ELECTRON TRANSFER FROM PLASTOCYANIN TO P700

Function of a subunit of photosystem I reaction center

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1. Introduction

P700, the reaction center chlorophyll in PS I [1], is reduced in the photosynthetic electron flow system after an ultrashort flash with 3 phases [2,3]. The half-times of the phases are 10–20 μs, 200 μs and ~10 ms in spinach chloroplasts [2–6]. The two rapid phases were also observed in blue-green algae [7] and recently in *Chlorella* [8,9]. The slow phase is due to a reduction of $P700^+$ via the rate-limiting step of linear electron transport by electrons from photosystem II [10]. The phase with 200 μs halftime coincides with the oxidation kinetics of PC, and should therefore originate from a reduction of $P700^+$ by PC [11,12]. The electron transfer with the 20 μs halftime could not yet be attributed to a known electron donor of $P700^+$. This electron donor functions between PC and P700 [9].

Purified chloroplast PSI reaction center is composed of 6 subunits [13]. The presence of subunit III is prerequisite for electron transfer from PC to $P700^+$ [13]. Thus, subunit III may function as the unknown immediate electron donor of P700 [9]. We have investigated the electron transfer from PC to $P700^+$ after a short flash at high time resolution using the PSI reaction center preparation with and without subunit III and digitonin particles enriched in PSI.

2. Materials and methods

Chloroplasts were isolated from spinach leaves as in [14] and used fresh. Digitonin particles and PSI reaction centers were prepared according to [15,16] except that DE-23 (Whatman) instead of DE-11 was used for the DEAE-cellulose chromatography in the presence of 0.2% Triton X-100. The subunit composition of the preparations was tested by SDS–gel electrophoresis as in [13,17]. Spinach plastocyanin was isolated similarly to the procedure in [18]. The reaction mixture usually contained chloroplasts or PSI particles at 10–25 μM chlorophyll, 20 mM *N*-tris (hydroxymethyl) methylglycine–NaOH buffer (pH 7.6), 20 mM KCl, 3 mM $MgCl_2$, 2 mM sodium ascorbate and 0.2 mM anthraquinone-2-sulfonate as electron acceptor. The temperature was 21–23°C. Further additions and deviating conditions are noted.

ΔA were measured with a single beam flash photometer [19] of high time resolution. The monitoring light of 703 nm had an intensity of 0.8 W/m^{-2}. A distance of 50 cm between the 1 × 1 cm cuvette and the silicon photocell (EG and G, type SGD 444), a narrow-band interference filter (703 nm, $\Delta\lambda$ = 2.2 nm) in front of the photocell and subtraction without monitoring light [19] were used to minimize disturbance of the signals by flash-induced fluorescence. Repetitive excitation of the sample with blue flashes (Schott-filter BG23/6 mm) of saturating intensity was either by Xe flashes (Xenon corp. 2 μs at halfheight) or by long flashes controlled by a Compur-electronic m-1 shutter (250 W tungsten halide lamp from Osram). The signals were digitized with a transient recorder (Biomation, type 805) and averaged in a signal processor TN 1500 from Tracor. The electrical bandwidth of the whole setup was limited by the dwelltime of 1 μs/address.

Abbreviations: $DCPIPH_2$, reduced form of 2,6-dichlorophenol indophenol; PC, plastocyanin; PSI, photosystem I; SDS, sodium dodecyl sulfate

3. Results

During separation of PSI reaction centers the concentration of Triton X-100 was critical for preserving subunit III [13]. To estimate the amount of PSI still connected with subunit III in our preparations we measured the P700 ΔA induced by long flashes. Fig.1 shows the kinetics in the presence of reduced PC on the left and after addition of reduced $DCPIPH_2$ on the right. During the flashes of saturating intensity total P700 was oxidized. Reduction in the subsequent dark period by PC should only be possible if subunit III is associated with the reaction center. Hence, the amplitude of the rapid reduction enables a quantitative and more direct determination of intact reaction centers than earlier methods. However, the amplitude found in the presence of $DCPIPH_2$ is proportional to total P700, because $P700^+$ is directly reduced [20]. This allowed the estimation of the relative amount of total P700 associated with subunit III. This was 97% in digitonin particles (fig.1, above) and only 26% in our PSI reaction center preparation (fig.1, below). We could not avoid this loss of subunit III during purification of the reaction centers which was caused by the column chromatography as well as by the sucrose gradient centrifugation in the presence of 0.2% Triton X-100. Consistent with [13] we found depletion of subunit III at 1% Triton X-100. Although the reaction centers are characterized in more detail than the digitonin particles of PSI, it is obvious that the latter are advantageous for investigations of subunit III.

In looking for large amplitudes of the fast $P700^+$ reduction in chloroplasts, we found that in the presence of 3 mM $MgCl_2$, 20 mM KCl and 200 mM sorbitol >60% of total $P700^+$ were reduced with 20 μs halftime after a short flash. This is shown in fig.2 (details will be reported elsewhere). Previous investigations reported a relative amplitude of only 30% [2] or 45% [4] for the 20 μs phase.

Fig.3 shows the time course of P700 in digitonin particles in the presence of 0.5 μM PC. The experimental conditions were very similar to those in fig.2. None of the fast electron-transfer reactions to $P700^+$ was observed. This led us to test if digitonin treatment may have slowed down the anticipated fast electron transfer from subunit III to $P700^+$.

For that we followed the complete time course of $P700^+$ reduction at different concentrations of PC as shown in fig.4. The halftime of the exponential decay decreased from 15.2 ms by a factor of 4 to 3.8 ms at an increase of PC from 0.25 μM by a factor of 4 to

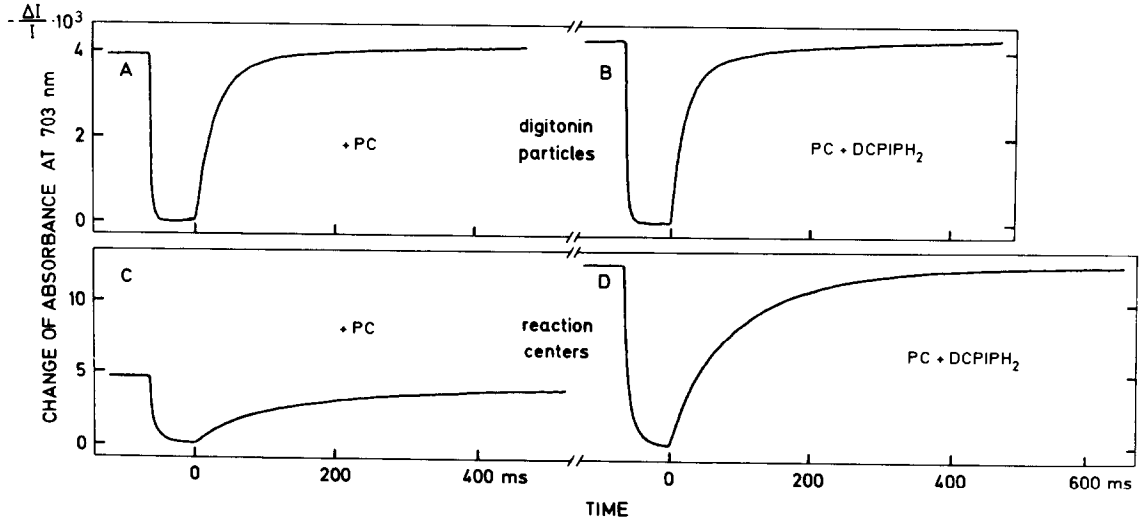

Fig.1. P700 ΔA_{703} in preparations of PSI particles induced by long flashes. Top (A,B) digitonin particles; bottom (C,D) purified reaction centers. Left (A,C) 0.25 μM PC; right (B,D) 0.25 μM PC plus 50 μM $DCPIPH_2$. The reaction mixture contained in addition 10 μM chlorophyll, 1 mM sodium ascorbate, 10 mM phosphate buffer (pH 7.0), 10 mM NaCl, 1 mM $MgCl_2$, 0.1 mM diaminodurene and 0.2 mM methylviologen. The time scale origin is at the end of the 67 ms flashes. 5 signals were averaged with a repetition rate of 0.2 Hz.

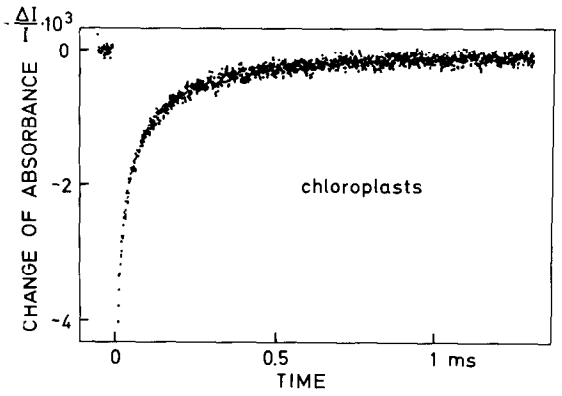

Fig.2. P700 ΔA_{703} in chloroplasts induced by a short flash as a function of time. The standard reaction mixture contained 15 μM chlorophyll and in addition 200 mM sorbitol, 1.5 μM gramicidin D and 0.2 mM $DCPIPH_2$. 2000 signals were averaged at 1 μs/address with a repetition rate of 4 Hz. The sample was changed after 1000 signals.

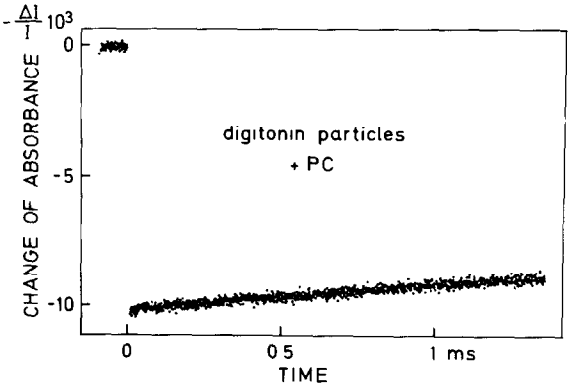

Fig.3. P700 ΔA_{703} in PSI particles induced by a short flash. The standard reaction mixture contained 24 μM chlorophyll and in addition 0.5 μM PC and 0.1 mM diaminodurene. 50 signals were averaged as in fig.2.

1 μM. The relationship was still linear at 2 μM PC. This indicates a pseudo first-order reaction between reduced PC and $P700^+$. The second-order rate constant of the reaction PC + $P700^+ \rightarrow PC^+ + P700$ was estimated from the equation:

$$k = \frac{\ln 2}{t_{1/2}} \times \frac{1}{[PC]} \quad \text{as } k = 1.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$$

The effect of KCl and $MgCl_2$ on the electron transfer from PC to $P700^+$ is shown in table 1. The stimulation by rather low concentrations, in particular by $MgCl_2$, was up to an order of magnitude. The halftime at 50 mM KCl and the rate constant determined at ~70 mM NaCl [21] are in good agreement.

Table 1
Effect of salt on the electron transfer from plastocyanin to P700

Added salt	Half-time (ms) of $P700^+$ reduction
–	43
KCl, 10 mM	14
KCl, 50 mM	7.8
KCl, 20 mM + $MgCl_2$, 3 mM	3.8

Measurement and other conditions as in fig.4, 1 μM PC

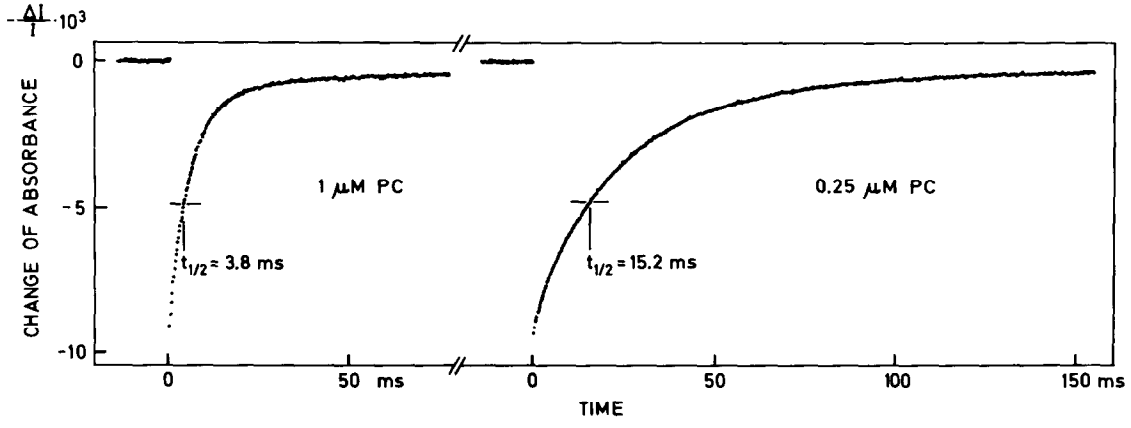

Fig.4. Time course of the P700 ΔA_{703} in PSI particles after a short flash in the presence of reduced PC. Conditions as for fig.3 except that PC was present at 1 μM and 0.25 μM during the measurement of the left and the right signal, respectively. 10 signals were averaged at 200 μs/address with a repetition rate of 1.1 Hz.

4. Discussion

Subunit III of PSI reaction center has been proposed to function as the immediate electron donor of $P700^+$ [9] responsible for the 20 μs phase of its reduction kinetics [2]. Accordingly in a reaction sequence PC → subunit III → P700, oxidation of P700 by a short flash would be followed by an electron transfer from subunit III to $P700^+$. Therefore we have looked for fast reduction kinetics of $P700^+$ in PSI particles. In contrast to our expectation we did not observe the fast reduction of $P700^+$ in the particles as found in chloroplasts (cf. fig.2,3), although in the used digitonin particles ~97% of total P700 was associated with subunit III and accessible to PC (fig.1, above). This result could indicate that the fast reduction of $P700^+$ by subunit III was artificially slowed down by the isolation procedure. Still an electron transfer from subunit III to $P700^+$ in the reaction center should be independent of [PC]. The reciprocal halftime of $P700^+$ reduction, however, increased linearly with the concentration of added PC (cf. fig.4). From this follows, that subunit III of PSI reaction center does not contain an electron carrier.

Nevertheless, our measurements with different PSI particles are consistent with [13] in that subunit III is prerequisite for the electron transfer from PC to $P700^+$. We conclude that subunit III is necessary for a conformation of the reaction center suitable for the electron transfer from PC to P700. Subunit III could enable the interaction between the highly acidic molecule of PC [22] and P700, located in a hydrophobic environment. The effect of salt and in particular of Mg^{2+} (table 1), being in agreement with [23], indicates a stimulation of the electron transfer after compensation of negatively-charged groups.

The discussed function of subunit III suggests that the immediate electron donor of $P700^+$ responsible for the reduction with 20 μs halftime may be PC rather than a completely unknown electron carrier. Our suggestion that it may be cytochrome *f* [11] has to be corrected, because recent measurements of cytochrome *f* in *Chlorella* [12] and in spinach chloroplasts (W.H., H. Krause, unpublished) showed a slower oxidation with a halftime of 100 and 350 μs, respectively.

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References

[1] Kok, B. (1957) Acta Bot. Neerl. 6, 316–336.
[2] Haehnel, W., Doring, G. and Witt, H. T. (1971) Z. Naturforsch. 26b, 1171–1174.
[3] Haehnel, W. and Witt, H. T. (1972) in: 2nd Int. Congr. Photosynthesis Research, Stresa 1971 (Forti, G. et al. eds.) vol. 1, pp. 469–476, Junk, The Hague.
[4] Mathis, P., Haveman, J. and Yates, M. (1976) Brookhaven Symp. Biol. 28, 267–277.
[5] Van Best, J. A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188.
[6] Warden, J. T. and Bolton, J. R. (1974) Photochem. Photobiol. 20, 263–269.
[7] Hiyama, T. and Ke, B. (1971) Biochim. Biophys. Acta 226, 320–327.
[8] Delosme, R., Zickler, A. and Joliot, P. (1978) Biochim. Biophys. Acta 504, 165–174.
[9] Bouges-Bocquet, B. and Delosme, R. (1978) FEBS Lett. 94, 100–104.
[10] Witt, H. T. (1971) Quart. Rev. Biophys. 4, 365–477.
[11] Haehnel, W. (1977) Biochim. Biophys. Acta 459, 418–441.
[12] Bouges-Bocquet, B. (1977) Biochim. Biophys. Acta 462, 362–370.
[13] Bengis, C. and Nelson, N. (1977) J. Biol. Chem. 252, 4564–4569.
[14] Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151.
[15] Bengis, C. and Nelson, N. (1975) J. Biol. Chem. 250, 2783–2788.
[16] Nelson, N. and Bengis, C. (1975) Proc. 3rd. Int. Congr. Photosynthesis, Rehovot 1974 (Avron, M. ed) pp. 609–620, Elsevier, Amsterdam, New York.
[17] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[18] Selman, B. R., Johnson, G. L., Dilley, R. A. and Voegeli, K. K. (1975) Proc. 3rd Int. Congr. Photosynthesis, Rehovot 1974 (Avron, M. ed) pp. 897–909, Elsevier, Amsterdam, New York.
[19] Döring, G., Stiehl, H. H. and Witt, H. T. (1967) Z. Naturforsch. 22b, 639–644.
[20] Izawa, S., Kraayenhof, R., Ruuge, E. K. and DeVault, D. (1973) Biochim. Biophys. Acta 314, 328–339.
[21] Wood, P. M. and Bendall, D. S. (1975) Biochim. Biophys. Acta 387, 115–128.
[22] Katoh, S., Shiratori, I. and Takamiya, A. (1962) J. Biochem. 51, 32–40.
[23] Lockau, W. (1979) Eur. J. Biochem. 94, 365–373.