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FLASH-INDUCED CHARGE SEPARATION AND DARK RECOMBINATION IN A PHOTOSYSTEM-II SUBCHLOROPLAST PARTICLE

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Summary

The decay time of flash-induced absorption changes in a Photosystem-II subchloroplast fragment is very temperature sensitive down to 210 K, below which it remains constant at 1.25 ± 0.05 ms. The difference spectrum from the near-infra-red to the ultraviolet regions indicates that the monophasic decay represents charge recombination between $P\text{-}680^+$ and the reduced primary acceptor. The charge recombination proceeds by electron tunneling. The *P*-680 concentration in the TSF-IIa fragment was estimated to be one in 30 ± 5 total chlorophyll molecules.

It is known that the major photosynthetic reaction centers undergo rapid charge separation upon absorption of photons. For example, with the time resolution currently available, this initial charge separation in bacterial and Photosystem-I reaction centers is reported to take place in less than 10 ps [1—4], with subsequent electron transfer to a more stable acceptor in approx. 200 ps. If the stable primary electron acceptor is chemically reduced beforehand in Photosystem I, for instance, charge recombination occurs in 3 μs at 20°C and 1.3 ms at 5 K [5, 6].

Similar information has not yet been obtained for Photosystem II, however. Rapidly reversible absorption changes attributed to the photooxidation of the Photosystem-II donor (*P*-680) were reported earlier by Döring et al. [7] and Floyd et al. [8]. Mathis and coworkers later monitored flash-induced oxidation of *P*-680 at 820 nm and observed a recombination

Abbreviations: DCIP, dichlorophenolindophenol; TSF-IIa, Triton-fractionated Photosystem II subchloroplast fragments enriched in chlorophyll *a*.

time of 3.0 ms at 77 K [9—11]. The decay kinetics of *P*-680$^+$ at room temperature has been reported to be multiphasic, and the nature of the various pathways has not yet been completely resolved ([12—14], also cf. reviews 15, 16).

A subchloroplast fragment highly enriched by cytochrome *b*-559 and Photosystem-II reaction-center components has been isolated by Triton treatment of spinach chloroplasts [17]. The Photosystem-II properties of this fragment have been extensively investigated, particularly in regard to the nature of the electron carriers, their chemical reactions and redox properties [17—20], light-induced absorption changes, luminescence and EPR signals [20—22], as well as reconstitution [23] and membrane properties [24].

We report here a flash-induced absorption change which decays in 1.25 ms at cryogenic temperatures. The difference spectrum from the near-infrared to the ultraviolet regions indicates that this monophasic decay represents charge recombination between *P*-680$^+$ and the reduced primary acceptor. We further found the charge recombination to take place by way of electron tunneling.

Experimental. The Photosystem-II subchloroplast fragments (TSF-IIa) were prepared according to Refs. 19 and 20. The reaction-center components (*C* 550, cyt *b*-559, *P*-680, etc) were estimated to be about one in 30—40 Chl molecules. It contains no detectable *P*-700, and has a DCIP-reduction activity of 1000—2000 μmol/mg Chl per h [18, 20]. The reaction mixture contained TSF-IIa particles at Chl concentration of 60—80 μg/ml suspended in a 0.01 M phosphate buffer, pH 6.4, containing 55% glycerol but no other redox carriers. Samples were dark adapted for a few minutes before freezing.

The measuring-light source was either a tungsten-iodine lamp or a high-pressure mercury lamp (ST-75, Hanau), filtered by a Jobin Yvon monochromator (bandwidth 4 nm). The detector was either an EMI-9558Q photomultiplier or a photodiode (SDC, Newbury Park, CA), shielded by a Bausch and Lomb 500-mm monochromator and appropriate filters. A dye-laser pulse with a duration of 300 ns at 640 nm was used for excitation, the flash-induced absorbance changes being registered in a Biomation model 805 waveform recorder and recorded, or, if needed, accumulated in a Tracor Northern model 1710 signal averager. The cryostat and the low-temperature cuvettes (1 mm pathlength) were described previously [25, 26].

Results. As reported previously [18], steady illumination of TSF-IIa particles (in the absence of any added redox carriers) at room temperature produces absorption changes both at 560 nm and in the Soret region which can be ascribed to the reduction of cytochrome *b*-559. Short excitation flashes elicit rapidly-decaying absorption changes in the Soret region. The decay kinetics at room temperature is multiphasic and rather complex. However, the most rapid change constitutes the major phase.

The decay of the major phase becomes slower as the temperature decreases. Below approx. 200 K, the decay becomes nearly monophasic and the lifetime approaches a constant value. Absorption-change transients at 95 K at representative wavelengths are shown in Fig. 1. The semi-log plot in Fig. 1 shows that the half-life at 95 K was the same (1.25 ms) for all wavelengths examined.

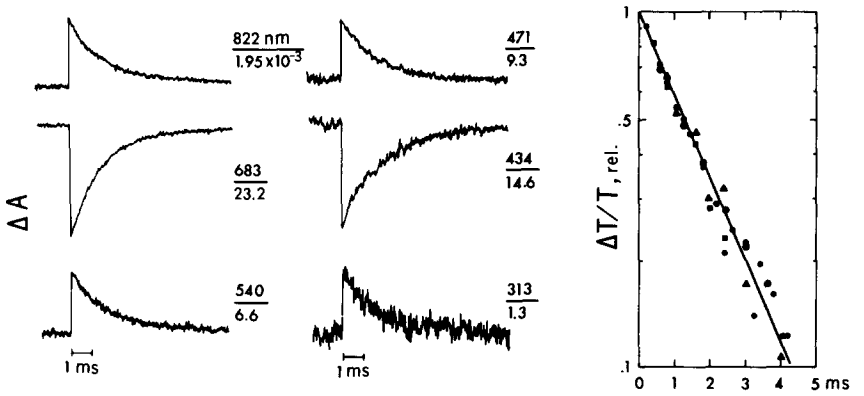

Fig. 1. Left and center: light-induced absorption-change transients in TSF-IIa particles at 95 K. The wavelength and the actual amplitude of change are indicated at the right of each transient. Time scale is shown at the bottom. The transients were obtained under different instrument conditions, and not presented on one single amplitude scale. The transients were obtained with one to eight flashes, with a dark interval of 5 s between flashes. Other experimental conditions are described in the text. Right: semi-log plot of the amplitude of absorption changes vs. time for the six wavelengths shown.

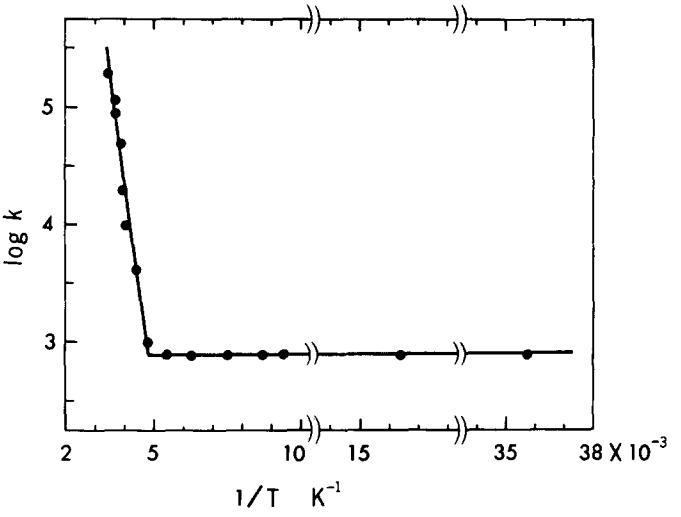

Fig. 2. Temperature dependence of the lifetime $t_{1/2}$ ($k = 1/t_{1/2}$) of the flash-induced absorption changes at 430 nm in TSF-IIa particles. Sample used in this experiment was first cooled down to the lowest temperature and then raised to desired temperatures. The reaction kinetics was reversible with respect to temperature. Other experimental conditions same as in Fig. 1.

Fig. 2 shows that the decay time of the absorption change is quite sensitive to temperature down to 210 K, below which it remains constant at 1.25 ± 0.05 ms. The activation energy calculated for the temperature-dependent region is 8.5 kcal·mol^{-1} ; that below 210 K is, within experimental error, practically zero.

The light-minus-dark difference spectrum constructed from these measurements is shown in Fig. 3. Major absorption decreases occur at 680 and 440 nm, with an apparent shoulder near 420 nm. Broad absorption increases occur between 545 and 600 nm, and above 700 nm. An absorption increase

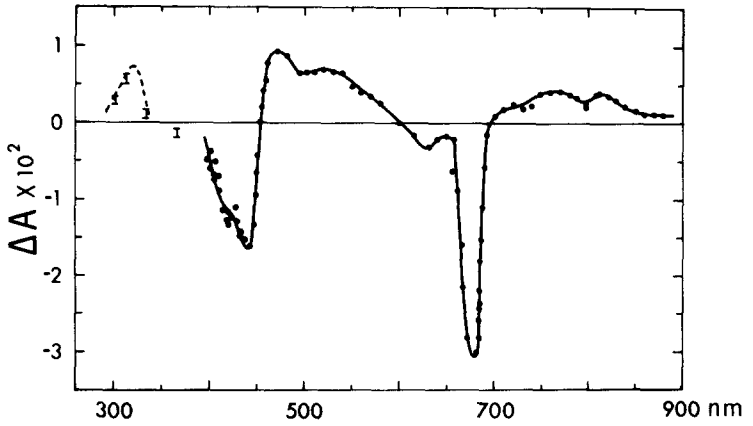

Fig. 3. Light-minus-dark difference spectrum of TSF-IIa particles at 95 K. Experimental conditions same as in Fig. 1.

typical of the cation radical of chlorophyll donor molecule can also be seen near 810 nm. In the near-ultraviolet region, absorbance changes at selected wavelengths indicate a positive difference band located at 320 nm.

Discussion. The light-minus-dark difference spectrum in Fig. 3 can be ascribed to the photooxidation of the primary donor and photo-reduction of the primary acceptor, i.e. $[(P\text{-}680^{+}\text{—}P\text{-}680)+(Q^{-}\text{—}Q)]$. This interpretation is supported by the resemblance to spectral features of similar reactions previously reported: the spectral changes for *P*-680 photooxidation [27], the near-infra-red absorption increase associated with the formation of chlorophyll cation radicals [9], the absorption changes of a quinone molecule accompanying its redox changes in vivo as well as in vitro [28—32]. In the Photosystem-II reaction center, as in other photosynthetic reaction centers, short flashes probably create charge separation followed by recombination at low temperatures:

$$P\cdot I\cdot Q \xrightarrow{h\nu} P^{*}\cdot I\cdot Q \rightarrow P^{+}\cdot I^{-}\cdot Q \rightarrow P^{+}\cdot I\cdot Q^{-} \rightarrow P\cdot I\cdot Q$$

where P is the donor molecule, Q the stable primary acceptor, and I the intermediary acceptor.

Klimov and coworkers [33] observed, under reducing conditions, absorption changes in Photosystem-II subchloroplast particles and attributed them to the reduction of a pheophytin molecule, which is presumably acting as the intermediary acceptor [33]. More recent measurements on fluorescence lifetimes of such systems suggest the recombination time between the oxidized donor and the reduced intermediary acceptor would fall in the ns time domain at room temperature [34]. We are currently looking for conditions, either by prior chemical reduction or removal of the stable primary acceptor Q, to directly obtain absorption changes of I^{-}.

The 1.25 ms for the monophasic decay at low temperatures appear to be in general good agreement with those reported by others. It is noted that the literature values range between 2 ms and 4.2—4.6 ms [9—11, 14, 35]. These variations suggest that the reaction time may be very sensitive to dis-

turbance of the architecture in the thylakoid membrane produced by different preparative procedures.

Note that the amplitude of absorption changes below and above 500 nm is slightly different from those reported by others. This cannot be attributed to additional changes associated with carotenoid triplet formation, as such reactions have been reported to have a much faster decay kinetics [35] at these temperatures. Furthermore, a single exponential decay observed here at different temperatures and at different wavelengths also suggests that the observed changes represent a simple recombination between the primary reactants.

The extinction coefficients of *P*-700 [36] and *P*-870 [37] are known with reasonable precision; they allow one to estimate the reaction-center concentrations in certain samples. No extinction coefficient has yet been determined for *P*-680. However, one may make an approximate estimate of *P*-680 concentration in the TSF-IIa particle by assuming that the extinction values of *P*-680 and the bulk chlorophyll to be nearly identical. By taking the ratio of the light-induced absorption change to the optical density of the red absorption band of the sample, one obtains approximately one *P*-680 per 30 ± 5 total chlorophyll molecules. Thus, TSF-IIa represents a particle highly enriched in PS-II reaction centers, and should provide a valuable material for further experimental use.

It is of interest to note that the temperature dependence shown in Fig. 3 bears a close resemblance to that for cytochrome *c* oxidation by *P*-890$^+$ in *Chromatium* [38] and the recombination of primary charges in Photosystem I [5, 6]. The finding of a temperature-independent charge recombination in Photosystem II adds to a large number of cases already known for various charge-recombination and electron-transport reactions in bacterial reaction centers and in Photosystem I which proceed by electron tunneling [5, 6, 38–42].

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