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Rapid report

Temperature dependence of biphasic forward electron transfer from the phylloquinone(s) A_1 in photosystem I: only the slower phase is activated

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Abstract

The temperature dependence of the biphasic electron transfer (ET) from the secondary acceptor A1 (phylloquinone) to iron–sulfur cluster F_X was investigated by flash absorption spectroscopy in photosystem I (PS I) isolated from *Synechocystis* sp. PCC 6803. While the slower phase ($\tau = 340$ ns at 295 K) slowed upon cooling according to an activation energy of 110 meV, the time constant of the faster phase ($\tau = 11$ ns at 295 K) was virtually independent of temperature. Following a suggestion in the literature that the two phases arise from bidirectional ET involving two symmetrically arranged phylloquinones, Q_K-A and Q_K-B, it is concluded that energetic parameters (most likely the driving forces) rather than the electronic couplings are different for ET from Q_K-A to F_X and from Q_K-B to F_X. Two alternative schemes of ET in PS I are presented and discussed.

Keywords: Electron transfer; Photosystem I; Phylloquinone; Activation energy; Transient absorption spectroscopy

The photosystem I (PS I) complex (for a collection of recent reviews, see Ref. [1]) is a light-driven oxidoreductase present in photosynthetic cyanobacteria, algae and higher plants. The physiological function of this membrane-bound complex is to oxidize the water-soluble proteins plastocyanin or cytochrome c_6 on the lumenal side and to reduce ferredoxin or flavodoxin on the stromal side of the thylakoid membrane. Within PS I, electron transfer (ET) occurs from an exited chlorophyll dimer (called P700) to two [4Fe–4S] clusters (F_A and F_B) that serve as terminal acceptors. This ET takes place via further cofactors called A_0 (chlorophyll *a*), A_1 (phylloquinone, Q_K, also known as vitamin K_1) and F_X ([4Fe–4S] cluster). The ET time constants τ are in the order of 1 ps for ET from excited P700 to A_0 and 30 ps from A_0^- to A_1. For ET from A_1^- to F_X, biphasic kinetics with $\tau = 10–30$ ns and $\tau \approx 300$ ns (at room temperature) have been reported [2–4]. The time constant of the faster phase and the amplitude ratio of the two phases varied considerably between PS I prepared from different organisms and by different methods. The ET steps from F_X to F_A and from F_A to F_B have not yet been resolved; however, the kinetics of ferredoxin reduction by PS I suggested that ET to F_B had occurred in less than about 500 ns [5].

The positions of the cofactors in PS I from *Synechococcus elongatus* have recently been accurately established by X-ray crystallography at a resolution of 2.5 Å [6]. P700 and F_X are about 31 Å apart and both located on the pseudo-C_2 symmetry axis relating the two core subunits PsaA and PsaB. Two copies of chlorophyll *a* (A_0) and phylloquinone (A_1) are related by the C_2 axis forming two highly symmetrical potential ET pathways from P700 to F_X. F_A and F_B are embedded in the stroma-exposed subunit PsaC.

To explain the biphasic reoxidation kinetics of A_1^-, Sétif and Brettel [2] initially favored a model where ET from A_1^- to F_X has only a very weak driving force so that back transfer from F_X^- to A_1 is not negligible. In this model, establishment of a quasi-equilibrium between A_1^- and F_X^- would give rise to the fast phase of A_1^- reoxidation, and depopulation of this quasi-equilibrium by ET from F_X^- to F_A would give rise to the slower phase. Recently, Guergova-Kuras et al. [7] provided strong support for an earlier suggestion by Joliot and Joliot [4] that the two phases of A_1^- reoxidation are in fact due to the implication of both of the two phylloquinone molecules. In whole cells of *Chlamydomonas reinhardtii*, mutation of PsaA

Abbreviations: ET, electron transfer; PS I, photosystem I; Q_K, phylloquinone

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W693 (homolog of W697 in *S. elongatus* that is stacked to the phylloquinone Q_K-A in PsaA) slowed selectively the slower phase of A_1^- reoxidation, while mutation of PsaB W673 (homolog of W677 that is stacked to the phylloquinone Q_K-B in PsaB) slowed selectively the faster phase. Both cofactor branches would be served by excited P700 with comparable probabilities, and the two phylloquinones would reduce F_X with different rates, namely $\sim (250\ \text{ns})^{-1}$ for Q_K-A and $\sim (20\ \text{ns})^{-1}$ for Q_K-B [7]. The structure of PS I from *Synechococcus elongatus* does not reveal obvious differences between the two putative ET branches at the level of the phylloquinones and F_X, which might explain such different ET rates; in particular, the edge-to-edge distances between F_X and the phylloquinones are very much the same (6.8 Å), and the binding pockets of the phylloquinones show no obvious differences [8].

Here we studied the temperature dependencies of the two phases of A_1^- reoxidation in order to check whether different energetic parameters might account for the different ET rates. In a previous study [9], it was found that the slower phase in *Synechococcus elongatus* PS I slowed with decreasing temperature, corresponding to an activation energy of about 220 meV. The faster phase was, however, not resolved in Ref. [9].

We monitored the reoxidation kinetics of A_1^- in monomeric PS I complexes from cyanobacteria *Synechocystis* sp. PCC 6803 [10] by flash absorption spectroscopy with a time resolution of about 2 ns essentially as described [3]. In brief, the sample was exited at a repetition rate of 1 Hz by 300-ps laser flashes of about 1 mJ/cm^2 at 532 nm. The measuring light provided by the relatively flat top of a 50-μs Xe flash passed through a combination of interference and colored glass filters placed before and behind the sample for wavelength selection, and was detected by a Si photodiode. Its output was pre-amplified and transmitted to a digitizing oscilloscope DSA 602 with plug-in 11A52 (Tektronix). Samples were kept in 1×1-cm plastic cuvettes and contained 65% v/v glycerol in order to obtain a transparent glass at low temperatures. For measurements from 295 to 253 K, the temperature of the sample was adjusted by a water–ethylene glycol mixture flowing through the cuvette holder. Lower temperatures were achieved in a He flow cryostat. A Marquardt least squares algorithm program was used for signal fitting. Reaction Scheme 2 was numerically simulated with GEPASI [11].

Fig. 1A shows kinetic traces at different temperatures of the absorbance changes at 380 nm that are essentially attributed [12] to the flash-induced formation of the phyllosemiquinone anion A_1^- (instrument limited absorption rise at time zero) and to the oxidation of A_1^- (absorption decay). The transient at 295 K (lowest trace in Fig. 1A) was satisfactorily fitted by a two-exponential decay (solid line) with $\tau = 14$ ns (28% of total decay amplitude) and $\tau = 340$ ns (72%). These kinetic parameters are similar to a previous study at room temperature, where no glycerol was present [3]. As expected, decreasing the temperature (see Fig. 1A for examples) led to a pronounced slowing of A_1^- reoxidation. According to two-exponential fits (solid lines in Fig. 1A; the fit parameters for the 380-nm traces are depicted as open symbols in Fig. 2A and B), only the rate of the slower phase (open circles in Fig. 2A) decreased with decreasing temperature. The rate of the faster phase (open triangles) was virtually unchanged down to 223 K, while the relative amplitude of the faster phase (open triangles in Fig. 2B) decreased moderately. Below 223 K, we were unable to

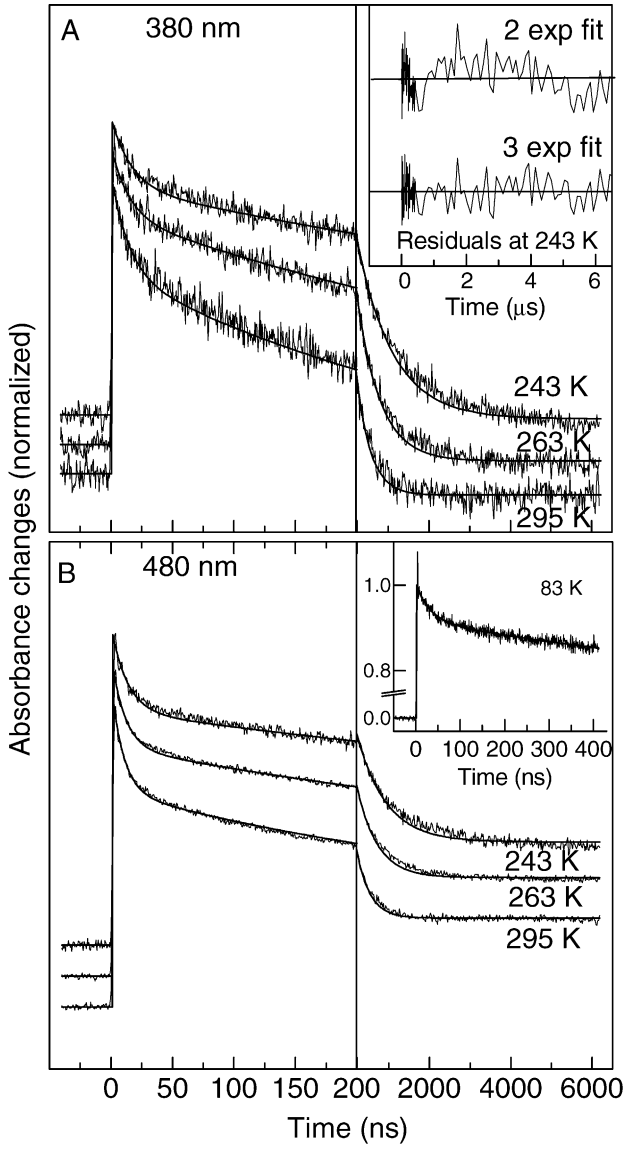


Fig. 1. Flash-induced absorbance changes reflecting the kinetics of A_1^- in PS I complexes from *Synechocystis* sp. PCC 6803 at different temperatures. Panel A: measurements at 380 nm. The inset shows the residuals of two- and three-exponential fits to the kinetic trace at 243 K. Panel B: measurements at 480 nm. The inset shows a kinetic trace at 83 K. Solid lines in both panels represent two-exponential fits with a constant accounting for long lived ($\tau > 10$ μs) absorption changes. For clearness, traces are offset. The samples contained 20 mM Tricine pH 8, 20 μM 2,6-dichlorophenolindophenol, 5 mM sodium ascorbate, 65% (v/v) glycerol and PS I complexes at a chlorophyll concentration of about 15 μM (panel A) or 40 μM (panel B).

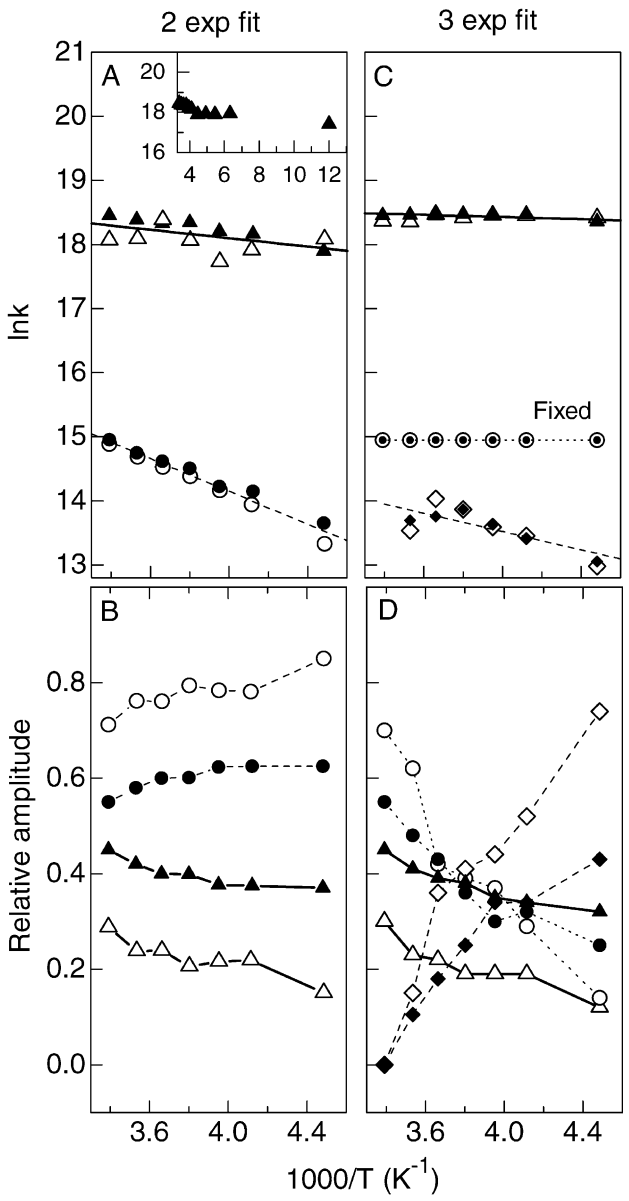


Fig. 2. Temperature dependence of the decay constants k and relative amplitudes of the kinetic phases of A_1^- reoxidation. Shown are the parameters obtained from fitting transient absorption changes at 380 nm (open symbols) and 480 nm (closed symbols) at various temperatures (see Fig. 1 for examples). Panel A: Arrhenius plot of the decay constants from two-exponential fits. Triangles, faster phase. Circles, slower phase. Solid and broken line, linear regressions for the faster and slower phase, corresponding to activation energies of 15 and 110 meV, respectively. The inset shows the rate of the faster phase at 480 nm down to 83 K. Panel B: relative amplitudes of the phases from two-exponential fits. Symbols as in panel A. Panel C: Arrhenius plot of the decay constants from three-exponential fits with an intermediate rate fixed at $(315\ ns)^{-1}$. Triangles, fastest phase. Circles, intermediate phase. Diamonds, slowest phase. Solid and broken line, linear regressions for the fastest and slowest phase, corresponding to activation energies of 7 and 65 meV, respectively. Panel D: relative amplitudes of the phases from three-exponential fits. Symbols as in panel C. All relative amplitudes refer to the total decay on this time scale (constant excluded).

resolve the faster phase reliably at 380 nm (at these temperatures, the total amplitude decreased because of an increasing accumulation of the photochemically inactive state $P700^+$ $(F_AF_B)^-$ under repetitive excitation [9]; see also Ref. [12] for a review on the peculiar effects of cryogenic temperatures on ET in PS I).

It has been shown previously [3] that reoxidation of A_1^- can be monitored with a better signal-to-noise ratio at 480 nm (presumably, the negative charge on A_1 induces an electrochromic bandshift of a nearby carotenoid that gives rise to an absorption increase at 480 nm Ref. [12]). The kinetic traces at 480 nm (Fig. 1B) were similar to those at 380 nm, except for a long-lived positive contribution due to $P700^+$. Bi-exponential fits of the transients at 480 nm at various temperatures yielded very similar rate constants (closed symbols in Fig. 2A) as at 380 nm (open symbols). Linear regression of the Arrhenius plots in Fig. 2A for the data collected at both wavelengths yielded activation energies of 15 meV for the faster phase (with a mean time constant of 11 ns at room temperature) and 110 meV for the slower phase. The relative amplitude of the faster phase at 480 nm (closed triangles in Fig. 2B) was significantly larger than at 380 nm (open triangles), as noted earlier [3], but followed the same trend with temperature as at 380 nm. We were able to resolve the faster phase at 480 nm down to 83 K (insets of Figs. 1B and 2A). Its rate decreased only by a factor of 3 between room temperature and 83 K.

We noticed that at temperatures below 283 K, the two-exponential fits deviated slightly but systematically from the experimental data (see, e.g., residuals shown in the inset of Fig. 1A, upper trace). Fits with three free running exponentials (not shown) yielded excellent fits with a fast phase similar to that of the two-exponential fits. For the two slower phases, however, the obtained rate constants and relative amplitudes scattered strongly with temperature and wavelength, indicating a too large number of fit parameters at the given signal-to-noise ratio. It turned out that fixing the rate of the intermediate phase in the three-exponential fits to $(315\ ns)^{-1}$ at all temperatures yielded both good fits (see residuals in the inset of Fig. 1A, lower trace) and a relatively smooth temperature dependence of the fit parameters (Fig. 2C and D). Again, the fastest phase was virtually temperature independent (triangles in Fig. 2C; the linear regression line corresponds to an activation energy of 7 meV). In this description, the temperature dependence of A_1-reoxidation is accounted for by a slowing of the slowest phase (diamonds in Fig. 2C) and a strong redistribution of relative amplitudes from the intermediate phase (circles in Fig. 2D) to the slowest phase (diamonds) with decreasing temperature.

We consider these three-exponential fits with fixed intermediate rate as not more than one among other possible mathematical descriptions of our experimental data at the present signal-to-noise ratio. Nevertheless, it appears that, below room temperature, the slower phase in the two-exponential description becomes more complex than a simple exponential decay.

The activation energy of the slower phase in the two-exponential description (110 meV) is lower than the previously published value of 220 meV [9]. We cannot exclude that this difference is due to the use of different biological material (monomeric PS I complexes from *Synechocystis* 6803 in the present study versus trimeric PS I complexes from *Synechococcus elongatus* in Ref. [7]). We consider it as likely, however, that at least part of the difference results from different experimental time resolutions and time windows. As the present study was aimed to resolve the faster phase of A_1^- reoxidation, a setup with high time resolution (2 ns), but restricted time window (up to 6 μs after excitation), was used at all temperatures. Hence, kinetic components with lifetimes exceeding several microseconds that might contribute preferentially at the lower temperatures could not be determined reliably and may have been underestimated. In the previous study [9], the time resolution was about 10 ns for measurements above 250 K, and about 2 μs below 250 K. Hence, if components faster than a few microseconds were present below 250 K, their contribution to the overall lifetime (only mono-exponential fits were performed) could have been underestimated.

These ambiguities do not affect, however, the principal new result of the present study, namely that the rate of the faster phase of A_1^- reoxidation is virtually independent of temperature, in contrast to the thermally activated slower phase. This result suggests a straightforward explanation why ET from Q_K-A to F_X is slower than ET from Q_K-B to F_X. The different ET rates may be primarily due to different energetic parameters in the two branches rather than to different distances and hence electronic couplings. An activation energy of 110 meV as derived from our results on the slower phase would slow the reaction at 293 K about 70 times compared to the same reaction at vanishing activation energy. This is the same order of magnitude as the ratio of rates of the two phases at room temperature: $(11\ \text{ns})^{-1}$:$(340\ \text{ns})^{-1} = 31$. A 2higher activation energy may be due to a lower driving force and/or to a higher reorganization energy (see below). As phylloquinone in the A_1 site(s) appears to be pushed to an unusually low reduction potential by its protein environment [13], it is easy to imagine that minor structural differences between the two branches (e.g., in *Synechococcus elongatus* Trp673 in PsaB is replaced by Gly693 in PsaA, and some water molecules are arranged differently Ref. [6]) might cause a significant difference in reduction potentials of the two phylloquinones.

According to Marcus theory [14] that treats vibrations coupled to ET classically, the temperature dependence of ET is essentially due to the factor $\exp[-(\Delta G^0+\lambda)^2/4\lambda k_B T]$ in the rate expression, where ΔG^o is the standard reaction free energy, λ the reorganization energy, k_B the Boltzmann constant, and T the absolute temperature. An essentially temperature-independent rate as observed for the faster phase of A_1^- reoxidation would imply $\Delta G^o \approx -\lambda$. The reorganization energy for ET from either phylloquinone to F_X can hardly be lower than 0.4 eV [2]. Hence, ΔG^o would have to be ≤ -0.4 eV, a value that is inconsistent with the energetic scheme of PS I [15]. Furthermore, for the given edge-to-edge distance of 6.8 Å between phylloquinone and F_X [6], the optimized ($\Delta G^o = -\lambda$) ET rate is expected to be in the order of $(10\ \text{ps})^{-1}$ [16], i.e. three orders of magnitude faster than observed. Hence, Marcus theory seems not adequate to describe the faster phase of ET from A_1^- to F_X.

Temperature-independent ET in the case that $\Delta G^o \neq -\lambda$ can be explained by theories that treat nuclear vibrations quantum mechanically, provided that the characteristic quantum energy $\hbar\omega$ of vibrations coupled to ET exceeds thermal energy $k_B T$. Assuming this case for intraprotein ET, a semi-empirical rule for the rate k_{et} of downhill ($\Delta G^o < 0$) ET has been suggested [16]:

$$\log_{10} k_{et} = 15 - 0.6R - 3.1(\Delta G^o + \lambda)^2/\lambda \quad (1)$$

R is the edge-to-edge distance in Å between electron donor and acceptor, ΔG^o and λ are in eV, and k_{et} is obtained in s^{-1}. Noteworthy, k_{et} does not depend on temperature. With $R = 6.8$ Å, the observed $k_{et} = (11\ \text{ns})^{-1}$ for the faster phase of ET from A_1^- to F_X with $\Delta G^o < 0$ can only be obtained for $\lambda > 0.95$ eV. Possible energetic parameters are, e.g., $\Delta G^o = -23$ meV with $\lambda = 1.0$ eV, $\Delta G^o = -130$ meV with $\lambda = 1.2$ eV, or $\Delta G^o = -244$ meV with $\lambda = 1.4$ eV. In the framework of this highly simplified description, activated ET as observed for the slower phase of A_1^- reoxidation requires that the reaction is energetically uphill ($\Delta G^o > 0$). Neglecting entropic contributions, the observed activation energy of 110 meV would imply $\Delta G^o = 110$ meV. The rate of uphill ET is obtained by multiplying the rate of the reverse (downhill) ET (obtained from Eq. (1) with inverted ΔG^o) with the equilibrium constant $\exp(-\Delta G^o/k_B T)$. Accordingly, the observed rate of $(340\ \text{ns})^{-1}$ at room temperature requires $\lambda = 1.08$ eV, a value within the range of reasonable reorganization energies for the faster phase (see above).

Summarizing, it is possible to account for the different kinetic behaviors of the two phases of A_1^- reoxidation in a particular simple model where only the driving force varies significantly between the two branches. We would like to point out that this description is certainly oversimplified, as

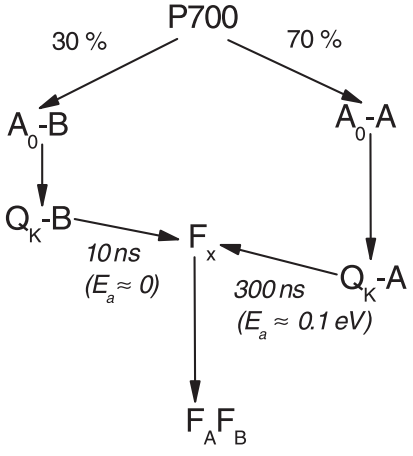
- differences between the two branches that affect ΔG^0 are likely to affect λ as well;
- ΔG^o for uphill ET would deviate from the activation energy if entropy changed in the reaction; entropy changes are common for the reduction of [4Fe–4S] clusters in proteins [17];
- Eq. (1) is a very crude approximation as it was assumed that the electronic coupling between donor and acceptor depends only on distance, and that a unique vibration ($\hbar\omega = 70$ meV, i.e., nearly three times kT at room temperature) is coupled to ET. Allowing for lower frequency vibrations, the quantum mechanical treatment predicts thermal activation for downhill ET as

well. It has been shown in Ref. [9] that the temperature dependence of the slower phase of A_1^- reoxidation reported there can be described by a quantum mechanical treatment assuming $h\omega = 25$ meV and, e.g., $\Delta G^o = 60$ meV with $\lambda = 0.8$ eV or $\Delta G^o = -100$ meV with $\lambda = 1.1$ eV.

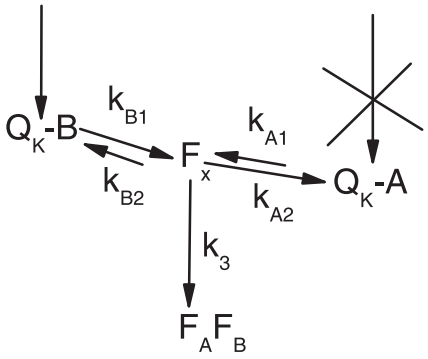
Obviously, the available data do not allow to determine the reaction free energies (and other parameters) of the ET reactions represented by the two phases of A_1^- reoxidation unambiguously. We favor a model (Scheme 1) where ET from phylloquinone Q_K-B to F_X is downhill and essentially independent of temperature (faster phase), and ET from phylloquinone Q_K-A to F_X slightly uphill and hence thermally activated (slower phase).

It has been argued [6,13] that the pronounced structural asymmetry of P700 [6] would suggest ET from excited P700 into one cofactor branch rather than bidirectional ET. Interestingly, it is possible to explain both the mutation effects mentioned above [7] and our present results in a model (Scheme 2) that assumes unidirectional ET from P700 into the B-branch. Q_K-B^- would transfer its electron to F_X in about 10 ns, followed by competing ET from F_X^- to Q_K-A and to F_A. Q_K-A^- would finally be reoxidized by thermally activated ET via F_X to F_A. Numerical simulations of Scheme 2 showed that phyllosemiquinone oxidation similar to the biphasic kinetics observed at room temperature can be obtained, e.g., with the following rate constants: $k_{B1} = (21\ \text{ns})^{-1}$, $k_{B2} = (100\ \text{ns})^{-1}$, $k_{A1} = (111\ \text{ns})^{-1}$, $k_{A2} = (8\ \text{ns})^{-1}$, $k_3 = (15\ \text{ns})^{-1}$. Furthermore, if mutation of the tryptophan stacked to Q_K-B slows k_{B1}, and if mutation of the tryptophan stacked to Q_K-A slows k_{A1}, Scheme 2 predicts the selective slowing of the fast and slow phase, respectively, of A_1^- reoxidation observed in *Chlamydomonas* [7].

A decision between Schemes 1 and 2 might become possible by studying mutants that selectively affect one or the other of the two chlorophylls functioning as primary electron acceptor(s) A_0. Results on such mutants presented



Scheme 1.



Scheme 2.

at a recent congress [18,19] indicate that both A_0-A and A_0-B participate in charge separation in wild-type PS I from *Chlamydomonas*. Yet we feel that time-resolved studies of all ET steps in such mutants are required to solve the issue of directionality of primary charge separation in PS I definitively.

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