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THE BACK REACTION IN THE PRIMARY ELECTRON TRANSFER COUPLE OF PHOTOSYSTEM II OF PHOTOSYNTHESIS

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SUMMARY

Changes of C-550, cytochrome b_{559} and fluorescence yield induced in chloroplasts by single saturating flashes were studied at low temperature. A single saturating flash at −196 °C was quite ineffective in reducing C-550, oxidizing cytochrome b_{559} or increasing the fluorescence yield, presumably because most of the charge separation induced by the flash was dissipated by a direct back reaction in the primary electron transfer couple. The back reaction, which competes with the dark reduction of the oxidized primary electron donor by a secondary electron donor, becomes increasingly important as the temperature is lowered because of the temperature coefficient of the reaction with the secondary donor. The effect of the back reaction is to lower the quantum yield for the production of stable photochemical products by steady irradiation Assuming a quantum yield of unity for the photoreduction of C-550 at room temperature, the quantum yield for the reaction is about 0.40 at −100 °C and 0.27 at −196 °C

INTRODUCTION

A previous paper[1] describing the kinetics of C-550, cytochrome b_{559} and fluorescence yield changes in chloroplasts following the onset of steady illumination at low temperature interpreted the results in terms of the following photochemical model for the Photosystem II reaction centers:

$$D.P.A \underset{k_{-1}}{\overset{k_1(I)}{\rightleftarrows}} D.P^+.A^- \xrightarrow{k_2} D^+.P.A^-$$

where the primary electron acceptor, A, is C-550, the primary electron donor, P, is the reaction center chlorophyll, P680, the secondary donor, D, is cytochrome b_{559} at −196 °C but unknown at temperatures above −100 °C, and the rate constant k_1 is a function of the light intensity I. The main point of that paper was to show that the

Abbreviation DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

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oxidized forms of both the primary donor and acceptor, P^+ and A, respectively, quenched chlorophyll fluorescence and that the kinetics of the light-induced fluorescence yield increase followed the dark reduction of P^+, reaction k_2, rather than the photochemical reduction of A. In the present paper the same photochemical model has been used to interpret absorbance and fluorescence yield changes induced by single saturating flashes of light at low temperatures.

It was noted previously[2] that a single saturating flash on a sample of algae or chloroplasts frozen to −196 °C caused a rather small increase of fluorescence yield; much smaller than that produced by continuous irradiation at low temperature or by the same flash at room temperature. It had been expected that a saturating flash should reduce all of the C-550 and produce the maximum fluorescence yield. In the present paper, the absorbance and fluorescence yield changes induced by saturating flashes at low temperature were examined. All of the measurements confirm that the extent of the photoreaction induced by such flashes at low temperature is a small fraction of that induced by continuous irradiation. The relative ineffectiveness of the flashes at low temperature appears to be due to the back reaction k_{-1}. The influence of the back reaction on the quantum yield of the photoreaction induced by steady illumination is also demonstrated.

METHODS

Spinach chloroplasts were prepared as previously described[3]. Chloroplast samples in 55 % glycerol were placed in 1 × 10 × 30-mm plastic cuvettes and frozen slowly in a Dewar with liquid N_2 to give a clear glass. A thermocouple mounted on the metal cuvette holder adjacent to the cuvette monitored the temperature. The sample was frozen to liquid N_2 temperature and temperatures above −196 °C were obtained by blowing N_2 gas through the Dewar. The concentration of chlorophyll in the samples was 0.6 mg/ml for the absorbance measurements (Fig. 2) and 0.1 mg/ml (Fig. 1) or 0.2 mg/ml (Fig. 3) for fluorescence measurements.

The measurements of fluorescence and absorbance were made with the same instrument and methods described previously[1]. The influence of a flash on fluorescence yield was determined by irradiating the frozen sample in the Dewar with an unfiltered xenon flash of 16-μs duration[4]. The Dewar was then placed in the instrument and fluorescence measurements begun within 30 s after the flash. The energy of the flash was several fold greater than that needed to saturate oxygen evolution. Decreasing the intensity of the flash by 50 % with a neutral density filter had no effect on the results obtained. Absorbance changes induced by a broad band red actinic beam were measured at 543, 547 or 556 nm with a weak monochromatic measuring beam, which had no detectable actinic effect in the frozen samples over the time-course of the measurements. Fluorescence measurements at 692 nm were made with broad band blue actinic light as described previously[1]. The actinic light was used to both excite the measured fluorescence and drive the photochemical reaction.

RESULTS AND DISCUSSION

The curves for the light-induced fluorescence yield increase of frozen dark adapted chloroplasts before and after a single saturating flash are shown in Fig. 1 as

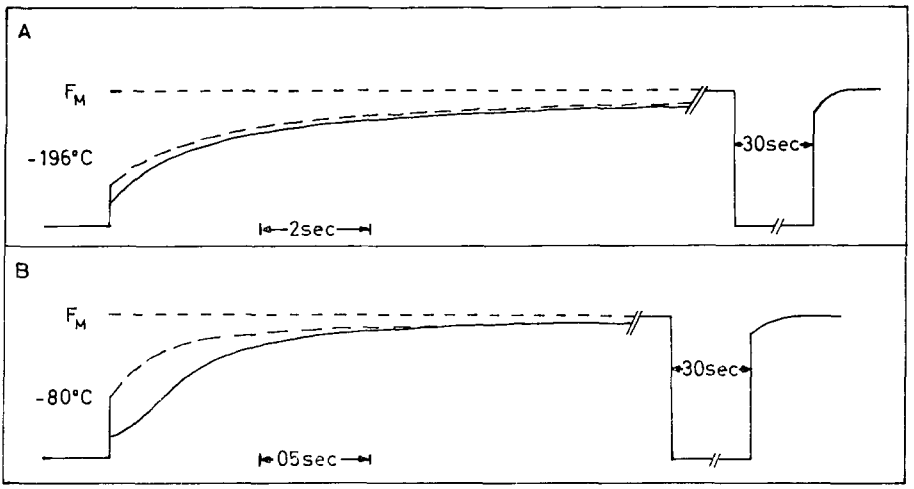

Fig 1 Time-course curves for the light-induced fluorescence yield increase of dark-adapted chloroplasts before (——) and after (– – –) a single saturating xenon flash A, at −196 °C and B, at −80 °C The dot-dash line indicates the maximal F_M level Note the time base in part B is four times faster than that in part A Some decay of the fluorescence yield (and oxidation of C-550 not shown) occurs during a 30-s dark period given after the F_M level was reached

the solid and dashed lines, respectively The dot-dash line indicates the level of the maximum light-induced fluorescence yield. It is clear that the flash at −196 °C (Fig. 1A) caused a rather small increase of yield. The flash was somewhat more effective at −80 °C (Fig. 1B) but still far from producing the maximum yield. Essentially the same results were obtained with flashes from a xenon lamp (16-μs duration) or from a Q-switched ruby laser (30-ns duration). The flash energy of both sources was far in excess of that needed to activate all Photosystem II reaction centers.

The dark decay of the high fluorescence yield condition at low temperatures is demonstrated also in Fig. 1. There is a slow decay of the high fluorescence yield even at −196 °C as shown by the effect of a 30-s dark period. A slow dark oxidation of the photoreduced C-550, consistent with the decrease of fluorescence yield, also occurs under these conditions (data not shown). This slow dark oxidation of C-550 at low temperatures is not thought to involve the back reaction.

Absorbance measurements were made to determine the effectiveness of single saturating flashes to reduce C-550 and oxidize cytochrome b_{559} at low temperatures (Fig 2). C-550 was assayed by absorbance changes at 543 or 547 nm and cytochrome b_{559} by the absorbance change at 556 nm. As in the case of the fluorescence measurements the action of the flash was ascertained by determining how much the light-induced absorbance changes were decreased by a prior single saturating flash. The flash at −190 °C (Fig. 2A) caused only a small part of the maximum light-inducible reduction of C-550 and oxidation of cytochrome b_{559} At −95 °C (Fig. 2B) only the change due to C-550 is shown because cytochrome b_{559} is not photooxidized either by a flash or by continuous irradiation (an unknown secondary donor appears to function at temperatures above −100 °C (ref 1) to the exclusion of cytochrome b_{559}). The flash at −95 °C is somewhat more effective in reducing C-550 than that at −190 °C

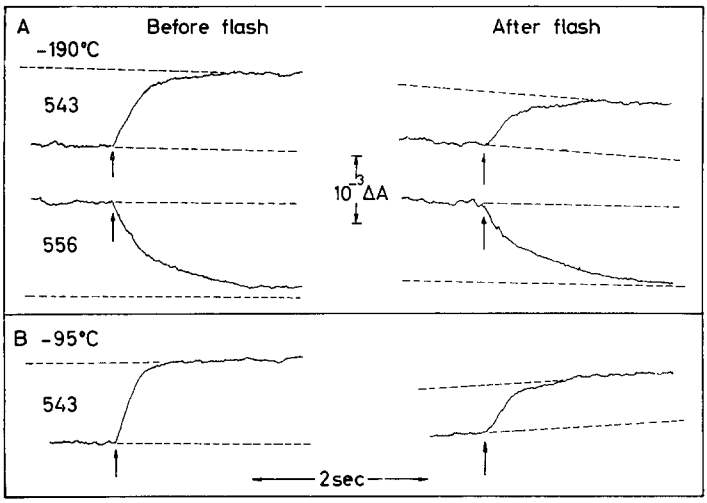

Fig. 2. Time-course of light-induced absorption changes of spinach chloroplasts (0.6 mg chlorophyll/ml in 1-mm cuvette) before (left hand curves) and after (right hand curves) a 16-μs saturating flash A, 543 and 556 nm at −190 °C and B, 543 nm at −95 °C. Actinic light (broad band red 2.0 mW/cm^2) on at upward arrows

but it is still far from maximum effectiveness. The extents of the flash-induced absorbance changes are consistent with the extents of the flash-induced fluorescence yield changes. Vermeglio and Mathis[5] also measured flash-induced changes of C-550 and cytochrome b_{559} at low temperatures and reported results similar to those reported here.

The explanation for the relative ineffectiveness of the flashes at low temperature is inherent in the photochemical model presented in Introduction. It is apparent that the reaction k_2 is needed to stabilize the charge separation in the reaction centers before it is dissipated by the back reaction k_{-1}. At room temperature k_2 competes favourably with k_{-1} and the stable photochemical products, D^+ and A^-, are formed in good yield (a saturating flash at room temperature causes a near-maximum increase of fluorescence yield[4,6]). As the temperature is lowered, however, k_2 appears to slow down relative to k_{-1} so that the back reaction becomes more important. At −196 °C we assume that a saturating flash effects the primary charge separation in all of the reaction centers but that most of the reduced C-550 back reacts with the oxidized P680. Only to the extent that P^+680 is reduced by a secondary electron donor can C-550 be found in the reduced state after a flash.

The photochemical model is consistent with the measurements of Floyd *et al.*[7] on the changes of P680 and cytochrome b_{559} induced by brief laser flashes at −196 °C but leads to somewhat different conclusions from those they presented. Their measurements at 680 nm showed a very rapid bleaching induced by the flash and a dark recovery with a half time of about 4.5 ms (presumably the photooxidation of P680 followed by the dark reduction). At 556 nm the flash caused a bleaching which had the same kinetics as the recovery of P680. They interpreted their results to indicate the photooxidized P680 was reduced in the dark by cytochrome b_{559} with a half

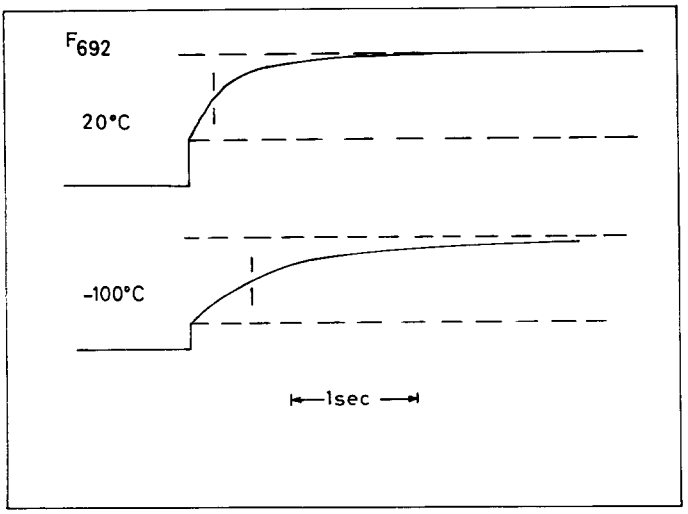

Fig 3. Time-course of fluorescence of chloroplasts at 692 nm during irradiation with blue actinic (excitation) light (50 μW/cm^2) in the presence of 10^{-5} M DCMU Upper curve. 20 °C Lower curve —100 °C Vertical lines on curves indicate half times

time of 4.5 ms at —196 °C. However, the magnitude of their absorbance changes indicated that more P680 recovered after the flash than could be accounted for by the amount of cytochrome b_{559} oxidized. We would interpret their results to indicate that, following a flash at —196 °C, most of the P^+680 is reduced by the back reaction and that only a small amount is reduced by cytochrome b_{559} According to that interpretation the measurements of Floyd *et al.*[7] would indicate primarily the kinetics of the back reaction; the kinetics of the oxidation of cytochrome b_{559} would, of necessity, follow the decay of P^+680 and show the same half time. In actuality, however, the rate constant for the oxidation of cytochrome b_{559} by P^+680 at —196 °C is probably considerably slower than that for the back reaction.

A consequence of the back reaction k_{-1} should be a lowering of the quantum yield of the photoreaction in continuous irradiation. If the back reaction becomes increasingly important as the temperature is lowered the quantum efficiency for the photoreduction of C-550 should show such a temperature dependence. We reported previously[1] that the kinetics of the light-induced fluorescence yield increase follows closely to the kinetics of the photoreduction of C-550 at temperatures above — 100 °C (at temperatures below —100 °C the fluorescence yield change lags behind the photoreduction of C-550 because of the quenching action of P^+680). Fig 3 shows the kinetics of the fluorescence yield increase measured at room temperature and at —100 °C in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) The half time for the light-induced fluorescence yield increase, which is taken as an index of the quantum requirement for the photoreduction of the primary electron acceptor, is 2 5 times longer at —100 °C than at room temperature.

Direct absorbance measurements were also made to determine the rate of photoreduction of C-550 at room temperature and —100 °C The problem encountered in these measurements was that the extent of the absorbance change of C-550 at room

temperature was less than one-third that at −100 °C so that an accurate ratio of half-times was difficult to determine. The rate of photoreduction of C-550 was faster at room temperature and the results, within the limits of experimental error, agreed with the more precise values measurements obtained by fluorescence. The magnitude of the absorbance change of C-550 is greater at low temperatures because of band sharpening. The redox change of C-550 at −196 °C results in only a slight shift of the absorption maximum from 547 nm for the oxidized form to 543 nm for the reduced form[8]. Spectral discrimination of differences between such closely overlapping bands is dependent markedly on band width.

The photochemical model would predict that the quantum yield of the primary photochemical reaction should be proportional to $k_2/(k_{-1}+k_2)$ If we assume that the quantum yield is unity at room temperature, the quantum yield at −100 °C would be 0.40 (from the results of Fig. 3) and k_{-1} should be 1.5 times greater than k_2 at that temperature These figures are consistent with our measurements which show that approximately one-third of the photoreaction, as assayed by the fluorescence yield or the C-550 change, is accomplished by a saturating flash at −100 °C.

In our previous paper[1] we reported that the rate of the photoreduction of C-550 was about 30 % faster at −100 °C than at −196 °C. We did not consider the effect of the back reaction on the quantum yield then but rather suggested that the difference in rates was due to a less efficient trapping of excitation energy in the Photosystem II reaction centers at the lower temperature because of a sharpening of the chlorophyll absorption bands. We would suggest now that that difference could also be due to the greater significance of the back reaction at the lower temperature. That data in conjunction with our estimate of the quantum yield of the photoreaction at −100 °C indicate that the quantum yield at −196 °C is about 0 27 assuming a value of unity at room temperature.

A direct estimate of the quantum yield of the photoreduction of C-550 at −196 °C was made from the rate of absorption of quanta of the 630-nm actinic light and the rate of the light-induced absorbance change at 543 nm by assuming an extinction coefficient for C-550 at 543 nm of 15 mM^{-1} cm^{-1} ★ The measurements were made with relatively low actinic intensities (0.15 $mW \cdot cm^{-2}$) where the rate of the reaction is linear with light intensity[1]. That calculation gave a value of 0 17 electron equivalents per quantum If we assume that 60 % of the actinic light is used by Photosystem II and 40 % by Photosystem I, the quantum yield for the Photosystem II reaction becomes 0.28 in good agreement with our previous estimate.

After this work was completed we learned (personal communication) that Dr N. Murata also measured the fluorescence yield changes induced by flashes at low temperatures and arrived at a photochemical model involving the back reaction which is essentially the same as that proposed here.

★ We assume from the photochemical model that equal molar amounts of C-550 and cytochrome b_{559} undergo redox change in the photoreaction at −196 °C The extinction coefficient of cytochrome b_{559} has been reported to be 16 $mM^{-1} \cdot cm^{-1}$ at room temperature[9], we assume this value increases to 20 $mM^{-1} \cdot cm^{-1}$ due to band sharpening on cooling to −196 C The average of a number of kinetic measurements at −196 °C indicates that the extent of the absorbance change at 543 nm is 75 % of that at 556 nm Thus, we obtain a value of 15 $mM^{-1} \cdot cm^{-1}$ for the extinction coefficient of C-550 at 543 nm From this value we also calculate one C-550 per 400 chlorophyll molecules

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