PRIMARY PHOTOCHEMISTRY OF THE REACTION CENTER OF PHOTOSYSTEM I

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Received 22 January 1979

1. Introduction

The earliest primary photochemical reaction of green plant photosynthesis has, thus far, remained unexplored as reaction center chlorophyll *a* (Chl *a*) complexes have not yet been isolated [1]. In contrast, primary photochemical steps have been successfully studied, with picosecond (ps) absorption spectroscopy [2,3] in isolated bacteriochlorophyll (B Chl)–reaction center complexes from photosynthetic bacteria. Here, the primary charge separation occurs within 10 ps followed by electron transfer, within 200 ps, to a relatively stable (>1 ms) acceptor (Q–Fe, a quinone–iron complex):

$$(\text{B Chl})_2 \cdot \text{I} \cdot \text{Q–Fe} \xrightarrow{h\nu} (\text{B Chl})_2{}^* \cdot \text{I} \cdot \text{Q–Fe} \xrightarrow{<10\ \text{ps}}$$

$$(\text{B Chl})_2^+\ \text{I}^- \cdot \text{Q–Fe} \xrightarrow{100\text{–}200\ \text{ps}} (\text{B Chl})_2^+ \cdot \text{I} \cdot \text{Q}^-\text{–Fe} \qquad (1)$$

where, $(\text{B Chl})_2$ is the primary electron donor, a dimer of B Chl, and I is the real primary electron acceptor; I has been associated with bacteriopheophytin (reviewed in [4]). Evidence for the production of $(\text{B Chl})_2^+$ came from the observation of an absorbance increase at 1250 nm [5] due to the cation.

In green plants, there are two photochemical systems and two light reactions [6,7]. Light reaction II, initiated by the reaction center chlorophyll *a* P680–690, leads to O_2 evolution and the reduction of the products oxidized by light reaction I. (Reviewed in [7], see pp. 149–185). Light reaction I oxidizes a reaction center chlorophyll *a*, P700 [8] and reduces an unknown electron acceptor X (see [9,10] for a discussion of electron acceptors of this system) producing the cation $P700^+$ and anion X^-. P700 has been suggested to contain a special pair of chlorophyll *a* molecules [11,12] and its oxidation can be monitored by an increase in absorbance in the 780–820 nm range [13]. The nature of X is unknown. The time for the primary reaction:

$$\text{P700 X} + h\nu \rightarrow \text{P700}^+\ \text{X}^-$$

has been only indirectly monitored through the accompanying electrochromic shift at 515 nm in the thylakoid membrane; furthermore, this time (<20 ns) was instrument limited [14].

In this letter, we report picosecond measurements of the time for the primary charge separation in photochemical system I, using particles enriched in P700 (Chl *a*/P700, ~30–40). This charge separation was shown to occur within 10 ps by observing a laser-induced increase in absorption around 800 nm. A transient absorption at 730 nm which was quenched by continuous illumination was also observed. A preliminary assignment of this transient to the reduced primary acceptor is made.

2. Materials and methods

Thylakoid membranes were isolated from market spinach by homogenization of leaves in a Waring Commercial blender; the suspension medium contained 0.05 M potassium phosphate buffer (pH 7.4),

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0.5 M sucrose and 0.01 M KCl and 5 mM $MgCl_2$. This suspension was passed through 4 and 12 layers of cheesecloth and centrifuged at 4000 × *g* for 10 min; the pellet of this step was given an osmotic shock by resuspension in the above buffer, but without sucrose. The osmotically shocked preparation was centrifuged, and its pellet was resuspended in 0.05 M potassium phosphate (pH 7.8), 10 mM KCl, 5 mM MgCl and 0.4 M sucrose. The chlorophyll concentration was determined as in [15] then adjusted by further addition of buffer and a 10% digitonin solution to give a final concentration of 1 mg Chl/ml and 1.0% digitonin. This suspension was incubated with stirring at 0°C for 2 h; then centrifuged at 40 000 × *g* for 0.5 h. The supernatant of the last step was centrifuged at 144 000 × *g* for 2 h and the resulting pellet (D144, cf. [16]) resuspended in 10 mM Tris–HCl (pH 7.6). The D144 pellets were further enriched in P700 activity by chromatography on DEAE-cellulose (Pharmacia Sephacel) in a manner similar to that in [17]. After washing the column with 10 mM Tris–HCl (pH 7.6), 50 mM NaCl and 0.1% LDAO (lauryl dimethylamine *N*-oxide), an additional wash was added to the procedure by substituting Triton X-100 for LDAO. The final elution was also accomplished in the presence of Triton X-100 rather than LDAO. Our final preparations contained Chl *a*/P700 ratios ranging from 30–40. These P700-containing particles were concentrated in an Amicon Ultrafiltration Cell (Model 202 with a UM2 filter) to provide an A_{530} of ~1.0 (pathlength, 1 mm).

For the picosecond measurements, the dual-beam picosecond absorption spectrophotometer in [18] was used. The wavelength of excitation was 528 nm; incident energy, 10 mJ; beam area, 2 × 4 mm (elliptical shape); and duration of the individual pulse, 8 ps. Continuous actinic light was obtained from a 5 mW HeNe laser (Spectra Physics Model 125).

3. Results and discussion

The laser-induced absorbance changes obtained 8 ps and 200 ps after excitation are shown in fig.1. The kinetics at 800 nm in the absence and the presence of the actinic light are shown in fig.2. The short-lived transient, $\tau < 100$ ps, appears to be present irrespective of the state of the reaction center. At 800 nm a small part of the transient may be quenched by the

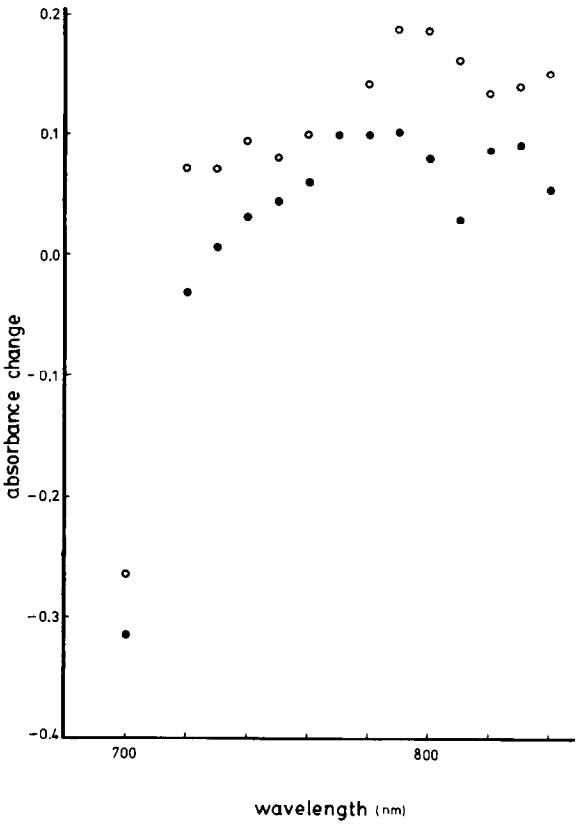

Fig.1. Absorbance changes observed 8 ps (○) and 200 ps (●) after an 8 ps exciting pulse; Chl:P700 ratio ≈ 38; Chl ≈ 1.5 mM.

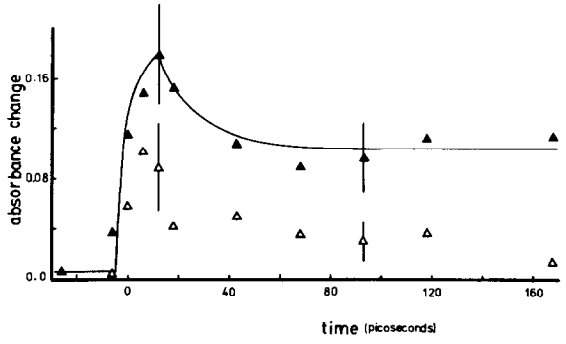

Fig.2. Kinetics of the positive absorbance changes at 800 nm measured in dark adapted samples (▲) and in samples exposed at least 20 s before, as well as during, the flash to the output of a 5 mW He–Ne laser (△); sample as in fig.1.

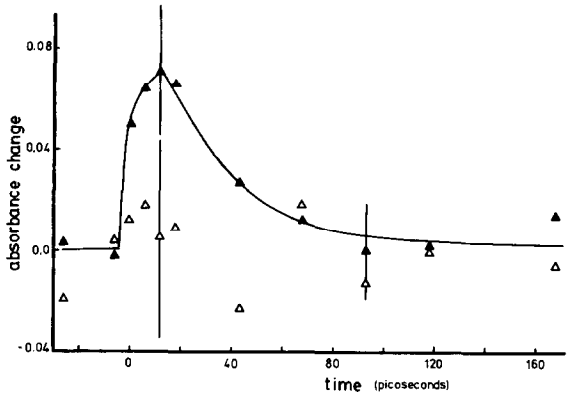

Fig.3. Kinetics at 730 nm, which is the isobestic point for the ($P700^+$–P700) difference spectrum, in dark-adapted samples (▲) and samples illuminated as in fig.2 (△); sample as in fig.1.

application of the continuous illumination. However, the errors in the measurements are larger than the portion of the transient that appears to be sensitive to the state of oxidation of the reaction centers. In contrast, at 730 nm a 40 ps transient (fig.3) is observed which disappears under steady state illumination. Since 730 nm is the isobestic of P700 and $P700^+$, this transient is most likely due to a reduced acceptor formed during the photochemical oxidation of the chlorophyll special pair. Transients were also observed at 700 nm (fig.4) and suggest the formation of a

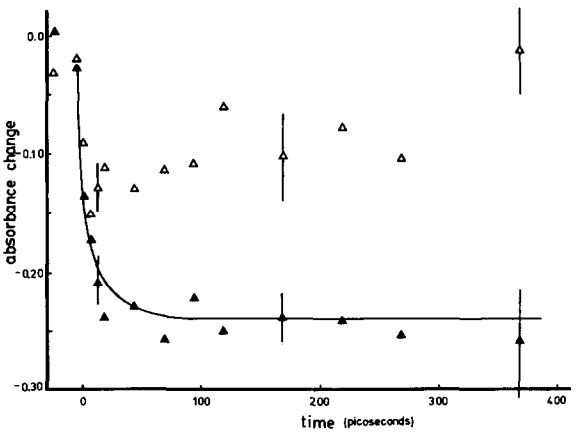

Fig.4. Kinetics at 700 nm for dark-adapted samples (▲) and samples illuminated as in fig.2 (△); Chl:P700 ratio ≈ 38; Chl ≈ 1.1 mM.

species which contributes a positive absorbance change to the difference spectrum, and another, namely $P700^+$, which contributes a negative change. Decay of the transient species which has a positive absorption at 700 nm is responsible for the increase in the bleaching at 700 nm. When the changes 200 ps after excitation (fig.1) are examined, the decrease in absorbance ($-\Delta A$) at 700 nm was 0.32 (±0.03), and the increase in absorbance ($+\Delta A$) at 800 nm was 0.07 (±0.03). In the same preparation, the ratio of the steady state changes at 700–800 nm was 6.8, as measured by a Cary spectrophotometer (model 14 R) equipped with a laboratory constructed side illuminator (intensity of incident light, 640 Wm^{-2}; filters, C.S. 4-76, 2 in. water). In view of the errors (±0.03) in the picosecond measurements, the ratios of $-\Delta A$ at 700 nm and $+\Delta A$ at 800 nm, obtained by the two methods are of almost the same magnitude. The ratio of ~4.5, obtained in the picosecond study, also compares favorably with the steady state ratios of 5–7, obtained [13,19] on P700 changes in system I fragments of blue–green algae and spinach chloroplasts. Furthermore, an absorbance change at 720 nm which is ~10% of that at 700 nm was also observed by other investigators. This similarity in absorbance profile of steady state P700 changes, with those of the picosecond measurements clearly indicate that we are looking at P700.

Work [20] on in vitro chlorophyll has suggested that a positive infrared band around 800 nm may also arise from excited singlet chlorophyll ($^1Chl^*$). Thus we attribute the majority of the transient changes at 800 nm (fig.1,2] to arise from antenna chlorophyll. The lifetimes of the 800 nm transient changes are much shorter than those observed in vitro [21], since even the oxidized reaction center is believed to be capable of trapping and quenching excitation energy. In addition, singlet–singlet annihilation is also expected to quench the excitation in the antenna. Except for the transients, the absorbance changes at both 800 nm and 700 nm were, within experimental error, abolished when P700 was kept oxidized by continuous illumination. Therefore, we were able to establish that the major portion of the absorbance change observed here is not due to singlet excited states of the antenna but to $P700^+$. Moreover, the absorbance changes at 700 nm and 800 nm last for >6 ns in contrast to excited antenna molecules which

should decay within ~200 ps [21] in photosystem I particles. Any changes due to free chlorophyll should decay in ~4–5 ns [22]. Since our preparations contain antenna pigments, it is likely that P700 may have also been indirectly excited by energy transfer from antenna pigments. There are, however, enough photons per P700 in the exciting beam for direct light absorption. The above experiment and arguments suggest that we indeed have observed the formation of the dimer cation, $P700^+$ within 10 ps of illumination and that a reduced electron acceptor absorbing at 700 nm and 730 nm may be formed.

Further work is needed to determine the absorption spectrum of the observed intermediate and to confirm that these changes are not due to a spurious source, such as the excited singlet state of P700, but to physiologically meaningful electron acceptor. The dependence of the observed changes on the power of the excitation pulse, the redox state of the secondary acceptors and the size of the bound antenna protein are needed to verify our findings. These as well as fluorescence lifetime studies are presently underway. The observation of positive changes in the near infrared make it tempting to assign the transient to the anion of pheophytin or chlorophyll based on the spectra [23] and also by analogy with the bacterial reaction.

4. Concluding remarks

This paper presents preliminary picosecond measurements on the primary photochemistry of the reaction center chlorophyll *a* P700. These show that the dimer, P700, is oxidized to $P700^+$ within 10 ps, reducing an electron acceptor. This acceptor appears to be reoxidized in tens of picoseconds.

Acknowledgements

We are grateful to NSF grants PCM 76-21388 and PCM 78-15295 to K.J.K. and PCM 76-11657 to Govindjee. J.F. acknowledges the support of a training grant (HEW PHS GM 07283). K.J.K. is an Alfred P. Sloan Fellow.

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