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LIGHT-INDUCED REACTIONS OF FERREDOXIN AND P700 AT LOW TEMPERATURES

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SUMMARY

Absorbance difference spectra and kinetics of the photooxidation of the primary electron donor of System 1 of photosynthesis, P700, were measured at low temperatures in spinach chloroplasts and in intact cells of *Chlorella vulgaris*, *Porphyridium aerugineum* and *Anacystis nidulans*, and the redox state of P700 was compared with that of ferredoxin by use of electron paramagnetic resonance techniques.

Analysis of the light-induced absorbance difference spectra of chloroplasts at 110 °K in the region 670–720 nm showed two components. The first one was due to Photosystem 1 and reflected the oxidation of P700. The shape of the spectrum indicated that at least three chlorophyll molecules are involved in this reaction. The second component indicated a shift to shorter wavelength of an absorbance band at 686 nm, presumably associated with the reduction of the primary electron acceptor of Photosystem 2.

The photooxidation of P700 was found to be partly reversible at temperatures between 10 and 200 °K. The extent of the irreversible part increased with decreasing temperature. Below 150 °K, the decay of the reversible part was biphasic. The first-order rate constants obtained were 2.0 and 0.03 s^{-1}, independent of the temperature; the extents of both phases of the decay were about equal. The photoreduction of ferredoxin was also partially reversible and the kinetics were identical to those of P700. These results indicate that below 150 °K three types of reaction centers of Photosystem 1 occur. Ferredoxin is the only electron acceptor, but the rates of the back reaction of reduced ferredoxin with oxidized P700 are different for each type of reaction center.

INTRODUCTION

Around 1960 it was found [1, 2] that P700, the primary electron donor of System 1 of photosynthesis, can be photooxidized at 77 °K. Information about the low temperature reactions of the electron acceptor, however, became available only rather recently when Malkin and Bearden [3, 4] observed an electron paramagnetic

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; P700, the primary electron donor of Photosystem 1.

resonance (EPR) signal which could be induced by light at temperatures of 77 °K and lower. They concluded that the signal was due to reduced ferredoxin, and suggested that it was the primary electron acceptor of Photosystem 1.

With respect to the reversibility of the primary reaction the available data are contradictory. Some authors observed a partial reversibility of P700 oxidation in spinach chloroplasts at low temperature [1, 5, 6], but there are also reports that the reaction is completely or nearly completely irreversible [2, 7–12]. In whole cells of blue-green algae about 50 % of P700 was found to be reversibly oxidized [9, 13]. For ferredoxin a very slow reoxidation in the dark was reported [3].

The main purpose of this study was a comparative study of the kinetics of P700 and ferredoxin, both by EPR and optical methods at temperatures between 200 and 10 °K. The results are in agreement with the hypothesis that ferredoxin is the primary electron acceptor of Photosystem 1. They showed a stoichiometry between $P\,700^+$ and reduced ferredoxin under various conditions, and indicated that the only dark processes that occur are two independent back reactions between these two compounds, with two different time constants. Each of these reactions takes place at a different type of reaction center. With decreasing temperature in an increasing fraction of the reaction centers no back reaction occurred at all.

MATERIALS AND METHODS

Materials

Algae were cultured and prepared as described in ref. 14. Chloroplasts were obtained from market spinach as described elsewhere [15], suspended in a medium containing 50 mM tricine (pH 7.8), 0.4 M sucrose, 10 mM KCl and 2 mM $MgCl_2$ and stored in the dark on ice until use.

Shortly before measurements of light-induced absorbance changes samples were diluted and suspended in glycerol (final concentration: 55 %, v/v) in order to obtain clear samples upon freezing; EPR measurements were performed without glycerol. 2,5-Dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) was a kind gift of Dr. A. Trebst (Ruhr-Universität, Bochum).

Methods

The photometric apparatus to measure light-induced fluorescence yield changes and flash-induced absorbance changes at low temperature was described earlier [16]. A Xenon flash lamp provided the actinic light. The duration of the flash was 8 μs at one-third of the peak. The flash light was filtered by a broad green filter combination consisting of a Balzers Filtraflex DT interference and a Schott OG 4 cut-off filter. For the absorbance measurements, suitable interference filters placed in front of the photomultiplier served to minimize stray light from the monochromator and to diminish the fluorescence signal excited by the flash.

Absorbance changes induced by continuous light were measured with an apparatus equipped with two independent measuring beams, as described in ref. 18. For measurements at low temperature, the suspension was contained between two plexiglas plates, pinched at a brass block. The two sample compartments were 1mm × 10mm × 25mm, the optical pathway being 1 mm. By means of a controlled flow of liquid N_2 through the block the suspension could be cooled to any desired temperature

above 80 °K. The flow rate was adjusted by a valve that was driven by an automatic temperature controller (TR) from Cryoson B.V.. The temperature was measured with a thermocouple positioned in the sample. The sample holder was surrounded by a box of polystyrene foam with quartz windows at the sample positions. The windows were kept free of condensation by a stream of N_2 gas.

The actinic light was filtered by a blue filter combination, consisting of Schott BG 12 and BG 18 glass filters to give a band with a maximum at 460 nm and bandwidth 80 nm. In some experiments a filter combination consisting of a Schott RG 695 cut-off and AL 718 interference filter was used to provide Photosystem 1 light. A Balzers heat reflecting Calflex C filter was added to each filter combination. The photomultipliers were protected with suitable interference filters.

Absorbance measurements in the red spectral region were performed in the presence of DBMIB, which quenches chlorophyll fluorescence [17] in order to diminish artifacts due to changes in the fluorescence yield of pigment System 2.

Electron paramagnetic resonance measurements were recorded using a Varian E-9 Spectrometer, operating near 9.1 GHz, that was calibrated as described before [15]. Samples in standard quartz tubes (3 mm inner diameter) were cooled to the desired temperature by a stream of N_2, provided by a Varian variable temperature accessory (E-257) for measurements at $90 < T < 200$ °K, and by a stream of He as described in ref. 5 for measurements at $10 < T < 90$ °K.

The sample was illuminated in the cavity by an Aldis slide projector (500 W). The beam passed through a cuvette (5-mm pathway) filled with water and through suitable lenses to illuminate the slotted front side of the cavity. The intensity in the cavity was about 100 $mW \cdot cm^{-2}$.

RESULTS

Low-temperature absorbance difference spectra

Fig. 1 shows absorbance difference spectra of spinach chloroplasts, illuminated at low temperature. Fig. 2 shows the kinetics of the changes at a number of wavelengths. The chloroplasts were kept in the dark at 0 °C for at least 10 min, frozen in the dark, and then illuminated. In the red region the first illumination at 110 °K induced absorbance increases at around 683 and 690 nm and decreases near 676, 686 and 702 nm (spectrum A). After several light–dark periods, however, illumination caused a decrease in the region 680–687 nm and the band at 702 nm was about 35 % smaller (spectrum B). These results show that at least two different reactions are responsible for the absorbance changes in the spectral region between 670 and 715 nm. Chloroplasts illuminated in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU)(10^{-5} M) and hydroxylamine (10^{-2} M) shortly before freezing gave a light-induced difference spectrum similar to spectrum B. Since photoreactions of System 2 are blocked by this treatment, due to accumulation of the reduced electron acceptor [19], this indicates that spectrum B is caused by System 1 only, and is apparently due to photooxidation of P700. The kinetics at 703 nm were the same with and without hydroxylamine and DCMU (Fig. 2). In both cases the first illumination at 110 °K produced an absorbance decrease that was about 35–40 % larger than that obtained upon subsequent illumination, even with an intervening dark period as long as 40 min. This indicates that about two-third of the available P700

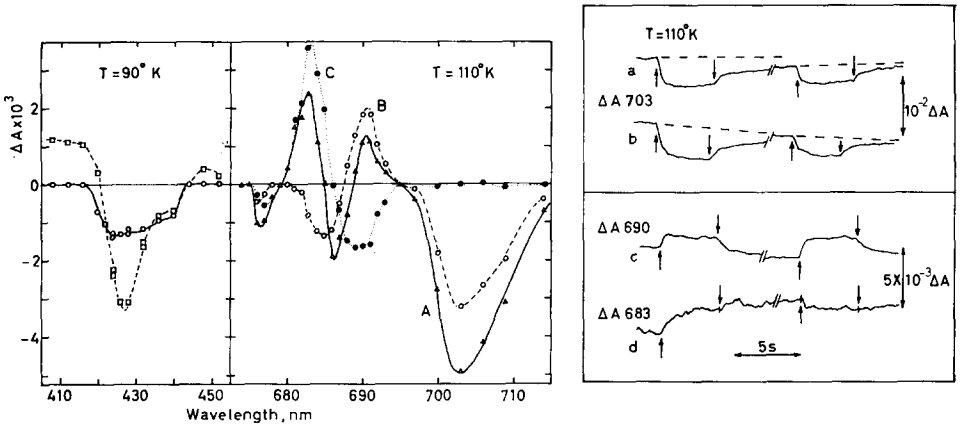


Fig. 1. Absorbance difference spectra upon illumination of dark-adapted spinach chloroplasts (0.22 mg chlorophyll per ml) at low temperature. Left hand spectra at 90 °K; actinic illumination: circles, 718 nm, 60 nEinstein · $cm^{-2} \cdot s^{-1}$; squares, absorbance changes induced by 660 nm light (40 nEinstein · $cm^{-2} \cdot s^{-1}$) with far-red (718 nm) background illumination. Right hand spectra at 110 °K and in the presence of DBMIB (50 μM); actinic illumination: 420–500 nm, 10 nEinstein · $cm^{-2} \cdot s^{-1}$. A, spectrum obtained upon the first illumination; B, spectrum of a 5-s light–1-min dark illumination cycle; C, spectrum of the changes induced by System 2 (see text).

Fig. 2. Time courses of light-induced absorbance changes at 703, 690 and 683 nm, a, c, d, see Fig. 1; b, as (a) without DBMIB, but in the presence of 10^{-5} M DCMU and 10^{-2} M NH_2OH, and with blue pre-illumination (5 s) before freezing. Actinic light on at upwards, off at downward pointing arrows.

was reversibly photooxidized; the remaining reaction centers were irreversibly photoconverted.

On basis of these observations the contribution of Photosystem 2-driven changes to spectrum A was calculated by subtracting spectrum B, normalized at 703 nm. Spectrum C, thus obtained, could also be recorded directly, if Photosystem 1 activity was eliminated by far-red background illumination. Recent measurements of Lozier and Butler [6] at 77 °K and of van Gorkom [20] at room temperature in the presence of deoxycholate indicate that absorbance changes in the region 680–690 nm are caused by reduction of the primary acceptor of Photosystem 2. The shape of the absorbance difference spectrum obtained by Lozier and Butler [6], however is different from that shown here, and was interpreted as a narrowing and increase of an absorption band at 683 nm. According to our spectrum (C) however, the increase at 683 nm is mainly due to a shift of an absorption band near 686 nm toward shorter wavelength. The spectrum obtained by van Gorkom can be similarly interpreted as the blue-shift of an absorption band. The small decrease at 677 nm in spectrum C is probably caused by fluorescence yield changes, that remained in the presence of DBMIB. Ommission of this quencher enhanced both the variable fluorescence yield and the 677 nm absorbance change by a factor of five, but had a smaller effect upon the changes at 683 and 690 nm.

Unexpectedly the absorbance changes induced by far red illumination (718 nm) were rather small in the 430 nm region at low temperatures (Fig. 1). At 90 °K a broad bleaching around 430 nm was observed, amounting to one-third of the 702-nm

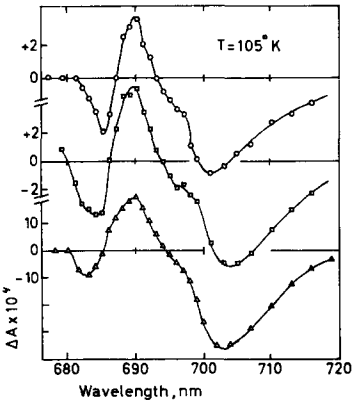
decrease. The changes were partly reversible, like those in the red region. Since both P700 and ferredoxin ("P430", [21]) may contribute to absorbance changes near 430 nm, and since their separate difference spectra at low temperatures are not known, a precise analysis of the spectrum is not possible.

Illumination with red light on a background of far-red light (Fig. 1) caused additional absorbance changes apparently due to excitation of Photosystem 2. The band at 427 nm, which was not observed in the presence of 3 mM ferricyanide (not shown) clearly reflects oxidation of cytochrome b_{559}. Relatively small absorbance changes around 444 nm suggest the red shift of a band peaking at 444 nm.

Repeated illumination of intact cells of *Chlorella vulgaris*, *Porphyridium aerugineum* or *Anacystis nidulans* at 90 °K induced absorbance difference spectra similar to spectrum B (Fig. 3). Just like with chloroplasts, only two-third of P700 could be reversibly oxidized. Apparently this phenomenon reflects a basic property of the Photosystem 1 reaction center.

EPR spectra of P700 and ferredoxin

The most obvious way to explain the partial irreversibility of P700 oxidation would be by means of a dark reaction that stabilized the charge separation (cf. ref. 6) in an analogous way as proposed for Photosystem 2 [22]. For System 2 a secondary electron donor was postulated in order to explain the irreversibility of C550 reduction; for System 1 a secondary electron acceptor might be involved, that oxidized the reduced acceptor in some of the reaction centers, thus preventing a back reaction with $P700^+$. As ferredoxin was reported to be reduced practically irreversibly at low temperatures



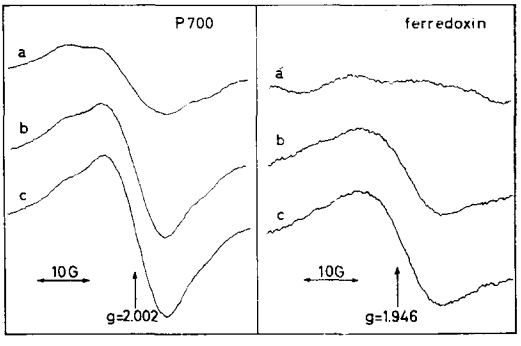


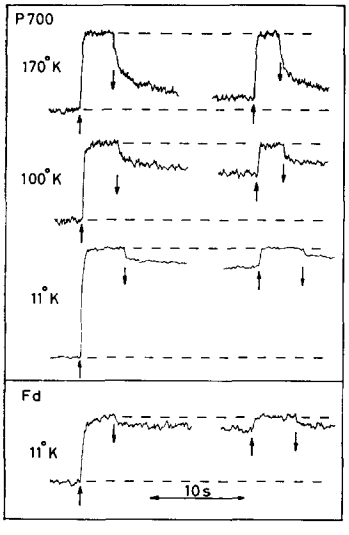
Fig. 3. Difference spectra light minus dark of the reversible absorbance changes at 105 °K in the presence of DBMIB (150 μM) of intact cells of ○, *C. vulgaris*; □, *P. aerugineum*; △, *A. nidulans*. Actinic illumination: 420–500 nm, 90 nEinstein · cm^{-2} · s^{-1}, in a 5-s light–1-min dark cycle. Absorbance per mm at 680 nm, corrected for scattering: ○, 0.7; □, 0.3; △, 1.0.

Fig. 4. EPR spectra at 11 °K of spinach chloroplasts (2 mg chlorophyll per ml) near $g = 2.00$ and $g = 1.94$. a, sample frozen in the dark; b, sample frozen in the dark and illuminated for about 30 s with white light at 77 °K; c, sample b during subsequent illumination with white light at 11 °K. Instrument settings: frequency: 9.080 GHz; power: 2 mW (P700) and 10 mW (ferredoxin); modulation amplitude: 5 G (P700) and 10 G (ferredoxin); time constant: 0.3 s; scan time: 1 G/s. The right hand spectra (ferredoxin) were recorded with four times increased sensitivity. Control experiments showed the same behavior for the ferredoxin bands at $g = 2.05$ and 1.86 as at $g = 1.94$.

[3] this compound could be the secondary acceptor in such a model. Therefore, we studied the kinetics of the Photosystem 1 reactions at low temperature in more detail by means of EPR measurements of Signal I (P700) and of the ferredoxin signal at $g = 1.94$. Fig. 4 shows spectra, measured with a sample that was subsequently illuminated at 77 and at 11 °K. The spectra of a dark-frozen sample are also given to show the extent of Signal II [23]. If we take the signal height in the spectra which were measured during illumination at 11 °K, to indicate 100 % reduction and oxidation of ferredoxin and P700 respectively, it is clear from Fig. 4 that at 77 °K about 70 % of the total amount of P700 is irreversibly photooxidized and also about 70 % of the ferredoxin photoreduced.

The spectra b of Fig. 4 were obtained with a dark time of 3 min at 77 °K after the illumination. More P700 and ferredoxin were found to be in the oxidized and reduced state, respectively, when samples were cooled to 11 °K immediately after the illumination. The relative amounts of $P700^+$ and reduced ferredoxin were always equal in the same sample.

The reversibility of P700 oxidation and ferredoxin reduction was found to be temperature dependent (Fig. 5). At 11 °K only 20 % of the amount of P700 was photooxidized reversibly. Measurement of the EPR signal at $g = 1.95$ indicated that



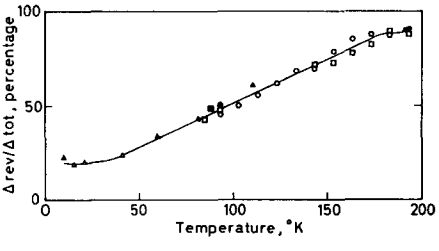


Fig. 5. Time courses of light-induced changes of the low-field maxima of the signals at $g = 2.002$ and 1.946 of spinach chloroplasts (1 mg chlorophyll per ml) at the temperature indicated. White light on at upward, off at downward pointing arrows. Left hand curves: changes induced by the first illumination; right hand curves: changes after several 5-s light–1-min dark cycles. Instrument settings: at 170 and 100 °K: frequency: 9.084 GHz; power: 2 mW; modulation amplitudes: 5 G; time constant: 0.1 s; at 11 °K: as in Fig. 4. Receiver gain: at 170 and 100 °K: $8 \cdot 10^3$; at 11 °K for P700: $5 \cdot 10^3$, and for ferredoxin: 10^4. Fd, ferredoxin.

Fig. 6. Temperature dependence of the fraction of reversibly photooxidized P700, measured as in Fig. 4. Δ_{tot}: extent of the change at first illumination; Δ_{rev}: extent of the dark decay. Open symbols refer to different batches of spinach chloroplasts, solid symbols were obtained with *C. vulgaris*. (●), *A. nidulans* (▲) and *P. aerugineum* (■).

similarly 20 % of the amount of ferredoxin was reversibly photoreduced. Comparison of the kinetics at much lower intensities showed that also the light-on signals of ferredoxin and P700 were the same.

The experiments described above show that both at 77 and 11 °K there was always a stoichiometric relation between the amount of $P700^+$ and reduced ferredoxin, both in the light and in the dark. This indicates, in contrast to the above-mentioned model that ferredoxin is the only electron acceptor and that secondary reactions do not occur at these temperatures. Apparently in some reaction centers reduced ferredoxin and $P700^+$ are able to react back with each other, in others they are not.

The temperature dependence of the fraction of reversibly photooxidized P700, measured from the EPR kinetics as in Fig. 5, is depicted in Fig. 6. With increasing temperature gradually more P700 was reversibly photooxidized between 40 and 170 °K. Between 10 and 40 °K and between 170 and 195 °K this fraction remained constant at 20 and 90 %, respectively. Experiments at 90 and 180 °K indicated the same temperature dependence for intact cells of *C. vulgaris*, *P. aerugineum* and *A. nidulans* (Fig. 6). The same temperature dependence was also obtained with optical measurements. A typical experiment with chloroplasts at 110 °K is shown in Fig. 2. These results demonstrate that the phenomenon is not affected by the presence of glycerol. Remarkably, the fraction of irreversibly photoconverted P700 and ferredoxin seemed to depend on the temperature only and not on the preceding treatment of the sample. Chloroplasts illuminated at 10 °K showed 80 % irreversibly converted P700 and ferredoxin, but after subsequent warming to 77 °K, this amount decreased to 70 %, the same number as given in Fig. 6 for this temperature. At 90 °K, a saturating flash oxidized 65 % of the available P700, half of which was converted reversibly (Fig. 7), like with continuous light (Fig. 6).

Rate constants of the back reaction

At temperatures below 150 °K the back reaction between $P700^+$ and reduced ferredoxin was biphasic (Fig. 5). 50–70 % decayed with a first-order rate constant

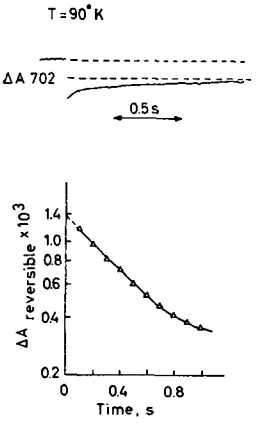


Fig. 7. Time course of the decay of the absorbance change at 702 nm after the first (saturating) flash at 90 °K, of dark frozen spinach chloroplasts (0.44 mg chlorophyll per ml), and semilogarithmic plot of the reversible part of the absorbance change.

of 2.0 s^{-1} (Fig. 7), the remaining part with a rate constant of 0.03 s^{-1}. Between 10 and 150 °K, this ratio, as well as the rate constants were independent of temperature and of the duration of the preceding illumination (up to 20 s). These results show that two types of reaction centers can be distinguished, which are different with respect to the rate constant of the back reaction. Otherwise reaction centers with a low rate constant would accumulate during prolonged illumination. For *C. vulgaris*, *P. aerugineum* and *A. nidulans*, measurement of the dark reduction of $P700^+$ at 100 °K similarly showed a biphasic decay with roughly the same rate constants as for chloroplasts.

The experiments reported above and in the preceding section are thus in agreement with the following model for the reaction centers of Photosystem 1:

$$P700 \cdot \text{ferredoxin} \underset{k_{-1}}{\overset{k_1(\Phi, I)}{\rightleftharpoons}} P700^+ \cdot \text{reduced ferredoxin}$$

Three kinds of reaction centers can be distinguished with respect to the values of k_{-1}: 2.0, 0.03 and $< 4 \cdot 10^{-4}$ s^{-1}. The efficiency of the charge separation, ϕ, was found to equal 0.65 equiv./*hv*.

The intensity dependence of the steady-state deflection in blue light of the absorbance change at 702 nm was found to agree with this model, when it was assumed that 40 % of absorbed actinic illumination (460 nm) was used by Photosystem 1, that no energy transfer between photosynthetic units occurred and that the differential extinction coefficient of P700 oxidation at 702 nm is 70 $mM^{-1} \cdot cm^{-1}$ at 110 °K. The latter value gave a ratio of 390 chlorophyll molecules per oxidized P700 in spinach chloroplasts (see Fig. 1).

A fourth component, which was temperature dependent, was observed in the decay of the changes at temperatures between 150 and 200 °K. The Arrhenius constants of this back reaction were $A = 6 \cdot 10^4 \cdot s^{-1}$, and $E = 1.7 \cdot 10^4$ J. A calculation shows that the half time of this reaction will be 20 ms at 300 °K in reasonable agreement with the rate of back reaction reported by Ke [21] for System 1 particles.

DISCUSSION

The results described above support the hypothesis of Malkin and Bearden [3, 4] that the primary electron acceptor of Photosystem 1 is a ferredoxin with EPR signals at $g = 2.05$, 1.94 and 1.86. Below 150 °K, the only dark reaction that was observed in our preparation was a back reaction of $P700^+$ and reduced ferredoxin: even though we observed that the reaction centers of Photosystem 1 are heterogeneous with respect to this back reaction, in all types of centers the redox state of ferredoxin was stoichiometrically related to that of P700. Yang and Blumberg [5], by comparison of the rate of $P700^+$ decay with that reported for ferredoxin by Malkin and Bearden [3] arrived at a different conclusion, and suggested that ferredoxin might not be the primary acceptor of System 1. Our results, however, show a clear stoichiometry for dark times of up to several minutes. For longer times the possibility cannot be ruled out that secondary dark reactions might affect the redox states of P700 and ferredoxin. Yang and Blumberg [5] also observed that the initial decay of P700 at 77 °K is not of first order. We showed above, that the initial decay is described by two first order

rate constants: 2.0 and 0.03 s^{-1}, reflecting independent reactions between $P700^+$ and reduced ferredoxin.

A possible explanation for these two time constants might be based on the assumption that the distances between ferredoxin and P700 are not the same in all reaction centers. The temperature-independence of the two rate constants suggests that the back reaction occurs by way of a tunneling process. The tunneling rate is known to depend exponentially on the distance between the reacting species. According to a classical approximation (e.g. ref. 24)

$$\frac{1}{\tau} = \nu_0 \exp\left[-\frac{4\pi d}{h}(2m\Delta E)^{\frac{1}{2}}\right],$$

where τ is the half time of the reaction, ν_0 is the frequency of the electron approaching the barrier, h is Planck's constant, d and ΔE are the width and height of the barrier, respectively, and m is the mass of an electron. Substituting for ΔE the estimated excess energy of the absorbed photon, 0.8 eV and for ν_0, $10^{15} \cdot s^{-1}$ (this number is not very critical as the exponential term dominates the expression), distances of 37 and 41 Å, respectively are obtained for P700 and ferredoxin for $\tau = 0.3$ and 25 s, respectively. Because of the approximations involved in the derivation of the equation used, these numbers are only rough approximations. Nevertheless, they are quite compatible with present theories about the structure of the thylakoid membrane. The difference in distance, 4 Å, would be more reliable. On the other hand the different half times might also be due to a difference in height (ΔE) or in both height and width of the barrier. A difference of 0.2 eV would account for the difference of the half times for $d = 40$ Å. Such a difference might be due to different surroundings or orientations of the primary reactants.

For spinach chloroplasts it is known that two types of System 1 reaction centers can be distinguished, viz. those located in the grana and in the stroma lamellae [25, 26]. However, we observed about the same rate constants for the two first-order back reactions in spinach chloroplasts, *C. vulgaris*, *P. aerugineum* and *A. nidulans*, which have different structures of the thylakoid systems.

The heterogeneity of the reaction centers with respect to the irreversibility of the photoconversion has been discussed above in terms of a model involving a secondary reaction in part of the centers. It was concluded that such a model does not explain our results, since the redox state of ferredoxin was stoichiometrically related to that of P700. We have no ready explanation for the temperature dependence of the heterogeneity (Fig. 6), especially in view of the observation that the effect is independent of the preceding treatment. One might speculate that the effect is related to the temperature dependence of the magnetic susceptibility of ferredoxin [27], which is due to antiferromagnetic coupling between the two (high spin) iron atoms. Since it is known that the EPR signal of ferredoxin may reflect both iron atoms of the molecule [28], it might be postulated that a different iron atom is reduced in the reaction centers that are irreversibly photoconverted than in those in which a back reaction between $P700^+$ and reduced ferredoxin occurs.

Conflicting values have been reported for the fraction of reversibly photoconverted reaction centers in chloroplasts at low temperature [1, 2, 5–12]. Since most authors did not specify the experimental conditions into sufficient detail, it is impossible to explain these differences with certainty. However it should be pointed out

that the use of low actinic intensities and of strong measuring light will affect the (apparent) fraction of irreversibly converted reaction centers.

Floyd et al. [29] reported that, after a short laser flash, about two-third of P700 decayed very rapidly at liquid N_2 temperature, with a half time of 30 μs. Whatever the cause of this rapid reaction, it seemed to be absent in our conditions, judging from the fact that the total amount of oxidizable P700 we observed was the same as that observed by Floyd et al. [29], relative to that of cytochrome b_{559}.

A final point that should be discussed here is the shape of the difference spectrum of P700 at low temperature. Negative bands at 700–705 nm have been observed repeatedly [2, 9, 10, 13]. More recently, bands at shorter wavelengths were also observed. Our spectrum B is similar to that obtained by Lozier and Butler [6] with a scanning method. This also applies to the spectrum reported by Floyd et al. [29] except that they noted a negative band at 680–682 nm instead of 685 nm, which they attributed to P680, the primary electron donor of System 2. Witt [12] recently reported an increase at 695 nm, instead of 690 nm, and a decrease at 710 instead of 703 nm. The reason for this discrepancy is not clear. He proposed that the increase would reflect the reduction of a chlorophyll molecule. This hypothesis is contradicted by at least two observations: firstly that there is a stoichiometry between $P700^+$ and reduced ferredoxin, and secondly that the increase is also present in the difference spectrum of chemically oxidized P700 [6].

Two proposals have been put forward to explain the shape of spectra of P700 obtained at room temperature. According to the first one [30, 32] the spectrum is composed of a bleaching centered at about 700 nm and a red shift of a band located at shorter wavelength. In a second proposal of van Gorkom et al. [33] the spectrum was explained by a bleaching near 680 nm and a blue-shift of a pigment absorbing near 693 nm. The bleaching was thought to be due to the conversion of the P700 dimer to its monocation. Analysis of the difference spectrum obtained at 110 °K by the first method was found to be impossible, unless the formation of a third band at about 690 nm (broken line) was postulated (Fig. 8A). The second type of analysis gave the result shown in Fig. 8B. In this case in addition to a blue shift centered at 698 nm, a two-banded absorbance decrease at 680–700 nm (dotted line) was obtained, which could conceivably be explained by the assumption that the absorption band

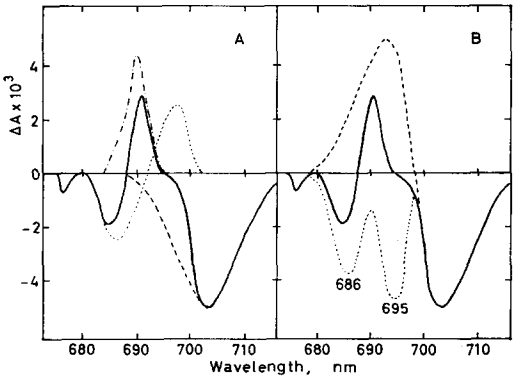


Fig. 8. Analysis of spectrum B of Fig. 1 (see Discussion).

of the dimercation is narrower and somewhat lower than that of the reduced dimer (as also seems to be true at room temperature [33]), and that both bands are located near 690 nm. According to this analysis the bands of the P700 dimer, the dimercation and of the species causing the band shift at 698 nm would all be shifted to longer wavelength by lowering the temperature viz. by 24, 28 and by 5 nm, respectively. Red shifts of the absorption bands of bulk [34, 35] and reaction center [34, 36, 37] bacteriochlorophyll, and of chlorophyll in solution [38] upon cooling have been observed earlier. It thus appears that the hypothesis of van Gorkom fits both the room and low temperature data.

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