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Use of Ruthenium Photooxidation Techniques to Study Electron Transfer in the Cytochrome bc₁ Complex

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Abstract

Ruthenium photooxidation methods are presented to study electron transfer between the cytochrome bc₁ complex and cytochrome c, and within the cytochrome bc₁ complex. Methods are described to prepare a ruthenium cytochrome c derivative, Ru_Z-39-Cc, by labeling the single sulfhydryl on yeast H39C;C102T iso-1-Cc with the reagent Ru(bpz)₂(4-bromomethyl-4'-methylbipyridine). The ruthenium complex attached to Cys-39 on the opposite side of Cc from the heme crevice does not affect the interaction with cyt bc₁. Laser excitation of reduced Ru_Z-39-Cc results in photooxidation of heme c within 1 μs with a yield of 20%. Flash photolysis of a 1:1 complex between reduced yeast cytochrome bc₁ and Ru_Z-39-Cc leads to electron transfer from heme c₁ to heme c with a rate constant of $1.4 \times 10^4 \text{ s}^{-1}$. Methods are described for the use of the ruthenium dimer, Ru₂D, to photooxidize cyt c₁ in the cytochrome bc₁ complex within 1 μs with a yield of 20%. Electron transfer from the Rieske iron-sulfur center [2Fe2S] to cyt c₁ was detected with a rate constant of $6 \times 10^4 \text{ s}^{-1}$ in *R. sphaeroides* cyt bc₁ using this method. This electron transfer step is rate-limited by the rotation of the Rieske iron-sulfur protein in a conformational gating mechanism. This method provides critical information on the dynamics of rotation of the iron-sulfur protein (ISP) as it transfers electrons from QH₂ in the Q_o site to cyt c₁. These ruthenium photooxidation methods can be used to measure many of the electron transfer reactions in cytochrome bc₁ complexes from any source.

1. Introduction

Cytochrome bc₁ (cyt bc₁), an integral membrane protein in the energy-conserving electron transport chains of mitochondria and many prokaryotes, contains two b-type hemes (b_L and b_H) in the cyt b subunit, the Rieske iron-sulfur protein, and cyt c₁ (Trumpower and Gennis, 1994). The complex translocates four protons to the positive side of the membrane per two electrons transferred from ubiquinol to Cc in a widely accepted Q-cycle mechanism (Scheme 1) (Trumpower, 1990). A bifurcated electron transfer reaction occurs at the Q_o-site, where the first electron is transferred from ubiquinol to the Rieske iron-sulfur center [2Fe2S], and then to cyt c₁ and Cc. The second electron is transferred from semiquinone in the Q_o site to cyt b_L and then to cyt b_H and ubiquinone in the Q_i site. X-ray crystallographic studies have shown that the conformation for the Rieske iron-sulfur protein (ISP) depends on the crystal form and the presence of Q_o site inhibitors (Xia et al., 1997; Zhang et al., 1998; Iwata et al., 1998; Kim et al., 1998). The ISP is in a conformation with [2Fe2S] close to cyt c₁, called the c₁ state, in native chicken or beef P6522 crystals (Zhang et al., 1998; Iwata et al., 1998). However, the ISP is in a conformation with [2Fe2S] proximal to the cyt b_L heme, called the b state in beef, chicken and yeast cyt bc₁ crystals grown in the presence of stigmatellin (Zhang et al., 1998; Iwata et al., 1998; Hunte et al., 2000). A rotational shuttle mechanism has been proposed for the ISP during electron transfer based on these structural studies (Scheme 1). QH₂ in the Q_o site transfers an electron to the oxidized [2Fe2S] center when the ISP is initially in the b state.

The ISP then rotates by 57° to the c_1 state and reduced [2Fe2S] transfers an electron to cyt c_1 (Zhang et al., 1998; Iwata et al., 1998). This mobile shuttle mechanism has received support from cross-linking and mutational studies that immobilize the ISP or alter the conformation of the neck region (Xiao et al., 2000; Darrouzet and Daldal, 2002)

Detection of the rapid electron transfer reactions in cytochrome bc_1 has been challenging, and a number of different kinetics techniques have been utilized. Stopped-flow spectroscopy and rapid-mix/freeze-quench EPR spectroscopy have been used to study the kinetics of reduction of cytochrome bc_1 by QH_2 (Zhu et al., 2007). Extensive kinetic studies have also been carried out in chromatophores of photosynthetic bacteria including *Rhodobacter sphaeroides* and *Rb. capsulatus* (Hong et al., 1999; Darrouzet and Daldal, 2002). Photoexcitation of the photosynthetic reaction center in these systems leads to oxidation of cyt c_2 and reduction of Q to QH_2 .

Our laboratory has developed a new ruthenium photooxidation technique to study electron transfer in cytochrome bc_1 with greater time resolution than previously possible (Millett and Durham, 2002). In one method, a polypyridyl ruthenium complex [Ru(II)] is covalently attached to cytochrome c to form Ru-Cc (Engstrom et al., 2003). Photoexcitation of Ru(II) to the metal-to-ligand charge-transfer state, Ru(II*), a strong oxidant, leads to rapid oxidation of the ferrous heme group in Cc. Subsequent electron transfer from cytochrome c_1 to the Ru-Cc heme can be measured on a time scale as fast as 50 nanoseconds (Scheme 1). This new technique has been used to measure intracomplex electron transfer between cytochrome bc_1 and Cc for the first time (Engstrom et al., 2003). In a second method, a new ruthenium dimer has been developed which binds with high affinity to cytochrome bc_1 and can photooxidize cyt c_1 within 1 μ s (Sadoski et al., 2000). This new technique has been used to measure the rate constant for electron transfer from the iron-sulfur center to cyt c_1 for the first time, and provides critical information on the dynamics of rotation of the iron-sulfur protein (ISP) as it transfers electrons from QH_2 in the Q_o site to cyt c_1 (Scheme 2).

2. Design and Synthesis of Ruthenium-labeled Proteins

Several different methods have been developed for covalently attaching a polypyridyl ruthenium complex to a protein. One method involving the attachment of a Ru (bpy)₂(dicarboxybipyridine) complex to a lysine amino group by an amide linkage has been used to prepare over 10 different singly-labeled Ru-Cc derivatives (Durham et al., 1989). This ruthenium complex is useful for photoreduction of the heme in Ru-Cc, followed by electron transfer from Ru-Cc heme to cyt c_1 , which is the reverse direction of physiological electron transfer. Another method involves the formation of a thioether link between a protein cysteine residue and a ruthenium complex containing a bromomethyl group (Geren et al., 1995). The location of the cysteine residue on the protein can be genetically engineered to address specific questions. All three chelating ligands can be altered to tune the redox potentials over a wide range, allowing measurement of the reorganization energy, and optimization of the rate and yield of photoinduced electron transfer (Table 1). The Ru(bpz)₂(dmb) complex can be used to photooxidize the heme in Ru-Cc, allowing the measurement of electron transfer from cyt c_1 to Ru-Cc in the physiological direction.

2.1. Method for the Synthesis of Ruthenium-labeled Cytochrome c

The new brominated Ru(bpz)₂(dmb) reagent can be used to covalently attach a ruthenium complex to a protein containing a single cysteine sulfhydryl group on its surface (Engstrom et al., 2003). In order to label yeast Cc, the single Cys-102 in wild-type yeast iso-1-Cc is first mutated to Thr, and then His-39 is mutated to Cys to form H39C;C102T yeast iso-1-Cc. The H39C;C102T yeast iso-1-Cc is first treated with a two-fold excess of dithiothreitol for 15 minutes under anaerobic conditions to reduce any disulfide dimers that may have formed. 1.8

mM Ru(bpz)₂(4-bromomethyl-4'-methylbipyridine) is then added to the solution containing 530 μ M H39C;C102T yeast iso-1-Cc and 260 μ M dithiothreitol in 50 mM sodium borate, pH 9.0 under anaerobic conditions and allowed to react for 3 hours at 37 °C. Amincon concentrators are used to exchange the reaction mixture into 5 mM sodium phosphate, pH 7.0 and remove excess reagent. The Ru_Z-39-Cc derivative is purified using a high-resolution Waters 1 $\times$ 10 cm Sp 8HR cation exchange column in a Waters 625 HPLC (30). Usually there is a single major band containing Ru_Z-39-Cc, a smaller band of unlabeled Cc, and several small bands of derivatives labeled at lysines. It is important to determine that the derivative is singly labeled with ruthenium. The UV/visible spectrum of the Ru_Z-39-Cc band should be the sum of the spectra of one equivalent of Ru(bpz)₂(dmb) and one equivalent of native yeast iso-1-Cc. The yield of Ru_Z-39-Cc is typically 70%, provided that fresh reagent is used under anaerobic conditions. Another important step in the characterization of the derivative is to confirm the location of the ruthenium complex at Cys-39 by tryptic digestion, HPLC separation of the resulting peptides, and sequencing the ruthenium labeled peptide (Engstrom et al., 2003). Finally, steady-state kinetics is used to determine that yeast Ru_Z-39-Cc has the same steady-state activity with yeast cytochrome bc₁ as wild-type yeast Cc. This important characterization provides evidence that the ruthenium complex has no effect on the interaction with cyt bc₁.

3. Measurement of Interprotein Electron Transfer in a Ruthenium-labeled Protein

Flash photolysis methods are used to measure internal electron transfer between the ruthenium complex and the heme in a ruthenium-labeled protein (Engstrom et al., 2003). The third harmonic of a Nd:YAG laser, with a pulse width of 20 ns and wavelength of 356 nm is used for the excitation pulse, the probe source is a pulsed 75 W xenon arc lamp, and the photomultiplier detector has a response time of 10 ns. Photoexcitation of Ru(II) in 5 μ M reduced yeast Ru_Z-39-Cc under anaerobic conditions leads to electron transfer from Fe(II) to Ru(II*) to form Ru(I) and Fe(III) (Figure 1A). The oxidation of Fe(II) is detected at 550 nm and the reduction of Ru(II*) is detected at 434 nm, an isobestic for the Cc heme. The back reaction from Ru(I) to Fe(III) is observed with a rate constant of $k_4 = 7,000 \pm 1000 \text{ s}^{-1}$ (Figure 1B). The kinetics are fit to Scheme 3 with $k_3 = (1.5 \pm 0.2) \times 10^6 \text{ s}^{-1}$, $k_4 = 7000 \pm 1000 \text{ s}^{-1}$, and $k_d = (3.5 \pm 0.6) \times 10^6 \text{ s}^{-1}$ (Engstrom et al., 2003). The back electron transfer reaction from Ru(I) to Fe(III) is prevented by aerating the solution (Figure 1B), indicating that Ru(I) was oxidized by oxygen in the solution. [Co(NH₃)₅Cl]²⁺ can also be used to irreversibly oxidize Ru(I), preventing the back reaction under anaerobic conditions.

The yeast Ru_Z-39-Cc derivative is optimized to study electron transfer with the cyt bc₁ complex in the physiological direction. The ruthenium complex attached to Cys-39 on the opposite side of Cc from the heme crevice does not affect the interaction with cyt bc₁. The distance between the heme and the ruthenium complex is 12.6 Å, and there is an electron transfer pathway consisting of 13 covalent bonds and one hydrogen bond. The Ru(bpz)₂(dmb) complex has a reduction potential of 1.22 V for the Ru(II*)/Ru(I) transition (Table 1), providing a driving force $\Delta G^{\circ'}$ of -1.0 V for the Ru(II*)-Fe(II) $\rightarrow$ Ru(I)-Fe(III) reaction. The value of $\Delta G^{\circ'}$ is thus nearly the same as the reorganization energy λ of 0.8 V providing an optimal electron transfer rate. The rate constant for the forward reaction k_3 is very large, $k_3 = 1.5 \times 10^6 \text{ s}^{-1}$, while the back electron transfer reaction from Ru(I) to Fe(III) is quite slow, with a rate constant of $k_4 = 7000 \text{ s}^{-1}$. The yield of photooxidized heme *c* is quite large, 20% in a single flash, which is important for the electron transfer studies with cyt bc₁.

4. Measurement of Electron Transfer between Ru_z-39-Cc and Cytochrome bc₁

Flash photolysis is used to study electron transfer from cyt c₁ in the yeast cyt bc₁ complex to Ru_z-39-Cc in the forward, physiological direction (Engstrom et al., 2003). A Phase R model DL1400 flash lamp-pumped dye laser emitting a 480 nm light flash of < 0.5 μs duration is used as the excitation source. The reactions are studied in 300 μL samples containing 3 - 6 μM cyt bc₁, 3-10 μM Ru_z-39-Cc, 0.01% lauryl maltoside in 5 mM sodium phosphate, pH 7.0 and 0-800 mM NaCl. Cyt c₁ and Ru_z-39-Cc are reduced with 10 μM sodium ascorbate and 2 μM TMPD. The reaction is first studied at high ionic strength where there is no stable complex between Ru_z-39-Cc and cyt bc₁. Flash photolysis of 5.2 μM reduced yeast cyt bc₁ and yeast Ru_z-39-Cc in 260 mM ionic strength buffer leads to a decrease in the 550 nm absorbance, indicating rapid photooxidation of heme c Fe(II) by Ru(II*) in Ru_z-39-Cc (Figure 2). This is followed by an exponential increase in the 550 nm absorbance and decrease in the 557 nm absorbance with a rate constant of $3,900 \pm 600 \text{ s}^{-1}$, indicating electron transfer from cyt c₁ Fe(II) to heme Fe(III) in Ru_z-39-Cc (Figure 2). 557 nm is an isobestic point for Cc, allowing observation of the oxidation of cyt c₁. The very small difference between the absorbance spectra of cyt c₁ and Cc makes detection of the reaction between them difficult. The rate constant increased linearly with the concentration of cyt bc₁, indicating a bimolecular reaction between solution Ru_z-39-Cc and solution cyt bc₁ with a second order rate constant of $8.9 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$. Therefore, at the high ionic strength of 260 mM, there is no tight complex between Ru_z-39-Cc and cyt bc₁.

In order to study intracomplex electron transfer, flash photolysis is carried out on a stable 1:1 complex of 5.2 μM yeast Ru_z-39-Cc and 5.2 μM yeast cyt bc₁ in low 5mM ionic strength buffer. The rate constant for electron transfer from cyt c₁ to Ru_z-39-Cc, $14,000 \pm 3000 \text{ s}^{-1}$, is independent of protein concentration, indicating intracomplex electron transfer according to the top line of Scheme 4. The rate constant of this fast, intracomplex phase of electron transfer does not change as the ionic strength is increased to 110 mM (Figure 3). However, the amplitude of the intracomplex phase decreases to 60% and a new slow phase appears with an amplitude of 40% and a rate constant of 2500 s^{-1} . These results indicate that the complex begins to dissociate at 110 mM ionic strength, and the slow phase is due to bimolecular reaction between solution Ru_z-39-Cc and cyt bc₁. An equilibrium constant of $K_d = 0.85 \text{ μM}$ can be calculated from the relative amplitudes of the intracomplex and bimolecular phases. Since both intracomplex and bimolecular phases are present at 110 mM ionic strength, the bimolecular reaction involves formation of a 1:1 complex followed by intracomplex electron transfer according to Scheme 4 with $k_d \ll k_{et}$. The observed bimolecular phase rate constant is given by equation 2:

$$k_{obs} = k_f \left(E_o - \frac{1}{2} \left(\frac{k_d}{k_f + E_o + C_o} - \left(\frac{k_d}{k_f + E_o + C_o} \right)^2 - 4E_o C_o \right)^{1/2} \right) \quad (2)$$

where E_o is the concentration of cyt bc₁ and C_o is the concentration of Ru_z-39-Cc (41,44). The formation and dissociation rate constants from this equation are $k_f = 2.0 \times 10^9 \text{ s}^{-1}$ and $k_d = 1.7 \times 10^3 \text{ s}^{-1}$, using the relation $K_d = k_d/k_f$. The rate constant k_{obs} increases to a maximum of 5200 s^{-1} as the ionic strength is increased to 200 mM, and the fast intracomplex phase disappears, indicating complex dissociation (Figure 3). Assuming rapid equilibrium conditions apply to Scheme 4, the second-order rate constant is given by equation 3:

$$k_{2nd} = k_f k_{et} / (k_d + k_{et}) \quad (3)$$

The second-order rate constant decreases with increasing ionic strength above 250 mM, indicating a reaction between oppositely charged proteins (Figure 3). Since k_{et} remained unchanged from 5 to 100 mM ionic strength, it is likely that it also remains constant at higher ionic strength. The decrease in k_{2nd} with increasing ionic strength is therefore most likely due to a decrease in k_f and an increase in k_d . The results indicate that the mechanism occurs according to Scheme 4 at all ionic strengths. At low ionic strength electron transfer takes place within a preformed complex according to the top line of Scheme 4, while at high ionic strength uncomplexed Ru_z -39-Cc must first bind to cyt bc_1 before intracomplex electron transfer can take place.

5. Measurement of Electron Transfer within the Cytochrome bc_1 Complex

In order to understand the mechanism of bifurcated electron transfer in cyt bc_1 , it is important to determine the kinetics of each step in the reaction, as well as structural linkages between the conformation of the Q_o site and the conformation and dynamics of the Rieske iron-sulfur protein (ISP). We have developed a new ruthenium technique to measure electron transfer between the ISP and cyt c_1 for the first time (Sadoski et al., 2000; Engstrom et al., 2002; Xiao et al., 2003). The binuclear ruthenium complex Ru_2D has a charge of 4+, which allows it to bind with high affinity to the negatively charged binding domain on the surface of cyt c_1 as a surrogate for Cc (Scheme 2). In order to study electron transfer in the forward direction, the ruthenium dimer Ru_2D is used to rapidly photooxidize cyt c_1 in a solution containing 5 μM *Rsp.* cyt bc_1 with cyt c_1 and [2Fe2S] initially reduced with QH_2 , and cyt b_L and cyt b_H oxidized (Figure 4). The metal-to-ligand charge-transfer excited state of Ru_2D is a strong oxidant, and oxidizes cyt c_1 within 1 μs as indicated by the decrease in absorbance at 552 nm. A sacrificial electron acceptor A must be present in the solution to oxidize Ru^{II*} and/or Ru^I according to the mechanism shown in Scheme 3. The photooxidation of cyt c_1 Fe(II) may involve either the top or bottom pathway of Scheme 3. The sacrificial electron acceptor $[Co(NH_3)_5Cl]^{2-}$ rapidly decomposes following reduction, providing an irreversible reaction which is advantageous. The sacrificial electron acceptor paraquat is used in aerobic buffer, since photoreduced paraquat is rapidly oxidized by oxygen. Concentration dependence studies have indicated that Ru_2D binds to cyt bc_1 with a dissociation constant of about 10 μM , so Ru_2D concentrations of 20 μM are adequate for efficient photooxidation of cyt c_1 with a yield of 20% in a single flash.

Following rapid photooxidation by Ru_2D , cyt c_1 in *Rsp.* cyt bc_1 is subsequently reduced in a biphasic reaction with rate constants of $k_1 = 60,000 s^{-1}$ and $k_2 = 2,300 s^{-1}$, as indicated by the increase in absorbance at 552 nm (Figure 4, top trace). The fast phase k_1 is assigned to direct electron transfer from [2Fe2S] to cyt c_1 , since it is inhibited by stigmatellin, which locks the ISP in the b position near the Q_o site (38). The slow phase of reduction of cyt c_1 has the same rate constant as the reduction of cyt b_H observed in the 561-569 nm difference transient (Figure 4, bottom trace). These results indicate rate-limiting electron transfer from QH_2 to [2Fe2S] with rate constant k_2 followed by rapid electron transfer from [2Fe2S] to cyt c_1 , and from the semiquinone to cyt b_L and cyt b_H (Scheme 2). Electron transfer from the semiquinone to cyt b_L is very fast and has never been detected, while the rate constant for electron transfer from cyt b_L to cyt b_H is approximately $1 \times 10^4 s^{-1}$ (Shinkarev et al., 2001). One of the advantages of the ruthenium method is that it can be applied to cyt bc_1 from any organism. The rate constants for electron transfer in bovine cyt bc_1 have been found to be $k_1 = 16,000 s^{-1}$ and $k_2 = 250 s^{-1}$ (Sadoski et al., 2000).

An important question about the mechanism of cyt bc_1 is whether the fast phase of electron transfer from [2Fe2S] to cyt c_1 is rate-limited by pure electron transfer or instead by the rate of rotation of the ISP from the b position to the c_1 position in a conformational gating process. The best way to test this is to vary the driving force of the reaction, which depends on the difference in redox potentials of [2Fe2S] and cyt c_1 . If k_1 is rate-limited by true electron transfer

from [2Fe2S] to cyt c_1 , then Marcus theory would predict a large dependence on the driving force of the reaction. As an example, it was found that the rate constant was not affected by the ISP mutations Y156W, S154A, or Y156F/S154A which decrease the redox potential of [2Fe2S] by 62 mV, 109 mV, and 159 mV, respectively (Engstrom et al., 2002). This experiment provides evidence that the reaction is not rate-limited by electron transfer, since Marcus theory predicts that the increase in the driving force of the reaction for these mutants would increase the rate constant by up to 15-fold. It therefore appears that the rate constant k_1 for electron transfer from [2Fe2S] to cyt c_1 is rate limited by the rotation of the ISP from the b-state to the c_1 state (Scheme 2).

Ruthenium kinetics studies of the effects of Q_o site inhibitors on electron transfer in cyt bc_1 have revealed a crucial linkage between the conformations of the Q_o site and the ISP (Xiao et al., 2003). The Q_o site inhibitors stigmatellin and famoxadone have many similarities to the natural ubiquinol substrate, and lead to conformational changes in the Q_o pocket which stabilize the ISP in the b state. X-ray crystallographic studies have shown that both inhibitors displace the cd1 helix and the PEWY sequence in the ef helix outward to expand the Q_o pocket (Esser et al., 2006). These changes in the Q_o pocket are relayed to the surface of cyt b to form a docking crater which captures the ISP in the b-state conformation. Stigmatellin forms a hydrogen bond with the His-161 ligand of the reduced [2Fe2S] center, thereby increasing its redox potential by 250 mV and immobilizing the ISP in the b conformation (Zhang et al., 1998; Iwata et al., 1998). Famoxadone binds somewhat deeper in the Q_o pocket than stigmatellin, does not contact the ISP or form a hydrogen bond with His-161, and only increases the redox potential of [2Fe2S] by 30 mV (Gao et al., 2002). Famoxadone binding to *Rsp.* cyt bc_1 decreases the rate of forward electron transfer from [2Fe2S] to cyt c_1 from $60,000\text{ s}^{-1}$ to $4,800\text{ s}^{-1}$ (Xiao et al., 2003). This indicates that famoxadone does not completely lock the ISP in the b state, but instead decreases the rate of release of the ISP from the b state to the c_1 state to $4,800\text{ s}^{-1}$. The linkage between the conformation of the Q_o site and the conformation and dynamics of the ISP could be a key to how the enzyme promotes bifurcated electron transfer from QH_2 to [2Fe2S] and cyt b_L , and minimizes short-circuit reactions such as delivery of both electrons to the ISP.

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Abbreviations

Cc, cytochrome c
 cyt bc_1 , cytochrome bc_1
 [2Fe2S], Rieske iron-sulfur center
 ISP, iron-sulfur protein
 bpy, 2,2'-bipyridine
 dmb, 4,4'-dimethyl-2,2'-bipyridine
 bpz, 2,2'-bipyrazine
 bpd, 3,3'-bipyridazine
 qpy, 2,2':4',4'':2'',2'''-quaterpyridine
 Ru₂D, [Ru(bpy)₂]₂qpy⁴⁺
 Ru₂Z, [Ru(bpz)₂]₂qpy⁴⁺
 Rsp., Rhodobacter sphaeroides

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A

B

Figure 1.

Photoinduced electron transfer within yeast Ru_Z-39-Cc. A: A solution of reduced 5 μM Ru_Z-39-Cc in 5 mM sodium phosphate, pH 7.0 was photoexcited with a 356 nm Nd-Yag laser flash, and the absorbance at 434 nm and 550 nm was monitored. B: The same sample as in A at a longer time scale in the presence and absence of atmospheric oxygen.

Figure 2.

Photoinduced electron transfer between yeast Ru_z-39-Cc and yeast cyt *bc*₁. A solution containing reduced 5.2 μ M yeast Ru_z-39-Cc and yeast cyt *bc*₁ in 5 mM sodium phosphate, pH 7.0, 250 mM NaCl, and 0.1% lauryl maltoside was photoexcited with a 480 nm laser flash. The 550 nm transient shows Cc photooxidation and reduction, while the 557 nm transient shows cyt *c*₁ oxidation.

Figure 3.

Ionic strength dependence of photoinduced electron transfer between yeast Ru_Z-39-Cc and yeast cyt *bc*₁. The reaction was measured under the same conditions as in Figure 3 with 0 to 800 mM NaCl.

Figure 4.

Photoinduced electron transfer within wild-type *R. sphaeroides* cyt bc₁. A sample containing 5 μ M cyt bc₁, 20 μ M Ru₂D, 5 mM [Co(NH₃)₅Cl]²⁺, in 20 mM sodium borate, pH 9.0 with 0.01% dodecylmaltoside was treated with 10 μ M Q_oC₁₀BrH₂, 1 mM succinate, and 50 nM SCR to reduce [2Fe2S] and cyt c₁, and reduce cyt b_H by about 30%. Excitation of Ru₂D with 480 nm laser flash led to photooxidation of cyt c₁ within 1 μ s, followed by reduction in a biphasic reaction with rate constants of 60,000 s⁻¹ and 2,000 s⁻¹ as shown in the 552 nm transient. The 561 - 569 nm transient shows reduction of cyt b_H with a rate constant of 2,300 s⁻¹.

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Table 1
Standard reduction potentials of ruthenium complexes

Complex	(II)/(III)	(II*)/(III)	(II)/(I)	(II*)/(I)
Ru(bpy) ₃	1.27	-0.87	-1.31	0.83
Ru(bpy) ₂ (dmb)	1.27	-0.83	-1.36	0.79
Ru(bpz) ₂ (dmb)	1.76	-0.25	-0.79	1.22
Ru(bpd) ₂ (dmb)	1.49	-0.49	-1.00	0.98