Biochimica et Biophysica Acta, 449 (1976) 84–94

BBA 47172

LIGHT-INDUCED ABSORPTION CHANGES IN PHOTOSYSTEM I AT LOW TEMPERATURES*

BACON KE, KIYOSHI SUGAHARA** and SAURA SAHU***

Charles F. Kettering Research Laboratory, Yellow Springs, Ohio 45387 (U.S.A.)

(Received March 2nd, 1976)

SUMMARY

Light-induced absorption changes associated with the primary photochemical reaction and dark relaxation in Photosystem I were measured at various low temperatures. A possible temperature-dependent long-range electron tunneling process was suggested to account for the unique temperature dependence of the dark decay process. The kinetics of the light-induced absorption changes are in good agreement with the light-induced EPR changes reported earlier (Ke, B., Sugahara, K., Shaw, E.R., Hansen, R. E., Hamilton, W. D. and Beinert, H. (1974) Biochim. Biophys. Acta 368, 401–408) for the same Photosystem I subchloroplast fragments at comparable temperatures.

All absorption changes between 400 and 725 nm at 86 °K have identical kinetics. The light-minus-dark difference spectrum is very similar to that of *P*-700 at room temperature, with an additional prominent positive change at 690 nm. Possible contributions by *P*-430 to the blue and red spectral changes were discussed.

It was demonstrated that the intensity of the measuring beam has a drastic effect on the light-induced absorption changes of Photosystem I at low temperatures. Various pretreatments of the Photosystem I fragments such as those that photochemically (or chemically) oxidize the primary donor or photoreduce the primary acceptor abolish the subsequent photochemical reaction. Continuous illumination of the Photosystem I fragments before and during freezing has the same effect.

In the temperature range of −20 to −60 °C, an unusual counter absorption change as well as a counter EPR change were observed.

INTRODUCTION

Low temperature photooxidation of *P*-700 was reported earlier by Calvin and Sogo [1] from EPR measurements and by Müller and Witt [2] from measurements of

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; TMPD, *N,N,N',N'*-tetramethylphenylenediamine.

* Contribution No. 534 from the Charles F. Kettering Research Laboratory.

** Present address: Section of Physiology and Biochemistry, Division of Environmental Biology, The National Institute for Environmental Studies, Yatabe, Ibaraki-ken 300-21, Japan.

*** Present address: Duke University Medical Center, Durham, N.C., U.S.A.

absorption changes; the reaction was considered as irreversible at −150 °C. A subsequent EPR study by Cost et al. [3] and a study of absorption changes by Witt [4] also showed that *P*-700 photooxidation was practically irreversible near 110 °K. In some of the more recent reports [5–7] on the EPR signals of photoreduced iron-sulfur proteins, it was implied that the photoreaction of Photosystem I was largely irreversible at liquid nitrogen temperature. However, Malkin and Bearden [5] mentioned that the EPR signal of photoreduced ferredoxin had a half-life of about 8 h, and Yang and Blumberg [8] showed that the EPR signal of *P*-700 was partially reversible upon cessation of illumination at 77 °K, and that it took days for the signal to decay to the 50 % level.

Low temperature reactions of *P*-700 had also been reported earlier by others [9, 10]. While we observed a completely reversible light-induced absorption change due to *P*-700 in the Triton Photosystem I subchloroplasts at 77 °K [9], Mayne and Rubinstein [10] observed that photobleaching of *P*-700 at 77 °K was partially reversible in the blue-green alga, *Anacystis nidulans* and in spinach chloroplasts. Warden et al. [11] examined the temperature dependence of $P\text{-}700^+$ decay by EPR spectroscopy and reported that in the temperature range 150–270 °K it follows first-order kinetics, whereas below approx. 150 °K only 10–30 % of the signal was reversible. Lozier and Butler [12] estimated from *P*-700 absorption changes that approx. 50 % of the change was reversible at 77 °K, and the EPR signal was approx. 35 % reversible at 93 °K.

More recently, Visser et al. [13] and Ke et al. [14] made refined EPR and optic spectroscopic measurements of the photooxidation of *P*-700 and its decay in the dark over a wide temperature range. The former group studied these changes in spinach chloroplasts and in intact algal cells [13], the latter group used the Triton-fractionated Photosystem I subchloroplasts from spinach [14]. While there exist small differences in details, both groups found *P*-700 photooxidation to be partially reversible at low temperatures, and a greater proportion of the change became irreversible with lowering temperatures. With the subchloroplasts, Ke et al. [14] found the light-induced EPR signals due to $P\text{-}700^+$ as well as reduced iron-sulfur protein to be completely irreversible at 13 °K. Thus, at intermediate low temperatures, the decay appears multiphasic and the rates of the separate decay courses appear to be temperature independent. Visser et al. [13] and Ke et al. [14] correlated the decay kinetics of $P\text{-}700^+$ and the reduced iron-sulfur protein and found the kinetic data to be consistent with the view that the iron-sulfur protein acts as the primary electron acceptor of Photosystem I.

We report here further details of the light-induced absorption changes associated with *P*-700 photooxidation and its dark decay over a wide temperature range, extended to below 77 °K, since such changes have not yet been reported in the literature to date. Some details on the decay kinetics and their temperature dependence, the extreme sensitivity of the low temperature absorption changes to the measuring beam intensity, and an unusual phenomenon of decay under illumination within the temperature range of −20 to −60 °C will be reported.

EXPERIMENTAL

Triton-fractionated Photosystem I subchloroplast particles were prepared

according to ref. 15. All chemicals were reagent grade whenever available, and were used without further purifications.

Light-induced absorption changes at temperatures above 77 °K were measured in a metal cuvette attached to a cold finger and placed inside a glass dewar as described in ref. 16. For measurements below 77 °K, a modified quartz dewar adapted to a helium refrigerator and an associated transfer line (Air Products Heli-Tran System LTD-3-110) was used. The temperature of the sample was measured with a copper-Constantan thermocouple and an iron-doped gold-Chromel thermocouple for temperatures above and below 77 °K, respectively. The subchloroplast was suspended in a buffer medium containing glycerol at a 50 % final concentration.

The majority of the light-induced absorption changes were measured in a modified dual-wavelength spectrophotometer with provisions to eliminate the interferences of fluorescence when measurements were made in the red region [17]. More rapid decay kinetics were examined by flash excitation, and the signals were averaged to improve the signal/noise ratio [16].

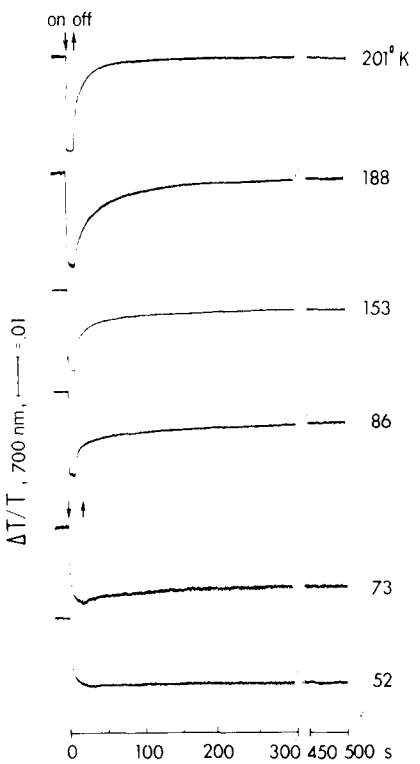

Fig. 1. Light-induced absorption changes in Triton-fractionated Photosystem I subchloroplast fragments at various low temperatures. The fragments were suspended in 0.04 M Tricine buffer containing 50 % glycerol, pH 8.0. The sample also contained ascorbate (1 mM) and TMPD (40 μM). Cuvette pathlength, 1 mm. All measurements were taken at 700 nm vs. 725 nm. A polychromatic blue (400–460 nm) actinic light at an intensity of $5 \cdot 10^4$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1} was used for excitation. Light on, downward arrow; light off, upward arrow. See Experimental for other experimental details.

RESULTS AND DISCUSSION

Light-induced absorption changes due to P-700 photooxidation at low temperatures

As reported previously, the decay of the light-induced absorption changes at low temperatures is highly temperature dependent [13, 14]. Fig. 1 shows these changes at several temperatures above and below 77 °K. Although not shown in the figure, the decay rate was decreased substantially when the sample temperature was lowered from ambient to slightly above freezing. For instance, at 0 °C, the apparent $t_{\frac{1}{2}}$ was approx. 0.5 s (vs. approx. 50 ms at room temperature). As the temperature was lowered, a biphasic decay profile resulted, as examplified by the absorption change at 201 °K shown in Fig. 1. In the meantime, a small fraction (approx. 5 %) of the absorption change appeared irreversible at the end of 500 s.

As the temperature was lowered further, only a slow decay phase remained. For instance, the decay at 188 °K (see Fig. 1) has an apparent $t_{\frac{1}{2}}$ of approx. 20 s, and approx. 10 % of the absorption change was irreversible at the end of 500 s. This decay pattern persisted to approx. 160–170 °K. Below this temperature, the decay clearly consisted of a rapid portion and two slower portions, as shown by the change at 153 °K in Fig. 1. We previously reported $t_{\frac{1}{2}}$ values of 3 and 75 s for the two slow decay phases [14]. These values compare reasonably with the values of 0.5 and 33 s reported by Visser et al. [13]. At 86 °K, the decay course maintained the complex multiphasic profile as at 153 °K, and the fraction of irreversible change increased to approx. 40 %. The $t_{\frac{1}{2}}$ of the rapid decay phase was separately measured to be approx. 2 ms. At 73 °K, only about 20 % of the total change decayed slowly; only a little rapid decay could be seen. At 52 °K, practically no decay occurred for the 500-s recording period. It should be noted that the decay kinetics measured here by optic spectroscopy agree well with those measured by EPR spectroscopy (cf. Fig. 1 in ref. 14). It was found previously that 14 % of the EPR change recovered at 75 °K, and no recovery at 13 °K for 20 min.

Light-minus-dark difference spectrum of the Photosystem-I reaction at 86 °K

The light-induced absorption changes were measured at other wavelengths and the complete difference spectrum was constructed. Absorption changes at three characteristic wavelengths, namely, one near the Soret maximum, 430 nm, one at the red band maximum, 700 nm, and one representing an absorption increase at 690 nm, are shown in Fig. 2. The onset of all changes is instantaneous at the time resolution used; the decay consists of an irreversible and a reversible portion, with the latter slightly greater than one half of the total change. The proportions of reversible and irreversible changes and the decay kinetics at all three wavelengths are consistent for the temperature used in the measurements (cf. Fig. 1). Although the absorption changes in some other spectral regions are smaller, all absorption changes measured between 400 and 725 nm appear to have the same kinetic profile.

The difference spectrum constructed from these changes, as shown in Fig. 3, thus represents the species involved in the primary photochemical events of Photosystem I. The absorption changes, especially those in the blue region, probably include contributions from both *P*-700 and *P*-430. As the extinction of *P*-430 is much lower than that of *P*-700, and since the recombination between the two charged species at low temperatures provide no kinetic differentiation, it is not feasible to isolate the spectra

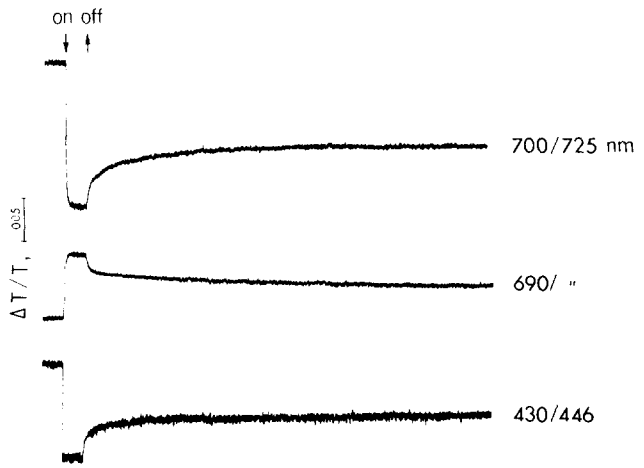

Fig. 2. Light-induced absorption changes in Triton-fractionated subchloroplast fragments at three wavelengths and at 86 °K. Other conditions were the same as in Fig. 1.

for the two separate species. It is interesting to note that because of the small size of the Photosystem I subchloroplast fragments used here, and consequently an unlikelihood of light scattering, the ratio of the blue to red band heights is nearly the same as found at room temperature for the same fragments. If any measurable "flattening" effect [18] occurred in the blue region, it is conceivable that some of the absorption changes in this region would be contributed by *P*-430.

The spectral changes in the red region are complex and the low temperature

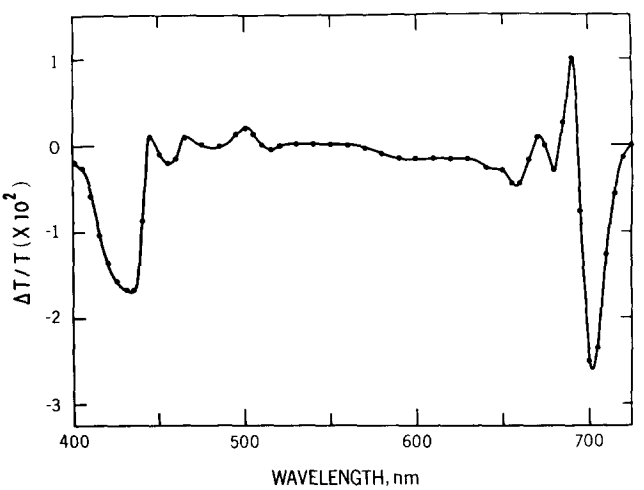

Fig. 3. The difference spectrum of the light-induced photoreactions of Photosystem I. Excitation conditions the same as in Fig. 1, except a polychromatic red (650–750 nm) actinic light was used for measuring absorption changes in the blue region. In the orange/green and blue regions, 610, 550 and 446 nm were used as the reference wavelengths. The different segments were then normalized with respect to 725 nm as the reference wavelength to yield the complete spectrum.

difference spectrum appears quite different from the room temperature one [19]. In addition to the usual bleaching at 701 nm and the band shift at 680 nm [20], a positive band located at 690 nm, which was first observed by Lozier and Butler [12], has been confirmed in the present work. The 690 nm band was accounted for by a band narrowing and extinction increase, and was interpreted as an indicator response for the redox state of the primary electron donor of Photosystem I [12]. Witt [4] observed a similar positive band (at 695 nm, however) and interpreted it as due to the reduction of an electron acceptor chlorophyll molecule. However, this appears inconsistent with the observation that the ferricyanide-oxidized difference spectrum of *P*-700 also showed a 690 nm-positive band [12].

P-430 may also make a small contribution to the absorption changes in the red spectral region. Recent measurements by Shuvalov et al. [21] showed that in addition to the absorption decrease in the Soret region found earlier [22], photoreduction of *P*-430 is also accompanied by an electrochromic spectral shift at 677 nm (decrease) and 690 nm (increase), and also a small absorption decrease at 717 nm. The 720 nm absorption change has been independently observed previously for soluble ferredoxin during its reduction [23].

Sensitivity of the low temperature Photosystem I absorption changes to the measuring beam intensity

In kinetic spectrophotometry the intensity of the measuring beam is usually kept sufficiently low, so that it does not bring about a photochemical change of the system under investigation before the actinic light is applied [16]. This requirement becomes even more important for a photochemical reaction at low temperatures, since part or all of it may be slowly reversible or irreversible. Prolonged exposure of the system to a measuring beam of moderate intensity may have a dramatic effect on the subsequent light-induced absorption changes, as illustrated by low temperature *P*-700 absorption changes shown in Fig. 4.

Signals A and B in Fig. 4 are absorption changes due to *P*-700 photooxidation at 86 °K monitored at 700 nm, one at a very weak and one at a relatively strong measuring beam intensity. With a weak measuring beam, the decay is partly reversible and partly irreversible, whereas with the stronger measuring beam, a predominantly reversible dark reaction was observed. One might suspect that the irreversible portion of the absorption change was already effected by the strong measuring beam itself, as signal B coincides well with the reversible portion of signal A.

The effect of a strong measuring beam on the absorption change may be directly demonstrated, as shown by signal C in Fig. 4: a polychromatic blue actinic beam of 20 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}, with sufficient time, brought about the irreversible change; a subsequent stronger actinic beam ($3.2 \cdot 10^4$ ergs $\cdot$ cm^{-2} $\cdot$ s^{-1}) immediately produced a rapid and predominantly reversible absorption decrease, with a magnitude comparable to that of signal B.

In Fig. 5 the magnitude of the total initial absorption change at 700 nm and that of the irreversible portion are plotted against the measuring beam intensity. It can be seen that the measuring beam had a detectable effect even at an extremely low intensity. For instance, at an intensity of 1 erg $\cdot$ cm^{-2} $\cdot$ s^{-1}, nearly one-fourth of the irreversible change was effected. Of course, this magnitude also depends on the time for which the sample was exposed to the measuring beam.

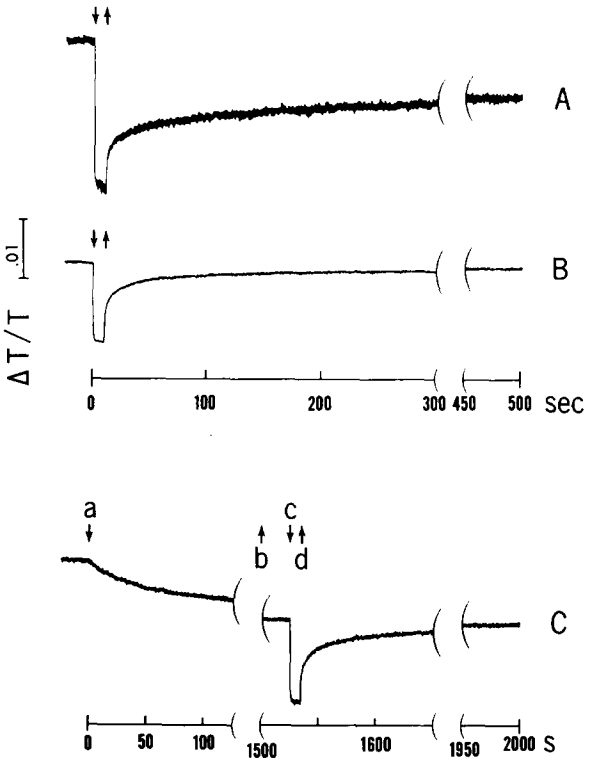

Fig. 4. Effect of measuring beam intensity on the light-induced absorption changes in Photosystem I fragments at 86 °K. Other experimental conditions were the same as in Fig. 1. Measuring beam intensities for curves A and B were 0.63 and 20 ergs · $cm^{-2} \cdot s^{-1}$, respectively. Both changes were excited with a polychromatic (400–460 nm) beam at an intensity of $5 \cdot 10^4$ ergs · $cm^{-2} \cdot s^{-1}$ for ×10 s. (The difference in the noise level between signals A and B was due to a high photomultiplier voltage required in recording signal A because of a lower transmitted light level from the weak measuring beam). Measuring beam intensity for curve C was 3 ergs · $cm^{-2} \cdot s^{-1}$. At point a, a blue actinic beam at an intensity of approx. 20 ergs · $cm^{-2} \cdot s^{-1}$ was turned on. At point b, the weak blue actinic light was turned off. At point c, a strong blue actinic light ($3.2 \cdot 10^4$ ergs · $cm^{-2} \cdot s^{-1}$) was turned on, and off at point d.

Two earlier reports on light-induced absorption changes or EPR changes in Photosystem I at low temperatures showed the entire change was reversible [9, 11]. The completely reversible absorption change [9] was presumably the result of having used a strong measuring beam which bleached the irreversible portion prior to the measurement, and in the EPR study [11], the sample was bleached deliberately prior to measuring the reversible portion only.

Effects of various pre-treatments

Various treatments of the Photosystem I reaction mixture prior to freezing the sample, especially those that alter the redox state of one or both of the primary reactants, would be expected to affect the primary photochemical events. For instance, when the reaction mixture was frozen during the photochemical accumulation of $P\text{-}700^+$, i.e. by illuminating the reaction mixture containing methyl viologen, or, when

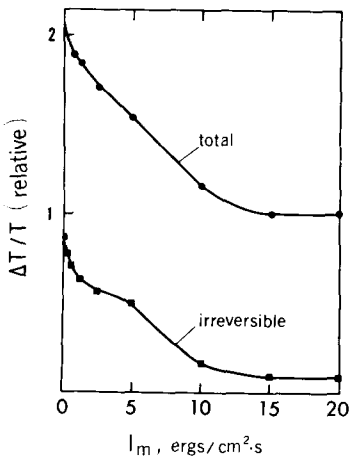

Fig. 5. Plot of the attenuation of light-induced absorption changes (total change and the irreversible portion) as a function of the measuring beam intensity (I_m). Experimental conditions similar to those in Fig. 4. At intensities of 0.3 and 0.15 ergs · cm^{-2} · s^{-1}, the required photomultiplier voltage exceeded 700 V, and fluorescence artifacts appeared. The irreversible portion could be measured, but the total absorption change at zero measuring intensity could only be estimated by extrapolation.

it was frozen during the photochemical accumulation of reduced *P*-430, i.e. by illuminating an anaerobic reaction mixture containing phenazine methosulfate in the presence of excess dithiothreitol, no light-induced absorption changes could be observed upon subsequent actinic illumination. When *P*-700 in the reaction mixture was chemically oxidized by ferricyanide prior to freezing, subsequent light-induced absorption change was similarly abolished.

When the primary acceptor is gradually reduced chemically, as in a redox titration, the absorption change is attenuated, and the extent of attenuation depends on the fraction of the primary acceptor being reduced. This is the basis for the determination of the redox potential of the primary acceptor of Photosystem I [24]. However, one anomaly was associated with this titration, namely, a residual rapid-decaying absorption change appears to persist even at the lowest potential used. This phenomenon is being examined further.

One interesting case of the abolition of the primary photochemical event (monitored by absorption changes) was by freezing the reaction mixture (which was poised with ascorbate and TMPD) during illumination. Such a situation was observed previously by other workers for chloroplasts [12] as well as photosysthetic bacterial reaction centers [25]. McElroy et al. [25] suggested two possible explanations for this so called "freezing in" of the light-generated charged primary donor, (1) when electron is removed from the $P^+ \cdot X^-$ complex, leaving the $P^+ \cdot X$ complex frozen in, or (2) when a conformational change occurs in the reaction centers during illumination at room temperature, and that the new conformational state that is frozen in requires a very long time for the charge recombination to take place. Since under our experimental conditions the reduced primary acceptor is not expected to lose an electron but rather to recombine with $P\text{-}700^+$, a conformational change in the reaction centers as suggested above [25] may be applicable.

With a similarly treated chloroplast sample, namely, that frozen under illumination, Evans et al. [26] reported a different observation by monitoring the EPR changes. They observed a rapidly decaying free radical signal when the frozen sample was illuminated again. However, no concomitant EPR change due to the reduction of iron-sulfur proteins could be observed. This observation led the authors to suggest that a component other than the iron-sulfur proteins is the primary acceptor.

A partial decay during illumination in the temperature range −20 to −60 °C

When the temperature of the Photosystem I reaction mixture was lowered from ambient to about 20 °C, a small counter absorption change began to appear while the sample was still under illumination. We have previously also observed a similar kinetic anomaly in the light-induced EPR changes of $P\text{-}700^+$ in Photosystem I in this same temperature range (ref. 14, and unpublished experiments of Ke and Beinert). This counter change, which may be attributed to the reversal of the oxidized $P\text{-}700^+$, caused a truncation in the absorption change transient, as shown for the change at −25 °C in Fig. 6. The rate of this counter reaction increased progressively as the temperature was lowered further, and then gradually decreased again, and the counter reaction finally ceased at slightly below −60 °C.

It was subsequently found that such counter changes during illumination were not observed if only ascorbate or one of the oxidized dyes, DCIP or TMPD, was present alone in the reaction mixture. The counter change was observed only in the presence of the reduced dye (with excess ascorbate). Furthermore, at a given ascorbate concentration, the rate of the counter change apparently increased with increasing concentration of the reduced secondary donor dye, as shown in the third column of Fig. 6. These data suggest that within this temperature range, the reduced dye can efficiently donate electrons to some photooxidized $P\text{-}700^+$, and consequently a small

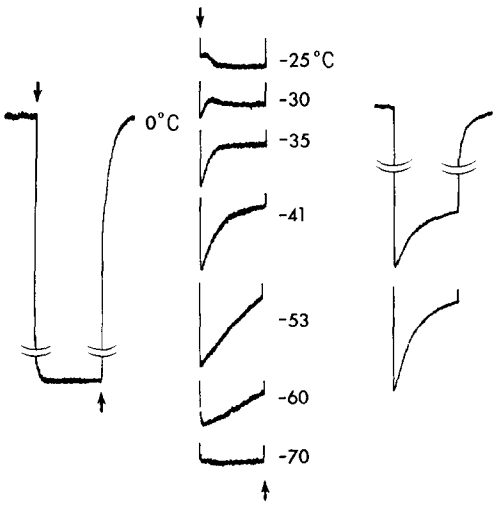

Fig. 6. A partial decay of the absorption change in Photosystem I fragments during illumination in the temperature range of −20 to −60 °C. All samples also contained ascorbate (1 mM) and TMPD (40 μM). Righthand side column: absorption changes measured at −45 °C. The samples contained 1 mM ascorbate and 20 μM DCIP (top) or 100 μM DCIP (bottom).

fraction of reduced $P\text{-}430^-$ would be accumulated. The counter reaction occurs possibly because of some phase transitions in the reaction medium within this temperature range which facilitated the electron transport from the artificial donor.

CONCLUDING REMARKS

The close agreement between the kinetics of light-induced absorption changes reported here and the light-induced EPR changes [14] obtained earlier with the same Photosystem I subchloroplasts at low temperatures indicate that the same primary photochemical events were measured by the two techniques. Furthermore, the close agreement between our measurements with subchloroplasts and those of Visser et al. [13] with unfractionated chloroplasts as well as whole algal cells indicate that the primary photochemical process remains unaltered in the detergent-fractionated subchloroplast fragments.

The unusual decay kinetics in Photosystem I at low temperatures have been observed by various workers [5, 8, 10–14, 27]. Over a short observation period, approximately equal portions of reversible and irreversible changes have been observed at 77 °K. This result had led to a conclusion that two kinds of Photosystem I reaction centers exist, thus accounting for the reversible and irreversible decays. It was further suggested [12] that the bound iron-sulfur protein(s) might be secondary acceptor(s) which, upon reduction, could serve to stabilize the photochemical charge separation, thus accounting for the irreversible portion of the reaction.

When the temperature was varied, a dependence of the irreversible fraction was subsequently observed, and the multiphasic nature of the decay was also recognized [13, 14]. By studying the dark decay of Photosystem I at low temperatures, three groups of workers [13, 14, 27] have independently observed that the dark reaction involved the recombination of $P\text{-}700^+$ and a reduced iron-sulfur protein with g-values at 2.05, 1.94 and 1.86, and that the two species bear a stoichiometric relationship.

The multi-phasic decay kinetics and the temperature dependence of the irreversible fraction may best be interpreted by a temperature-dependent long-range electron tunneling process. Electron tunneling in Photosystem I has been suggested previously by other workers [13, 28]. Visser et al. [13] suggested three different types of reaction centers to account for the three first-order reactions. It is possible that there may be a wider distribution of donor-acceptor distances among the Photosystem I reaction centers, and, in that case, the long-range electron tunneling should be accountable by the logarithmic decay kinetics [29]. Dark decays over longer periods of time must be measured to confirm this possibility.

Although the kinetic correlation between $P\text{-}700^+$ and reduced iron-sulfur protein during dark decay at low temperatures established the involvement of these two species in the recombination and that the observation is consistent with the view that the iron-sulfur protein functions as the primary acceptor [13, 14, 27], recent reports suggest that other possibilities may also exist. The free-radical signal, attributed to $P\text{-}700$ photooxidation and observed upon illuminating a sample previously frozen under illumination [26] or a sample previously reduced chemically by dithionite [30], however, contradicts the view that one of the iron-sulfur proteins is the primary electron acceptor. Evans et al. [30] further showed that under the above

conditions, a change in the EPR spectrum at $g = 1.75$ [28] was also observed. More recently, Blankenship et al. [31] reported the observation of a transient EPR emission signal upon flash excitation of chloroplasts at room temperature, and they suggested that at room temperature the primary photochemistry of Photosystem I proceeds via a triplet state of chlorophyll, and that the emission signal represents the counter radical produced by *P*-700 photooxidation, and would thus correspond to the primary electron acceptor of Photosystem I. It appears that a clarification and reconciliation of the diverse findings on the Photosystem I primary photochemical process are much needed.

ACKNOWLEDGEMENTS

This work was supported in part by a National Science Foundation Grant GB-29161. The authors thank E. R. Shaw for preparing the subchloroplast fragments used in these experiments.

REFERENCES

1 Calvin, M. and Sogo, P. (1957) Science 175, 499–500
2 Müller, A. and Witt, H. T. (1961) Nature 189, 944–945
3 Cost, K., Bolton, J. R. and Frenkel, A. W. (1969) Photochem. Photobiol. 10, 251–258
4 Witt, K. (1973) FEBS Lett. 39, 112–115
5 Malkin, R. and Bearden, A. J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 16–19
6 Leigh, Jr., J. S. and Dutton, P. L. (1972) Biochem. Biophys. Res. Commun. 46, 414–421
7 Evans, M. C. W., Telfer, A. and Lord, A. V. (1972) Biochim. Biophys. Acta 267, 530–537
8 Yang, C. S. and Blumberg, W. E. (1972) Biochem. Biophys. Res. Commun. 46, 422–428
9 Vernon, L. P., Ke, B. and Shaw, E. R. (1967) Biochemistry 6, 2210–2220
10 Mayne, B. C. and Rubinstein, D. (1966) Nature 210, 734–735
11 Warden, Jr., J. T., Mohanty, P. and Bolton, J. R. (1974) Biochem. Biophys. Res. Commun. 59 872–878
12 Lozier, R. H. and Butler, W. L. (1974) Biochim. Biophys. Acta 333, 465–480
13 Visser, J. W. M., Rijgersberg, K. P. and Amesz, J. (1974) Biochim. Biophys. Acta 368, 235–246
14 Ke, B., Sugahara, K., Shaw, E. R., Hansen, R. E., Hamilton, W. D. and Beinert, H. (1974) Biochim. Biophys. Acta 368, 401–408
15 Vernon, L. P. and Shaw, E. R. (1971) Methods Enzymol. 23, 277–289
16 Ke, B. (1972) Methods Enzymol. 24, 25–53
17 Ke, B., Sahu, S., Shaw, E. R. and Beinert, H. (1974) Biochim. Biophys. Acta 347, 36–48
18 Duysens, L. N. M. (1956) Biochim. Biophys. Acta 19, 1–12
19 Ke, B. (1973) Biochim. Biophys. Acta 301, 1–33
20 Vernon, L. P., Yamamoto, H. and Ogawa, T. (1969) Proc. Natl. Acad. Sci. U.S. 63, 911–917
21 Shuvalov, V. A., Klimov, V. V. and Krasnovsky, A. A. (1976) Mol. Biol. 10, 326–339
22 Hiyama, T. and Ke, B. (1971) Arch. Biochem. Biophys. 147, 99–108
23 Rawlings, J., Siiman, O. and Gray, H. B. (1974) Proc. Natl. Acad. Sci. U.S. 71, 125–127
24 Ke, B. (1975) Proc. 3rd Int. Congr. Photosynth. (Avron, M., ed.), Vol. 1, pp. 373–382, Elsevier Sci. Publ. Co., Amsterdam
25 McElroy, J. D., Mauzerall, D. C. and Feher, G. (1974) Biochim. Biophys. Acta 333, 261–277
26 Evans, M. C. W., Cammack, R. and Reeves, S. G. (1975) Proc. 3rd Int. Congr. Photosynth. (Avron, M., ed.), Vol. 1, pp. 383–388, Elsevier Sci. Publ. Co., Amsterdam
27 Bearden, A. J. and Malkin, R. (1974) Fed. Proc. 33, 378
28 McIntosh, A. R., Chu, M. and Bolton, J. R. (1975) Biochim. Biophys. Acta 376, 308–314
29 Zamaraev, K. I. and Khairutdinov, R. F. (1974) Chem. Phys. 4, 181–195
30 Evans, M. C. W., Sihra, C. K., Bolton, J. R. and Cammack, R. (1975) Nature 256, 668–670
31 Blankenship, R., McGuire, A. and Sauer, K. (1975) Proc. Natl. Acad. Sci. U.S. 72, 4943–4947