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STUDIES ON *P*-700 PHOTO-OXIDATION AND REDUCTION IN PHOTOSYSTEM I SUBCHLOROPLAST PARTICLES

J.S.C. WESSELS and F.W.J.M. SPIJKERBOER

Philips Research Laboratories, 5600 MD Eindhoven (The Netherlands)

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The photochemical oxidation and reduction of *P*-700 were studied in digitonin- and in sodium dodecyl sulphate (SDS)-Photosystem I (PS I) particles in the presence of ascorbate. In digitonin-PS I particles, reduction of *P*-700$^+$ occurs by the bound iron-sulphur protein (*P*-430) and by ascorbate. The relative contribution of these back reactions depends on the length of the exposure to light and on the temperature and pH of the reaction medium. Experiments performed under anaerobic conditions demonstrate that some endogenous component may serve as the electron acceptor of *P*-430$^-$. The rate of the latter reaction is also dependent upon the temperature and pH of the sample. At pH 9 and lower temperatures the rate of this reaction is so much reduced that the reduction of *P*-700$^+$ by ascorbate, which increases rapidly at high pH, can be observed even during illumination. The effects of secondary electron acceptors and of the presence of SDS on the absorption changes due to *P*-700 are also reported. Low concentrations of SDS are shown to retard the back reaction of *P*-700$^+$ with *P*-430$^-$. Studies with SDS-PS I particles (CP_I) confirm the absence of the iron-sulphur centres in this preparation. Three larger *P*-700-chlorophyll *a*-protein complexes prepared by mild electrophoresis in the presence of SDS plus Triton X-100, however, still contain *P*-430.

Introduction

The light reaction of PS I in chloroplasts involves the transfer of an electron from the reaction centre chlorophyll *P*-700 to the bound iron-sulphur protein *P*-430 [1,2]. The back reaction of *P*-700$^+$ with *P*-430$^-$ has a half-time of 30–45 ms at room temperature and does not occur at cryogenic temperatures [1,3]. A recent study [3] of the kinetics of flash-induced absorption changes in Triton-PS I particles in which *P*-430 was reduced beforehand revealed more rapid decays of *P*-700$^+$ (3 and 250 μs at 20°C). These results pointed to the existence of two intermediary electron acceptors, which have been designated A_1 and A_2. A_1 was identified with Chl *a*, either in dimeric [4] or monomeric form [5,6], and A_2 with an iron-sulphur protein [4,7,8].

In the present work, light-induced absorption changes due to *P*-700 photo-oxidation and the dark decay of *P*-700$^+$ were measured in both digitonin- and SDS-PS I particles. Most of the experiments were carried out in the absence of exogenous secondary electron donors or acceptors, except for some ascorbate or dithiothreitol, which was added to bring about a complete reduction of *P*-700$^+$ prior to illumination. As the fast charge recombinations between *P*-700$^+$ and A_1^- and between *P*-700$^+$ and A_2^- would not be expected to be observable in our measurements, due to the limited time resolution of the measuring instrument (100 ms), the absorption change kinetics are discussed in terms of the following scheme:

$$\begin{array}{ccc} & h\nu & \\ P\text{-}700 & \rightleftharpoons & P\text{-}430 \ (\rightarrow \text{methyl viologen}) \\ \uparrow & & \downarrow \\ \text{ascorbate} & & \text{endogenous electron acceptor} \end{array}$$

Abbreviations: SDS, sodium dodecyl sulphate; PS I, Photosystem I; Chl, Chlorophyll.

The kinetics of the back reactions between $P\text{-}700^+$ and ascorbate and between $P\text{-}700^+$ and $P\text{-}430^-$ and of the electron transfer from $P\text{-}430^-$ to an endogenous electron acceptor were studied by varying the temperature, illumination period and pH of the reaction mixture. The effect of secondary electron acceptors and of the presence of SDS was also investigated. Experiments carried out under anaerobic conditions demonstrated that oxygen does not necessarily serve as the terminal electron acceptor in this system. In contrast with the SDS-PS I particle CP_I, the larger *P*-700-Chl *a*-protein complexes prepared by electrophoresis in the presence of SDS plus Triton X-100 were found to contain *P*-430.

Methods

The isolation of digitonin-PS I particles (F_I) and the determination of the PS I activities were carried out as reported previously [9,10]. However, in order to achieve a more rapid isolation and a higher yield, we also performed experiments with a PS I-enriched preparation, the isolation and properties of which have been described in a previous paper [11]. Essentially identical results were obtained with the two preparations, except for a somewhat faster decay of $P\text{-}700^+$ in the absence of ascorbate in the PS I-enriched preparation. The SDS-PS I particles CP_I and the high molecular weight *P*-700-Chl *a*-protein complexes CP_{Ia}, CP_{Ib} and CP_{Ic} were prepared by electrophoresis as described previously [11].

Chlorophyll was measured spectrophotometrically by the method of Arnon [12]. *P*-700 was determined from the light-induced absorption change at 700 nm versus 730 nm as the reference wavelength in the presence of 1–2 mM ascorbate and 50 μM methyl viologen, using an Aminco DW-2 dual-wavelength spectrophotometer fitted with a side-illumination attachment and appropriate filters. The oxidation of *P*-700 was performed with blue actinic light (400–530 nm) at an intensity of 1.5 mW/cm^2. An extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used for calculating the amount of *P*-700 [13].

For low-temperature measurements, the reaction mixture contained, in a total volume of 1.5 ml, about 15 μg Chl, 5 mM potassium phosphate buffer, pH 7.2, 15 mM ascorbate and 65% (v/v) glycerol. The 1 cm path lucite cuvette was placed in a cryostat (Cryoson, type XL-500) that could be cooled to any desired temperature above 77 K. Control measurements were carried out in the presence of glycerol at room temperature. The sample was illuminated through a shutter for 250 ms or longer periods as indicated. The measuring light was admitted to the sample only during the time when recordings were made. As far as possible rapid absorption changes were measured at a faster chart speed.

Anaerobic experiments were performed in a Thunberg cuvette. In order to remove dissolved oxygen the cuvette was degassed under vacuum three to five times and filled with pure argon. The sample contained an oxygen-trapping system consisting of 10 mM glucose, 15 U glucose oxidase (Boehringer, grade I) and 0.04 mg (1400 U) bovine catalase (Sigma); in some experiments 10 μl of ethanol were also included. Moreover, the effect of consecutive light-dark periods, which may also result in depletion of remaining oxygen, was studied.

Results and Discussion

Light-induced absorption changes at low temperature

It is well known that PS I particles, at room tem-

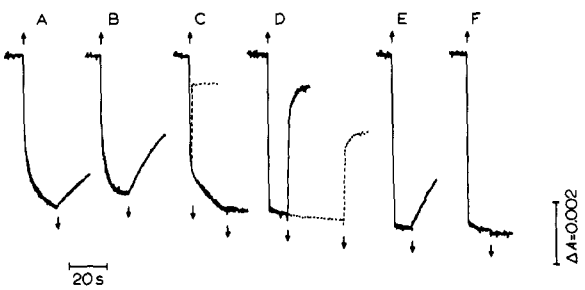

Fig. 1. Light-induced absorption changes at 700 nm in digitonin-PS I particles in the presence of ascorbate. The reaction mixture (1.5 ml) contained digitonin-PS I particles (15 μg Chl), 5 mM potassium phosphate buffer (pH 7.2), 65% glycerol (v/v) and ascorbate as indicated. The absorption changes were measured in an Aminco DW-2 dual-wavelength spectrophotometer at 700 nm vs. 730 nm as the reference wavelength. Light on at upward, off at downward pointing arrows. A, 7.5 mM ascorbate, room temperature; B, 15 mM ascorbate, room temperature; C, 15 mM ascorbate, −20°C; dashed line, $P\text{-}700^+$ decay after an illumination period of 1 s; D, 15 mM ascorbate, −60°C; dashed line, $P\text{-}700^+$ decay after an illumination period of 40 s; E, 15 mM ascorbate and 50 μM methyl viologen, room temperature; F, 15 mM ascorbate and 50 μM methyl viologen, −60°C.

perature and in the presence of ascorbate as an electron donor, exhibit a light-induced absorption change at 700 nm that reverses in the dark (Fig. 1, curve A). We found the decay of $P\text{-}700^+$ in digitonin-PS I particles to be very slow if no ascorbate was added (half-time at room temperature at least 5 min), so that long dark periods were required to obtain a maximum absorption change in the light. The rate of $P\text{-}700^+$ reduction was dependent on the amount of ascorbate added (cf. Ref. 14). As shown in Table I, the half-time of this reaction proved to be approximately inversely proportional to the ascorbate concentration in the 3–90 mM region. This is easily understood as the concentration of ascorbate is much higher than that of *P*-700. Due to the enhanced reduction rate the extent of the *P*-700 photo-oxidation became less at higher ascorbate concentrations (Fig. 1, curve B).

When the temperature of the reaction mixture was lowered, the decay rate decreased substantially and at $-20°C$ the reduction of $P\text{-}700^+$ by ascorbate virtually stopped (Fig. 1, curve C). However, at $-60°C$ a fast back reaction, with a half-time of 0.5 s, became apparent (Fig. 1, curve D; see also Fig. 3, curve C), and the recovery was more complete as the illumination period was shorter. It may be assumed that this fast decay is due to charge recombination between $P\text{-}700^+$ and $P\text{-}430^-$. During a long exposure, however, $P\text{-}430^-$ is slowly oxidized by some endogenous electron acceptor and $P\text{-}700^+$ can no longer be reduced in a subsequent dark period. At higher temperature, the fast back reaction could only be observed when the illumination period was shortened (Fig. 1, curve C, dashed line), which can be explained by the more rapid electron transfer from $P\text{-}430^-$ to the endogenous electron acceptor.

We generally noticed that the rate and extent of the *P*-700 oxidation increased somewhat at lower temperatures, which may be due to a decreased rate of the back reactions. At room temperature such a stimulating effect was observed when the oxidation of $P\text{-}430^-$ was accelerated by an efficient electron acceptor such as methyl viologen [15] (Fig. 1, curve E) or, naturally, when the light intensity was increased. The presence of methyl viologen also prevented the detection of the fast charge recombination between $P\text{-}700^+$ and $P\text{-}430^-$ at $-60°C$ (Fig. 1, curve F).

TABLE I

REDUCTION HALF-TIMES OF $P\text{-}700^+$ IN DIGITONIN-PS I PARTICLES AS A FUNCTION OF ASCORBATE CONCENTRATION AT pH 7.2

Each value represents an average of three different samples.

Ascorbate concentration (mM)	Reduction half-time (s)
3	134
6	65
9	46
12	35
15	29
20	21
30	18
60	9
90	7

Effect of pH on P-700 photo-oxidation and reduction

The reduction rate of $P\text{-}700^+$ by ascorbate was found to increase very rapidly at pH values higher than 8 (Fig. 2, curve A) (cf. Refs. 14 and 16). This pH effect was not observed when dithiothreitol was used as an electron donor. At pH 9, the back reaction in the dark between $P\text{-}700^+$ and ascorbate could be observed (in the presence of methyl viologen) even at

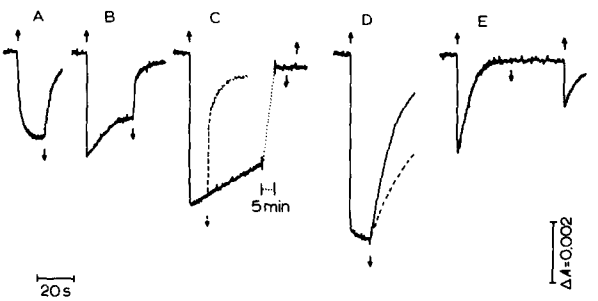

Fig. 2. *P*-700 photo-oxidation and reduction in digitonin-PS I particles at pH 9. Same conditions as in Fig. 1 except for the replacement of potassium phosphate buffer (pH 7.2) by 30 mM Tris-acetate buffer (pH 9.0). The ascorbate concentration was 15 mM with the exception of curve E. A, room temperature; B, $-20°C$; C, $-60°C$; dashed line, $P\text{-}700^+$ decay after an illumination period of 10 s; after completion of the $P\text{-}700^+$ decay in the light a second light exposure only results in an oxidation of *P*-700 after a relatively long dark period; D, $-20°C$, 50 μM methyl viologen added; dashed line, the same at $-60°C$; E, $-20°C$, 30 mM ascorbate (the same curve was obtained when the ascorbate concentration was 15 mM and 10 mM $MgCl_2$ was added).

–20 and –60°C, though the decay rate was decreased when the sample temperature was lowered (Fig. 2, curve D). At pH 4.5 and 6.0, on the other hand, the reduction rate of $P\text{-}700^+$ by ascorbate was not very different from that at pH 7.2. The increased decay rate at high pH may be ascribed to the fact that the divalent ascorbate ion could be a more efficient electron donor than the monovalent ion or the undissociated ascorbic acid.

The electron transfer from $P\text{-}430^-$ to some endogenous electron acceptor was found to decrease at higher and to increase at lower pH values. At pH 9, the fast $P\text{-}700^+$ decay could be observed (in the presence of low ascorbate concentrations) after much longer exposure times and at higher temperatures than at pH 7.2 (e.g., 50% reduction of $P\text{-}700^+$ after 30 s illumination at –20°C). At pH 4.5, on the other hand, the fast back reaction was not even observed at –20°C, whereas at –60°C the *P*-700 recovery in the dark was decreased.

The finding that the rate and extent of the *P*-700 oxidation at room temperature, particularly at low light intensities, were increased by lowering the pH also reflects the enhanced rate of $P\text{-}430^-$ oxidation at low pH. The back reaction between $P\text{-}700^+$ and $P\text{-}430^-$ was found to be virtually independent of pH, the half-time of this reaction at –60°C being 0.5–1 s both at pH 4.5 and at pH 9.

At pH 9, a partial or complete decay of $P\text{-}700^+$ could be observed during the illumination period at –20 and –60°C (Fig. 2, curves B, C and E). This decay can be attributed to reduction of $P\text{-}700^+$ by ascorbate, as the reaction was dependent on the ascorbate concentration and did not occur when ascorbate was replaced by dithiothreitol. Moreover, the rate of this counter reaction increased upon addition of $MgCl_2$ (Fig. 2, curve E). Previously, it has been shown [16,17] that the reduction of $P\text{-}700^+$ by ascorbate above pH 8 is strongly stimulated by $MgCl_2$. In digitonin-PS I particles we found this stimmulation to occur at room temperature as well as at –20 and –60°C. The counter change during illumination resulted in a partial reduction of $P\text{-}700^+$ at –20°C and in a slower but complete reduction at –60°C (Fig. 2, curves B and C). In the presence of $MgCl_2$ and/or larger amounts of ascorbate, however, the complete reduction of $P\text{-}700^+$ could also be observed at –20°C (Fig. 2, curve E). Apparently, the reduction of $P\text{-}700^+$ by ascorbate then exceeds the oxidation of $P\text{-}430^-$ by the endogenous electron acceptor so that an accumulation of $P\text{-}430^-$ occurs. As seen in Fig. 2, curve D, the $P\text{-}700^+$ decay during illumination was not observed when $P\text{-}430^-$ was oxidized by methyl viologen.

When the illumination at –60°C was continued untill $P\text{-}700^+$ was completely reduced by ascorbate and the light was switched off for a short time, a second exposure did not cause any absorption change (Fig. 2, curve C). Apparently, *P*-700 cannot be oxidized in the light because *P*-430 is completely reduced. Not before 30 min of darkness could 50% of the *P*-700 be photo-oxidized again, indicating that at pH 9 and –60°C $P\text{-}430^-$ has a long lifetime. However, when after the first exposure the reaction mixture was quickly heated to room temperature, resulting in an enhanced rate of electron transfer from $P\text{-}430^-$ to the endogenous electron acceptor, complete oxidation of *P*-700 occurred during a second illumination period. At –20°C and in the presence of a high concentration of ascorbate (30 mM), 30 s of darkness were required to obtain 50% oxidation of *P*-700 during a second exposure to light (Fig. 2, curve E).

The partial decay of $P\text{-}700^+$, previously observed by Ke et al. [18] during illumination of Triton-PS I particles in the presence of ascorbate and 2,6-dichlorophenol-indophenol or *N,N,N',N'*-tetramethylphenylenediamine in the temperature range –20 to –60°C, may bear resemblance to that of our experiments with ascorbate at pH 9.

Absorption changes due to P-700 under anaerobic conditions

Though molecular oxygen must be considered as a poor acceptor for $P\text{-}430^-$ relative to electron acceptors such as methyl viologen [19], most authors assume that O_2 is the final electron acceptor at the reducing end of PS I. Comparative studies under aerobic and anaerobic conditions demonstrated, however, that O_2 does not necessarily serve as the terminal electron acceptor in digitonin-PS I particles. It was found that strict anaerobic conditions, or saturation of the reaction mixture with oxygen, did not affect the rate and extent of the fast back reaction of $P\text{-}700^+$ with $P\text{-}430^-$ in a series of experiments involving varying illumination periods at different temperatures both at pH 7.2 and at pH 9. Neither were dif-

ferent results obtained under anaerobic and aerobic conditions when ascorbate was replaced by dithiothreitol as an electron donor. It seems that some endogenous component in the preparation can compete favourably with oxygen for the electrons from $P\text{-}430^-$. Preliminary experiments by Lien and San Pietro [20] indicated that further purification of their subchloroplast preparation resulted in a significant reduction in the endogenous rate of light-induced O_2 uptake. This result may be in accordance with our finding that in digitonin-PS I particles $P\text{-}430^-$ has a relatively low affinity for molecular oxygen.

Effect of SDS

Though high SDS concentrations were inhibitory, low concentrations of SDS (0.05–0.1%) stimulated the rate and extent of *P*-700 photo-oxidation (Fig. 3, curves A and B). This effect was reversed by removal of the detergent by means of dialysis. The stimulation by SDS appeared to result from a reduction in the rate of the back reaction between $P\text{-}700^+$ and $P\text{-}430^-$. The half-time of this reaction at −60°C was found to be 5 s in the presence of 0.07% SDS as compared to 0.5 s in the absence of this detergent (Fig. 3, curves C and D). The electron transfer from $P\text{-}430^-$ to the endogenous acceptor as well as the reduction of $P\text{-}700^+$ by ascorbate were hardly affected by low SDS concentrations.

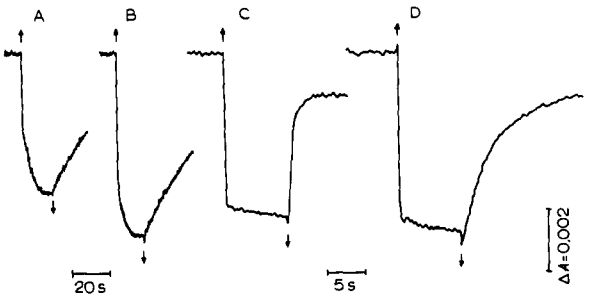

Fig. 3. Effect of 0.07% SDS on the kinetics of *P*-700 photo-oxidation and reduction. A and B, 20°C; C and D, −60°C, time course of the reaction recorded at a faster chart speed; A and C contained 15 mM ascorbate, B and D in addition 0.07% SDS. Other conditions as for Fig. 1.

P-700 photo-oxidation and reduction in electrophoretically prepared P-700-chlorophyll a-protein complexes

The *P*-700 photo-oxidation in SDS-PS I particles (CP_I) exhibited a low efficiency and was found to decrease strongly at lower temperatures (Fig. 4, curves D–F). The reaction had a higher light saturation than in digitonin-PS I particles and methyl viologen did not show any effect on the rate or extent of the *P*-700 oxidation. Since the *P*-700/Chl ratio is higher in the SDS- than in digitonin-PS I particles (1 : 40–1 : 80 as against 1 : 110–1 : 130 [11]) the absorption change per mg Chl was found to be larger. At −60°C no fast back reaction was observed, which is in accordance with the view that this preparation no longer contains iron-sulphur proteins [21,22]. The reduction of $P\text{-}700^+$ by ascorbate resembled that observed in the digitonin-PS I particles. Anaerobiosis or saturation of the sample with oxygen was not found to have any effect on the *P*-700 photo-oxidation and reduction.

Using more gentle procedures of solubilization and electrophoresis, we have recently [11] isolated three *P*-700-Chl *a*-protein complexes with mobilities lower than those of CP_I from spinach thylakoids and digitonin-PS I preparations. Just like the digitonin-PS I particles, but in contrast to CP_I, these complexes showed a very efficient photochemical oxidation of *P*-700 and a fast decay in the dark after short illumination periods and at lower temperatures, indicating the

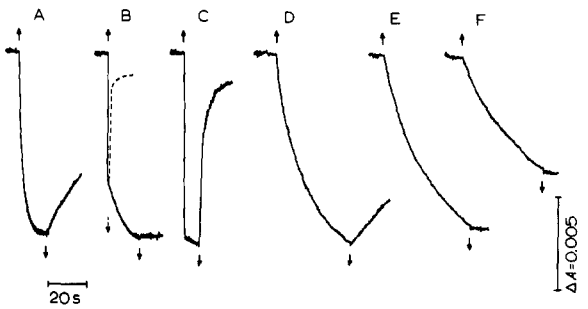

Fig. 4. *P*-700 photo-oxidation and reduction in SDS-PS I particles. A–C, CP_{Ia} (similar results were obtained with the other high molecular weight *P*-700-Chl *a*-protein complexes CP_{Ib} and CP_{Ic}); D–F, CP_I. A and D, room temperature; B and E, −20°C; C and F, −60°C. Dashed line in B, $P\text{-}700^+$ decay after an illumination period of 1 s. Ascorbate concentration 15 mM. Other conditions as for Fig. 1.

presence of *P*-430 (Fig. 4, curves A–C). Previously, we have shown [11] that the high molecular weight *P*-700-Chl *a*-protein complexes consist of CP_I plus five smaller polypeptides with molecular weights similar to those obtained from the digitonin subchloroplast particle F_I. It was suggested that the electron acceptors of PS I may be identified with some of these smaller polypeptides. Some evidence in support of this suggestion was recently provided by Møller and Høyer-Hansen [23], who have tentatively identified two low molecular weight polypeptides as iron-sulphur proteins.

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