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Journal of Molecular Catalysis A: Chemical 172 (2001) 1–7

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

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Photoinduced hydrogen evolution with cytochrome c_3-viologen-ruthenium(II) triad complex and hydrogenase

Noriyuki Asakura, Tomohiro Hiraishi, Toshiaki Kamachi, Ichiro Okura*

Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta-cho 4259, Midori-ku, Yokohama 226-8501, Japan

Received 12 March 2001; accepted 28 March 2001

Abstract

Cytochrome c_3-viologen-ruthenium(II) triad complex, Ru-V-cyt.c_3, was prepared and characterized by using spectroscopic techniques. Effective quenching of the photoexcited state of ruthenium complex moiety by the bound viologen was observed in Ru-V-cyt.c_3. When the system containing Ru-V-cyt.c_3 and hydrogenase was irradiated by visible light, photoinduced hydrogen evolution was observed, showing the effective two-step electron transfer from the photoexcited state of ruthenium complex moiety to cytochrome c_3 via bound viologen. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Photoinduced hydrogen evolution; Cytochrome c_3-viologen linked ruthenium(II) complex; Hydrogenase; Intramolecular electron transfer

1. Introduction

Photoinduced hydrogen evolution from water has been studied extensively using four component systems: an electron donor (D), a photosensitizer (P), an electron carrier (C) and a catalyst as shown in Scheme 1 [1–4]. Among the photosensitizers, ruthenium tris(2,2′-bipyridine) derivatives are suitable compounds for photoinduced hydrogen evolution system, because ruthenium complexes are photostable in water, and have high extinction coefficients in the visible region and relatively long-lived excited states. As a catalyst for hydrogen evolution, hydrogenase is useful. Hydrogenase is an enzyme that catalyzes the reaction $2H^+ + 2e^- \rightleftarrows H_2$.

In the system using hydrogenase, electron-mediation between non-enzymatic and enzymatic reaction is one of the important factors to determine all over-reactivity. In order to connect enzymatic reaction and non-enzymatic reaction, the combination of two types of electron carrier, such as methyl viologen and cytochrome c_3 are effective. Cytochrome c_3, a substrate of hydrogenase in vivo, has high affinity for hydrogenase. Methyl viologen is used to support the electron-mediation between photoexcited photosensitizer and cytochrome c_3. In the kinetic studies using stopped-flow technique, it is clarified that electron flows from reduced methyl viologen to cytochrome c_3, leading to hydrogenase [5]. As the redox potential of methyl viologen (−440 mV versus SHE) is lower than that of cytochrome c_3 (−220 to −360 mV versus SHE), back electron transfer from reduced cytochrome c_3 to methyl viologen is difficult to occur. Therefore, photoinduced hydrogen evolution using methyl viologen and cytochrome c_3 proceed effectively.

To enhance the reactivity of photoinduced hydrogen evolution, cytochrome c_3-viologen-ruthenium(II) triad complex (Ru-V-cyt.c_3) was designed. When

* Corresponding author. Tel.: +81-45-9245752; fax: +81-45-9245778.
E-mail address: iokura@bio.titech.ac.jp (I. Okura).

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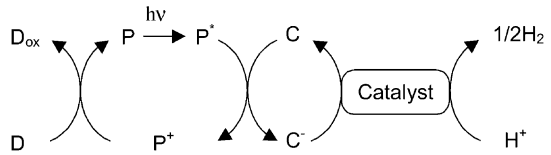
PII: S1381-1169(01)00159-5



Scheme 1. Photoinduced hydrogen evolution system.

two electron carriers, such as viologens with different redox potentials are connected to photosensitizer, effective photoinduced electron transfer has been proceeded due to the two-step intramolecurar electron transfer between photoexcited photosensitizer and viologen [6]. As Ru-V-cyt.c_3 may induce intramolecular electron transfer, like our previous report, high yield photoreduction of cytochrome c_3 can be established.

In this paper, we hope to describe the preparation and the photochemical properties of Ru-V-cyt.c_3. Ru-V-cyt.c_3 was applied to the photoinduced hydrogen evolution with hydrogenase.

2. Experimental

2.1. Materials

All the reagents used were of analytical or of the highest grade available. 4,4′-Dimethyl-2,2′-bipyridine (dmbpy), 4,4′-dicarboxy-2,2′-bipyridine (dcbpy) and $Ru(bpy)_2CO_3$ were prepared as described previously [7–9]. Cytochrome c_3 and hydrogenase were purified from *Desulfovibrio vulgaris* (Miyazaki) according to the literatures [10,11]. Cytochrome c_3 linked ruthenium(II) complex was synthesized according to the literature [12].

2.2. Preparation of cytochrome c_3-viologen-ruthenium(II) triad complex (Ru-V-cyt.c_3)

2.2.1. 1-(2-carboxyethyl)-4,4′-bipyridinium ($BpyC_2COOH$)

4,4′-Bipyridine (96 mmol) and benzyl bromide (98 mmol) were dissolved in 400 ml of acetone and stirred at room temperature for 24 h. White-yellow precipitate was filtered and washed with acetone. White-yellow precipitate was recrystallized from acetone and water, and dried under vacuum overnight. $BpyC_2COOH$ was dissolved in a minimum amount of water and then reprecipitated from water by the addition of ammonium hexafluorophosphate (NH_4PF_6). The white PF_6^- salt was filtered, washed with water and dried under vacuum overnight. ^{1}H NMR in DMSO-d_6: δ (ppm) 3.1–3.3 (t, 2H), 4.9–5.0 (t, 2H), 8.1–8.2 (d, 2H), 8.7–8.8 (d, 2H), 8.9–9.0 (d, 2H), 9.3–9.4 (d, 2H).

2.2.2. 4-(4-Bromobutyl)-4′-methyl-2,2′-bipyridine (dmbpy-C_4Br)

Dmbpy-C_4Br was synthesized analogously to the literature [13]. A total of 1.6 mol dm^{-3} *n*-butyl lithium (13 mmol) was added dropwise with stirring to di-iso-propylamine (14 mmol) in 10 ml of anhydrous tetrahydrofuran (THF) at −78°C under argon to yield a lithium di-iso-propylamide (LDA). LDA was added dropwise to dmbpy (10 mmol) in 130 ml of THF at 0°C under argon to yield a dark brown solution and stirred at 0°C for 1 h. 1,3-Dibromopropane (25 mmol) was added quickly with stirring to the dark brown solution, which then became blue. The solution was stirred at 0°C for an additional hour whereupon its color became yellow. The reaction was quenched by the addition of 100 ml of 100 mmol dm^{-3} phosphate buffer (pH 7.0) and the reaction mixture was extracted with diethyl ether for three times. The ether extract was dried over magnesium sulfate, and the ether was removed under vacuum at room temperature to obtain yellow-brown oily compound. The yellow-brown oily compound was dissolved in chloroform and applied to neutral alumina column with hexane. The alumina column was washed by hexane rich three times of the dead volume of the column and then eluted with hexane/ethyl acetate (4:1) to yield a product as a yellow band. The solvent was removed under vacuum leaving a yellow oil. ^{1}H NMR in DMSO-d_6: δ (ppm) 1.8–2.0 (multiplet, 4H), 2.6–2.8 (t, 2H), 3.35–3.45 (t, 2H), 7.05–7.15 (multiplet, 2H), 8.15–8.25 (s, 2H), 8.5–8.6 (multiplet, 2H).

2.2.3. 1-(4-(4′-Methyl-2,2′-bipyridine-4-yl)butyl)-1′-carboxyethyl-4,4′-bipyridinium (dmbpy-C_4VC_2COOH)

Dmbpy-C_4Br (2.4 mmol) and $BpyC_2COOH$ (3.1 mmol) were dissolved in 20 ml of dried acetonitrile (dry-MeCN) and refluxed under argon for 3 days. Yellow precipitate was filtered, washed with dry MeCN and dried under vacuum overnight. The yellow

product was dissolved in a minimum amount of water and the concentrated aqueous solution of NH_4PF_6 was added to obtained the PF_6^- salt. The PF_6^- salt was filtered and washed with water to remove excess NH_4PF_6. The white PF_6^- salt was recrystallized from water and dried under vacuum overnight. ^{1}H NMR in DMSO-d_6: δ (ppm) 1.6–1.8 (quintet, 2H), 1.95–2.1 (quintet, 5H), 2.8–2.9 (t, 2H), 3.1–3.2 (t, 2H), 4.7–4.8 (t, 2H), 7.4–7.5 (multiplet, 2H), 8.3–8.4 (s, 2H), 8.55–8.6 (d, 1H), 8.6–8.65 (d, 1H), 8.7–8.8 (multiplet, 4H), 9.3–9.4 (multiplet, 4H).

2.2.4. Preparation of cytochrome c_3-viologen-dmbpy triad (dmbpy-V-cyt.c_3)

Cytochrome c_3-viologen-dmbpy triad, dmbpy-V-cyt.c_3, was prepared as follows. Dmbpy-C_4VC_2COOH (6.7 μmol) was dissolved in 0.5 ml of dried *N,N*-dimethylformamide (dry-DMF). The compound was stirred with dicyclohexylcarbodiimide (7.3 μmol) and *N*-hydroxysuccinimide (7.4 μmol) at 30°C for 15 h. The white precipitate was removed by centrifugation and DMF was removed under vacuum. The white solid was redissolved in 100 μl of dry-DMF and the solution was centrifugated to remove precipitate.

The supernatant (50 μl) was added to 950 μl of 351 μmol dm^{-3} cytochrome c_3 in 10 mmol dm^{-3} EPPS buffer (pH 8.5) and then the solution was stirred at 30°C for 4 h. The reaction mixture was applied to Sephadex G-25 column (Pharmacia) to remove the excess mono-*N*-hydroxysuccinimide ester of dmbpy-C_4VC_2COOH. Dmbpy-V-cyt.c_3 was chromatographed on SP Sepharose Fast Flow HR 10/10 column (Pharmacia) with 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4) at 0.5 ml min^{-1}. The dmbpy-V-cyt.c_3 was eluted by using a linear gradient from 0 to 250 mmol dm^{-3} NaCl at 2.5 mmol dm^{-3} min^{-1}. After SP Sepharose Fast Flow column, the sample fractions were concentrated by using Centricon 10 (Pharmacia) and exchanged with 100 mmol dm^{-3} sodium acetate buffer (pH 4.0).

2.2.5. Preparation of cytochrome c_3-viologen-ruthenium triad complex (Ru-V-cyt.c_3)

Dmbpy-V-cyt.c_3 was treated with 10 equivalent of $Ru(bpy)_2CO_3$ in 100 mmol dm^{-3} sodium acetate buffer (pH 4.0) at 30°C for 15 h. The sample solution was applied to Sephadex G-25 column to remove the excess $Ru(bpy)_2CO_3$ and then chromatographed on CM Sepharose Fast Flow column (Pharmacia) with 20 mmol dm^{-3} phosphate buffer (pH 7.7) at 0.5 ml min^{-1}. Ru-V-cyt.c_3 was eluted with a linear gradient from 0 to 250 mmol dm^{-3} NaCl at 2.5 mmol dm^{-3} min^{-1}. The sample fractions were concentrated by using Centricon 10 and exchanged with 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4).

2.3. MALDI-TOF MS measurement

The molecular mass was determined by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) with sinapic acid as a matrix.

2.4. Spectroscopic measurements

UV–VIS absorption spectra were measured in 25 mmol dm^{-3} Tris–HCl buffer (Tris, tris(hydroxymethyl)aminomethane) (pH 7.4) using SHIMADZU MultiSpec-1500 spectrometer.

^{1}H NMR spectra were measured by Varian GEMINI-200 or Varian OXFORD NMR300. The chemical shifts were referenced to the solvent peak calibrated against tetramethylsilane (TMS).

The luminescence spectra were measured in 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4) at room temperature using HITACHI F-4010 spectrometer. The absorbance at the excitation wavelength was kept constant at 0.05 for all the sample solutions in these experiments.

2.5. Photoreduction of cytochrome c_3 in Ru-V-cyt.c_3

Photoreduction of cytochrome c_3 in Ru-V-cyt.c_3 and Ru-cyt.c_3 was studied in pyrex tubes which was evacuated by repeated freeze-pump-thaw cycles. The reaction mixture containing 2-mercaptoethanol (240 mmol dm^{-3}) and Ru-V-cyt.c_3 (1.0 μmol dm^{-3}) was prepared in 2.2 ml of 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4). The solutions were irradiated with 200 W tungsten lamp (Philips KP-8) at 30°C. Light of wavelength less than 370 nm was removed by Toshiba L-37 cut-off filter. The absorption spectra were measured by SHIMADZU MultiSpec-1500 spectrometer at intervals.

2.6. Photoinduced hydrogen evolution under steady state irradiation

For the photoinduced hydrogen evolution under steady state irradiation, the sample solution in a pyrex cell was irradiated using 200 W tungsten lamp cut-off less than 370 nm. The reaction was carried out at 30°C. The sample solution containing EDTA (7.0 mmol dm^{-3}), ruthenium complex derivatives (5.0 μmol dm^{-3}) and hydrogenase (0.2 μmol dm^{-3}) in 1.0 ml of 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4) was deaerated by repeated freeze-pump-thaw cycles and then incubated for 5 min under argon. Evolved hydrogen was detected by gas chromatography (Shimadzu GC-14B, detector: TCD, column: active carbon).

3. Results and discussion

3.1. Preparation of cytochrome c_3-viologen-ruthenium(II) triad complex (Ru-V-cyt.c_3)

Ru-V-cyt.c_3 was prepared by two-step procedures as shown in Fig. 1. In the first step, the prepared cytochrome c_3 mono-*N*-hydroxysuccinimide activated ester and dmbpy-C_4VC_2COOH was coupled to dmbpy-V-cyt.c_3. Dmbpy-V-cyt.c_3 was purified by SP Sepharose Fast Flow and fractionated in two fractions. From MALDI TOF-MS measurements of dmbpy-V-cyt.c_3, after SP Sepharose Fast Flow column, the molecular mass of dmbpy-V-cyt.c_3 (fractions 1 and 2) were ca. 14200 and 14750, respectively. Under these experiment conditions, the molecular mass of native cytochrome c_3 was ca. 14200. These results suggested that fraction 1 was native cytochrome c_3 and fraction 2 contained a single equivalent of dmbpy-C_4VC_2COOH. On the second step, dmbpy-V-cyt.c_3 treated with $Ru(bpy)_2CO_3$ to form Ru-V-cyt.c_3 derivative. Ru-V-cyt.c_3 was purified by using CM Sepharose Fast Flow cation exchange chromatography.

3.2. Photochemical properties of Ru-V-cyt.c_3

The absorption spectra of Ru-V-cyt.c_3 and native cytochrome c_3 are shown in Fig. 2. The absorption spectrum of Ru-V-cyt.c_3 was almost the same as the spectrum of equimolar mixture of native cytochrome c_3, methyl viologen and ruthenium complex. This result indicates no electronic interaction between the $Ru(bpy)_2(dcbpy)$ moiety and the viologen and/or cytochrome c_3 in the ground state of Ru-V-cyt.c_3.

The photoexcited state of Ru-V-cyt.c_3 was studied by luminescence emission spectra measurements. The luminescence spectra of Ru-V-cyt.c_3, Ru-cyt.c_3 and $Ru(bpy)_2(dcbpy)$ + cytochrome c_3 (1:1) are shown in Fig. 3. The relative luminescence quantum yields of Ru-V-cyt.c_3, Ru-cyt.c_3 and $Ru(bpy)_2(dcbpy)$ + cytochrome c_3 (1:1) are listed in Table 1. For Ru-cyt.c_3, the shape of the luminescence spectrum of Ru-cyt.c_3 was the same as that of $Ru(bpy)_2(dcbpy)$ + cytochrome c_3 (1:1). The luminescence intensity of Ru-cyt.c_3 was lower than that of $Ru(bpy)_2(dcbpy)$ + cytochrome c_3 (1:1). These results indicate that the photoexcited state of $Ru(bpy)_2(dcbpy)$ moiety is oxidatively quenched by the bound cytochrome c_3 and no electronic interaction occurs between the photoexcited state of $Ru(bpy)_2(dcbpy)$ moiety and bound cytochrome c_3. In the case of Ru-V-cyt.c_3, the luminescence intensity of Ru-V-cyt.c_3 was much lower than that of Ru-cyt.c_3, indicating the efficient quenching of photoexcited state of ruthenium complex moiety by the bound viologen in Ru-V-cyt.c_3 molecule.

3.3. Photoreduction of cytochrome c_3 in Ru-V-cyt.c_3 under steady state irradiation

The photoreduction of cytochrome c_3 in Ru-V-cyt.c_3 and Ru-cyt.c_3 was carried out at 30°C. Fig. 4 shows the absorption changes of Ru-V-cyt.c_3 and Ru-cyt.c_3. When the solution containing Ru-V-cyt.c_3 was irradiated, the rapid change of absorption spectrum shape was observed. The specific absorption maximum at 552 nm of reduced cytochrome c_3 increased with irradiation time. On the other hand, in the case of Ru-cyt.c_3, the absorption maximum at 552 nm

Table 1
Relative luminescence quantum yields (ϕ) of Ru-v-cyt.c_3 and Ru-cyt.c_3

Compound	ϕ
$Ru(bpy)_2(dcbpy)$ + cytochrome c_3	1
Ru-cyt.c_3	0.125
Ru-V-cyt.c_3	0.013

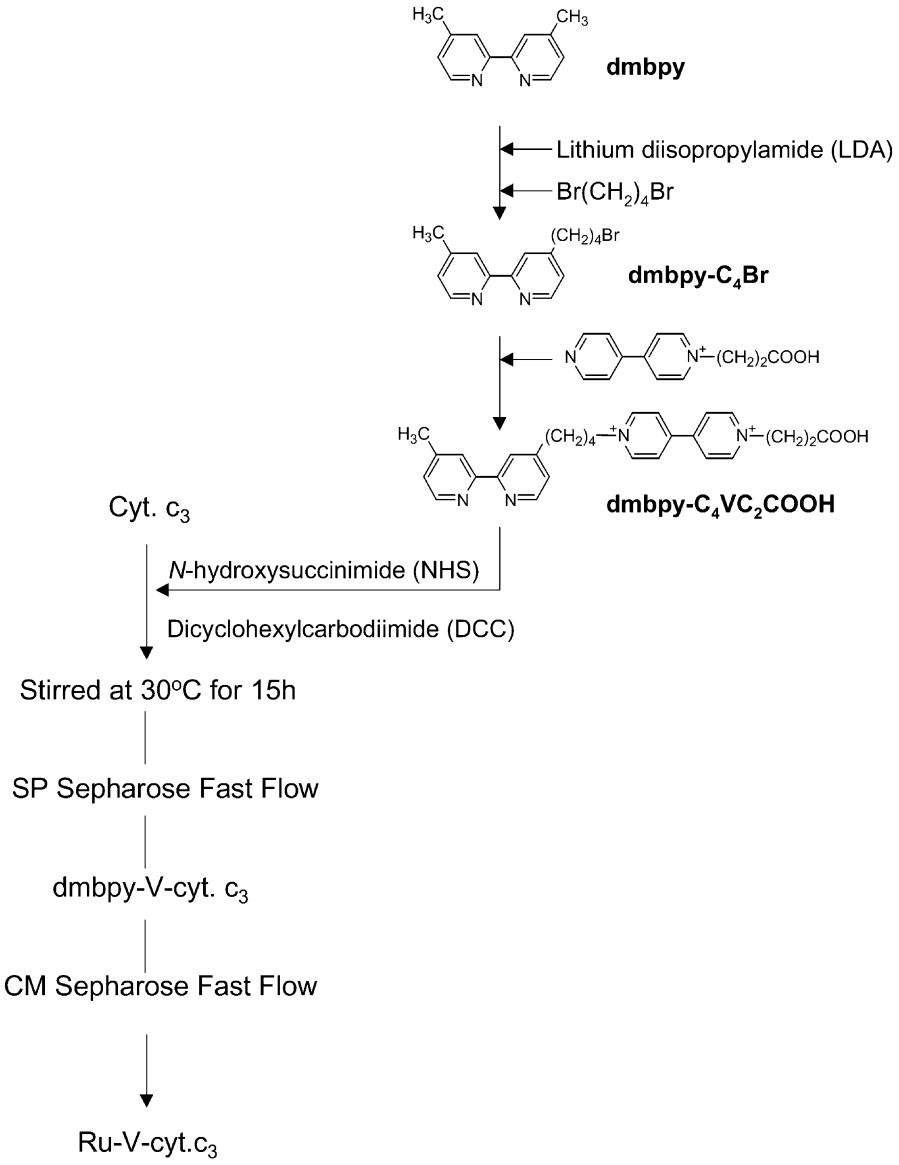


Fig. 1. Preparation of cytochrome c_3-viologen linked ruthenium(II) complex (Ru-V-cyt.c_3).

increased slowly. These results indicate that the rapid electron transfer from the photoexcited state of ruthenium(II) moiety to cytochrome c_3 via the bound viologen occurs in Ru-V-cyt.c_3. The first electron carrier, viologen moiety, probably supports electron transfer from photoexcited ruthenium(II) to cytochrome c_3 and accelerates production of reduced cytochrome c_3.

3.4. Photoinduced hydrogen evolution with Ru-V-cyt.c_3 and hydrogenase

Table 2 shows the photoinduced hydrogen evolution with Ru-V-cyt.c_3 and hydrogenase. When the solution containing Ru-cyt.c_3 and hydrogenase was irradiated, no hydrogen evolution was observed. On the other hand, in the case of Ru-V-cyt.c_3 hydrogen evolution

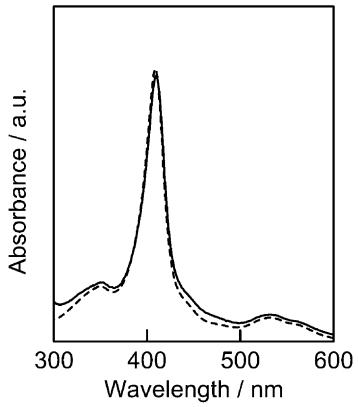


Fig. 2. Absorption spectra of Ru-V-cyt.c_3 (solid line) and native-cytochrome c_3 (dashed line) in 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4).

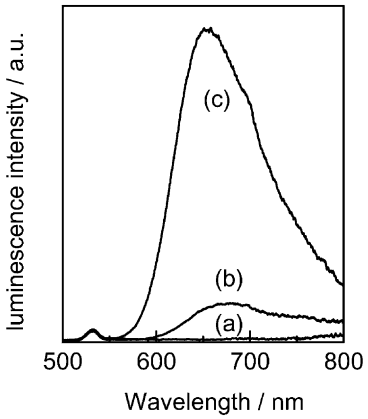


Fig. 3. Luminescence spectra of Ru-V-cyt.c_3 (a), Ru-cyt.c_3 (b), and Ru(bpy)$_2$(dcbpy) + cytochrome c_3 (1:1) (c) in 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4). The excitation wavelength was 450 nm.

was observed, indicating that Ru-V-cyt.c_3 can be a substrate of hydrogenase on the photoinduced hydrogen evolution. From the results, it is concluded that the effective electron transfer from the photoexcited state of ruthenium complex to cytochrome c_3 via the bound viologen occurs in Ru-V-cyt.c_3 and hydrogen evolves.

Table 2
Photoinduced hydrogen evolution with Ru-V-cyt.c_3 and Ru-cyt.c_3

Compound	Irradiation time (h)	H_2 evolved (10^{-8} mol)
Ru-cyt.c_3	20	0
Ru-V-cyt.c_3	20	1.66

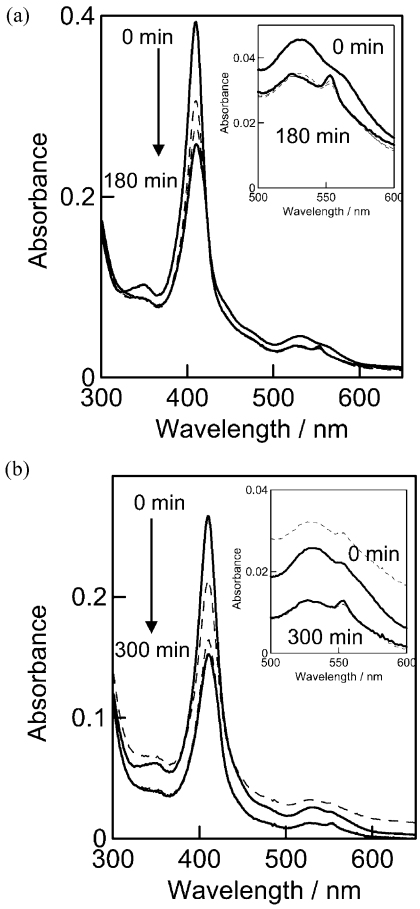


Fig. 4. Change in absorption spectra of Ru-V-cyt.c_3 (a) and Ru-cyt.c_3 (b) under steady state irradiation at 30°C. The solution contained 1.0 mmol dm^{-3} of Ru-V-cyt.c_3 and 240 mmol dm^{-3} 2-mercaptoethanol in 2.2 mdm^3 of 25 mmol dm^{-3} Tris–HCl buffer (pH 7.4).

Acknowledgements

The present work is partly defrayed by the Grant-in-Aid on Priory-Areas-Research from the Ministry of Education, Science, Sports and Culture of Japan (No.11167228, 12019219).

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