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INFLUENCE OF TEMPERATURE ON PHOTOSYSTEM II ELECTRON TRANSFER REACTIONS

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Summary

The influence of temperature on the rate of reduction of P-680$^+$, the primary donor of Photosystem II, has been studied in the range 5—294 K, in chloroplasts and subchloroplasts particles. P-680 was oxidized by a short laser flash. Its oxidation state was followed by the absorption level at 820 nm, and its reduction attributed to two mechanisms : electron donation from electron donor D_1 and electron return from the primary plastoquinone (back-reaction).

Between 294 and approx. 200 K, the rate of the back-reaction, on a logarithmic scale, is a linear function of the reciprocal of the absolute temperature, corresponding to an activation energy between 3.3 and 3.7 kcal · mol^{-1}, in all of the materials examined (chloroplasts treated at low pH or with Tris; particles prepared with digitonin). Between approx. 200 K and 5 K the rate of the back-reaction is temperature independent, with $t_{1/2}$ = 1.6 ms. In untreated chloroplasts we measured a $t_{1/2}$ of 1.7 ms for the back-reaction at 77 and 5 K.

The rate of electron donation from the donor D_1 has been measured in dark-adapted Tris-treated chloroplasts, in the range 294—260 K. This rate is strongly affected by temperature. An activation energy of 11 kcal · mol^{-1} was determined for this reaction.

In subchloroplast particles prepared with Triton X-100 the signals due to P-680$^+$ were contaminated by absorption changes due to the triplet state of chlorophyll *a*. This triplet state has been examined with pure chlorophyll *a* in Triton X-100. An Arrhenius plot of its rate of decay shows a temperature-dependent region (292—220 K) with an activation energy of 9 kcal · mol^{-1}, and a temperature-independent region (below 200 K) with $t_{1/2}$ = 1.1 ms.

Abbreviation: Tricine, *N*-tris(hydroxymethyl)methylglycine.

Introduction

The effect of temperature has long been used as a tool to study the photosynthetic electron transfer reactions in photosynthetic bacteria and in Photosystems I and II. In Photosystem II the reactions leading to the evolution of oxygen are influenced by temperature in a complex manner [1—4]. Between −30 and −60°C the electron transfer from the primary plastoquinone PQ_1 to the pool of plastoquinone is greatly slowed, in parallel with the accumulation of positive charges [5]. The physiological electron donor to P-680 appears to be sensitive to temperature and is progressively replaced by cytochrome *b*-559 between −50°C and −100°C; this donation is influenced by the 'S' state of Photosystem II (reviewed in Ref. 3).

The primary charge separation in the Photosystem II reaction center takes place at cryogenic temperatures, as evidenced by the accumulation of stable photoproducts [1] and by flash-induced signals which reflect the oxidation of P-680 or the reduction of the primary plastoquinone [6—11]. A model describing the Photosystem II reaction center at cryogenic temperatures has been proposed by Butler [1] and Murata et al. [12]. According to this model, oxidized P-680 can be re-reduced either by a back-reaction with a reduced acceptor molecule or by a slower electron donation. This model has been further substantiated and clarified by flash-induced absorption spectroscopy experiments [7—11].

A similar model can describe the reduction of $P\text{-}680^+$ at ambient temperature. After the initial photoinduced electron transfer the following scheme can be drawn:

$$D_1 \overset{k_d}{\rightarrow} P\text{-}680^+ \overset{k_{br}}{\leftarrow} PQ_1^- \rightarrow PQ_2$$

(arrows represent electron transfers; k_d and k_{br} are rates of transfer, D_1 is not cytochrome *b*-559). Under physiological conditions k_d is very high, over $2 \cdot 10^6$ s^{-1}, and is difficult to measure [13,14]. This rate can be slowed down to the microsecond range by inhibiting oxygen evolution, as with alkaline Tris [15]. After chloroplasts have been treated at low pH [16—18], or when D_1 is oxidized by preillumination in Tris-treated [15] and in detergent-treated chloroplasts, the reduction of $P\text{-}680^+$ is mainly by the back-reaction. By manipulating experimental conditions we were able to separately examine the effect of temperature on k_{br} and on k_d. The reaction rate k_d appears to be strongly slowed down by cooling and it could be studied only in a limited temperature range. The rate k_{br} varies less rapidly with temperature and it becomes temperature independent below approx. 200 K. Control experiments were also performed with chlorophyll *a* dissolved in the detergent Triton X-100.

Materials and Methods

Spinach leaves were homogenized for 10 s in 400 mM sucrose, 10 mM NaCl, 20 mM Tris (pH 7.8). The brei was filtered through a nylon mesh (10-μm openings) and the chloroplasts pelleted by centrifugation. Tris treatment of the chloroplasts consisted of resuspension of the pellet in 200 mM Tris (pH 9.0)

and incubation for 10 min under ambient light, at 4°C. For treatment at low pH the chloroplast pellet was resuspended and incubated for 5 min in 50 mM succinate (pH 3.9), at 4°C. After incubation the treated chloroplasts were centrifuged and the pellet was resuspended in 50 mM Tricine (pH 7.0), 10 mM NaCl, 5 mM $MgCl_2$. D-10 particles were prepared by treatment of chloroplasts with digitonin, following the procedure of Boardman [19]. TSF-II particles were obtained by action of Triton X-100, as described by Vernon and Shaw [20]. The particles pellets were stored at −16°C, whereas Tris-treated and low-pH-treated chloroplasts were freshly prepared. Chlorophyll *a*, extracted and purified from spinach, was kindly provided by J. Kleo. The lyophilized chlorophyll was solubilized in 10% Triton X-100.

A pellet, or chlorophyll *a* in Triton, was suspended in a small volume of 50 mM Tricine (pH 7.0), 10 mM NaCl, 5 mM $MgCl_2$ at the beginning of each experiment. The sample (200 μl), 300 μl glycerol and 10 μl of 200 mM ferricyanide were put into a 2 mm path plexiglass cuvette. Ferricyanide was added to chemically oxidize P-700; it was not added to pure chlorophyll *a*.

Absorption changes induced in the cuvette by a ruby (694 nm) or a dye (approx. 600 nm) laser flash were measured as previously described [13,15]. In most experiments the cuvette was placed in a cryostat cooled by a flow of helium gas; the temperature could be regulated between 5 and 294 K. For temperatures between 21 and 0°C a 10 × 10 mm cuvette with four clear windows was inserted in a cooled cuvette holder. Preillumination of the cuvette was effected by a xenon flash [15]. Other experimental conditions are given in Results. For a series of experiments the sample was progressively cooled; when the desired temperature was attained the sample was equilibrated for 20 min. The flash-induced absorption change was then measured, and the sample was further cooled. The sample was changed twice or three times in a complete series.

Results

Chloroplasts

Flash excitation of chloroplasts treated at low pH leads to an absorption increase at 820 nm, which displays nearly monophasic decay kinetics with $t_{1/2}$ = 140 μs at 21°C. Previous analysis led to the conclusion that the signal is due to P-680$^+$, which decays mainly by back-reaction, i.e. an electron return from PQ_1^- [18,21]. This signal (at two different temperatures) is illustrated in Fig. 1. A progressive decrease of the signal size was observed during each series of measurements, which can be attributed to an accumulation of PQ_1 in the reduced state. Illumination of the sample by continuous white light at 77 K or below almost completely suppressed the subsequent flash-induced signals. In Fig. 2 the rate of decay (reciprocal of the half-time $t_{1/2}$) is plotted vs. the reciprocal of absolute temperature, in an Arrhenius plot. There are two domains in the plot, separated by a clear break around 210 K: the decay becomes temperature independent below 210 K (with $t_{1/2}$ = 1.6 ms) whereas it requires an activation energy E_A = 3.7 kcal · mol^{-1} (0.16 eV) above 210 K.

Chloroplasts treated with Tris display a more complex kinetic behavior. Previous experiments at ambient temperature have shown that the kinetics of

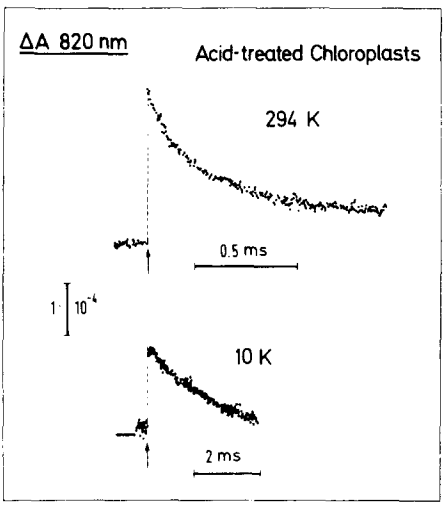

Fig. 1. Kinetics of absorption changes at 820 nm induced in a suspension of acid-treated chloroplasts (chlorophyll concentration: $7 \cdot 10^{-5}$ M; optical path: 2.4 mm) at 294 and 10 K. Averaged effect of two dye laser flashes.

recovery of the absorbance change at 820 nm are different if the first flash follows a period of dark adaptation or if the flash is given after preillumination [15]. The kinetics are also influenced by pH [22] and these experiments were performed at pH 7.6. As shown in Fig. 3, the kinetics are nearly monophasic after the first flash, the major phase decaying with $t_{1/2} \approx 8$ μs (at 21°C). After the second flash, the major phase is slower, with $t_{1/2} \approx 80$ μs. The fast decay is believed to occur in reaction centers where the secondary donor D_1 is reduced, and to represent the rate of electron transfer from D_1 to P-680$^+$ (rate k_d). The slow decay is attributed to the reduction of P-680$^+$ by an electron from PQ_1^- (back-reaction with a rate k_{br}) which occurs in reaction centers where D_1 is oxidized. D_1 must remain oxidized a rather long time (over 1 s) after a flash [15]. The influence of temperature on k_{br} is shown in Fig. 4 (open symbols).

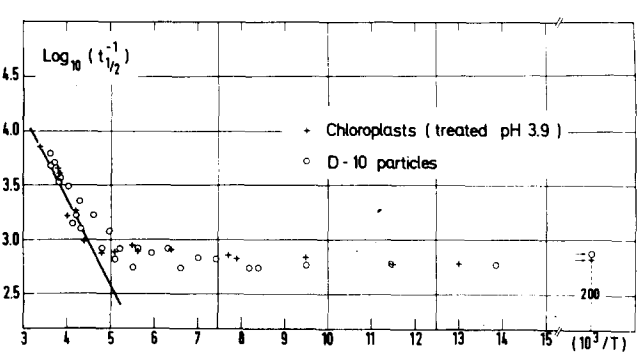

Fig. 2. Arrhenius plot of the decay of flash-induced absorption changes in acid-treated chloroplasts and in preilluminated D-10 particles. Slow phases in the decay (see Fig. 1 at 294 K) have been subtracted.

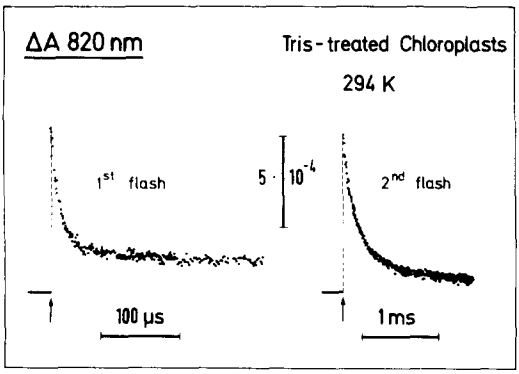

Fig. 3. Kinetics of absorption changes at 820 nm induced in a suspension of Tris-treated chloroplasts (chlorophyll concentration: $4 \cdot 10^{-5}$ M; optical path: 10 mm) at 294 K. Averaged effect of two ruby laser flashes. Left trace: first flash given after 1 min of darkness. Right trace: second flash (one saturating xenon flash was given 50 ms before the laser flash).

The behavior is very similar to that of the back-reaction in chloroplasts treated at low pH: a break at 200 K separates the domain of a temperature-insensitive reaction ($t_{1/2}$ = 1.7 ms) and a region with an activation energy E_A = 3.4 kcal · mol^{-1} (0.15 eV).

Without preillumination a more strongly temperature-dependent re-reduction was observed above approx. 270 K, corresponding to E_A = 11.4 kcal · mol^{-1} (0.50 eV), presumably representing the reaction with D_1 (k_d). Data are shown in Fig. 4 (full symbols). Measurements were not systematically performed below 230 K since it was not possible to decide if P-680$^+$ was reduced by D_1 or by the back-reaction. A $t_{1/2}$ of 1.7 ± 0.2 ms was observed at 77 and at 5 K with Tris-treated chloroplasts cooled in the dark.

Several experiments were also performed with untreated chloroplasts. Chlo-

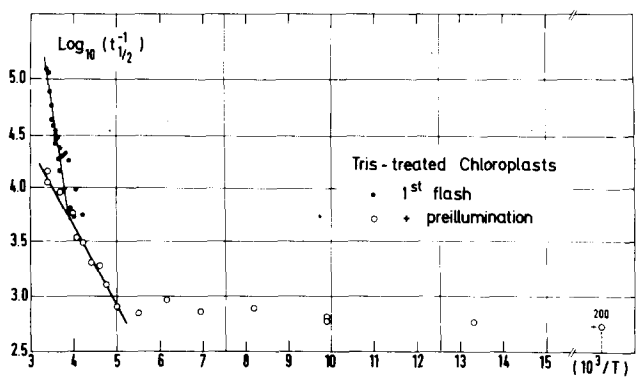

Fig. 4. Arrhenius plot of the decay of flash-induced absorption changes in Tris-treated chloroplasts. Slow phases in the decay (see Fig. 3) have been subtracted. First flash: the time of dark adaptation was progressively increased from 1 min at 294 K to 20 min at 230 K. Preillumination: the time between the xenon flash and the laser flash was 50 ms at 294 K; below 240 K, the sample was permanently illuminated by weak xenon flashes, at the frequency of 0.1 Hz.

roplast pellets were suspended in 50 mM Tricine, pH 7.6, supplemented with 5 mM ferricyanide, with glycerol, and cooled in the dark. Upon excitation with the dye laser, the flash-induced ΔA at 820 nm decayed with $t_{1/2} = 1.7 \pm 0.1$ ms at 77 and 5 K.

Subchloroplast particles

The effect of temperature on the absorption recovery at 820 nm has been studied with preilluminated D-10 particles, prepared with digitonin. The results, as shown in Fig. 2, are similar to those obtained with Tris-treated chloroplasts. The decay is monophasic with $t_{1/2} = 120$ μs at 21°C. It is temperature dependent to 198 K and is then practically temperature independent ($t_{1/2} =$ 1.6 ms). In the temperature-sensitive domain the activation energy is $E_A = 3.7$ kcal · mol^{-1} (0.16 eV).

Particles prepared with Triton X-100 (TSF-II) were also examined. At ambient temperature the kinetics of decay of the flash-induced absorbance increase at 820 nm were rather complex. Fast phases ($t_{1/2} = 2$—8 μs) were only partially transformed into a slow phase ($t_{1/2} \approx 100$—200 μs) after illumination. We suspect that a large fraction of the signal was due to the triplet state of chlorophyll *a* molecules disorganized by the detergent. At present the kinetics were too complex for further study at low temperature.

Chlorophyll a in Triton

After flash excitation of an aerated sample of chlorophyll *a* dissolved in Triton, transient absorption changes were observed in several spectral regions. The kinetics of decay were the same at all wavelengths: $t_{1/2} = 3.0$ μs (measured at 430, 515 and 820 nm, at 21°C). In deaerated samples, the signals have the

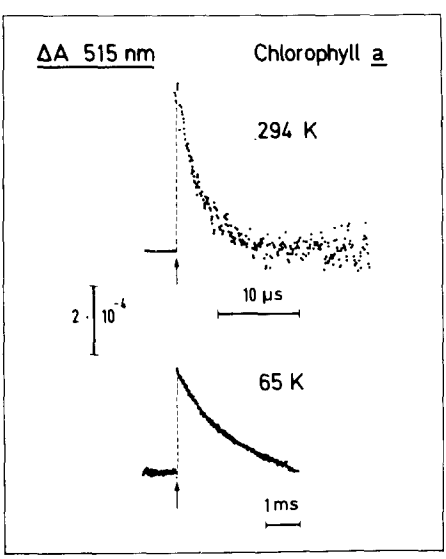

Fig. 5. Kinetics of absorption changes at 515 nm induced in chlorophyll *a* (concentration: approx. $2 \cdot 10^{-5}$ M, optical path: 2.4 mm) by ruby laser flashes at 294 K, and by dye laser flashes at 65 K. Averaged effect of four flashes.

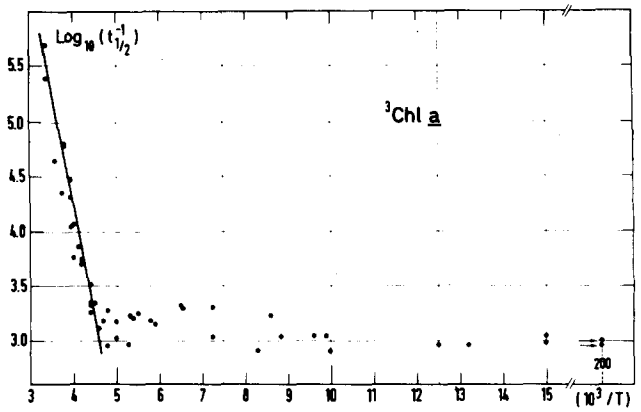

Fig. 6. Arrhenius plot of the decay of flash-induced absorption changes at 515 nm in chlorophyll *a*. Between 294 and 200 K the ruby laser was used, the dye laser was used below 200 K. Around 200 K the two types of flashes led to undistinguishable kinetics.

same size (within 5%) but the decay is much slower ($t_{1/2}$ = 480 μs) at all wavelengths. The signals are thus attributed to the triplet state of chlorophyll *a*. The absorption increase at 820 nm is a residual of a broad maximum around 750 nm. In aerated samples the decay of the triplet state is slowed down at lower temperatures, until 220 K where it is no longer temperature sensitive and the $t_{1/2}$ is 1.1 ms. The effect of temperature on the kinetics of the decay is the same at 515 nm (Fig. 5) and 820 nm. Between 294 and 220 K the reaction has an activation energy of 9.2 kcal · mol^{-1} (0.4 eV) (Fig. 6). At ambient temperature the decay of the triplet state is mainly determined by the rate of collisional quenching by oxygen. The observed slower decay at lower temperature can thus be primarily attributed to a decrease of that rate resulting from an increase of the solvent viscosity. Activation energies similar to the value we found have been reported for the quenching of other triplet states by oxygen, in glycerol [23]. Our system is rather complex (glycerol/water mixture, detergent) and a strict correspondence cannot be expected. At low temperature the decay of triplet states becomes nearly temperature independent and the observed $t_{1/2}$ of the chlorophyll *a* triplet is in agreement with those previously reported [24].

Discussion

There is a striking similarity in the kinetic and energetic properties of the back-reaction, when P-680$^+$ is reduced by an electron returning from the primary stable acceptor PQ_1^-, in acid-treated chloroplasts, preilluminated Tris-treated chloroplasts and preilluminated D-10 particles. In the low-temperature range, where the decay is not influenced by temperature, electron transfer may occur by the tunneling mechanism [25]. The rate of tunneling is critically dependent upon the distance between the reactants PQ_1^- and P-680$^+$. Since the half-time of the back-reaction in untreated chloroplasts is 1.7 ms, similar to the measured rates in the examined materials, the various preparations must not have disturbed the geometry of the reaction center (see Refs. 26—28 for the

effect of various treatments on the rate of the back-reaction in the bacterial and the Photosystem I reaction center). The $t_{1/2}$ in untreated-chloroplasts is shorter than previously reported, ranging between 2 and 5 ms [6—11] probably due to improved time resolution. The activation energy for the electron return (approx. 3.5 kcal · mol^{-1}) is somewhat lower than that reported by Jursinic and Govindjee [29] on the basis of luminescence decay measurements.

The rate of electron transfer from D_1 to P-680$^+$ is strongly affected by temperature (E_A = 11 kcal · mol^{-1}), in the narrow temperature range (294—270 K) where it could be studied. These measurements were with Tris-treated chloroplasts in which D_1 may be a modified state of the physiological donor which reduces P-680$^+$ in approx 30 ns in dark-adapted chloroplasts [13]. If the activation energy is the same for this electron transfer in untreated and in Tris-treated chloroplasts, a calculation of the $t_{1/2}$ of the reaction in untreated chloroplasts gives 1 ms at 185 K, 3 ms at 179 K and 10 ms at 173 K. The $t_{1/2}$ of the back-reaction is approx. 1.6 ms at these temperatures. Thus, in this temperature domain, electron donation from D_1 should become slower than the back-reaction. Actual measurements [30,31] indicate that the back-reaction does predominate below 170 K. It is thus possible to propose that the inactivation of the donor D_1 at low temperature is mostly based on kinetic properties. The rate of electron transfer from D_1 to P-680$^+$ decreases at lower pH in Tris-treated chloroplasts [22]. Since lowering the temperature tends to stabilize protonated states, it is possible that the strong effect of temperature observed for k_D is partially a pH effect (see Ref. 32 for a description of this type of effect). The effect of temperature on k_D has been previously discussed by Jursinic and Govindjee [29] who found no effect of temperature (between 3 and 25°C) on the $t_{1/2}$ of a fluorescence rise (4—5 μs) which they attributed to the electron transfer from D_1 to P-680$^+$. This does not agree with our data. However, the fluorescence rise may not be solely due to the reduction of P-680$^+$, but also to the decay of the triplet state of carotenoids which is only mildly sensitive to temperature (its $t_{1/2}$ is 2—3 μs at 21°C and 7 μs at 77 K [33]).

Ke and Dolan [34] recently studied the effect of temperature on flash-induced absorption changes in Photosystem II subchloroplast particles prepared with Triton. The decay kinetics were attributed to a back-reaction between PQ_1^- and P-680$^+$. Their characterization of the back-reaction is at variance with ours in many respects: $t_{1/2}$ at 21°C (5 μs, instead of approx. 120 μs) and at low temperature (1.25 ms instead of 1.6—1.7 ms), activation energy (8.5 kcal · mol^{-1} instead of 3.5 kcal · mol^{-1}). The differences may originate in the biological material examined. It is possible that some triplet chlorophyll *a* may have been formed following flash excitation in their particles since the kinetic and energetic properties that we found for that triplet state (Fig. 6) bear a definite similarity with those reported by Ke and Dolan for the back-reaction. The difference spectraum also indicates such a contamination, with a broad absorption band at 760 nm, and the near-infrared difference spectrum is different from that of the couple (P-680$^+$, PQ_1^-) [35]. The formation of the chlorophyll triplet state in subchloroplast particles may result from chlorophyll molecules becoming unable to transfer their energy to carotenoids after intersystem crossing.

The back-reaction between PQ_I^- and P-680$^+$ in Photosystem II greatly differs from the similar back-reaction occurring in bacterial reaction centers, between the primary quinone Q_I and the bacteriochlorophyll pair, which is slower at ambient temperature (80 ms) than at low temperature (approx. 20 ms below 150 K) [26,27,36,37]. The equivalent back-reaction has also been studied in Photosystem I [38,39]: its behavior vs temperature is similar to that in Photosystem II. Hopefully the knowledge of the effect of temperature on the kinetics of the back-reactions, along with recent advances in the theories of electron transfer processes [25,40,41], will further the physical understanding of photosynthetic charge separation.

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