Biochimica et Biophysica Acta, 460 (1977) 36–46

BBA 47272

PHOTOOXIDATION OF CHLOROPHYLL IN SPINACH CHLOROPLASTS BETWEEN 10 AND 180 K

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(Received September 13th, 1976)

SUMMARY

Electron paramagnetic resonance (EPR) and optical absorbance difference spectra and kinetics upon illumination by saturating flashes and continuous light of spinach chloroplasts frozen under various conditions were measured between 10 and 180 K.

1. At 100 K illumination with continuous light caused an EPR signal which decayed during the light in about 30 ms. This change is probably due to the reduction of P^+-680, the oxidized primary electron donor of Photosystem II, by a secondary electron donor, cytochrome *b*-559. Flash illumination yielded the previously observed rapid (2 ms) transient. This transient has been ascribed to a back-reaction of the two primary reagents of Photosystem II (Malkin, R. and Bearden, A. J. (1975) Biochim. Biophys. Acta 396, 250–259; Visser, J. W. M. (1975) Thesis, Leiden).

2. Between 10 and 40 K, illumination with continuous light showed a transient which decayed in about 500 ms. The extent decreased with increasing temperature. However, the half time appeared to be temperature independent. This signal was also attributed to P^+-680.

3. At 180 K it appeared to be impossible to observe the 2 and 30 ms components in dark frozen chloroplasts. However, they could be observed again if two short saturating flashes were given shortly before freezing. These changes seem to be dependent on the S-state of the reaction center.

4. After oxidizing the sample with ferricyanide (E_h = 540 mV), the light-induced absorbance difference spectrum showed a bleaching near 676 nm. This change is ascribed to the irreversible oxidation of a dimeric chlorophyll molecule which acts as electron donor to P^+-680 under these conditions.

5. Titration curves of the irreversible light-induced absorbance change at 676 nm and the irreversible light-induced EPR change near g = 2.00 provide strong evidence that these two changes reflect the same compound.

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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Finally, a model is given to explain the observed reactions of Photosystem II at 10–180 K. The model involves three different ultimate and one intermediate electron donor to P^+-680 at these temperatures.

INTRODUCTION

The occurrence of a backreaction between the photoconverted primary reactants of Photosystem II, Q^- and P^+-680, at low temperatures, has been demonstrated by several investigators [1–5]. The half time of the back-reaction (3–5 ms) seems to be independent of the temperature between 35 and 110 K (cf. refs. 3, 4 and 5). Other observations, however, were explained by an irreversible photooxidation of *P*-680 below 170 K [6–8].

We reported recently [9] the observation of a partly reversible light-induced EPR signal at 110 K with spinach chloroplasts in the presence of ferricyanide. The EPR parameters of the signal were similar to those of the signal of P^+-700, the oxidized primary electron donor of Photosystem I. The signal was found to be partly reversible with a half time less than 100 ms after a short illumination period. After prolonged illumination, an irreversible signal with the same EPR characteristics was observed. From these experiments, it was concluded that in spinach chloroplasts in the presence of ferricyanide at 110 K, there is a secondary reaction between P^+-680 and another chlorophyll molecule, in addition to the back reaction.

In the present work, we investigate the kinetics of the light-induced EPR signals near $g = 2$ in more detail. This revealed a new transient EPR signal, the significance of which will be discussed. We have also measured light-induced absorbance changes near 676 nm, indicating the photooxidation of a Photosystem II reaction center chlorophyll in spinach chloroplasts at 110 K in the presence of ferricyanide.

MATERIALS AND METHODS

Chloroplasts were obtained from market spinach as described earlier [10] and stored on ice in the dark in a solution containing 0.05 M *N*-tris-(hydroxymethyl)-methyl-glycine (tricine), 10^{-2} M KCl, $2 \cdot 10^{-3}$ M $MgCl_2$ and 0.4 M sucrose, pH 7.8. The concentration of chlorophyll was determined according to Arnon [11]. Prior to freezing, the chloroplasts were suspended in glycerol (final concentration: 55 % v/v) in order to avoid crystallization of the sample at low temperatures.

Light-induced absorbance and fluorescence yield changes at low temperatures were measured using the split-beam spectrophotometer described in Ref. 12. The actinic light was filtered by a blue-green filter combination (420–580 nm), consisting of a Corning 4-96 glass filter, a 585 nm short pass interference filter, and a Calflex C heat reflecting filter. Appropriate combinations of Schott RG filters and Balzers interference filters (band width 12 nm) were placed in front of the photomultipliers in order to block stray light from the monochromators and to diminish artifacts due to changes in the fluorescence yield of pigment system 2. For the same purpose absorbance measurements in the red spectral region were performed in the presence of dibromothymoquinone (DBMIB), which quenches chlorophyll fluorescence [13, 14]. DBMIB was a gift of Dr. A. Trébst, Ruhr-Universität, Bochum. The measurements of

fluorescence were performed as described earlier [15]. The photomultiplier was provided with a Corning 4-77 colored glass filter and a Schott 692 nm interference filter.

EPR measurements at temperatures between 80 and 200 K were performed by use of a Varian E-9 spectrometer, operating near 9.1 GHz. A signal averager (Nuclear Chicago, model 7100) was connected to the apparatus. First derivative spectra were obtained using 100 kHz modulation of the magnetic field. Kinetic measurements were performed with the magnetic field positioned at the low field maxima of the first derivative signals. Samples in standard EPR quartz tubes (3 mm inner diameter) were cooled to the desired temperature by use of a stream of cold nitrogen gas provided by a Varian variable temperature accessory (E-257). The sample was illuminated with white light through the slotted front side of the cavity by a saturating, 8 μs xenon flash or by a tungsten halogen lamp (250 W), which was provided with a mechanical shutter. The half time of the opening of the shutter was 4 ms. The maximal intensity of continuous light at the sample position was about 300 $mW \cdot cm^{-2}$. EPR measurements at temperatures below 80 K were made with a Varian E-3 spectrometer [10]. The sample was cooled by a stream of helium as described by Lundin and Aasa [16]. The sample was illuminated in the cavity [17].

Oxidation-reduction potentials of the suspensions were determined as follows. Dark-adapted spinach chloroplasts were suspended in darkness in 2-ml isolation buffer, a mixture of potassium ferri- and ferrocyanide (2 ml) and glycerol (5 ml). This suspension was stirred in an open beaker at 25 °C in the dark. The redox potential of the medium was measured with a combination of a platinum and a calomel (saturated KCl) electrode (Radiometer type P101 and K401, respectively). The electrode was calibrated against a saturated quinhydrone electrode at 25 °C. The potential of the suspension was found to be stable after about 2 min. A sample of the suspension was then taken and frozen in darkness. A different suspension was used for each measurement. The rate of cooling was kept the same for each sample, to avoid differences between samples caused by the temperature dependence of the potential of the ferri-ferrocyanide redox buffer [18]. Care was taken to perform measurements involving the absorption of light at wavelengths where the contribution of the ferri- and ferrocyanide to the absorption was negligible.

RESULTS

EPR signals at 100 and 180 K

Fig. 1 shows the EPR kinetics near $g = 2.00$ upon illumination with strong, continuous light of dark-adapted spinach chloroplasts at 100 K. A transient signal can be seen which is superimposed on the steady EPR change due to *P*-700 photooxidation. The signal decayed in the light with a half time of 30 ms (Fig. 1). It was observed only at the first illumination period at 100 K. The signal observed in subsequent light periods can be attributed to P^+-700 [17]. Fig. 1B shows that the transient is absent in spinach chloroplasts in the presence of ferricyanide. Fig. 1C shows that the transient is also absent in spinach chloroplasts which were illuminated in the presence of DCMU and hydroxylamine prior to freezing in order to block Photosystem II activity [19]. This indicates that the transient is due to Photosystem II and can be attributed to a rereduction of P^+-680 by a secondary donor. The reduced extent of the signal and

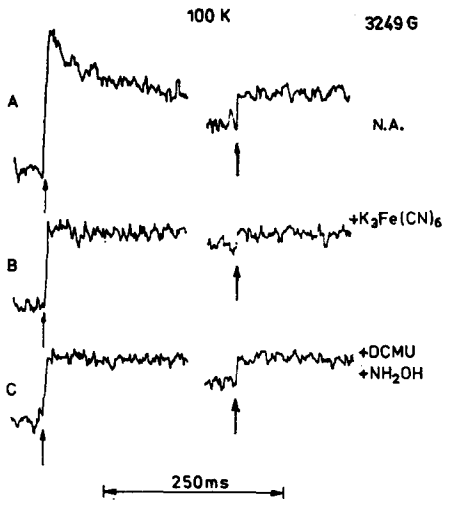

Fig. 1. Time course of EPR signals of chloroplasts at 100 K illuminated with strong (200 mW · cm^{-2}) continuous light. Upward arrows indicate light on. Left, first illumination period; right, subsequent illumination after 20 s dark. A, control; B, +0.1 M $K_3Fe(CN)_6$; C, $+10^{-5}$ M DCMU+10^{-2} M hydroxylamine preilluminated for 5 s with white light (100 mW · cm^{-2}) before freezing. Instrument settings: power, 8 mW; modulation amplitude, 10 G.

the absence of the transient in Fig. 1B can be explained by the oxidation of *P*-700 in the dark and the generation of an ESR signal due to oxidized chlorophyll (see below). In addition to the measurements with strong continuous light, experiments were done with short saturating flashes as excitation light. The results of these experiments confirmed those of Malkin and Bearden, except that we observed a decay with a half time of 2.0 ms (see ref. 12).

The spectrum of the 2.0 and 30 ms transients were identical and had the same *g*-value as the P^+-700 spectrum (signal I; ref. 20), however, the peak-to-peak bandwidth, ΔH_{pp} appeared to be somewhat smaller (6–7 G) than that of P^+-700 [12]. In previous measurements [17] the transient signal was not observed, since the opening of the shutter was too slow and the intensity of the actinic irradiation was too low in those experiments. With the maximal intensity of illumination (300 mW · cm^{-2}) in

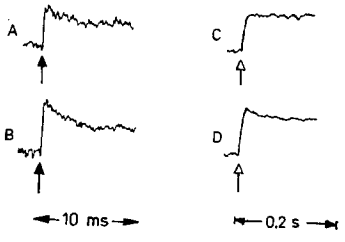

Fig. 2. Time course of EPR signals at 180 K with samples (curves A and C) as for Fig. 1A, and with similar samples preilluminated with two flashes 10 s before freezing (curves B and D). Curves A and B are the average of the change induced by the first three flashes; C and D were obtained with continuous light as curve A in Fig. 1. Amplification for curve A was twice that for curve B.

the present experiments the extent of the transient was found to be only 30 % of that of the 2.0 ms change, the half time being 30 ms.

The 2 and 30-ms EPR signals were not observed in dark-adapted spinach chloroplasts at 180 K (Fig. 2). With chloroplasts, which were illuminated with two saturating flashes shortly before freezing, both transients could be observed again. This may indicate that at 180 K with dark-adapted chloroplasts the back-reaction as well as the secondary reaction occurred too fast to be measured. The half times of the back-reaction and of the secondary reactions have to be shorter than 0.5 and 5 ms, respectively.

EPR measurements at 10–50 K

At the onset of strong, continuous actinic light at temperatures below 50 K with dark-adapted chloroplasts, EPR kinetics as shown in Fig. 3 were observed near $g = 2.00$. Superimposed on the EPR signal of P^+-700, a transient signal can be seen (curve A), which decays during illumination with a half time of 500 ± 30 ms (average of 13 measurements). The transient occurred only at the first illumination period. Therefore, these kinetics can not be attributed to a signal decrease due to heating by light absorption. Curve B was measured with spinach chloroplasts, which were illuminated in the presence of DCMU and hydroxylamine prior to freezing. The transient signal is abolished by this treatment; the kinetics shown in curve B are due to photooxidation of *P*-700 only [17]. This demonstrates that the transient is due to Photosystem II activity. Curve C shows the kinetics of the EPR signal upon a short light period (about 0.1 s). As can be seen most of the signal decays rapidly upon darkening ($\tau_{\frac{1}{2}} < 0.1$ s). In a subsequent light period, the kinetics of the signal are similar to those shown in curve A. The spectrum of the transient and of the P^+-700 EPR changes are shown in Fig. 4.

Since this 500 ms transient was not observed at 100 K, one would expect a

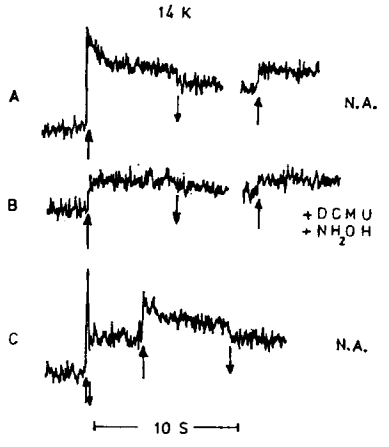

Fig. 3. Time course of EPR signals at 3230 G of chloroplasts at 14 K. A and C, no additions; B with DCMU and hydroxylamine as for curve C of Fig. 1. Left and right, first and subsequent light periods, respectively. For recording C, the first light period was 0.1 s. Upward arrows, light on; downward arrows, light off. Instrument settings: power, 2 mW; modulation amplitude, 5 G; frequency, 9.077 GHz.

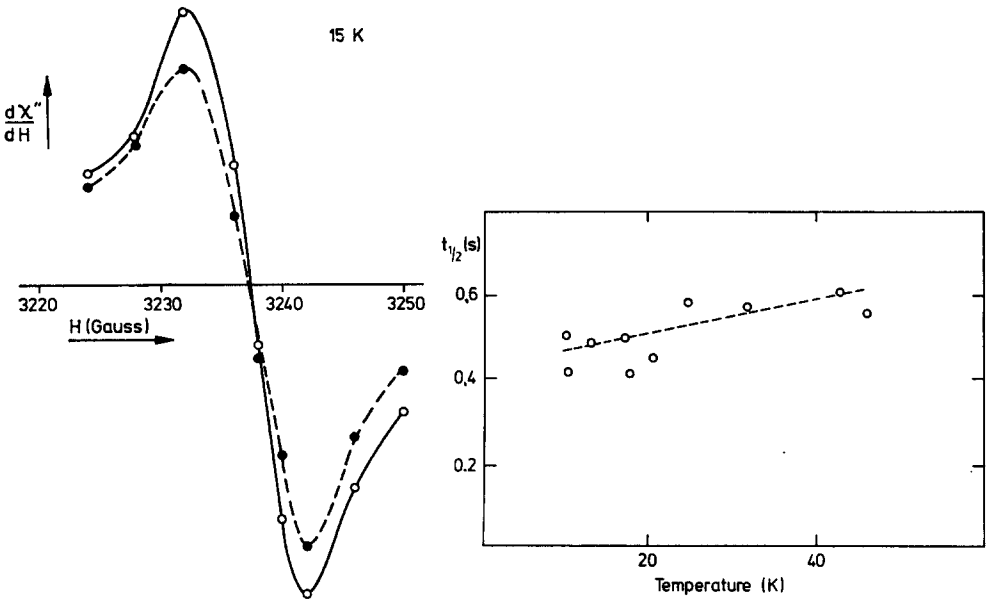

Fig. 4. Spectrum (closed circles) of the transient EPR signal with decays with a half time of 0.5 s during illumination of dark-adapted chloroplasts at 15 K. The spectrum of P^+-700 is also given (open circles, solid line). Instrument setting as for Fig. 3.

Fig. 5. Temperature dependence of the half time of decay of the transient EPR signal at 3230 G. Conditions and instrument setting as for Fig. 3A.

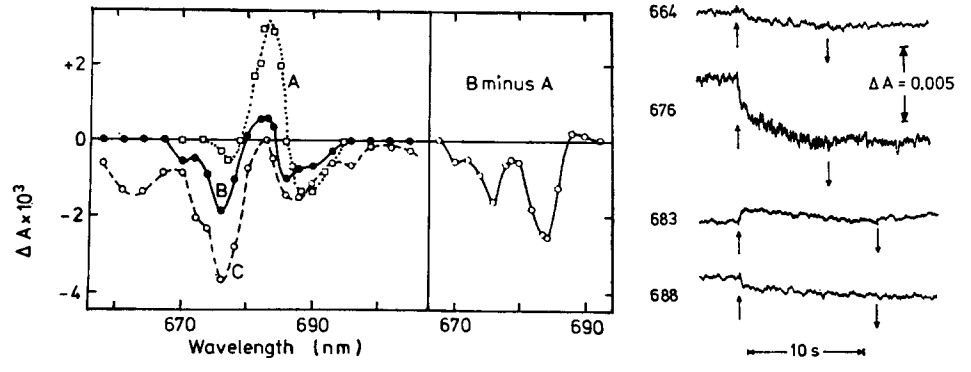

Fig. 6. Spectra of light-induced absorbance changes at 100 K with chloroplasts (0.2 mg chlorophyll/ml) in the presence of 10^{-4} M DBMIB and with (B and C) or without (A) 85 mM $K_3Fe(CN)_6$. Spectrum A was taken from Ref. 17. Actinic irradiation: 440–570 nm, 7 mW · cm^{-2}. Curves B and C are the spectra of the changes after 1 and 10 s of illumination, respectively.

Fig. 7. Time course of absorbance at 664, 676, 683 and 688 nm at 110 K measured for Fig. 6.

temperature-dependent half time for this reaction. However, the half time of the decay appeared to be more or less independent of temperature between 10 and 45 K (Fig. 5). The extent of the transient decreased with increasing temperatures; above 60 K, the transient was not observed. Unfortunately, the time resolution with the EPR measurements at temperatures below 80 K was too poor to detect possibly faster decaying transients. The available data suggest that on lowering the temperature in an increasing number of reaction centers of Photosystem II the 30 ms secondary electron donation is replaced by one with a half time of 500 ms.

Absorbance changes of chlorophyll at 110 K

Figs. 6 and 7 show spectra and kinetics or irreversible absorbance changes between 655 and 705 nm induced by continuous illumination at 110 K with chloroplasts in the presence of DBMIB and ferricyanide. Fig. 6 also shows (spectrum A) the band shift due to reduction of Q as reported previously [17]. With ferricyanide present, the shape of the spectrum was found to depend on the intensity and duration of actinic irradiation. Generally, at high light intensities (above 5 $mW \cdot cm^{-2}$) a fast and slow phase could be observed between 668 and 695 nm (Fig. 7). The shape of the spectrum of the fast phase was different from that of the slow phase (spectra B and C). The absorbance decrease at 676 nm was not due to fluorescence yield changes since, in contrast to the fluorescence, it was greater with than without ferricyanide (e.g. ref. 21).

The extent of the absorbance increase at 683 nm was smaller with ferricyanide present than without (Fig. 6). The absorbance changes of *c*-550 in the green region were not affected by the presence of the oxidant (not shown). Therefore, we assume that the band shift around 686 nm, which is also due to *c*-550 [17, 22, 23], occurred to the same extent with and without ferricyanide. The difference between spectra measured with and without ferricyanide is shown at the right hand side of Fig. 6 (B minus A). This difference spectrum resembles that of P^+-700 as obtained by the analysis given in Ref. 17 at 110 K except for its position and for the absence of the band shift. Therefore, the difference spectrum (B minus A) in Fig. 6 may be interpreted in a similar way as the spectrum of P^+-700 as being due to the oxidation of a chlorophyll dimer at 110 K (see also Ref. 13). Since the absorbance changes were irreversible, they cannot be attributed to P^+-680. We interpret this chlorophyll bleaching as being due to secondary electron donation by a chlorophyll dimer to P^+-680 [9].

EPR measurements indicated that the presence of DBMIB in the samples affects the kinetics of the chlorophyll oxidation. Fig. 8 shows a biphasic time course for this reaction in the presence of DMBIB. The fast phase occurred to a smaller extent with DBMIB than without. Since the extent of the fast phase was strongly dependent on the light intensity, this may be explained by the assumption that with DBMIB, which is a potent fluorescence quencher, the amount of light available for Photosystem II reaction is less than without DBMIB. The rate of the slow phase was linearly dependent on the light intensity (not shown). This was also observed for the slow phase of the absorbance changes shown in Fig. 6 and 7. The slow phase also went on for at least several minutes during continuous illumination. The reaction proceeded after closure of all Photosystem II traps, as indicated by the absorbance changes due to the reduction of Q (683 nm increase). This suggests that the reaction occurs directly between (bulk) chlorophyll and (oxidized) DBMIB molecules. As can be seen in Fig. 6, a slow bleaching occurred also at the absorbance band of monomeric chlorophyll

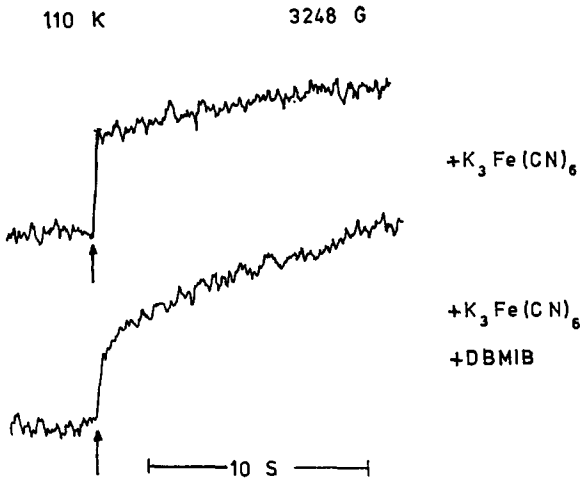

Fig. 8. Time course of EPR change upon illumination at 110 K in the presence of $K_3Fe(CN)_6$ (0.17 M) with and without 10^{-4} M DBMIB. The magnetic field was set at the maximum of the signal of P^+-700 (3248 G). Light on at upward arrows. Instrument setting: power: 2 MW; modulation amplitude: 5 G.

(664 nm; Refs. 24 and 25). We interpret this slow change at 664 nm and at other wavelengths as being due to oxidation of chlorophyll by DBMIB. The fast rise phase at 676 nm then is attributed to chlorophyll molecules donating electrons to Photosystem II.

If a flattening factor of 2.5 (Pulles, M.P.J., personal communication; Ref. 26) for absorbance changes at 676 nm is taken into account, the extent of the fast chlorophyll bleaching in Fig. 6 (B minus A) is about equal to that due to complete photooxidation of *P*-700 at 703 nm [17].

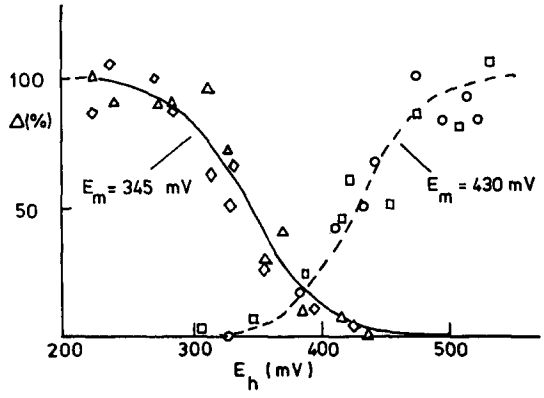

Fig. 9. Effect of oxidation-reduction potential on change of absorbance at 556 (◇) and 676 nm (○), of the fluorescence (△) and of the irreversible EPR signal near $g = 2.00$ (□) of dark-adapted chloroplasts at 110 K. Contribution of change due to *P*-700 oxidation are subtracted from the results of EPR measurements and of optical measurements at 676 nm. The fluorescence at $E_h = 450$ mW was subtracted from the fluorescence yield measured at lower E_h. The solid and broken lines are one-electron Nernst curves.

Fig. 9 shows redox titration curves for the extents of the light-induced changes at 110 K of the absorbance at 556 and 676 nm, of the fluorescence yield, and of the irreversible Photosystem II EPR signal near $g = 2.00$. The figure shows, firstly, that the extent of the variable fluorescence yield follows closely that of the amount of photooxidizable cytochrome *b*-559. Our E_m value is lower than that reported by Okayama and Butler [21] due to the presence of glycerol. In addition, Fig. 9 shows that the change of the absorbance at 676 nm and of the EPR signal at $g = 2.00$ are similar with respect to their extents at various redox potentials ($n = 1$, $E_m = 430$ mV). This provides strong evidence that these two changes reflect the photoconversion of the same compound. Both the position of the optical bleaching and the shape and *g*-value of the EPR signal suggest that this compound is a dimeric chlorophyll *a* molecule, which is photooxidized irreversibly at 77–100 K in spinach chloroplasts in the presence of ferricyanide.

DISCUSSION

The transient EPR signal observed upon illumination of chloroplasts at low temperature with strong continuous light (Figs. 1 and 2) presumably originates from Photosystem II. The main evidence for this is its insensitivity to preillumination in the presence of hydroxylamine and DCMU prior to freezing. As discussed already, this conclusion is supported by the kinetics in the presence of a high concentration of ferricyanide. The signal is obviously not due to Q^-. (cf. Refs. 15 and 17).

The conformity of the spectrum of the transient and that of P^+-700 appears to indicate that the change is due to a chlorophyll dimer radical. The EPR signal is probably caused by the oxidized form of the reaction center pigment *P*-680. The decay then would represent the reduction of P^+-680 by a secondary donor. In the temperature region from 10–50 K, this donation is obviously much slower. It is not known whether the species acting at 100 K as secondary electron donor is the same as the one at 10–50 K. The data, obtained at 100 and 180 K and displayed in Figs. 1 and 2 respectively, indicate that the half time of the reaction between P^+-680 and the secondary donor is much faster at 180 K than at 100 K. After preillumination of the sample with two saturating flashes at room temperature, resulting in the generation of states S_2 and S_3, the difference in half time disappeared. This indicates that the rapid secondary reaction is absent in states S_2 and S_3 at 100 K. Measurements of the kinetics of the variable fluorescence yield [27–29] and those of cytochrome b_{559} oxidation [27, 30] have also suggested similar properties of chloroplasts at temperatures below 130 K and those in states S_2 and S_3 at higher temperatures.

Our data, and those of other investigators, indicate that there are at least three ultimate electron donors at low temperatures. Under normal conditions cytochrome b_{559} is photooxidized [1, 3, 4, 15, 21, 22, 30, 31]. However, from the data given in Refs. 1 and 15, it can be calculated [12] that only about one cytochrome *b*-559 per two *P*-680 is oxidized. Since the charge separation is irreversible after prolonged illumination, this calculation indicates that half of the reaction centers have an unknown ultimate electron donor (D_2 in the scheme given below). Malkin and Bearden [32, 33] suggested that an unknown substance with an E_m of 465 mV donates electrons to P^+-680. This is in agreement with the data shown in Fig. 9. This donor may be identical to D_2. Finally, in the presence of ferricyanide electron donation

from a dimeric chlorophyll to P^+-680 occurs (Figs. 6 and 7). At $E_h > 500$ mV EPR [23] and optical [4] data indicate that at 77–100 K the amount of irreversibly photooxidized chlorophyll is equal to the amount of oxidizable *P*-700. Since the concentration of *P*-700 is equal to that of *P*-680 [4, 5, 28] it may be concluded that chlorophyll is the only ultimate donor at 77–110 K under these conditions.

To summarize the above considerations the following model is introduced:

$$ZPQ \underset{-1}{\overset{(h\nu)\ 1}{\rightleftharpoons}} ZP^+Q^- \underset{-2}{\overset{2}{\rightleftharpoons}} Z^+PQ^-$$

with in half of the centers at 77–110 K:

$$\text{cytochrome } b\text{-}559 \cdot Z^+ \xrightarrow{3} (\text{cytochrome } b\text{-}559)^+ \cdot Z$$

and in the other half:

$$D_2 \cdot Z^+ \xrightarrow{4} D^+{}_2 \cdot Z$$

Under strongly oxidizing conditions:

$$(\text{chlorophyll}) \cdot Z^+ \xrightarrow{5} (\text{chlorophyll})^+ \cdot Z$$

P and Q are the primary electron donor (*P*-680) and acceptor respectively, Z is an intermediary reactant. The intermediate Z is introduced to explain the fluorescence measurements of Den Haan et al. [34] who demonstrated that the fluorescence yield of chloroplasts upon flash illumination at 77 K increases with a half time of less than 20 μs. The fluorescence yield did not change between 40 μs and several seconds. These data indicate that a very rapid electron donation from an intermediate Z to P^+-680 occurs in the system 2 reaction center.

The spectra shown in Fig. 8 (B and C) are significantly different from those reported by Lozier and Butler [22]. Probably, these authors did not detect an absorbance decrease near 676 nm in the presence of ferricyanide at 77 K due to an important contribution of the fluorescence excited by the measuring light in their single beam scanning apparatus. Our spectra were plotted from kinetics recorded with a double beam apparatus using narrow band interference filters in front of the photomultiplier to diminish fluorescence. Recently, Mathis and Vermeglio [4] observed an irreversible light-induced absorbance increase near 820 nm with chloroplasts in the presence of ferricyanide, at 100 K. Since chlorophyll cations are known to absorb near 820 nm [24, 25] these changes are probably due to the photooxidation of the same chlorophyll which causes the 676 nm change and the EPR signal.

Assuming that the reactions of the model given above are first or pseudo-first order reactions, the rate constants at 77–110 K can be calculated to be: $k_{-1} = 350\ s^{-1}$ [3–5, 12]; $k_2 > 3 \cdot 10^4\ s^{-1}$ [34]; $k_{-2} > k_2/4$ [12, 34]; $k_3 = 20\ s^{-1}$ (Refs. 1, 2 and Fig. 1), k_4, $2 \cdot k_3$ (see below); and $k_5 = k_3/2$ (see below). The rate constant k_4 was estimated from Fig. 9 in Ref. 15 assuming that at temperatures between 110 and 180 K, D_2 competes with cytochrome *b*-559 in the electron donation to the oxidized $Z \cdot P$-680 complex. From Fig. 5, k_4 may be calculated to be equal to $1.4\ s^{-1}$ at 10–20 K. Finally, k_5 was calculated from Fig. 3 in Ref. 22 which showed that the efficiency to induce an irreversible charge separation in system 2 at 77 K by flashes is decreased

by a factor of two with chloroplasts frozen at $E_h > 500$ mV with respect to those frozen at lower oxidation-reduction potentials.

ACKNOWLEDGEMENTS

We are indebted to Dr. J. Amesz for his stimulating interest in the course of the experiments and for his valuable help with the preparation of this manuscript. The investigation was supported in part by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

1 Butler, W. L., Visser, J. W. M. and Simons, H. L. (1973) Biochim. Biophys. Acta 325, 539–545
2 Murata, N., Itoh, S. and Okada, M. (1973) Biochim. Biophys. Acta 325, 463–471
3 Mathis, P. and Vermeglio, A. (1974) Biochim. Biophys. Acta 368, 130–134
4 Mathis, P. and Vermeglio, A. (1975) Biochim. Biophys. Acta 396, 371–381
5 Malkin, R. and Bearden, A. J. (1975) Biochim. Biophys. Acta 396, 250–259
6 Ke, B., Sahu, S., Shaw, E. and Beinert, H. (1974) Biochim. Biophys. Acta 347, 36–48
7 Knaff, D. B. and Malkin, R. (1974) Biochim. Biophys. Acta 347, 395–403
8 Witt, K. (1973) FEBS Lett. 38, 116–118
9 Visser, J. W. M. and Rijgersberg, C. P. (1975) in: Proceedings of the IIIrd International Congress on Photosynthesis, Rehovot (Avron, M., ed.), Vol. 1, pp. 399–408, North-Holland, Amsterdam
10 Visser, J. W. M., Amesz, J. and van Gelder, B. F. (1974) Biochim. Biophys. Acta 333, 279–287
11 Arnon, D. I. (1949) Plant Physiol. 24, 1–15
12 Visser, J. W. M. (1975) Thesis, Leiden
13 Kitajima, M. and Butler, W. L. (1975) Biochim. Biophys. Acta 376, 105–115
14 Lozier, R. H. and Butler, W. L. (1972) FEBS Lett. 26, 161–164
15 Butler, W. L., Visser, J. W. M. and Simons, H. L. (1973) Biochim. Biophys. Acta 292, 140–151
16 Lundin, A. and Aasa, R. (1972) J. Magn. Res. 8, 70–73
17 Visser, J. W. M., Rijgersberg, C. P. and Amesz, J. (1974) Biochim. Biophys. Acta 368, 235–246
18 O'Reilly, J. E. (1973) Biochim. Biophys. Acta 292, 509–515
19 Bennoun, P. (1970) Biochim. Biophys. Acta 216, 357–363
20 Commoner, B. (1961) in A Symposium on Light and Life (McElroy, W. D. and Glass, B., eds.), pp. 356–377, John Hopkins Press, Baltimore
21 Okayama, S. and Butler, W. L. (1972) Biochim. Biophys. Acta 267, 523–529
22 Lozier, R. H. and Butler, W. L. (1974) Biochim. Biophys. Acta 333, 465–480
23 Van Gorkom, H. J., Tamminga, J. J., Haveman, J. and van der Linden, I. K. (1974) Biochim. Biophys. Acta 347, 417–438
24 Borg, D. C., Fajer, J., Felton, R. H. and Dolphin, D. (1970) Proc. Natl. Acad. Sci. U.S. 67, 813–820
25 Fajer, J., Borg, D. C., Forman, A., Felton, R. H., Dolphin, D. and Vegh, L. (1974) Proc. Natl. Acad. Sci. U.S. 71, 994–998
26 Latimer, P. and Eubanks, C. A. H. (1962) Arch. Biochem. Biophys. 98, 274–285
27 Amesz, J., Pulles, M. P. J. and Velthuys, B. R. (1973) Biochim. Biophys. Acta 325, 472–482
28 Joliot, P. and Joliot, A. (1972) in: Proceedings of the IInd International Congress on Photosynthesis Research, Stresa (Forti, G., Avron, M. and Melandri, A., eds.), Vol. 1, pp. 26–38, Dr. W. Junk, The Hague
29 Joliot, P. and Joliot, A. (1973) Biochim. Biophys. Acta 305, 302–316
30 Vermeglio, A. and Mathis, P. (1973) Biochim. Biophys. Acta 314, 57–65
31 Knaff, D. B. and Arnon, D. I. (1969) Proc. Natl. Acad. Sci. U.S. 63, 956–962
32 Malkin, R. and Bearden, A. J. (1973) Proc. Natl. Acad. Sci. U.S. 70, 294–297
33 Bearden, A. J. and Malkin, R. (1973) Biochim. Biophys. Acta 325, 266–274
34 Den Haan, G. A., Warden, J. T. and Duysens, L. N. M. (1973) Biochim. Biophys. Acta 325, 120–125