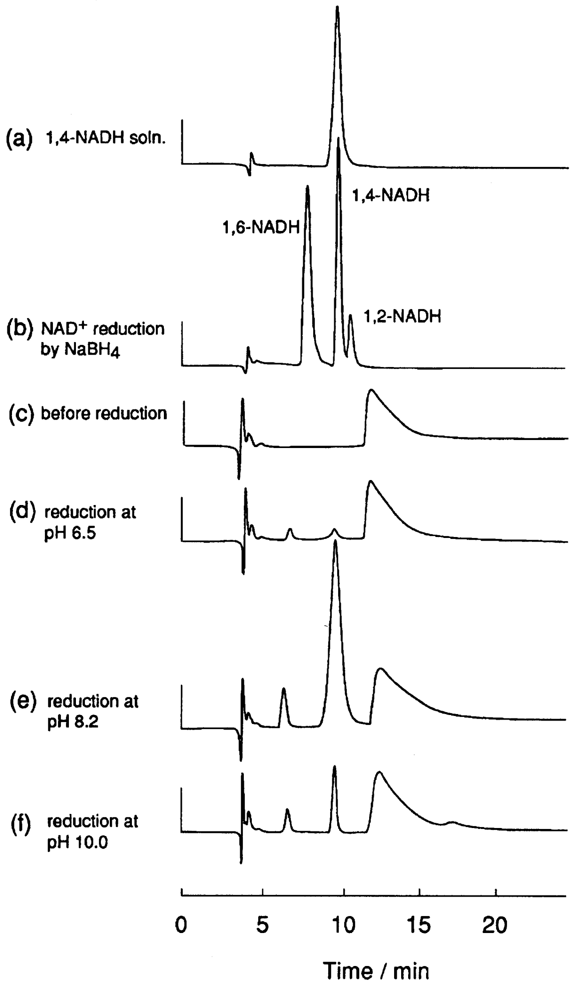


Fig. 8. HPLC analysis of (a) 125 µM NADH, (b) $\mathrm{NAD^+}$ reduced by $\mathrm{NaBH_4}$, (c) the mixture of 6 mM $\mathrm{NAD^+}$ and 250 µM $\mathrm{Rh(terpy)_2^{3+}}$, (d) the reaction mixture of 6 mM $\mathrm{NAD^+}$ and 250 µM $\mathrm{Rh(terpy)_2^{3+}}$ reduced by $\mathrm{NaBH_4}$ at pH 6.5 after 45 min incubation at 25 °C, (e) the reaction mixture same to (d) except at pH 8.2, and (f) the reaction mixture at pH 10.0. The peak assignment of (b) was based on results in Ref. 9. The mobile phase was composed of $\mathrm{H_2O}$–0.1 M $\mathrm{NH_4HCO_3}$–MeOH (39.2 : 60 : 0.8) and flow rate was 0.8 mL $\mathrm{min^{-1}}$.

systems, and that the $\mathrm{Rh(terpy)_2^+}$ species presumably reduces $\mathrm{NAD^+}$, as was mentioned before. Considering these matters, the mechanism is elucidated. One of the possible mechanism is described as follows:

$$\mathrm{NAD^+ + Rh(terpy)_2^+ \rightharpoonup NAD\cdot + Rh(terpy)_2^+} \qquad (6)$$

$$\mathrm{NAD\cdot + H^+ \rightharpoonup NADH^{+\cdot}} \qquad (7)$$

$$\mathrm{NADH^{+\cdot} + Rh(terpy)_2^+\ (or\ Rh(terpy)_2^{2+}) \rightharpoonup NADH + Rh(terpy)_2^{2+}\ (or\ Rh(terpy)_2^{3+})} \qquad (8)$$

However, these reactions are unlikely, since the redox potential of $\mathrm{NAD^+/NAD\cdot}$ is −1.1 V (vs. SCE),[9,10,24] and Ep of $\mathrm{Rh(terpy)_2^{3+}/Rh(terpy)_2^{2+}}$ is −0.88 V (vs. SCE). Further, that of $\mathrm{Rh(terpy)_2^{2+}/Rh(terpy)_2^+}$ seems to be close to −0.88 V (vs. SCE) (Fig. 6). In addition, when NAD· is produced (Eq. 7), the dimmer species ($\mathrm{NAD_2}$) should be produced. Nevertheless, in our previous work, no formation of $\mathrm{NAD_2}$ was observed.[14] Accordingly, this mechanism should be excluded.

Another possible mechanism is as follows:

$$\mathrm{NAD^+ + Rh(H^-)(terpy)_2^{3+} \rightharpoonup NADH + Rh(terpy)_2^{3+}}. \qquad (9)$$

Support for this mechanism requires evidence concerning $\mathrm{Rh(H^-)(terpy)_2^{3+}}$. In order to confirm the existence or $\mathrm{Rh(H^-)(terpy)_2^{3+}}$, the IR spectra (Fig. 9) were measured for reduced-$\mathrm{Rh(terpy)_2^{3+}}$, which was obtained by freeze drying after reduction by $\mathrm{NaBH_4}$ at several pH. The characteristic peak at 2100 $\mathrm{cm^{-1}}$ was observed in the spectrum of a sample prepared at pH 8.0. On the contrary, the spectra for a pH 6.5 sample and a pH 10.0 sample showed a small peak at around 2100 $\mathrm{cm^{-1}}$. The fact that the IR absorption by metal-hydride stretching appears in this range of wavenumbers[21,26] demonstrates that the rhodium-hydride compound exists in reduced species of $\mathrm{Rh(terpy)_2^{3+}}$ at pH 8.2, but does not exist at pH 7.0 or pH 10.0.

The results that NADH was produced at pH 8.0, but

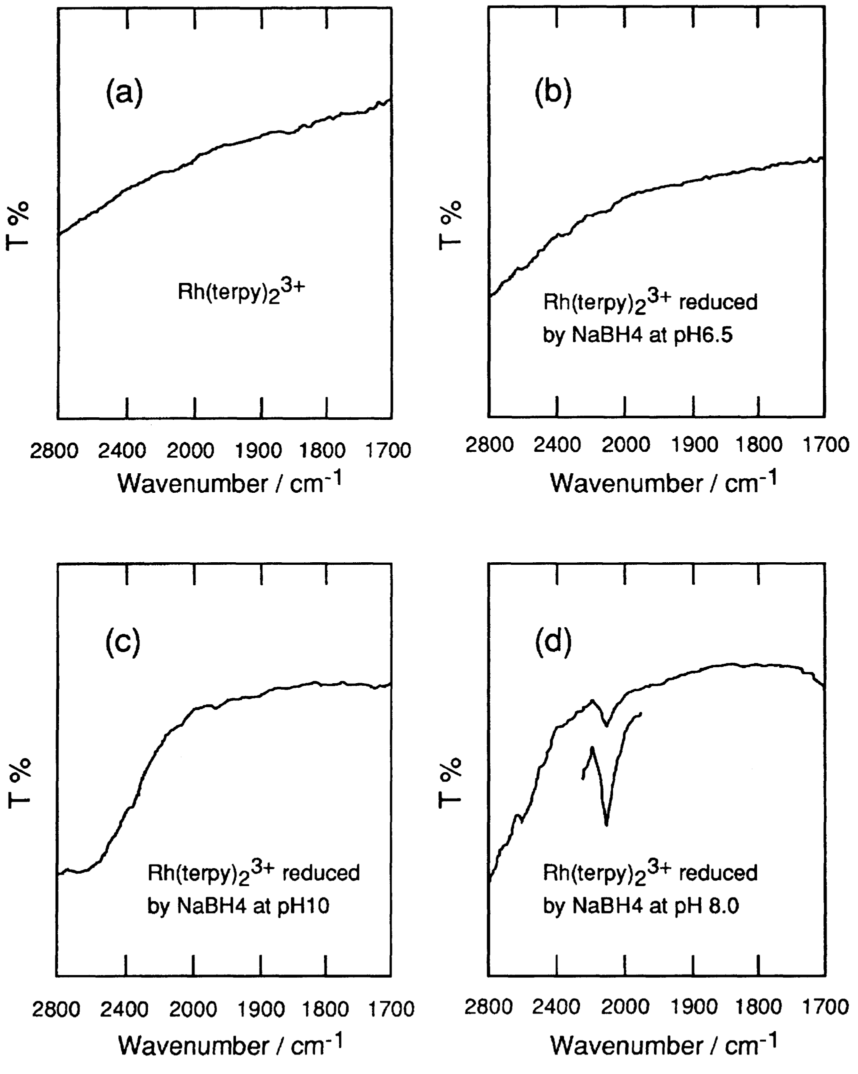
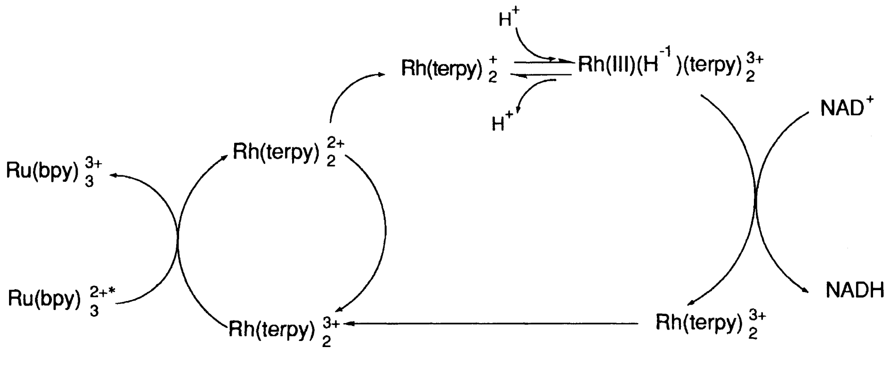


Fig. 9. IR spectra of (a) $\mathrm{Rh(terpy)_2^{3+}}$, (b) $\mathrm{Rh(terpy)_2^{3+}}$ reduced by $\mathrm{NaBH_4}$ at pH 10, (c) at pH 8.2, and (d) at pH 6.5.



Scheme 1. Proposed reaction mechanism of the $\mathrm{NAD^+}$-photoreduction system.

not at pH 6.5 or 10.0 and $\mathrm{Rh(H^-)(terpy)_2^{3+}}$ was present at pH 8.0, demonstrate that the reduction of $\mathrm{NAD^+}$ proceeds by a hydride-transfer. In the photochemical reduction system, $\mathrm{Rh(H^-)(terpy)_2^{3+}}$ seems to be generated according to the following series:

$$\mathrm{Ru(bpy)_3^{2+*} + Rh(terpy)_2^{3+} \rightarrow Ru(bpy)_3^{3+} + Rh(terpy)_2^{2+}} \qquad (10)$$

$$\mathrm{2Rh(terpy)_2^{2+} \rightarrow Rh(terpy)_2^{3+} + Rh(terpy)_2^{+}} \qquad (11)$$

$$\mathrm{Rh(terpy)_2^{+} + H^+ \ (or\ H_3O^+) \rightarrow Rh(H^-)(terpy)_2^{3+} \ (+\ H_2O)} \qquad (12)$$

In Eq. 12, although this reaction should be favorable at pH 6.5, rather than at pH 8.0, $\mathrm{Rh(H^-)(terpy)_2^{3+}}$ was not observed at pH 6.5. The reason for this is still obscure, and has been under investigation. Note that its consumption, such as H_2 evolution at pH 6.5, does not occur under our experimental conditions, in addition to a previous report.[3)]

Conclusion

We examined the photosensitized reduction of NAD^+ into NADH with $Rh(terpy)_2^{3+}$ used as a catalyst, showing that it has a catalytic activity with a pH dependence. Only in the narrow range of pH (ca. 8.0) was NAD^+ catalyzed by $Rh(terpy)_2^{3+}$ reduced photochemically and chemically; the existence of the $Rh^{III}(H^-)$ complex was recognized in the reduced species of $Rh(terpy)_2^{3+}$ within this pH range. From these results, we conclude that NAD^+-reduction into NADH proceed by a hydride-transfer route. (Scheme 1).

Experimental

Materials: $[Rh(terpy)_2]Cl_3 \cdot 5H_2O$ was prepared[3,27)] according to a previously reported method. The product was confirmed by means of the UV spectrum (λ_{max} (ε): 324 (15400), 337 (23300), 354 (25600)). Rhodium trichloride and 2,2′:6′,2″-tripyridine were purchased commercially. $[Rh(bpy)_3]Cl_3 \cdot 5H_2O$ was prepared by a previously described method, and was confirmed by means of the UV spectrum (λ_{max} (ε): 305 (43800), 318 (46700)). 2,2′-bipyridine (bpy) was purchased commercially. $[Ru(bpy)_3]Cl_2 \cdot 6H_2O$ was prepared by means of a previously described method. TEOA·HCl, EDTA (ethylenediaminetetraacetic acid), NAD^+, and NADH were purchased commercially and were used without further purification.

Methods and Apparatus: Steady state irradiation was carried out at 25 °C with a 500-W xenon-arc lamp, fitted with appropriate glass cutoff filters. Light was produced in the 420—700 nm range. 4 ml of the reaction mixtures in a quartz cell was deaerated and enclosed with argon prior to irradiation. Reaction mixtures containing NAD^+ (4 mM), $Rh(terpy)_2^{3+}$ (0.25 mM), $Ru(bpy)_3^{2+}$ (50 μM), and TEOA (250 mM) (or EDTA in pH 10.0 solution) in the presence of 2 M NaCl were adjusted to appropriate pH by 0.1 M tris(hydroxymethyl)aminomethane-hydrohcloride. The UV-visible absorption spectra were recorded on a Hitachi 220-A spectrophotometer. The luminescent lifetimes were measured at 25 °C by a Horiba NAES 550 nanosecond fluorometer. The concentrations of NADH produced were determined by reversed-phase HPLC analyses with the mobile phase of H_2O–0.1 M NH_4HCO_3–MeOH (30:69—69.2:1—0.8) and with a radial PAK cartridge C-18 (Waters). They were with precolumn (GUARD-PAK (Waters)) in front of the main column and the eluents were monitored at 340 nm by UVDEC-100 (JASCO). The flow rate was 0.8—1.0 ml min^{-1}. IR spectra was recorded on a JASCO A-102 diffraction grating infrared spectrophotometer.

The preparation of samples for IR spectral measurements was as follows: 4 mM of a $Rh(terpy)_2^{3+}$ (0.25 mM) buffer solution was reduced by $NaBH_4$ (above mentioned) and freeze-dried. A small amount of the freeze-dried product was then treated with KBr to make a tablet.

The reaction of NAD^+ with the reduced species of $Rh(terpy)_2^{3+}$ was performed as follows: 1 ml of a $Rh(terpy)_2^{3+}$ (1 mM) buffer solution bubbled by Ar for over 2.5 h was mixed with 8 μl of a $NaBH_4$–0.1 M NaOH solution, which was also treated with Ar. The amount of $NaBH_4$ was 80% equivalent to $Rh(terpy)_2^{3+}$. Then, 4—5 min later, a NAD^+ buffer solution bubbled by Ar for over 2.5 h was added to the reduced $Rh(terpy)_2^{3+}$ solution while carefully avoiding exposure to air. The mixture was then incubated at 25 °C. The final concentrations of $Rh(terpy)_2^{3+}$ and NAD^+ were 0.25 and 6 mM, respectively.

References

1) J. M. Ford, J. W. Otvos, and M. Calvin, *Nature*, **274**, 507 (1978); C. Loane, I. Willner, J. W. Otvos, and M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 5928 (1981); H. Ti Tien and N. B. Joshi, *Photobiochem. Photobiophys.*, **10**, 241 (1986); W. E. Ford and G. Tollin, *Photochem. Photobiol.*, **36**, 647 (1982); W. E. Ford and G. Tollin, *Photochem. Photobiol.*, **35**, 809 (1982); M. A. J. Rodgers and J. C. Becker, *J. Phys. Chem.*, **84**, 2762 (1980); J. S. Krueger, J. E. Mayer, and T. E. Mallouk, *J. Am. Chem. Soc.*, **110**, 8232 (1988); S. Rong and G. Tollin, *Photochem. Photobiol.*, **47**, 277 (1988); Y. Fang and G. Tollin, *Photochem. Photobiol.*, **47**, 751 (1988); J. N. Robinson, D. J. C.-Hamilton, M. K. Whittlesey, and P. Camilleri, *J. Chem. Soc., Faraday Trans. 2*, **86**, 2897 (1990); P. Douglas, G. Waechter, and A. Mills, *Photochem. Photobiol.*, **52**, 473 (1990); A. Mills, C. Lawrence, and P. Douglas, *J. Chem. Soc., Faraday Trans. 2*, **82**, 2291 (1986); J. R. Darwent and A. Lepre, *J. Chem. Soc., Faraday Trans. 2*, **82**, 2323 (1986); H. Rau, R. Frank, and G. Greiner, *J. Phys. Chem.*, **90**, 2476 (1986).

2) K. Kalynasundaram and D. Dung, *J. Phys. Chem.*, **84**, 2551 (1980); V. E. Maier and V. Ya. Shafirovich, *J. Chem. Soc., Chem. Commun.*, **1985**, 1063.

3) M. Kirch, J.-M. Lehn, and J.-P. Sauvage, *Helv. Chim. Acta*, **62**, 1345 (1979).

4) S. -F. Chan, M. Chou, T. Matsubara, and N. Sutin, *J. Am. Chem. Soc.*, **103**, 369 (1981).

5) L. P. Vernon, A. S. Pietro, and D. A. Linbach, *Arch. Biochem. Biophys.*, **109**, 92 (1965); D. Mandler and I. Willner, *J. Chem. Soc., Perkin Trans. 2*, **1986**, 805; D. Mandler and I. Willner, *J. Am. Chem. Soc.*, **106**, 5352 (1984); J. Handman, A. Harriman, and G. Porter, *Nature*, **307**, 534 (1984); D. Mandler and I. Willner, *J. Chem. Soc., Perkin Trans. 2*, **1988**, 997; Z. Goren, N. Lapidot, and I. Willner, *J. Mol. Catal.*, **47**, 21 (1988); M. Julliard, J. Le. Petit, and P. Ritz, *Biotechnol. Bioeng.*, **28**, 1774 (1986); K. Umeda, A. Nakamura, and F. Toda, *Chem. Lett.*, **1990**, 1443.

6) A. G. Volkov, V. D. Kolev, A. L. Levin, and L. I. Boguslavsky, *Photobiochem. Photobiophys.*, **10**, 105 (1985); R. Maidan and I. Willner, *J. Am. Chem. Soc.*, **108**, 8100 (1986).

7) C. -H. Wong and G. M. Whitesides, *J. Am. Chem. Soc.*, **103**, 4890 (1981); H. Giinther, C. Frank, H. -J. Schuetz, J. Barder, and H. Simon, *Angew. Chem., Int. Ed. Engl.*, **22**, 322 (1983); C. -H. Wong, L. Daniels, W. H. O. -Johnson, and G. M. Whitesides, *J. Am. Chem. Soc.*, **103**, 6227 (1981); I. Thanos and H. Simon, *Angew. Chem., Int. Ed. Engl.*, **25**, 462 (1986); M. Masoom and A. Townshend, *Anal. Chim. Acta*, **185**, 49 (1986); Z. Shaked and M. Whitesides, *J. Am. Chem. Soc.*, **102**, 7105 (1980).

8) J. Kiwi, *J. Photochemistry*, **16**, 193 (1981).

9) H. Jaegfeldt, *Bioelectrochem. Bioenerg.*, **8**, 355 (1981).

10) M. A. Jensen and P. J. Elving, *Biochim. Biophys. Acta*, **764**, 310 (1984); W. T. Brensnaham and P. J. Elving, *J. Am. Chem. Soc.*, **103**, 2379 (1981); F. G. Drakesmith and B. Gibson, *J. Chem. Soc., Chem. Commun.*, **1988**, 1493; M. Studnicková, H. P-Klukanová, J. Turánek, and J. Kovár, *J. Electroanal. Chem.*, **252**, 383 (1988).

11) R. Wienkamp and E. Steckhan, *Angew. Chem., Int Ed. Engl.*, **22**, 497 (1983).

12) R. Wienkamp and E. Steckhan, *Angew. Chem., Int. Ed. Engl.*, **22**, 782 (1983).

13) S. Grammenudi, M. Franke, F. Vögtle, and E. Steckhan, *J. Inclusion Phenom.*, **5**, 695 (1987); R. Ruppert, S. Herrmann, and E. Steckhan, *J. Chem. Soc., Chem. Commun.*, **1988**, 1150; E. Steckhan, S. Herrmann, R. Ruppert, J. Thövnmes, and C. Wandrey, *Angew. Chem., Int. Ed. Engl.*, **29**, 388 (1990).

14) K. Umeda, A. Nakamura, and F. Toda, *J. Chem. Soc., Chem. Commun.*, **1990**, 885.

15) J. B. Jones, D. W. Sveddon, W. Higgins, and A. J. Lewis, *J. Chem. Soc., Chem. Commun.*, **1972**, 856; L. J. Haynes and A. R. Todd, *J. Chem. Soc.*, **1950**, 303.

16) C. C. Johnston, J. L. Gardner, C. H. Suelter, and D. E. Metzler, *Biochemistry*, **2**, 689 (1963); C. S. Y. Kim and S. Chaykin, *Biochemistry*, **7**, 2339 (1968).

17) S. Aono, T. Kita, I. Okura, and A. Ymada, *Photochem. Photobiol.*, **43**, 1 (1986).

18) J. Handman, A. Harriman, and G. Porter, *Nature*, **307**, 534 (1984); K. Kalyanasundaram, *J. Photochem. Photobiol.*, **42A**, 87 (1988).

19) G. Greiner, P. Pasquini, R. Weiland, H. Orthwein, and H. Rau, *J. Photochem. Photobiol.*, **51A**, 179 (1990).

20) C. Chiorboli, M. T. Indelli, M. A. R. Scandola, and F. Scandola, *J. Phys. Chem.*, **92**, 156 (1988).

21) Only when TEOA is deprotonated, and electron is released from the lone pair on nitrogen atom (in Ref. 3) to Ru(bpy)$_3^{2+*}$, resulting reductive quenching. When TEOA is protonated, the lone pair does not exisist any longer.

22) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," 3rd ed, Interscience, New York (1972), pp. 1017—1029.

23) G. M. Brown, S. -F. Chan, C. Creutz, H. A. Schwarz, and N. Sutin, *J. Am. Chem. Soc.*, **101**, 7638 (1979).

24) M. Chou, C. Creutz, D. Mahajan, N. Sutin, and A. P. Zipp, *Inorg. Chem.*, **21**, 3989 (1982).

25) NAD$^+$ can be detected at 340 nm because of a weak absorption at 340 nm ($\varepsilon \approx 50$ M^{-1} cm^{-1}) in our experimental condition.

26) For example: C. -Y. Hsu and M. Orchin, *J. Am. Chem. Soc.*, **97**, 3553 (1975); T. H. Tulip and D. L. Thorn, *J. Am. Chem. Soc.*, **103**, 2448 (1981).

27) C. M. Harris and E. D. Mckenzie, *J. Inorg. Nucl. Chem.*, **25**, 171 (1963).