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Stoichiometries and rates of electron transfer and charge recombination in *Heliobacterium chlorum*

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Flash-induced kinetics of the primary electron donor, P-798 and cytochrome *c*-553 were measured in membranes of *Heliobacterium chlorum* with and without added vitamin K-3. Without vitamin K-3, cytochrome *c* oxidation occurred in only part of the reaction centers, whereas in most of the other reaction centers a back-reaction of P-798$^+$ with reduced acceptors occurred with time constants of 8 and about 30 ms. Vitamin K-3 appeared to act as an efficient artificial electron acceptor: upon its addition the back-reactions disappeared and in most reaction centers cytochrome *c* oxidation and P-798$^+$ re-reduction occurred with an approximate time constant of 20 ms. The $\Delta A_{798}/\Delta A_{553}$ ratio of 10 indicated a differential extinction coefficient as high as 160–200 mM^{-1} cm^{-1} for P-798/P-798$^+$. This high number was confirmed by extraction experiments, which yielded a differential specific extinction coefficient of 180 mM^{-1} cm^{-1} at 798 nm. When oxidized cytochrome *c* was present before the flash, rapid reduction of cytochrome c^+ occurred in a small fraction of the reaction centers with a time constant of 1 ms. The reduction was not affected by antimycin A or stigmatellin, suggesting that it is not mediated by a cytochrome *bc*-complex. Evidence is presented for recombination reactions between P-798$^+$ and three functional secondary electron acceptors.

Introduction

The reaction center of heliobacteria is thought to be functionally [1–7] and stucturally [8] similar to that of Photosystem I (see Ref. 9 for a comprehensive review of the electron transport properties of PS I). As in PS I, evidence was found for the presence of iron-sulfur centers as terminal electron acceptors in heliobacteria [1–5]. The involvement of menaquinone as electron acceptor has also been suggested [3–7], in analogy with the first secondary acceptor A_1 in PS I, but this idea has recently been challenged [10].

The secondary electron donor to the primary donor, P-798, in heliobacteria is thought to be a membrane-bound cytochrome of the *c*-type, which has an α-absorption maximum at 553 nm [11]. Oxidation of cytochrome *c*-553 by the oxidized primary donor P-798$^+$ in whole cells of *Heliobacterium chlorum* occurs with major time constants of 0.11 and 0.7 ms [12]. This is considerably faster than in membrane fragments, where a major time constant of around 6 ms [1,2,13] was observed. Values between 10 and 40 were found for the ratio of the amplitudes of the bleachings at 798 nm and at 553 nm, due to the formation of P-798$^+$ and cytochrome *c*-553$^+$, respectively. The differential extinction coefficients of cytochrome *c* and (bacterio) chlorophyll oxidation are usually assumed to be 20 mM^{-1} cm^{-1} and 100 mM^{-1} cm^{-1}, respectively. This has prompted some speculation about the stoichiometry of cytochrome *c* oxidation and about the differential extinction coefficients of P-798 and cytochrome *c*-553 in heliobacteria [11].

In this paper, flash-induced absorbance difference kinetics will be presented of P-798$^+$ re-reduction and cytochrome *c*-553 oxidation in membrane fragments of *H. chlorum* under various conditions. It will be shown that the rate constant of cytochrome oxidation is $(20\ ms)^{-1}$, and that competing recombination reactions between P-798$^+$ and reduced secondary acceptors with rate constants of about $(8\ ms)^{-1}$ and $(30\ ms)^{-1}$ are the cause of the low yield that has been reported for cytochrome *c* oxidation [1]. We also report some observations on the light-induced reduction of cytochrome *c*-553$^+$, presumably by a 'cyclic' proces. Evidence will

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Abbreviations: A_0, primary electron acceptor; (B)Chl, (bacterio)chlorophyll; P-798, primary electron donor; PS, photosystem; X_1, X_2, X_3, secondary electron acceptors.

be given that the specific differential extinction coefficient of P-798 oxidation is as high as 180 mM^{-1} cm^{-1}.

Materials and Methods

Heliobacterium chlorum was grown and membrane fragments were prepared as described in Ref. 14. Flash-induced absorbance kinetics and difference spectra on millisecond time-scales were measured with the single beam spectrophotometer described in Ref. 1. The kinetics upon series of flashes were the average of ten separate measurements with an intervening dark time of 30 s. A shutter blocked the measuring beam until a few tens of milliseconds before the first flash, in order to minimize the actinic effects of the measuring light. Measurements with nanosecond resolution were performed as described in Ref. 15. In all experiments, saturating excitation flashes were provided by the frequency doubled output (532 nm) of a Q-switched Nd-YAG laser (15 ns fwhm). Absorbance spectra were recorded on a Shimadzu UV-360 spectrophotometer. For experiments in the presence of dithionite, the sample was sealed with a layer of paraffin.

Results

Cytochrome oxidation and reduction kinetics

Absorbance difference kinetics at 798 nm in membrane fragments of *H. chlorum* induced by a series of saturating laser flashes spaced at about 120 ms were measured at room temperature. In the presence of 10 mM ascorbate (Fig. 1A), a bleaching was observed upon the first flash, reflecting photo-oxidation of the primary donor P-798. The kinetics of cytochrome *c*-553 oxidation were measured at 553 nm (Fig. 1B). It may be noted that the P-798$^+$/P-798 difference spectrum has an isosbestic point at this wavelength [1]. The kinetics of P-798$^+$ re-reduction as well as those of cytochrome *c* oxidation (Fig. 1B) showed a major component of 6 ms, together with a slower one of about 25 ms. However, the amount of cytochrome oxidized was small, as compared to the amount of P-798 re-reduced, with an amplitude ratio $\Delta A_{798}/\Delta A_{553}$ of about 25. This indicates that most of P-798$^+$ is re-reduced by competing back-reactions with reduced electron acceptors [1]. A small fraction of P-798$^+$ ($< 10\%$ of the total bleaching) did not decay in the time between two flashes.

Whereas the first few flashes in Fig. 1B resulted in a bleaching caused by cytochrome oxidation, the later flashes gave a rapid (1 ms) absorption increase, which reversed again within the 120 ms until the next flash. The corresponding absorbance changes at 798 nm (Fig. 1A) show that the kinetics of P-798$^+$ formation and reduction were only slightly altered upon the later flashes; the amplitude of the bleaching decreased somewhat with increasing flash number because of incomplete reduction of P-798$^+$ between flashes, and the 6 ms component slowed down to about 8 ms.

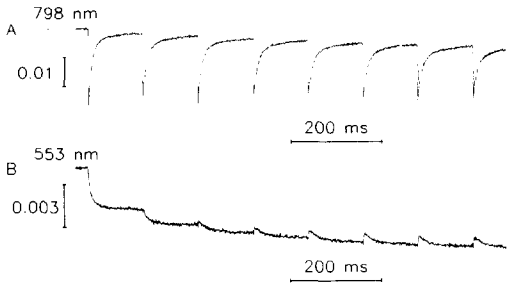

Fig. 1. Absorbance difference kinetics of membrane fragments of *H. chlorum* at 798 nm (A) and 553 nm (B) at 300 K upon a series of saturating laser flashes at 532 nm. The membrane fragments were suspended in a buffer containing 10 mM Tris and 10 mM ascorbate at pH 8.0. The absorbance of the sample was 0.65 (A) or 2.0 (B) at 788 nm.

Re-reduction of cytochrome *c*-553$^+$ in the dark did not occur on the time-scale of Fig. 1 but required several seconds in the presence of 10 mM ascorbate [1]. It could be accelerated considerably (to less than a second) by the addition of more ascorbate to the sample. At an ascorbate concentration of 200 μM, cytochrome oxidation was virtually irreversible. Kinetics upon single flashes at 1 Hz at 553 nm under these conditions are shown in Fig. 2. As upon the later flashes in Fig. 1B, an absorbance increase with a time constant of 1 ms was observed of which a major part (90%) decayed again with a time constant of about 40 ms. The spectrum of this absorbance increase is shown in Fig. 3 (solid circles), together with the spectrum caused by cytochrome *c* oxidation (open circles) at high ascorbate concentration. The two spectra have the same shape, indicating that the positive absorbance changes at 553 nm reflect reduction of oxidized cytochrome *c*-553. This reduction may be ascribed to 'cyclic' electron transport. However, the kinetics were not affected by the addition of up to 200 μM stigmatellin or antimycin A (not shown), suggesting that a cytochrome *bc* complex was not involved.

At low ascorbate concentration, cytochrome *c* could also be irreversibly oxidized in continuous light. The

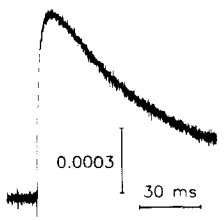

Fig. 2. Absorbance difference kinetics at 553 nm upon single flashes in the presence of 200 μM ascorbate. About ten preflashes were given to the sample before the measurements in order to oxidize all cytochrome irreversibly. The dark time between the flashes was 1 s. Other conditions as in Fig. 1.

drawn line in Fig. 3 shows the difference between the absorbance spectra of a sample containing 200 μM ascorbate that had been illuminated and an identical sample to which ascorbate had been added to a final concentration of about 500 mM. The difference spectrum resembles the flash-induced cytochrome spectra (Fig. 3, circles). Exposing the samples to higher levels of continuous illumination did not increase the amplitude of the difference spectrum and even if the samples were kept in the dark for several minutes after the illumination the amplitude of the bleaching remained unchanged. This indicates that the spectrum of Fig. 3 (drawn line) represents the maximum amount of photooxidizable cytochrome *c* in our *H. chlorum* membrane fragments.

Vitamin K-3 (menadione) as artificial electron acceptor

The kinetics at 798 nm and 553 nm as shown in Fig. 1 were altered significantly upon addition of vitamin K-3. Cytochrome *c* oxidation (Fig. 4B) now occurred upon the first flash with an approximately exponential time constant of 20 ms. The corresponding kinetics of P-798$^+$ (Fig. 4A) showed a major component with the same time constant, whereas a smaller component decayed with a time constant of about 500 ms. The former component comprised about 60% of the total amplitude of the bleaching upon the first flash, but became much smaller upon the later flashes, and was replaced by the 500 ms component (Fig. 4A). The amplitude of the corresponding cytochrome bleaching (Fig. 4B) decreased in proportion to the amplitude of the 20 ms component at 798 nm. As in Fig. 1B, flash induced reduction of oxidized cytochrome *c* was observed upon the later flashes. The small rapid component that is seen upon the later flashes at 798 nm (Fig. 4A) indicates that the cytochrome is subsequently oxidized by P-798$^+$.

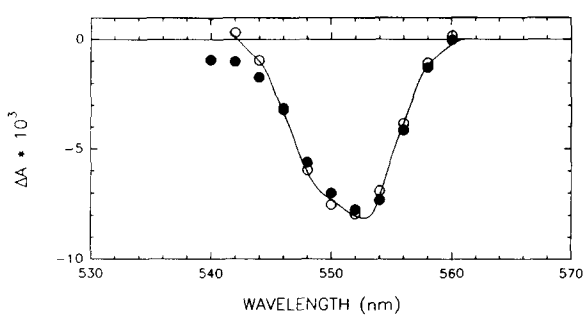

Fig. 3. Spectra of absorbance changes due to cytochrome oxidation and reduction. Open circles: Absorbance difference spectrum at 40 ms after a flash in the presence of 500 mM ascorbate. Solid circles: Inverted absorbance difference spectrum at 40 ms after a flash under the conditions of Fig. 2. The spectrum was corrected for a contribution by P-798$^+$ by using the kinetics at 798 nm and the P-798$^+$/P-798 difference spectrum [1]. Solid line: Steady-state difference spectrum between a sample that had been exposed to about 1000 lux of white light and an identical sample to which ascorbate had been added to a final concentration of about 500 mM. The vertical scale refers to spectrum of the solid line ($A_{788} = 1.5$); the other spectra were normalized at the maximum bleaching.

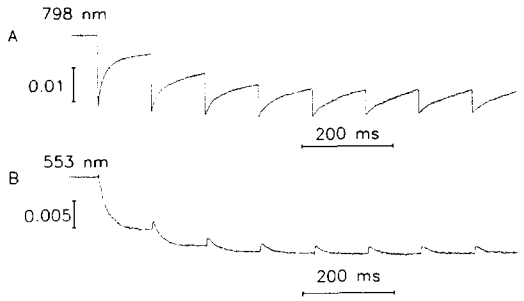

Fig. 4. Same as Fig. 1, with additional 100 μM vitamin K-3. The absorbance of the sample was 0.55 (A) or 4.2 (B) at 788 nm.

The above observations indicate that vitamin K-3 acts as an artificial electron acceptor for a component of the electron acceptor chain. The 8 ms and 25 ms back-reactions are thus abolished and we assume that P-798$^+$ and cytochrome *c* now react in stoichiometric amounts with each other, with an approximate time constant of 20 ms. At the first flash about 40% of P-798$^+$ does not react with cytochrome *c*-553 but is re-reduced in a slow reaction (about 500 ms). Addition of methylviologen, an electron acceptor for PS I [16], did not alter the kinetics of P-798 (not shown), indicating that it did not function as an artificial electron acceptor in *H. chlorum*.

The ratio of the amplitudes of the 20 ms components at 798 nm and 553 nm in the presence of vitamin K-3 was about 10. This would indicate that the differential extinction coefficient of P-798 is 10-times higher that that of cytochrome *c*-553 (see below).

Reduction of secondary acceptors

Addition of dithionite (at pH 8.0) to a final concentration of 40 mM led to an even faster reduction of oxidized cytochrome after a flash than did ascorbate at high concentration (Fig. 5B). Under these conditions, reduced cytochrome was present before each flash of a series. Cytochrome reduction occurred with a time constant of 6 ms, but the amplitude of the signal was about twice as low as in Fig. 1, presumably due to a rapid re-reduction by dithionite. The kinetics at 798 nm under these conditions are shown in Fig. 5A. The bleaching that was observed at 798 nm decayed with a major component (80% of the initial amplitude) of about 6 ms and a second component with a time constant of 30 ms. The amplitude of the bleaching decreased after six flashes to about one third of the initial amplitude, whereas the 30 ms component was virtually absent upon the later flashes. This phenomenon was further investigated on a faster time-scale at 450 nm, where P-798$^+$ formation causes an increase in the absorbance [1,14]. Figs. 6A and B show the

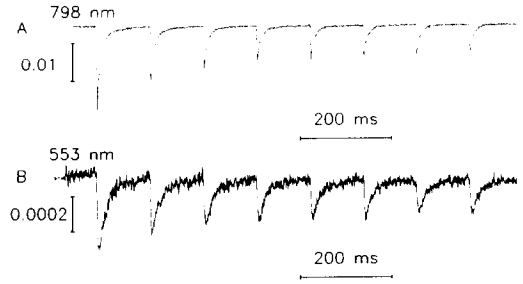

Fig. 5. Same as Fig. 1, but in the presence of 30 mM dithionite. $A_{788} = 0.65$ for both (A) and (B).

nanosecond kinetics on the 1st and the 3rd flash under the conditions of Fig. 1 (in the presence of 10 mM ascorbate). No decay of the absorbance increase occurred on a nanosecond time-scale. The amplitude of the signal was slightly reduced on the 3rd flash, due to incomplete reduction of $P\text{-}798^+$ between the flashes, as observed earlier (Fig. 1A). After addition of dithionite, the absorbance increase on the first flash (Fig. 6C) was again essentially irreversible on a nanosecond time-scale. However, after the later flashes (Figs. 6D and E) a fast decay of the absorbance increase was observed, that could be fitted with a single exponential of 17 ns. This decay time has earlier been observed upon single flashes in the presence of 30 mM dithionite at pH 9.5 [15] and was attributed to charge recombination between $P\text{-}798^+$ and A_0^-, the reduced primary acceptor. The decrease in the amplitude of the bleaching on a millisecond time-scale in Fig. 5 can thus be explained by the occurrence of the 17 ns decay phase (which is not resolved) upon the later flashes. These results indicate that dithionite causes a gradual photoaccumulation in the reduced state of all electron acceptors, except for the primary acceptor A_0, in a series of flashes. For the first secondary acceptor, X_1, this photoaccumulation occurred to an extent of only 60–70%.

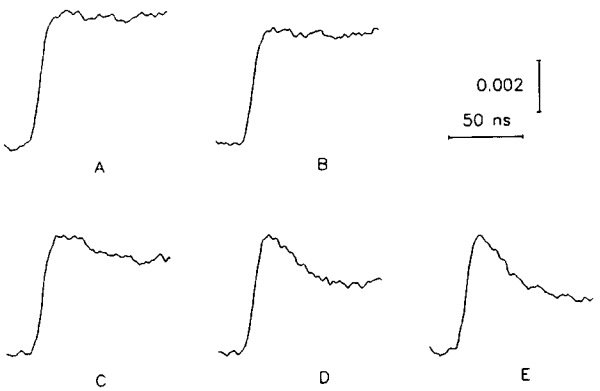

Fig. 6. Absorbance difference kinetics at 450 nm on nanosecond timescale upon the 1st (A and C), 3rd (B and D) and 7th (E) flash of a flash series. Traces A and B were recorded in the presence of ascorbate under the conditions of Fig. 1, traces C, D and E in the presence of dithionite under the conditions of Fig. 5.

The amplitudes of the P-798 and cytochrome signals on the fifth and later flashes (Fig. 5) indicated that about one-third of X_1^- was reoxidized between flashes, and was available for photoreduction on the next flash.

The differential extinction coefficient of P-798

The ratio of the amplitudes of the 20 ms components at 798 nm and 553 nm in the presence of vitamin K-3 (Fig. 4) is about 10. This is in agreement with the number found in whole cells (Ref. 12 and Nitschke, W., personal communication). However, on the assumption that the differential extinction coefficient of bacterial cytochrome *c* in its α-band is about 16–20 mM^{-1} cm^{-1} [17], this would indicate a differential extinction coefficient in the Q_y maximum of P-798 of 160–200 mM^{-1} cm^{-1}, which is unusually high.

In order to investigate this point further, we extracted BChl *g* from membranes of *H. chlorum* with acetone. Small aliquots of an aqueous suspension of sonicated cells of *H. chlorum* were added to a volume of acetone and sonicated for 1–2 min. After centrifugation, the supernatant was removed and its absorption spectrum was recorded. A fresh volume of acetone was added and after stirring of the sediment the sonication and centrifugation steps were repeated. After a third extraction step, a virtually colorless sediment remained. The absorption of the supernatant after the second and third extractions were 10% and less than 3% of that after the first extraction, respectively.

Fig. 7 shows the absorbance spectra of the combined extracts in acetone (solid line) and of an equivalent amount of sonicated cells in aqueous buffer (dashed line). The absorbance of the extract at the Q_y maximum (at 761 nm) was 70% of that of the corresponding maximum in sonicated cells (at 788 nm). Similar results were obtained upon extraction of isolated membrane fragments and whole cells. The amplitude of the absorption band around 670 nm remained about the same during the extraction, indicating the absence of significant BChl *g* degradation during the extraction procedure [18]. With a molar extinction coefficient of

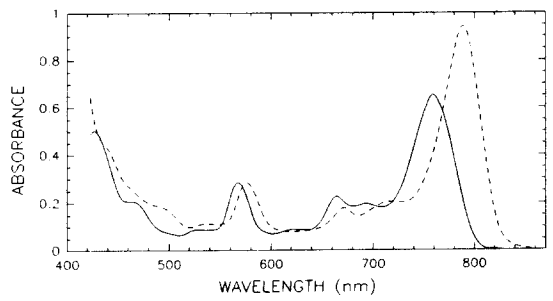

Fig. 7. Absorption spectra of an extract of sonicated cells of *H. chlorum* in acetone (solid line) and of an equivalent amount of sonicated cells in aqueous buffer (dashed line).

76 mM^{-1} cm^{-1} for BChl *g* in acetone at 761 nm [19] it can be calculated that the specific extinction coefficient of antenna BChl *g* at 788 nm is 109 mM^{-1} cm^{-1}. We may now use this value to estimate the differential extinction coefficient of P-798. Values of 20–22 have been found for the ratio between the maximum absorption in the Q_y maximum of *H. chlorum* membranes and the bleaching at 798 nm upon oxidation of P-798 [14,20]. On the assumption that 35 BChls are present for each P-798 [14,19], this indicates that the differential extinction coefficient of P-798 is 35/21 = 1.67-times that of antenna BChl *g* in vivo. This gives a value of 180 mM^{-1} cm^{-1} for the differential extinction coefficient for the photooxidation of P-798. To our knowledge, this would be the highest value found for the primary donor of any photosynthetic system.

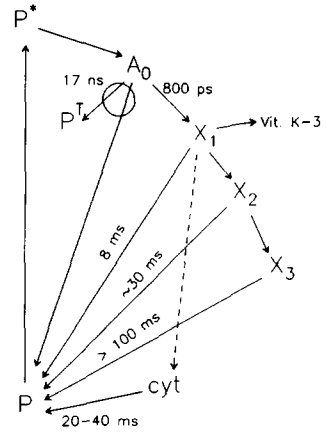

Fig. 8. Proposed scheme for electron transfer in membrane fragments of *H. chlorum* at room temperature. The electron acceptors and time constants are given as found in this work and elsewhere [1,15,25].

Discussion

Cytochrome oxidation

The kinetics of P-798$^+$ reduction and cytochrome *c* oxidation in the presence of vitamin K-3 show a decay component of 20 ms (Fig. 4). The 20 ms component in the P-798 kinetics disappears in the presence of oxidized cytochrome *c*, and is replaced by a much slower 500 ms component. This indicates that vitamin K-3 acts as an efficient acceptor for the electrons on the acceptor chain of *H. chlorum*, resulting in a much longer lifetime of 500 ms for P-798$^+$ than under conditions where recombination between P-798$^+$ and reduced secondary acceptors is allowed (Fig. 1). The 20 ms component can be ascribed to the oxidation, in stoichiometric amounts, of cytochrome by P-798$^+$, with a ratio of the amplitudes at 798 nm and 553 nm of about 10. In the presence of vitamin K-3, P-798$^+$ is reduced by cytochrome *c* in about 60% of the reaction centers upon the first flash (Fig. 4). This would indicate that no cytochrome is present in the remaining fraction of reaction centers. The occurrence of cytochrome oxidation upon the second flash (Fig. 4B) then suggests that in some reaction centers two photo-oxidizable molecules of cytochrome *c* are present. The same applied to reaction centers is the absence of vitamin K-3, but now the amount of cytochrome oxidized in a flash was significantly smaller, due to competing back reactions of P-798$^+$ with reduced electron acceptors.

Using a ratio of 10 between the differential extinction coefficients of P-798 and cytochrome *c*-553 at 798 nm and 553 nm, respectively, we can calculate that only about 40% of the P-798$^+$ decaying with a time constant of 6 ms in the presence of ascorbate (Fig. 1) is reduced by cytochrome. This can be explained by assuming that competition between the 20 ms reduction of P-798$^+$ by cytochrome oxidation and an 8 ms charge recombination with a reduced acceptor (see Fig. 8) yields an overall decay time of 6 ms for P-798$^+$ [1].

Cytochrome reduction and cyclic electron transport

In our measurements, clear evidence was found for the flash-induced reduction of oxidized cytochrome *c*-553. Under conditions where part of the cytochrome was in the oxidized state before a flash, an absorbance increase with a time constant of 1 ms was observed at 553 nm, which decayed again with a time constant of 40 ms (Fig. 2). The electron that reduces the oxidized cytochrome probably comes from an intermediate in the electron acceptor chain. The amplitude of the absorbance increase indicates that this reaction occurs in about 10% of the reaction centers. Oxidized cytochrome that is reduced in this manner is presumably oxidized again by P-798$^+$, suggesting that electron transport is cyclic in these reaction centers. Apparently, these reaction centers are not representative for the population as a whole, since the oxidation of cytochrome was somewhat slower than upon the first flash in the presence of vitamin K-3.

Our results suggest that the reaction is not mediated by a cytochrome *bc* complex [21], as appears to be the case in intact cells, where cytochrome c^+ reduction has been reported to be much slower and to be inhibited by stigmatellin [22], an inhibitor of quinol oxidation in cytochrome *bc* and *bf* complexes [23].

The electron acceptor chain

At least three components were observed in the decay kinetics of P-798$^+$. The main component had a 6 ms time constant in the presence of reduced cytochrome, and an 8 ms time constant when cytochrome was oxidized. We conclude that it can be ascribed to a recombination reaction between P-798$^+$ and a reduced secondary acceptor, X_1^-. If reduced cytochrome is present, cytochrome oxidation competes with this recombination reaction and the overall decay time of P-798$^+$ becomes shorter (6 ms). The second component has a

time constant of 25–30 ms. We attribute it to recombination between P-798$^+$ and a reduced electron acceptor X_2^-, beyond X_1 in the electron acceptor chain. A third decay component was virtually irreversible on the time scale of the experiment and is tentatively ascribed to reaction centers where charge separation has occurred between P-798 and an electron acceptor X_3, operating beyond X_2. The identities of these acceptors are not known, but it seems likely that at least some of them are iron-sulfur centers [1–5].

If dithionite was added to membrane fragments of *H. chlorum* at pH 8.0, no irreversible component was observed in the decay kinetics at 798 nm (Fig. 5), while components of about 6 ms and 30 ms were still observed. This indicates that acceptor X_3 is reduced by dithionite in the dark under these conditions, while X_1 and X_2 remain oxidized. The total amplitude of the bleaching at 798 nm that decayed on a time scale of milliseconds decreased by about 70% after the first six flashes and the 30 ms component ascribed to recombination between P-798$^+$ and X_2^- virtually disappeared (Fig. 5A). Instead, a 17 ns decay component was observed upon the later flashes (Fig. 6). This phenomenon was not observed in the absence of dithionite and can be ascribed to accumulation of reduced electron acceptors X_1^- and X_2^-. In the presence of dithionite, cytochrome *c* is still oxidized by P-798$^+$ (Fig. 5B), in competition with charge recombination between P-798$^+$ and X_1^- or X_2^-. We conclude that the electrons needed for the photo-accumulation of X_1^- and X_2^- come from cytochrome *c*, which is reduced by dithionite between flashes. Thus, upon successive flashes, the 30 ms component (associated with recombination between P-798$^+$ and X_2^-) disappears and the 6 ms component (recombination between P-798$^+$ and X_1^-) is reduced in amplitude and partially replaced by a 17 ns component (Figs. 5 and 6). The 17 ns component can be ascribed to recombination between P-798$^+$ and the reduced primary acceptor A_0^- [15], suggesting that X_1 is the first electron acceptor beyond A_0.

The interpretation of the results can be summarized in the scheme for electron transport and charge recombination in membrane fragments of *H. chlorum* at room temperature given in Fig. 8. In this scheme vitamin K-3 accepts electrons from X_1^-. This can be concluded from the absence of the 8 and 30 ms recombination components in the kinetics of Fig. 4. Light-induced cytochrome *c*-553$^+$ reduction does still occur in the presence of vitamin K-3 (Fig. 4), which indicates that X_2 and X_3 are not part of the electron donor chain to cytochrome c^+.

If we compare the secondary electron acceptors in this scheme to those that are currently thought to function in Photosystem I [9], it should be noted that the time constants that have been reported for the recombination reactions in PS I between P-700$^+$ and the secondary electron acceptors A_1^- or X_2^- [9] are about an order of magnitude shorter than those between P-798$^+$ and X_1^- or X_2^- found here. Evidence has been presented recently [10] that makes it doubtful whether a quinone acceptor is functioning as X_1, analogous to acceptor A_1 in Photosystem I [9]. The low rate of recombination between P-798$^+$ and X_1^- found here might be a further indication that the first secondary acceptors in PS I and heliobacteria are of a different nature.

The differential extinction coefficient of P-798

Our results indicate that the differential extinction coefficient, ϵ, for the photo-oxidation of P-798 is as high as 180 mM^{-1} cm^{-1}, higher than found for the primary donor in any other photosystem. For antenna BChl *g* an extinction coefficient of 109 mM^{-1} cm^{-1} was obtained. The approximate oscillator strengths may be compared with that of BChl *g* in acetone, by multiplying the extinction coefficients with the half-widths of the absorption bands (in frequency units). In this way a ratio of 1 : 1.3 : 0.8 was obtained for BChl *g* in acetone, antenna BChl *g* and P-798, respectively. It thus appears that the high ϵ value of P-798 is related to its narrow bandwidth, while its oscillator strength is comparable to that of monomeric BChl *g* in solution.

A value of 180 mM^{-1} cm^{-1} for the differential extinction coefficient of P-798 explains the rather high ratio that was observed between the bleachings at 798 nm and 553 nm under conditions where cytochrome *c*-553 may be assumed to be oxidized by P-798$^+$ in stoichiometric amounts. This ratio was found to be about 10 in the presence of vitamin K-3 in membrane fragments (Fig. 4), whereas a similar value has been reported for whole cells (Ref. 12 and Nitschke, W., personal communication). A value of 18 mM^{-1} cm^{-1} seems quite reasonable for the differential extinction coefficient in the α-band of a bacterial cytochrome *c* [17].

Our data may also be used to estimate the extinction coefficient of the primary electron acceptor, A_0, 8^1-hydroxy Chl *a* [19]. From kinetics of P-798$^+$ in the picosecond time range, as found by Nuijs et al. [24], it may be concluded that the differential extinction coefficients for the state P-798$^+$ A_0^- and the state P-798$^+$ X_1^- are approximately the same. From the absorbance difference spectrum at 150 ps after a flash and the kinetics at 670 nm [24,25] it follows that the initial bleaching of A_0 at 670 nm is approx. 2.7-times smaller than that of P-798 at 798 nm. This yields a value of 67 mM^{-1} cm^{-1} for A_0.

Acknowledgements

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