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Electron Transfer in Reaction Center Core Complexes from the Green Sulfur Bacteria *Prosthecochloris aestuarii* and *Chlorobium tepidum*[†]

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ABSTRACT: Electron transfer in reaction center core (RCC) complexes from the green sulfur bacteria *Prosthecochloris aestuarii* and *Chlorobium tepidum* was studied by measuring flash-induced absorbance changes. The first preparation contained approximately three iron−sulfur centers, indicating that the three putative electron acceptors F_X, F_A, and F_B were present; the *Chl. tepidum* complex contained on the average only one. In the RCC complex of *Ptc. aestuarii* at 277 K essentially all of the oxidized primary donor ($P840^+$) created by a flash was rereduced in several seconds by *N*-methylphenazonium methosulfate. In RCC complexes of *Chl. tepidum* two decay components, one of 0.7 ms and a smaller one of about 2 s, with identical absorbance difference spectra were observed. The fast component might be due to a back reaction of $P840^+$ with a reduced electron acceptor, in agreement with the notion that the terminal electron acceptors, F_A and F_B, were lost in most of the *Chl. tepidum* complexes. In both complexes the terminal electron acceptor (F_A or F_B) could be reduced by dithionite, yielding a back reaction of 170 ms with $P840^+$. At 10 K in the RCC complexes of both species $P840^+$ was rereduced in 40 ms, presumably by a back reaction with F_X^-. In addition, a 350 μs component occurred that can be ascribed to decay of the triplet of P840, formed in part of the complexes. For $P840^+$ rereduction a pronounced temperature dependence was observed, indicating that electron transfer is blocked after F_X at temperatures below 200 K.

The reaction center core (RCC)[1] complex of green sulfur bacteria is an intrinsic membrane protein that contains the photosynthetic reaction center together with a number of antenna pigments, the so-called core antenna. Like the corresponding complex of heliobacteria (*1*), it resembles the core of photosystem I (PS I) of green plants (*2*). However, the bacterial complexes contain fewer antenna pigments than PS I, which is one reason that makes them attractive for spectroscopic studies of energy and electron transfer (*3*).

Recently fast procedures have been developed for isolating photoactive RCC complexes from the green sulfur bacteria *Prosthecochloris aestuarii* and *Chlorobium tepidum* (*4*, *5*). Both complexes are devoid of the Fenna−Matthews−Olson (FMO) protein and show a high photochemical activity. They contain two copies of the main reaction center protein, PscA, and one of the F_A-F_B iron−sulfur protein PscB; the RCC complex from *Chl. tepidum* contains additionally the PscC cytochrome c_{551}. From the amplitude of its α-band, a cytochrome c_{551} content of 1.6 molecules/RCC complex was estimated (*5*). From these numbers, the molecular masses of the RCC complexes are calculated to be 188 and 225 kDa for *Ptc. aestuarii* and *Chl. tepidum*, respectively.

The composition and optical properties of these RCC complexes were described in detail by Permentier et al. (*5*). The RCC complex contains 16 bacteriochlorophylls (BChls) *a* (*6*), two of which constitute the primary electron donor P840. In addition, both RCC complexes contain four molecules of the acceptor pigment chlorophyll (Chl) *a* 670 (*5*, *6*), recently identified as Chl *a* esterified with Δ2,6-phytadienol (*7*). Like in PS I (*8*), the electron acceptor chain presumably contains three 4Fe-4S centers, F_X, F_A, and F_B (*9*). EPR experiments showed a light-induced reduction of quinones in some preparations and in analogy to PS I menaquinone was proposed to function as secondary electron acceptor in green sulfur bacteria (*10*−*12*). However, none of our RCC preparations contained significant amounts of quinones (*5*, *13*), indicating that a quinone acceptor is not needed for a stable charge separation.

So far, only little is known about the kinetics of photosynthetic electron transfer in green sulfur bacteria. The trapping of excitation energy by formation of $P840^+ A_0^-$ occurs with a time constant of about 25 ps (*14*−*17*). The subsequent electron transfer from A_0 to a secondary electron acceptor was reported to occur in 600 ps (*16*, *18*). For the

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[1] Abbreviations: A_0, primary electron acceptor; A_1, secondary electron acceptor; (B)Chl, (bacterio)chlorophyll; *Chl.*, *Chlorobium*; F_X, F_A, and F_B, iron−sulfur centers; FMO protein, Fenna−Matthews−Olson protein; P840, primary electron donor; *Ptc.*, *Prosthecochloris*; PMS, *N*-methylphenazonium methosulfate; PS I, photosystem I; RCC, reaction center core.

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kinetics of charge recombination between $P840^+$ and reduced F_X various time constants were reported, ranging from 70 μs (*19*) to 14 ms or more (*9*). The time constants reported for charge recombination between $P840^+$ and the terminal electron acceptors, F_A and F_B, also vary substantially, with values of 10 ms (*19*), 55 ms (*20*), and 580 ms or more (*9*). Major difficulties in analyzing recombination kinetics are the possible interference with triplet decays as well as the occurrence of multiple recombination processes due to sample inhomogeneity. Part of these difficulties may be overcome by spectral analysis of the kinetic components.

This paper describes a study of flash-induced absorbance changes in the red and near-infrared region of RCC complexes of *Ptc. aestuarii* and *Chl. tepidum*. The rereduction kinetics of $P840^+$ were studied both at room and at cryogenic temperature as well as under reducing conditions, resulting in an improved model for the kinetics of electron transfer in RCC complexes of green sulfur bacteria.

MATERIALS AND METHODS

RCC complexes of *Ptc. aestuarii* and *Chl. tepidum* were isolated as described by Francke et al. (*4*), with a slight modification as described by Permentier et al. (*5*). For optical measurements the samples were diluted with a buffer containing 20 mM potassium phosphate (pH 6.5) and 2.5 mM Triton X-100. Unless indicated differently, 20 mM sodium ascorbate and 100 μM *N*-methylphenazonium methosulfate (PMS) were added to keep P840 reduced in the dark. Glycerol (66% v/v) was added to obtain clear samples at low temperature.

The non-heme iron content of the complexes was assayed by a color reaction with bathophenanthroline following the procedure described by Doeg and Ziegler (*21*). Iron powder dissolved in HCl (37%) was used for calibration (*22*). In control experiments the same analysis was performed with horse heart cytochrome *c* (Sigma) and spinach ferredoxin (Sigma) to ensure that only non-heme iron and not the total iron was assayed.

Absorption spectra, with a spectral resolution of 1 nm, were measured on a single-beam spectrophotometer (*5*). Flash-induced absorption changes in the microsecond to second time range were measured with the apparatus described by Franken and Amesz (*23*). Excitation flashes were provided by a frequency-doubled Q-switched Nd–YAG laser (532 nm, 15 ns pulse duration, up to 90 mJ/pulse). Transient absorption difference measurements in the (sub)-picosecond time range were performed as described by Neerken et al. (*15*). Pump and probe pulses were polarized at the magic angle with respect to each other. Measurements at cryogenic temperature were performed with a helium flow cryostat (Utrecs-LSO, Estonia).

RESULTS

Composition of RCC Complexes. To obtain information on the presence of Fe–S centers in our preparations, the content of non-heme iron was determined in the RCC complexes from both species. The results are summarized in Table 1. For *Ptc. aestuarii* 11.0 ± 4.3 non-heme irons/RCC complex were found, indicating an average number of 2.8 ± 1.1 Fe–S centers/complex [assuming 4Fe-4S clusters (*24*)]. The iron content of the *Chl. tepidum* RCC complex was considerably lower: only 4.1 ± 0.4 non-heme irons were found per RCC complex, corresponding to only 1 Fe–S center/complex. We conclude that for *Ptc. aestuarii* most of the RCC complexes still contain all three Fe–S centers, F_X, F_A, and F_B. In contrast, the *Chl. tepidum* RCC complex contains on the average only one Fe–S center (most likely F_X), whereas the terminal Fe–S centers F_A and F_B are probably lost from most complexes during the isolation procedure.

Table 1: Non-Heme Iron Content of the RCC Complexes from *Ptc. aestuarii* and *Chl. tepidum*

	Fe^a (μM)	total protein $content^b$ (μg/mL)	Fe/RCC^c (mol/mol)
Ptc. aestuarii	6.6 ± 2.6	113	11.0 ± 4.3
Chl. tepidum	2.7 ± 0.2	147	4.1 ± 0.4

[a] For samples with $A = 1\ cm^{-1}$ at the Q_y maximum. [b] For samples with $A = 1\ cm^{-1}$ at the Q_y maximum, as determined by Permentier et al. (*5*). [c] Assuming molecular masses of 188 and 225 kDa for the RCC complexes from *Ptc. aestuarii* and *Chl. tepidum*, respectively.

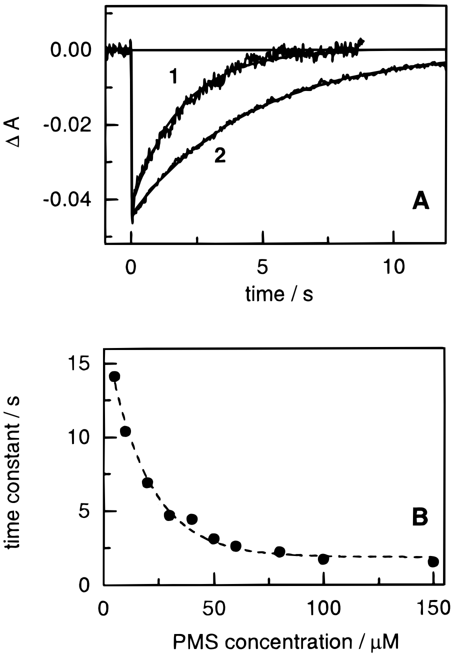

FIGURE 1: (A) Kinetics of flash-induced absorbance changes at 833 nm measured at 277 K in RCC complexes from *Chl. tepidum* (1) and *Ptc. aestuarii* (2) in the presence of 20 mM ascorbate and 100 μM PMS. Both traces were normalized for the same initial absorbance change and are the average of 10 recordings, with a low-pass filter to cut off frequencies above 100 Hz. The smooth lines represent monoexponential fits with the time constants mentioned in the text. (B) Time constant of $P840^+$ rereduction in *Ptc. aestuarii* RCC complexes as a function of the PMS concentration. The medium also contained 20 mM ascorbate (pH 6.5). The time constants were obtained from monoexponential fits of the decay kinetics measured at 833 nm, like those shown in panel A.

Room-Temperature Absorbance Changes. At 277 K, the RCC complexes of both species showed the same flash-induced absorption difference spectrum (except for the amplitude) and, at least in part, similar kinetics. For the *Ptc. aestuarii* RCC complex the spectrum, which is due to photooxidation of P840, has been published earlier (*4*). Kinetics of the absorbance changes at 833 nm induced by saturating laser flashes (532 nm) are shown in Figure 1A. Both preparations showed an immediate bleaching, followed by a very slow exponential decay with a time constant of several seconds. Monoexponential fits of the decay kinetics yielded time constants of 4 and 2 s for the RCC complexes of *Ptc. aestuarii* and *Chl. tepidum*, respectively. The complex from *Chl. tepidum* in addition showed a much faster decay component (see below).

The time constant of the kinetics was dependent on the PMS concentration in the medium (Figure 1B). This dependence indicates that the observed decay is caused by the (slow) reaction of P840$^+$ with reduced PMS and not by a back reaction with a reduced electron acceptor, suggesting that the electrons produced in the charge separation are rapidly taken up by the medium. Without PMS, no rereduction of P840$^+$ could be observed at all. Addition of the artificial electron acceptor methyl viologen (100 μM) did not affect the rate of P840$^+$ rereduction.

Recently, reduced neutral red was reported to be a very efficient electron donor in membranes of heliobacteria (*25*). In experiments with RCC complexes from *Ptc. aestuarii* we observed much faster P840$^+$ rereduction kinetics, with a time constant of 15 ms instead of several seconds, when PMS was replaced by 100 μM neutral red reduced with 10 mM sodium dithionite (pH 8.0). As in the experiments with PMS, the time constant of the decay was dependent on the concentration of the electron donor in the medium. In an attempt to bring about photoaccumulation of the reduced terminal electron acceptor, RCC complexes were excited with multiple flashes spaced by 100 ms (using neutral red and dithionite to rereduce P840$^+$). Even after five saturating flashes the kinetics and the amount of P840$^+$ produced in a flash were unchanged; i.e., no photoaccumulation of reduced electron acceptors was achieved. Therefore we conclude that electron transfer to the medium occurs in less than 100 ms.

In addition to the 2 s decay component described above, a prominent component with a time constant of 0.7 ms was found in RCC complexes of *Chl. tepidum* (Figure 2A). Such a fast component was absent in the preparation from *Ptc. aestuarii*. The relative amplitudes of the 0.7 ms and the 2 s component were approximately 0.7:0.3. The difference spectra of the fast and the slow component (Figure 2B) were essentially the same. Thus we conclude that the 0.7 ms component can be ascribed to rereduction of P840$^+$. Unlike the 2 s component, the time constant of the 0.7 ms component was independent of the concentration of PMS in the medium. We therefore conclude that the 0.7 ms component may be due to a back reaction between P840$^+$ and a reduced electron acceptor rather than to rereduction by PMS. In view of the absence of the terminal Fe−S centers in most of the *Chl. tepidum* RCC complexes, such a back reaction seems very likely. The time constant and amplitude of the 0.7 ms component did not change significantly upon addition of 20 mM dithionite (pH 10.5) or 0.5−5 mM methyl viologen nor upon replacement of PMS with neutral red and dithionite. For the 2 s component the effect of 20 mM dithionite (pH 10.5) or of neutral red reduced with dithionite was essentially the same as described elsewhere in the text for RCC complexes from *Ptc. aestuarii*.

Secondary Electron Transfer. Another difference between the RCC complex isolated from *Ptc. aestuarii* and the one from *Chl. tepidum* is the presence of cytochrome c_{551} in the latter (*5*). Cytochrome c_{551} was found to act as electron donor for P840$^+$ in reaction center preparations from *Chl. tepidum* (*20*, *26*). If no PMS but only 20 mM sodium ascorbate was added to the RCC complexes occasionally absorbance changes of the cytochrome were observed. In these measurements a small bleaching occurred at 551 nm, which developed with a time constant of approximately 1.5 ms and decayed on a time scale of seconds. The same time constant of ∼1.5 ms was observed in the decay of P840$^+$. A much faster oxidation of cytochrome c_{551} with time constants of 25−90 μs has been reported for other preparations (*20*, *23*, *27*−*29*). After a few flashes the signals at 551 and 833 nm both started to decrease and finally disappeared completely. Taken together, these observations indicate that in our preparation cytochrome c_{551} rapidly becomes inactive and does not act as an efficient electron donor for P840$^+$.

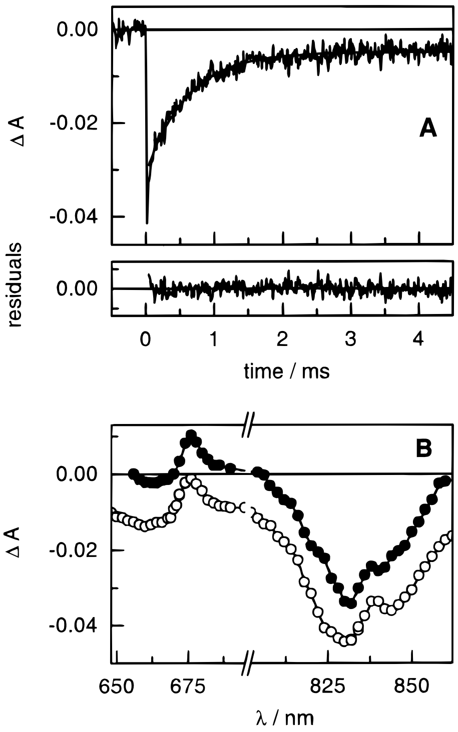

FIGURE 2: Fast component observed in RCC complexes from *Chl. tepidum* at 277 K. (A) Kinetics of flash-induced absorbance changes detected at 833 nm with microsecond time resolution. The experimental trace was the average of 30 recordings measured at intervals of 2 s, with a low-pass filter to cut off frequencies above 10 kHz. The initial spike is a fluorescence artifact. The smooth line shows a monoexponential fit with a time constant of 0.7 ms together with a component that did not decay in the millisecond range and is responsible for the 2 s decay shown in Figure 1A. The residuals of the fit are given in the lower graph. (B) Absorbance difference spectrum of the 0.7 ms component obtained by plotting the amplitude of the kinetic component as a function of the wavelength (●). For each point 10−20 recordings were averaged. For comparison, the absorption difference spectrum of the 2 s decay component (Figure 1A) is also shown (○). The spectrum is plotted with an offset and was normalized to that of the 0.7 ms component at 833 nm.

To chemically reduce the terminal Fe−S centers, sodium dithionite was added to the RCC complexes. As shown in Figure 3, after addition of 20 mM dithionite (pH 10.5), biphasic decay kinetics with time constants of 170 ms and 2 s occurred in *Ptc. aestuarii* RCC complexes. The relative amplitudes of the two phases were dependent on the pH of the medium as well as on the preparation. Both phases had very similar absorbance difference spectra. These results most likely indicate that in part of the reaction centers the terminal

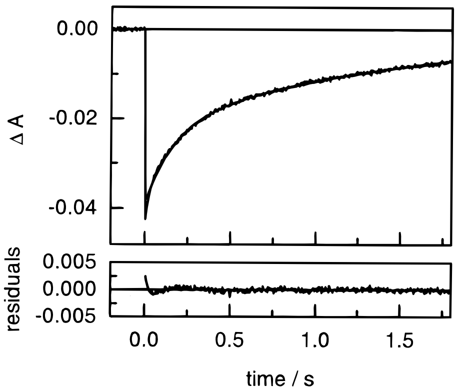

FIGURE 3: Kinetics of flash-induced absorbance changes at 833 nm in *Ptc. aestuarii* RCC complexes after addition of 20 mM dithionite (pH 10.5). The smooth line represents a fit with two exponential decay components with time constants of 170 ms and 2 s and a relative amplitude of 0.45 for the fast phase. Residuals of the fit are plotted in the lower graph. The experimental trace is the average of seven recordings measured at intervals of 25 s. A low-pass filter was used to cut off frequencies above 1 kHz.

electron acceptor was chemically reduced by dithionite and $P840^+$ was rereduced by a back reaction with the preceding electron acceptor (probably F_A or F_B). The partial chemical reducibility of the terminal electron acceptor indicates that its midpoint redox potential is near −620 mV (the midpoint potential of sodium dithionite at pH 10.5). From redox titrations of membrane preparations, midpoint potentials of −550 to −600 mV were reported for the terminal Fe−S centers in green sulfur bacteria (*24*, *30*, *31*).

Low-Temperature Absorbance Changes. Biphasic decay kinetics were observed at low temperature (10 K) for *Chl. tepidum* RCC complexes. This is illustrated by Figure 4. The slower component (A) could be fitted with a time constant of 44 ms and the faster one (B) with a time constant of 360 μs. The ratio of the amplitudes of the fast and the slow component was approximately 0.3:0.7, depending somewhat on the preparation. Essentially the same biphasic kinetics (350 μs and 40 ms) were observed in measurements with RCC complexes from *Ptc. aestuarii*, as already reported by Francke et al. (*4*), but with a lower relative amplitude of the 350 μs component (0.2:0.8). The time constants of both the slow and the fast component were independent of the concentration of PMS in the medium.

The absorption difference spectra of the 40 ms components observed in RCC complexes from *Ptc. aestuarii* and *Chl. tepidum* are shown in Figure 5A. These spectra can be ascribed to photooxidized $P840^+$. With a time constant of approximately 40 ms its rereduction was much faster than at 277 K, indicating the presence of a back reaction, presumably with the reduced Fe−S center F_X^- (*19*, *32*), at low temperature. In agreement with this interpretation, Vassiliev et al. (*33*) observed in EPR measurements that the electron transfer from F_X to F_A/F_B was blocked at temperatures below 200 K.

In both species the difference spectrum displays a maximal bleaching at 837 nm, which is probably due to the bleaching of P840, plus a multitude of bands in the Q_y region of both BChl *a* and Chl *a* 670, indicating electrochromism of the neighboring BChl *a* molecules. The difference spectra obtained from the complexes of the two species are nearly identical in the Chl *a* 670 region but they clearly differ in the BChl *a* Q_y region (790−830 nm). This indicates that there are structural differences in the environment of the BChls *a*, whereas the environment of the Chl *a* 670 molecules seems to be highly conserved. The same conclusion also follows from the low-temperature absorption, LD, and CD spectra (*5*).

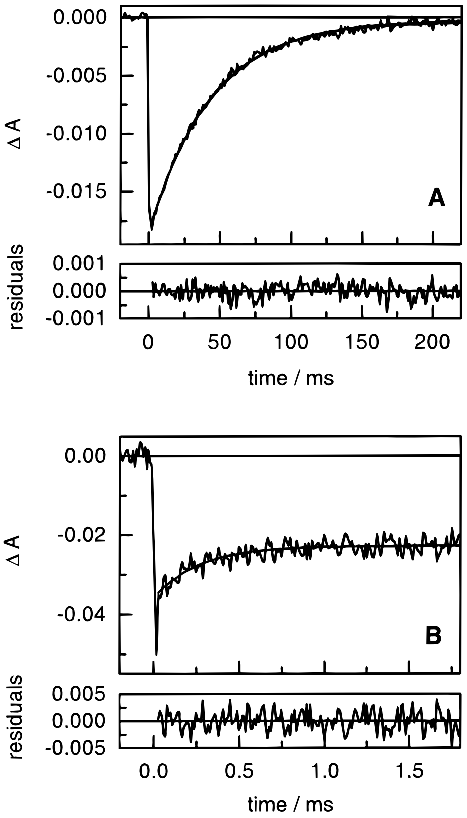

FIGURE 4: Kinetics of flash-induced absorbance changes observed in *Chl. tepidum* RCC complexes at 10 K. (A) Decay kinetics of the slow component detected at 837 nm. The experimental trace is the average of 25 recordings measured at intervals of 1 s, with a low-pass filter to cut off frequencies above 1 kHz. The smooth line shows a fit with a monoexponential decay constant of 44 ms. (B) Decay kinetics of the fast component detected at 837 nm. The experimental trace is the average of 30 recordings measured at intervals of 0.5 s, with a low-pass filter to cut off frequencies above 10 kHz. The smooth line represents a fit with a monoexponential decay constant of 360 μs together with a component that is constant on this time scale and corresponds to the 44 ms component. The initial spike is a fluorescence artifact. Residuals of the fits are plotted in the lower graphs. The samples contained 20 mM ascorbate, 100 μM PMS, and 66% glycerol.

Figure 5B shows the absorbance difference spectra of the 350 μs component. The difference spectra are nearly identical for *Ptc. aestuarii* and *Chl. tepidum*. With a main bleaching at 837 nm and only minor absorption changes in the Q_y region of Chl *a* 670, these spectra are probably due to formation of the triplet of P840 in some of the RCC complexes (*34*). Apparently electron transfer to the terminal acceptors is blocked in part of the complexes at low temperature, resulting in formation of a triplet state by the radical pair mechanism. The 350 μs decay component then reflects the decay of the triplet to the singlet state (*34*, *35*). In *Ptc. aestuarii* RCC complexes reduced with 20 mM dithionite the relative amplitude of the 350 μs component was about 3.5 times larger than without dithionite. For PS I it has been argued that the radical pair mechanism for triplet formation may involve A_0 as well as the secondary electron

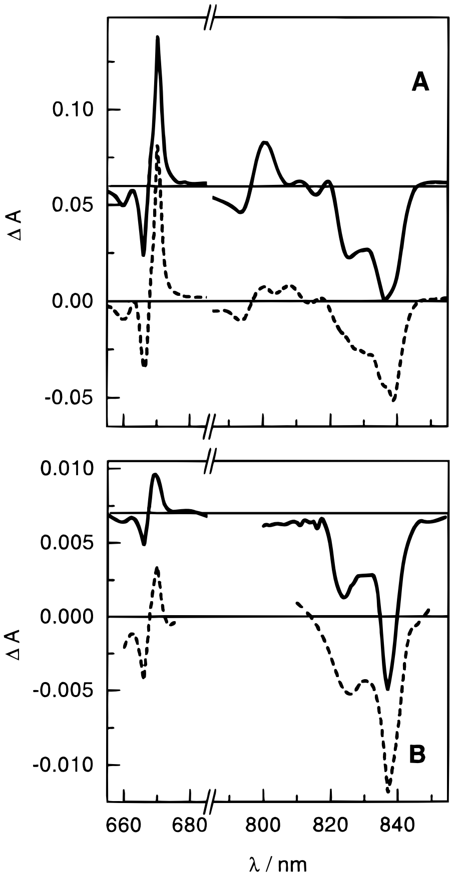

FIGURE 5: Low-temperature absorbance difference spectra of RCC complexes of *Ptc. aestuarii* (—) and *Chl. tepidum* (- - -). (A) Slow component (~40 ms). The spectrum of *Ptc. aestuarii* was plotted with an offset for clarity. (B) Fast component (~350 μs). The spectrum of *Ptc. aestuarii* was plotted with an offset. The spectra were recorded with a spectral resolution of 2 nm. In both panels the spectra were normalized to the spectrum of *Ptc. aestuarii* at 837 nm.

acceptor, A_1 (*8*). Since the extent of coupling between P840$^+$ and A_0^- is not known, it is not clear which mechanism applies to green sulfur bacteria. A second Chl *a* 670 molecule might function as secondary electron acceptor A_1; as discussed by Schmidt and Trissl (*16*) and Permentier et al. (*5*), one might envisage that two molecules of Chl *a* 670 are arranged in series.

Absorption difference spectra of P840 photooxidation at low temperature were also obtained by photoaccumulation of P840$^+$. The steady-state absorption spectrum of the RCC complexes was first recorded in the dark at 6 K in the presence of 50 μM PMS and 10 mM ascorbate. Then the sample was warmed to 130−150 K and cooled in 20−30 min again to 6 K under illumination with strong white light. Subsequently the absorption spectrum of the illuminated sample was recorded in the dark again, and the difference spectrum was obtained by subtracting the first from the second spectrum. The results are shown in Figure 6 for both RCC preparations. The difference spectra, especially that of *Ptc. aestuarii*, resemble those of the 40 ms component obtained with flash excitation (Figure 5A) and there is little doubt that they are at least largely due to photoaccumulation of P840$^+$. For both preparations the amplitude of the signal was nearly the same as with a saturating flash (see Table 2 and below).

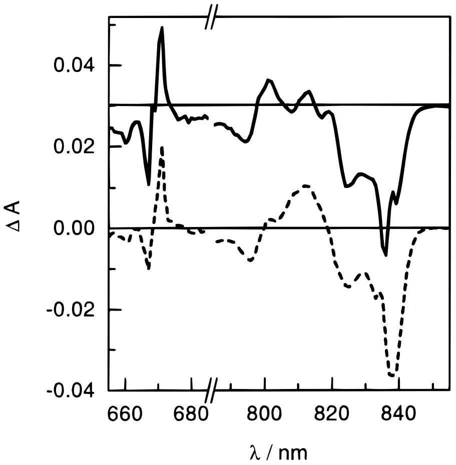

FIGURE 6: Low-temperature absorbance difference spectra obtained by photoaccumulation of P840$^+$ in RCC complexes of *Ptc. aestuarii* (—) and *Chl. tepidum* (- - -). The spectra were recorded with a spectral resolution of 1 nm. The spectrum of *Chl. tepidum* was normalized to the one of *Ptc. aestuarii* at 837 nm. The spectrum of *Ptc. aestuarii* was plotted with an offset.

Table 2: Photochemical Activities of RCC Complexes from *Ptc. aestuarii* and *Chl. tepidum*[a]

	T (K)	$\Delta A/A_{836}$[b] (flash)	$\Delta A/A_{836}$[c] (continuous)
Ptc. aestuarii RCC	277	0.087	0.093
Chl. tepidum RCC	277	0.041	
Ptc. aestuarii RCC	10	0.092	0.071
Chl. tepidum RCC	10	0.074	0.064

[a] As a measure for the relative photochemical activity, the bleaching, ΔA, at 840 nm (277 K) or 837 nm (10 K), divided by the absorbance in the long-wavelength BChl *a* Q_y maximum near 836 nm, A_{836}, was used (see text). [b] Absorbance changes induced by a saturating laser flash (15 ns) at 532 nm, as measured by the combined amplitudes of the decay components. [c] Absorbance changes induced by saturating continuous illumination (277 K) or by photoaccumulation of P840$^+$ upon cooling (10 K). At 277 K the bleaching could not be determined in the *Chl. tepidum* complex because of the rapid decay component in this preparation.

Temperature Dependence. Figure 7 shows the temperature dependence of the flash-induced absorption decay kinetics for the slow component (A) and for the fast component (B). For P840$^+$ rereduction a pronounced temperature dependence of the decay kinetics was observed (Figure 7A). Between 10 and 170 K the 40 ms time constant for both RCC complexes (●, ○) increased slowly with increasing temperature, reaching a value of 60 ms at 170 K. At the same time, its relative amplitude increased from about 0.8 to 0.9 in *Ptc. aestuarii* and from about 0.7 to 0.9 in *Chl. tepidum*. Between 170 and 200 K a much more rapid increase of the decay time occurred to a value of 100 ms, which remained approximately constant up to 230 K. The decay of P840$^+$ became biphasic above 230 K, and in addition to the 100 ms time constant a much slower component with a time constant of about 2 s was observed. The relative amplitude of this slower component increased with increasing temperature. It reached a value of 0.7 at 240 K, while at 277 K the kinetics of P840$^+$ rereduction were monophasic again with a time constant of 2 s. The temperature dependence of *Ptc. aestuarii* RCC complexes reduced with 20 mM dithionite (pH 10.5) (△) was essentially the same in the range of 10−230 K. However, in these complexes the 2 s component was

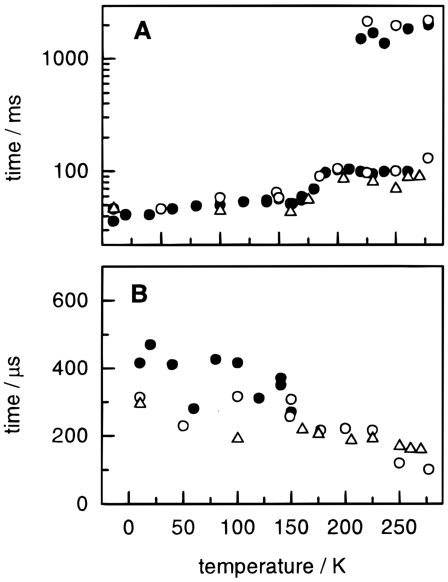

FIGURE 7: Decay constants of flash-induced absorption changes observed in RCC complexes of *Ptc. aestuarii* (●), and *Chl. tepidum* (○) and in RCC complexes of *Ptc. aestuarii* reduced with 20 mM dithionite (pH 10.5) (△) as a function of the temperature. The samples contained 20 mM ascorbate, 100 μM PMS, and 66% glycerol. (A) Time constants of the slow decay component (P840$^+$ rereduction). Above 225 K the decay of P840$^+$ was biexponential. (B) Time constants of the fast component (decay of the P840 triplet). The time constants were obtained from fits of the decay kinetics measured at 837 nm.

not observed and P840$^+$ was rereduced monophasically with a time constant of about 90 ms over the whole temperature range of 200−270 K.

The complex temperature dependence of the kinetics indicates that more than only one process is involved in rereduction of P840$^+$. At low temperature, electron transfer is presumably blocked after F_X (see above). The occurrence of the 100 ms phase above 180 K, which may correspond to the 170 ms component observed at 277 K with dithionite, might suggest that at this temperature electron transfer can progress from F_X to one of the terminal Fe−S centers, F_A or F_B, and P840$^+$ would be rereduced by a back reaction with this electron acceptor. Alternatively, this change in the kinetics might be associated with the phase transition of glycerol from an amorphous glass to a liquid phase (*36*). The appearance of the very slow rereduction component at temperatures higher than 230 K indicates that at these temperatures the electrons are transferred to the medium and therefore P840$^+$ is rereduced by PMS. In agreement with this interpretation, the slow component was absent after addition of dithionite, i.e., upon chemical reduction of the terminal electron acceptor.

The decay of the BChl *a* triplet formed in some of the complexes became faster with increasing temperature, reaching a time constant of about 100 μs at 277 K (Figure 7B), virtually the same as observed earlier at room temperature (*37*). The amplitude of the triplet signal decreased significantly with increasing temperature (data not shown). In the *Ptc. aestuarii* RCC complex at temperatures above 150 K, the relative amplitude of the triplet signal was less than 0.1 and hardly any triplet signal could be detected. For *Chl. tepidum* a similar decrease of the triplet signal from a relative amplitude of about 0.3 at 10 K to about 0.1 at temperatures higher than 185 K was observed.

Photochemical Activity. On the basis of the above observations, we are now in a position to compare the relative photochemical activities of the two preparations at 277 and 10 K. Such a comparison makes only sense if the differences in the absorption spectra, especially at low temperature, are taken into account. We therefore compared the P840 bleaching (ΔA, at 840 and 837 nm at 277 and 10 K, respectively) with the absorbance near 836 nm in the maximum of the long-wavelength BChl *a* Q_y absorbance band (A_{836}). At 10 K for both RCC complexes at this wavelength the extinction coefficient of BChl *a* is 116 mM^{-1} cm^{-1} (*5*). At 277 K the extinction coefficients at 836 nm are 66 and 61 mM^{-1} cm^{-1} for *Ptc. aestuarii* and *Chl. tepidum*, respectively. It is not clear if this difference is significant, but when comparing activities, one should keep in mind that those for *Chl. tepidum* at 277 K may have to be lowered by about 8%.

The results are summarized in Table 2. From the experiments with saturating laser flash excitation at 277 K we obtain a value of 0.087 for the *Ptc. aestuarii* RCC complex, which is close to the number of 0.093 obtained with continuous illumination (Table 2; *4*). The activity of the *Chl. tepidum* RCC complex, taking the combined amplitudes of the 0.7 ms and the 2 s component, was about 50% less, at least at 277 K, with $\Delta A/A_{836} = 0.041$. The relative photochemical activity of the RCC complex of *Ptc. aestuarii* with saturating laser flash excitation at 10 K (combining the amplitudes for P840$^+$ and for triplet formation) was about the same as at 277 K, assuming that the absorption band and the absorption difference signal show the same temperature dependence. The *Chl. tepidum* RCC complex showed again less activity, but the difference appeared to be smaller at 10 K than at 277 K. In the low-temperature photoaccumulation measurements the difference was even smaller. For the RCC complex of *Ptc. aestuarii* the absolute photochemical activity, i.e., the fraction of active reaction centers, at 277 K may be close to 100%, as indicated by the good agreement of the number of 16 BChl *a* molecules per RCC obtained by pigment analysis (*6*) with the ratio of 17 BChls *a* per P840 estimated by Francke et al. (*4*) from the light-induced bleaching.

An estimate of the relative photochemical activity in the RCC complexes of both species at 277 K was also obtained by comparing the amplitudes of the absorbance changes due to the formation of excited states with those due to photooxidized P840. Figure 8A,B shows some transient absorbance difference spectra of the RCC complex of *Chl. tepidum* in the femto- and picosecond time region, obtained upon excitation with (nonsaturating) 300 fs pulses at 800 nm. A negative band at 816−817 nm was rapidly formed, but already at 0.3 ps after the pulse a band at 835 nm was generated, which quickly developed into the largest one of the spectrum and reached a maximum after about 1 ps. Additional bands were seen at 820 and 804 nm, the latter being due to excited-state absorption. The amplitude of the negative band at 835 nm subsequently decreased, the excited-state absorption band disappeared, and the resulting spectrum, which was constant in the time range of 150−1200 ps, may be ascribed to P840$^+$. Similar results were obtained with *Ptc. aestuarii* [not shown; see also Neerken et al. (*15*)] and with an FMO-containing preparation from *Chl. tepidum* (*17*).

The amplitude of the bleaching due to P840$^+$, averaged in the time range of 300−500 ps, was divided by the

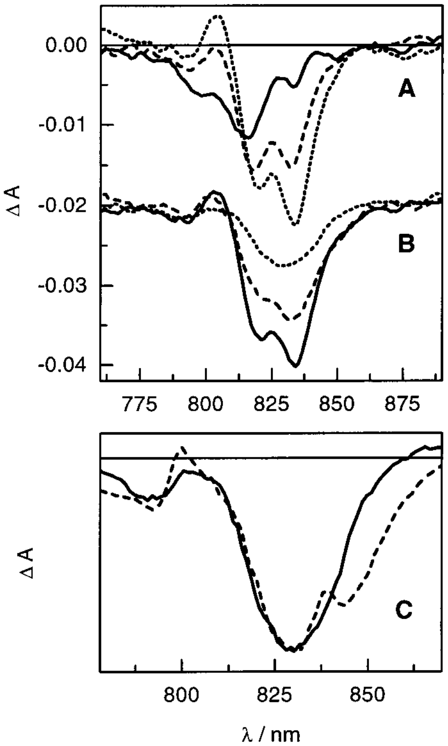

FIGURE 8: Time-resolved absorbance difference spectra of the *Chl. tepidum* RCC complex upon excitation with 300 fs laser pulses at 800 nm (bandwidth 8 nm). (A) Spectra at delays of 0.3 ps (—), 0.5 ps (- - -), and 1.4 ps (...) after the onset of the pulse. (B) Spectra at delays of 4 ps (—) and 20 ps (- - -) and spectrum averaged in the time range of 300−500 ps (...). The spectra were plotted with an offset for clarity. (C) Comparison of the P840 difference spectrum obtained by averaging the signal in the time range of 300−500 ps (—) with the spectrum of the 2 s decay component observed after excitation with a 15 ns 532 nm pulse (- - -). The amplitudes of both spectra were normalized at the 830 nm band.

maximum bleaching at 835 nm, measured at 1.4 ps after the pulse in both preparations, and this ratio was taken as a measure for the relative photochemical activity. For *Ptc. aestuarii* and *Chl. tepidum*, numbers of 0.32 and 0.28, respectively, were obtained in this way. This suggests that the relative photochemical activity may be a little less for *Chl. tepidum* than for *Ptc. aestuarii* RCC complexes, but the difference, if significant, appears to be much smaller than would follow from Table 2. This may indicate that at 277 K in the *Chl. tepidum* preparation in some reaction centers an even faster rereduction of P840$^+$ occurs than would be suggested by the 0.7 ms kinetics described above.

P840 Difference Spectra. In this context it is of interest to compare the P840 difference spectrum measured shortly after a laser pulse with that obtained at later times. Figure 8C (—) shows the difference spectrum of the *Chl. tepidum* RCC complexes obtained by averaging the signal in the time range of 300−500 ps. A similar but somewhat broader spectrum has been reported by Oh-oka et al. (*17*). The spectrum, which we ascribe to P840$^+$ A$_0^-$, is clearly different from that of the 2 s decay component (- - -). The latter appears to be more strongly affected by electrochromic bandshifts of neighboring BChl *a* molecules, whereas the early spectrum seems to consist of a bleaching band at 830 nm and only a small red shift around 800 nm. The most obvious difference is an additional band at 843 nm in the difference spectrum at 2 s, which can be ascribed to an electrochromic band shift of a pigment absorbing near 840 nm, while the differences in the 790−830 nm region suggest a larger band shift of a pigment absorbing near 800 nm. These observations are quite understandable in view of the fact that in the early spectrum the charge separation extends over a shorter distance.

DISCUSSION

The most important general conclusion that may be drawn from our experiments is that from green sulfur bacteria reaction center core complexes of high purity can be isolated that are devoid of FMO protein and still have a high and stable photochemical activity. Our data extend those reported earlier on the RCC complex from *Ptc. aestuarii* (*4*) and show that almost equally active RCC complexes can be obtained from the thermophilic species *Chl. tepidum*. Preparations of comparable composition, and likewise lacking the FMO complex, have also been described on some other occasions (*6*, *38*−*40*) but these showed no or only limited photoactivity or were not further examined in this respect.

Although in some respects the green sulfur bacteria offer significant advantages for optical studies of electron transfer, much less is known about the electron acceptor chain in these organisms than in PS I (*8*) or even in heliobacteria (*1*). This is undoubtedly due to problems in the preparation of photochemically active subcellular particles. Nevertheless, there is a general consensus that the electron acceptor chain contains Chl *a* 670 as primary acceptor A$_0$, and at least two, probably three, iron−sulfur centers, two of which (F$_A$ and F$_B$) are located on a separate protein, PscB (*2, 9*). Participation of a menaquinone as early electron acceptor is uncertain (*5*, *29*, *41*), but as we recently argued (*5*, *16*), a second Chl *a* 670 molecule may function as secondary electron acceptor, A$_1$. Thus, as a working hypothesis and in analogy to PS I, we may picture the electron acceptor chain as follows:

$$\mathrm{P840} \rightarrow \mathrm{A_0} \rightarrow \mathrm{A_1} \rightarrow \mathrm{F_X} \rightarrow \mathrm{F_A/F_B}$$

One of the most striking features of the RCC complex from *Ptc. aestuarii* is the unusually high stability of P840$^+$ produced by a flash at 277 K. With reduced PMS as electron donor the lifetime of P840$^+$ varied between 14 and 1.5 s, depending on the PMS concentration. There is no evidence for any back reaction with a reduced electron acceptor in this preparation, nor is there—in contrast to what was reported for membranes (*23*) or other reaction center preparations (*29*, *38*)—any significant formation of the triplet of P840 at 277 K. Other electron donors to P840$^+$ are neutral red and, in *Chl. tepidum* RCC complexes, cytochrome c_{551}. Both react several hundred times faster with P840$^+$ than PMS, but still cytochrome oxidation was much slower than reported for other preparations from *Chl. tepidum* (*20*, *29*).

Due to the high stability of P840$^+$ and the fast transfer of the electrons produced by the charge separation into the medium, it was difficult to photoaccumulate reduced electron acceptors. Still, in part of the complexes the terminal Fe−S centers could be reduced chemically by addition of 20 mM dithionite (pH 10.5). At 277 K a decay component with a time constant of 170 ms was observed, which we ascribe to the back reaction of P840$^+$ with F$_A^-$ or F$_B^-$. In the literature various time constants, ranging from about 10 ms to 600 ms, were reported for this process (*9*, *19*, *20*). As argued above, electron transfer from F_X to F_A and F_B appears to be blocked at temperatures below 200 K. The 40 ms decay component observed at 10 K therefore can be assigned to the back reaction of $P840^+$ with F_X^-. At 100 K this process takes approximately 50 ms, which is slightly slower than a time constant of about 30 ms observed by others (*19*, *32*).

The 0.7 ms component observed at room temperature in the preparation from *Chl. tepidum* may also be due to a charge recombination of $P840^+$ with a reduced electron acceptor, but the nature of this acceptor is not clear. No corresponding component was found in the *Ptc. aestuarii* complex, which still has the full complement of terminal Fe−S centers. One might speculate that the 0.7 ms decay could be caused by a back reaction with one of the early electron acceptors A_1 or A_0, but both processes would presumably also result in formation of the P840 triplet which was not observed at 277 K. Moreover, this would not explain the occurrence of the 40 ms decay component in the *Chl. tepidum* preparation at low temperature, which we ascribe to $P840^+F_X^-$ recombination, meaning that the electron transfer would proceed further at 10 K than at 277 K.

As mentioned above, after excitation with a flash $P840^+$ is rereduced in about 40 ms at 10 K by a back reaction with F_X^-. Our photoaccumulation experiments show that there is a slow leakage of electrons to another electron acceptor, leading to a stabilization of $P840^+$ at low temperature. Surprisingly, this process takes place in both *Ptc. aestuari* and *Chl. tepidum* RCC complexes, although in the latter the electron acceptor chain appears to be incomplete.

Quantitation of P840 oxidation in our preparations is difficult because the differential extinction coefficient of P840 is still not known precisely. Most authors use the old estimates of Olson et al. (*42*), 30 mM^{-1} cm^{-1} at 610 nm, or of Swarthoff and Amesz (*43*), 100 mM^{-1} cm^{-1} at 840 nm. Both estimates are based on the rather simple assumption that P840 has the same extinction coefficient as BChl *a* in the FMO protein, but the two values do not agree with each other—in the difference spectrum shown in ref *43* the $\Delta A_{840}/\Delta A_{610}$ ratio is about 6, while the ratio between the two extinction coefficients cited above is only about 3. Permentier et al. (*5*) recently determined the specific extinction coefficient for the *Ptc. aestuarii* RCC complex as a whole. They obtained a value of 1660 cm^{-1} mM^{-1} at 817 nm, corresponding to 1050 cm^{-1} mM^{-1} at 836 nm. If we use this number and the photochemical activity obtained from our measurements, $\Delta A/A_{836} = 0.090$ (Table 2), we calculate for P840 in *Ptc. aestuarii* at 277 K a differential extinction coefficient of $0.090 \times 1050 = 95$ mM^{-1} cm^{-1} at 840 nm. This calculation is only valid if all reaction centers are active, but as argued above, the activity of our preparation may indeed be close to 100%.

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