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Primary charge separation in photosystem II involves two electrogenic steps

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Similarities in the pigment and subunit composition and sequence homologies suggest a common evolutionary origin for the reaction centers (RCs) of photosynthetic purple bacteria and photosystem II (PS II) of green plants [1]. Besides these similarities, significant functional differences exist that make comparative studies between both types of RCs interesting. We report here (i) on the electric detection of two kinetically distinct phases of the primary charge separation in PS II with 100 and 500 ps reflecting trapping and charge stabilization, and (ii) the determination of the dielectrically weighted transmembrane distances between the primary donor (P), the pheophytin intermediary acceptor (I), and the first quinoic acceptor (Q_A). These data are compared with those for purple bacteria. In PS II the reduction of Q_A is 2–3-times slower, although the dielectric distance between I and Q_A appears to be significantly shorter than in purple bacteria.

Photosynthesis; Photosystem II; Dielectric distance; Primary charge separation; Fast photovoltage

1. INTRODUCTION

From recent progress in crystallization, X-ray analysis, and femtosecond spectroscopy of the reaction center (RC) of the photosynthetic purple bacteria *Rhodopseudomonas viridis* and *Rhodobacter sphaeroides*, a very detailed picture of the structure and primary photochemistry has emerged, demonstrating many similarities between both species [1–7]. The electron carriers essential for the primary photochemistry are bound to two polypeptide chains termed L and M. Upon excitation of the primary donor (P), a bacteriochlorophyll dimer, the intermediary acceptor (I), a bacteriopheophytin, is reduced with a time constant of 2.8 ps [5–7]. In a second step a quinone, Q_A, is reduced within ≈200 ps [8,9]. From the three-dimensional structure of the RC of *R. viridis* we know that the intermediary acceptor lies approximately halfway between P and Q_A [2–4].

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As the isolation and characterization of RC polypeptides of PS II from higher plants or cyanobacteria is less advanced, there is less structural information for the RC from PS II than for that of purple bacteria. The L and M subunits constituting the RC of bacteria appear to have their correspondence in the D1 and D2 subunits of PS II [1,10,11]. Furthermore, it is well established that the intermediary acceptor I in the PS II reaction center is a pheophytin [12,13], and that the charge is stabilized on a quinone-type acceptor [14–16]. In addition, both RCs have in common a non-heme iron atom at the terminal acceptor site. There is also evidence for the primary donor (P-680) to be a chlorophyll dimer. In analogy to the bacterial L and M subunit its binding site could be the two histidines 198 found on the D1 and D2 subunits at corresponding places (general discussion in [1,17,18]).

Differences between both species of RCs exist mainly at the donor site. Whereas in purple bacteria the primary donor P is reduced by cytochromes, in PS II P-680 is rereduced by a tyrosine residue [19]. To split water the midpoint potential of P-680 has to be more than 300 mV

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more positive than in purple bacteria. This draws the energy gap between P-680* and $P\text{-}680^+ \cdot Phe^-$ down to the order of kT and causes a reversibility of the primary charge separation [20].

The first step of charge separation in PS II, a 3 ps reduction of I [20,21], is much the same as in purple bacteria, whereas the second step, the reduction of Q_A, has been reported to occur with 250–650 ps in PS II [22,23], which is slower than in purple bacteria.

Recent methodological developments of photoelectric techniques make it possible to detect directly the growth of the membrane potential connected with the transmembrane charge separation in the sub-nanosecond range [24–26]. Applying this electric method to *R. viridis* whole cells, it could be demonstrated that the primary charge separation consists of two electrogenic steps with kinetics close to those mentioned. Furthermore, the dielectrically weighted position of I was found to be approximately in the middle between P and Q [26]. A similar result was recently reported for PS II of peas [24]. However, in both studies the given signal-to-noise ratio did not allow us to quote errors below ± 20%.

Here, we attempt to determine photoelectrically the position of the pheophytin intermediary acceptor in PS II with higher precision and compare it with the respective position of I in purple bacteria. In the case of PS II it is not practicable to measure the amplitudes with oxidised and reduced Q_A in order to evaluate the location of I, as was done for bacterial RCs [26,27], since in PS II the reduction of the quinone affects the yield of the state $P\text{-}680^+ \cdot Pheo^-$. In addition, the yield of the radical pair also depends on the size of the antenna pigment system connected with the RC [20,24].

2. EXPERIMENTAL AND RESULTS

To separate PS II signals from contributions of PS I, we have chosen two systems. First, chloroplasts from a barley mutant that is deficient in PS I (*viridis-zb*^{63}; lacking P-700 chlorophyll *a*-protein 1 (CP1) [28]). Second, grana membranes from pea chloroplasts that were pre-oriented by an electric field.

The chloroplasts from the barley mutant were prepared as osmotically swollen membranes ('blebs'), since chloroplasts with stacked grana thylakoids do not display a PS II photovoltage in the light-gradient technique. This lack of a PS II photovoltage was explained in a previous study by an excitonic short-circuit over the partition regions in the grana membranes [24]. The cancellation of the excitonic short circuit by destacking the grana membranes is a prerequisite for the observation of a PS II photovoltage.

In a second set of experiments we used BBY membranes (containing only PS II [?]) and eliminated the excitonic short circuit between the appressed grana membranes through separating the membranes by a mild trypsin treatment. The resulting single membranes can be oriented by small electric fields (~100 V/cm) giving the necessary asymmetry.

Fig.1a and b shows the photovoltage evoked by picosecond-flashes from the mutant blebs and from PS II membranes, respectively. The overall signal shape is largely determined by properties of the apparatus. To extract the molecular kinetic information, the time course of the signal was simulated by a convolution of the displacement current connected with two electrogenic reactions (schemes in fig.1) with the response function of the apparatus. The latter was obtained with purple membranes from *Halobacterium halobium* which are known to display a primary charge displacement that is faster than the time resolutions of currently available apparatus [26,29].

The analysis was done by varying the three parameters (γ_1, γ_2, A_2) so as to minimize the residuals between convoluted and experimental curves [25,26]. The best fit for a two-step irreversible consecutive electrogenic reaction was achieved within the following ranges of parameters: first reaction, γ_1 = 80–100 ps and A_1 = 1 (normalized to unity); second reaction, γ_2 = 450–550 ps and A_2 = 0.80–1.00. The first phase can be considered as trapping kinetics (specific for the given excitation energy) probed by the ultrafast $P^+ \cdot I^-$ formation [21], and the second phase can be ascribed to charge stabilization by the forward electron transfer from I to Q_A:

$$(\text{antenna})^* \cdot P \cdot I \cdot Q_A \xrightarrow[A_1 = 1.00]{\gamma_1} (\text{antenna}) \cdot P^+ \cdot I^- \cdot Q_A \qquad (1)$$

$$(\text{antenna}) \cdot P^+ \cdot I^- \cdot Q_A \xrightarrow[A_2]{\gamma_2} (\text{antenna}) \cdot P^+ \cdot I \cdot Q_A^- \qquad (2)$$

The reliability and convergence of the fitting procedure for the analysis of two consecutive reactions were tested by measuring the photovoltage from the purple bacteria *R. viridis* and *Rb. sphaeroides*. In this case, it is possible to measure independently the position of I by comparing the amplitude of the photovoltage with oxidised and reduced quinone. This method gave for both species a dielectrically weighted distance for P-I equal to 41 ± 2% of the total electrogenicity of $P^+Q_A^-$ formation, which agreed with the kinetic analysis at 7 GHz bandwidth (Trissl, H.-W. et al., in preparation). If the photovoltage from these bacteria was recorded at 1 GHz bandwidth and subjected to the same fitting procedure, the amplitude A_2 agreed within an error of ± 4%.

The data in fig.1 were also analysed by a reversible first reaction and an irreversible second reaction, since there is evidence that in PS II the charge-separated state $P^+ \cdot I^-$ is in equilibrium with the excited state, $P^* \cdot I$ [23]. This alternative treatment allows one to examine the influence of the assumed reaction scheme on the numerical determination of A_2. The analysis with both methods gave a value for A_2 that fell within the range quoted above. (Note that the time constants derived by the irreversible reaction scheme correspond to the apparent time constants of the reversible reaction scheme.)

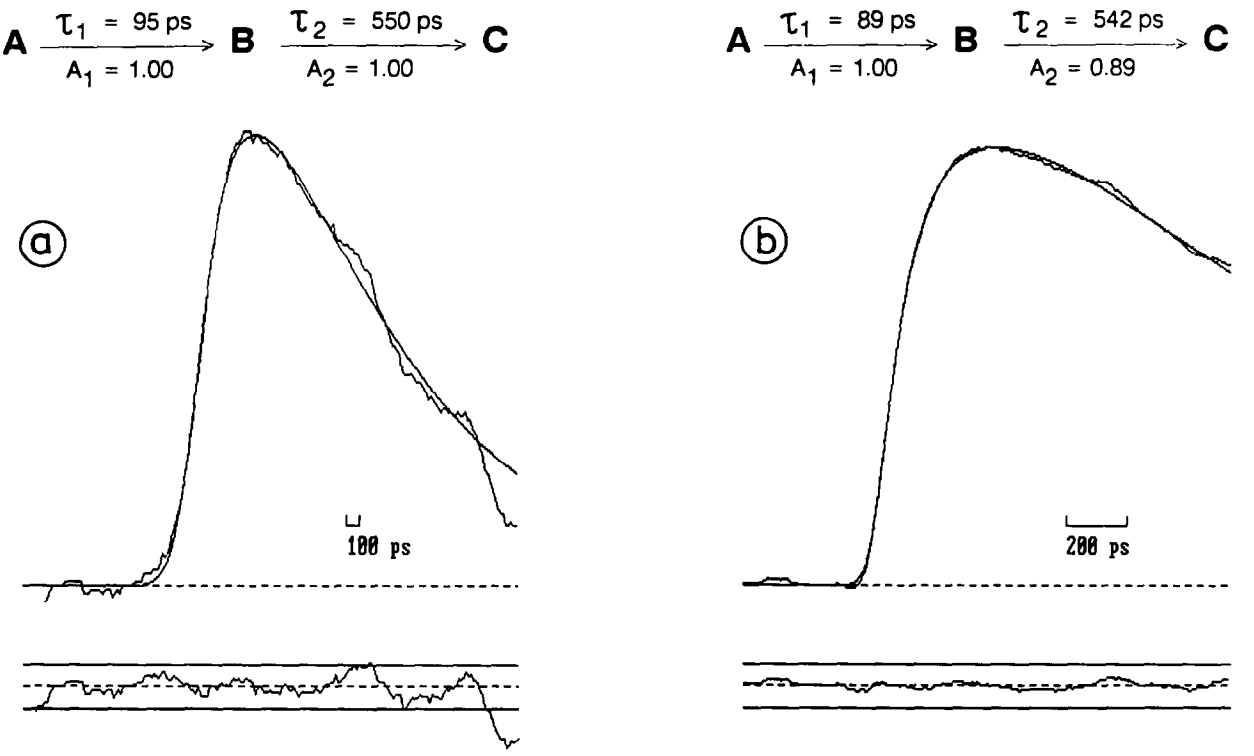

Fig.1. (a) Light-gradient photovoltage from blebs of a PS I-deficient barley mutant (*viridis zb*^{63}), evoked by 30 ps flashes (532 nm, 80 ± 15 μJ/cm^2). Recording bandwidth: 1 GHz. Average of 50 traces. Destacked chloroplasts were prepared as described [24]. (b) Photovoltage from oriented BBY membranes prepared from pea chloroplasts. Recording bandwidth: 7 GHz. Average of 10 traces. (Lower traces) Residuals between the experimental traces and calculated fits using the convolution parameters labeled in the figure. Chlorophyll concentration: 3.5 mM. Technical details are described in [24–26].

3. DISCUSSION

In the present experiments we have measured a trapping time of 80–100 ps. This apparent time constant ('trapping time') involves reversible processes like exciton transfer between the antenna pigments and the primary donor, formation of P*, and formation of $P^+ \cdot I^-$. The trapping time of 100 ps we observed is about half the duration of that found by time-resolved fluorescence decay analysis [20]. This is due to the different exciton density applied in these experiments: whereas the fluorescence is measured in the low-energy limit (much less than one exciton per trap), the photovoltage experiments are carried out at higher exciton densities (of the order of one exciton per trap).

In the purple bacterium *R. viridis* we found for the distance $d_{(P\text{-}I)} = 41 \pm 2\%$ and for $d_{(I\text{-}QA)} = 59 \pm 2\%$ (not shown; in preparation) and in PS II $d_{(P\text{-}I)} = 58 \pm 8\%$ and for $d_{(I\text{-}QA)} = 42 \pm 8\%$. This means that in PS II the intermediary acceptor I lies dielectrically closer to the quinone Q_A than it does in purple bacteria. This result partly explains why in PS II the negative charge on the reduced Q_A has a more pronounced effect on the kinetics and yield of the radical pair formation, $P^+ \cdot I^-$ (see also [20,23]).

A result contrasting with ours on the position of the intermediary acceptor has been reported [30]. From electric field-induced fluorescence yield measurements it was concluded that electron transfer from Pheo to Q_A spans 90% of the membrane dielectric. Such a location of I would indicate a significant difference between RCs from purple bacteria and PS II, that would not be supported by the amino acid sequence [1,10,11].

Both the similar relative location of the intermediary acceptor, Phe, with respect to the adjacent electron carriers P-680 and Q_A, and the

existence of a comparable second step in the forward electron transfer, are in line with the idea of a common evolutionary origin of the RCs of PS II and purple bacteria. However, there are small but significant differences between the two systems. If, as suggested by the amino acid sequences, the overall geometrical position of the electron carriers is the same in both species our results demonstrate a modified dielectric environment in PS II. On the one hand, the larger dielectric distance between P and I found in PS II, when compared to purple bacteria, is probably required to minimise the rate constant of the back-reaction, since in PS II the free energy gap between P* and $P^+ \cdot I^-$ is minute and the rate of the following Q_A reduction is relatively slow. On the other, the smaller dielectric distance between I and Q_A points to a larger reorganization energy which is also indicated by the decreased rate constant $k_2 = (550\ ps)^{-1}$. More general, specific structural and kinetic modifications must have been necessary to tune the RC to the high oxidising power for water splitting.

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