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THE KINETIC BEHAVIOR OF *P*-700 DURING THE INDUCTION OF PHOTOSYNTHESIS IN ALGAE

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SUMMARY

The kinetics of *P*-700 were examined spectrophotometrically during the induction of photosynthesis in algae. A pronounced oscillation was observed in the redox level of *P*-700 upon illumination of dark-adapted cells. The dark adaptation required approximately 1 min. The oscillation may be described as an initial rapid oxidation reaching a peak at approx. 50 ms followed by complete reduction of the pool of *P*-700. A subsequent slower oxidation resulted in attainment of the final state around 1 s. The main features of the oscillation were qualitatively the same in a wide variety of algae.

The modulation in redox level of *P*-700 required high intensity activation of both photosystems and was eliminated by pre-illumination of the cells with weak short wavelength light but not by longer wavelengths absorbed primarily by Photosystem I. We propose that the *P*-700 modulation is directly related to the fast redox changes in Photosystem II which occur during the induction of photosynthesis.

Cells incubated with methyl viologen did not show the *P*-700 oscillation confirming the suggestion previously advanced that exhaustion of Photosystem I acceptor and kinetic limitations in the carbon reduction cycle partially control the fast phase of photosynthetic induction.

INTRODUCTION

After a long period of darkness, the initiation of photosynthesis is accompanied by numerous changes in the redox status of intermediates in the electron transport chain as the final illuminated steady state is attained. Changes in some of the intermediates are quite complex and have been inferred from observations on the radiative emission of energy from the chlorophyll antenna and the rate of oxygen evolution [1]. The changes in fluorescence yield ϕO-I-D-P-S (denomination of Lavorel, ref. 2) during induction reflect the redox state of Q, the Photosystem II

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-(dimethylurea); DCPIP, 2,6,-dichlorophenol indophenol.

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acceptor [3], unidentified secondary quenchers [4] and the activation of Photosystem II units [5, 6]. The variations in rate of oxygen evolution [5] have been correlated with and attributed to the same events. Thus, most theories attempting to interpret the mechanism of induction [1] have considered in detail the behavior of Q and the oxygen-evolving assembly. However, no direct measurements on Photosystem I components have been reported although postulates have been advanced regarding the state of X, the Photosystem I acceptor, to account for the behavior of Q [7].

In a previous paper on the kinetics of *P*-700 in intact algae we reported a pronounced induction effect in the concentration of $P\text{-}700^+$ upon light activation [8]. The modulation in level of $P\text{-}700^+$ required relatively long periods of darkness prior to illumination, high excitation intensities and the activation of both photosystems. It was abolished by the addition of DCMU and was not observed in mutant strains which were blocked at Photosystem II but were otherwise competent in a cyclic turnover of *P*-700 driven by Photosystem I.

Induction effects on a different time scale and under different experimental conditions have been described for *P*-700 in algae [9, 10] and chloroplasts [11, 12, 13]. These effects represent lags in *P*-700 oxidation following various pre-illumination treatments and have been related to the pool size of carriers in the intersystem electron transport chain, $Q \rightarrow P$.

This paper reports in more detail induction effects at the level of P that may be correlated with the fast redox changes in Photosystem II. The data confirm the previous suggestion [7] that partial control in the induction mechanism is exerted by the inability of the units to initially oxidize XH, the Photosystem I reductant, and by kinetic limitations in the carbon reduction cycle.

EXPERIMENTAL

Unicellular algae were grown autotrophically as described previously [8]. The cells were harvested during the mid-logarithmic state of growth and resuspended in fresh growth medium at cell densities corresponding to between 2 and 10 μg chlorophyll/ml. The suspension was buffered with 10 mM bicarbonate/carbonate (pH 9.0).

Light-induced changes in whole cells were measured at 700 nm by means of the instrumentation utilized previously [8]. The combination of difference and phase sensitive signal detection effectively eliminated the interference due to fluorescence and delayed light in the measurement of *P*-700. Considerable signal/noise improvement was achieved by utilization of a measuring beam modulated at 1 kHz and signal averaging. The instrument time constant was 3 ms.

RESULTS

Timing sequences

Repetitive cycles of absorption transients attributable to *P*-700 in the cyanobacterium *Anacystis nidulans* are shown in Fig. 1. In all cases the flash times are identical (650 ms) and the effect of changes in the dark interval between flashes may be observed. The upper trace shows the behavior of *P*-700 in the quasi steady state where short, 200 ms, dark intervals permit observation of the complete relaxation of electron transport in the photosynthetic units. The lower traces show that as the dark

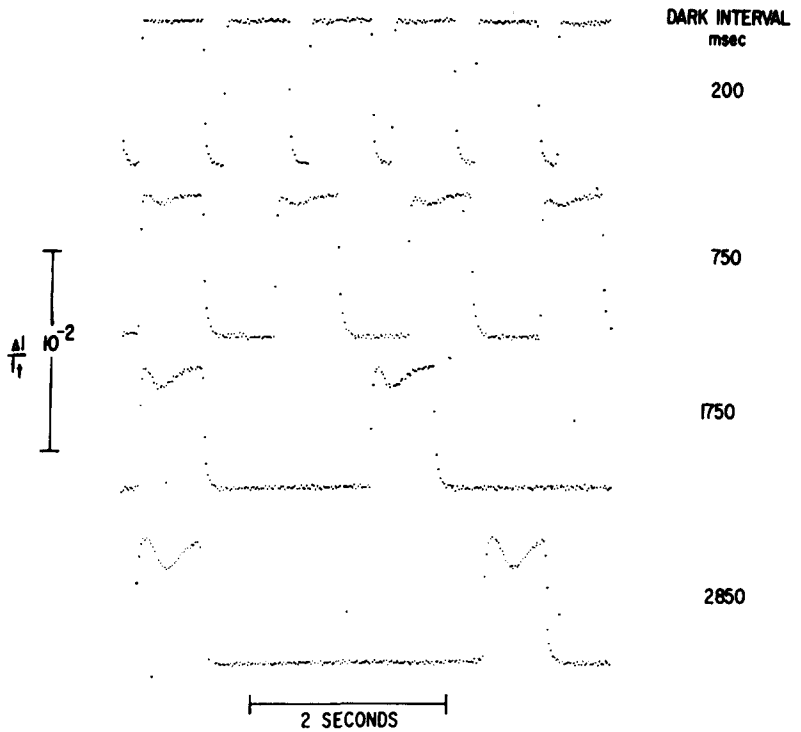

Fig. 1. Effect of dark interval on light-induced changes at 700 nm in *Anacystis nidulans*. An increase in transmission represents the oxidation of *P*-700. The chlorophyll concentration was 3.4 μg/ml, optical path length 10 mm. The white actinic light was saturating flashes of 650 ms duration. Each trace (5 s sweep time) shows the averaged signal after 128 repetitions. The dark interval between flashes was as indicated.

interval between flashes was extended an oscillation in concentration of $P\text{-}700^+$ occurred prior to attainment of the illuminated steady state. The oscillations became more pronounced as the dark interval was increased. As was previously noted [8] the half-time for the dark reduction of $P\text{-}700^+$ was approx. 5–10 ms and was independent of the flash frequency. The rate of dark reduction was also independent of flash duration even when the actinic light was extinguished during the oscillation.

The response of *P*-700 to individual long flashes is shown in Fig. 2 which also indicates the noise level in the signals prior to averaging in the other experiments. The first presentation of excitation to the cells (flash number 1) resulted in a fast transient oxidation and reduction of *P*-700. The *P*-700 remained fully reduced for 600 ms even in the presence of high intensity actinic light. The second flash, presented 1.5 s after cessation of the first, again resulted in the fast transient which peaked at 50 ms followed by a slow net oxidation of *P*-700. The third and fourth flashes resulted in qualitatively similar behavior but the slow oxidation accelerated and the final steady state was attained in a shorter time. Subsequent events were similar to flash number four and identical to the final absorption change obtained by averaging 128 transients.

Similar data for cells in anaerobic conditions as brought about by incubation of the suspension with glucose oxidase and glucose are shown in Fig. 3. Again the first

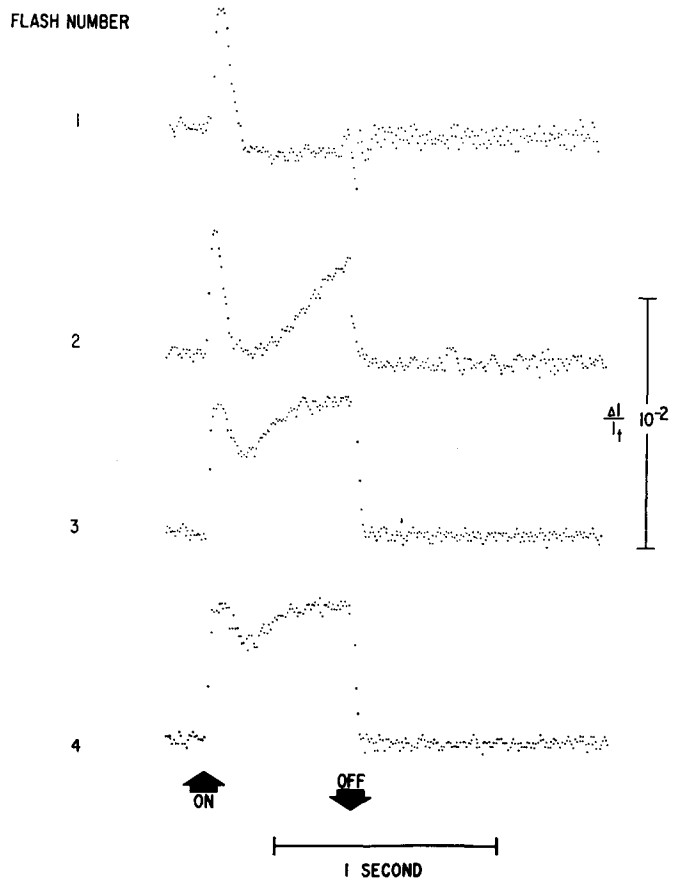

Fig. 2. Behavior of *P*-700 in response to individual actinic flashes in *Anacystis nidulans*. The chlorophyll concentration was 3.4 μg/ml. The white actinic light was at an incident intensity of 10^5 ergs/cm^2 · s and of 630 ms duration. The dark interval between the flashes was 1.5 s in all cases.

flash resulted in the fast transient oxidation but with a much diminished amplitude. No further changes were noted until the fifth flash that oxidized one quarter of the concentration of the pigment with a simple monotonous rise. Subsequent flashes led to increasing amplitudes of *P*-700$^+$, and no changes in the profile were noted after flash number seven. In addition to substantially modifying the behavior of *P*-700 in the flash sequence, anaerobiosis also brought about a slower rate of *P*-700$^+$ reduction in the dark and eliminated the oscillation.

Illumination conditions

The initial rate of *P*-700 oxidation is dependent upon light intensity and under our experimental conditions the peak was usually reached in 50 ms followed by complete reduction at 400 ms. The final oxidized steady state was reached by 1 s. The dark adaptation required approx. 1 min to invoke maximal modulation in the redox level of *P*-700. As was noted previously [8], the main features of the oscillation were observed in many species of algae (see Table I, ref. 8) but with quantitative differ-

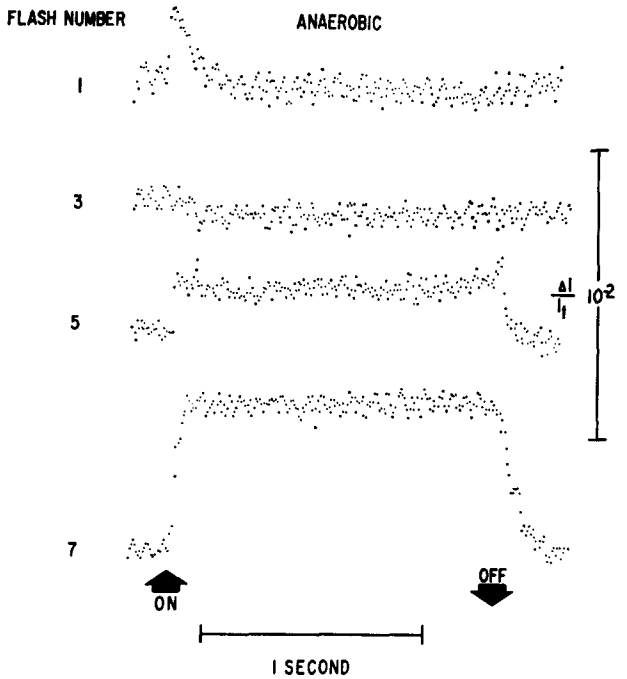

Fig. 3. Behavior of *P*-700 in response to individual actinic flashes in *Anacystis nidulans* under anaerobic conditions. The chlorophyll concentration was 2.5 μg/ml. The saturating white actinic light was of 1.5 s duration and the dark interval between flashes was 1.2 s. Anaerobiosis was achieved by incubating the cell suspension with β-D-glucose: O_2 oxidoreductase (0.1 I.U./ml) and 33 mM glucose for 10 min. Flash numbers 2, 3 and 4 did not result in any changes in *P*-700.

ences. For example, the second oxidation was always observed to be much slower in *Porphyridium cruentum* (cf. Fig. 4) and the first transient spike was less pronounced in green algae such as *Chlorella pyrenoidosa* (cf. Fig. 5).

The effect of supplementary continuous background illumination on the induction transient was explored in red and blue-green algae. The specific activation of the two photosystems is more readily achieved in these species by virtue of their accessory light harvesting pigments which absorb at wavelengths shorter than the main chlorophyll band in the red. The effects of continuous background illumination specific for Photosystem I and Photosystem II are shown in Fig. 4. Illumination of the cells with Photosystem I light prior to the flash raised the initial oxidation level of *P*-700 presumably due to Photosystem I activated cyclic turnover. The presentation of the flash resulted in oxidation of the remaining (45 %) *P*-700. Thereafter, the rate of reduction was retarded, but the second oxidation was considerably accelerated. Higher intensities of background Photosystem I light led to greater levels of conversion in the cyclic turnover prior to the flash but the modified oscillation persisted in the remainder of the *P*-700 pool which responded to the flash. We propose that the effect of background Photosystem I light may be simply accounted for by the increased activation of Photosystem I relative to Photosystem II.

The lower traces in Fig. 4 show the effect of two intensities of background Photosystem II light which primarily activated the phycobilins. The low intensity

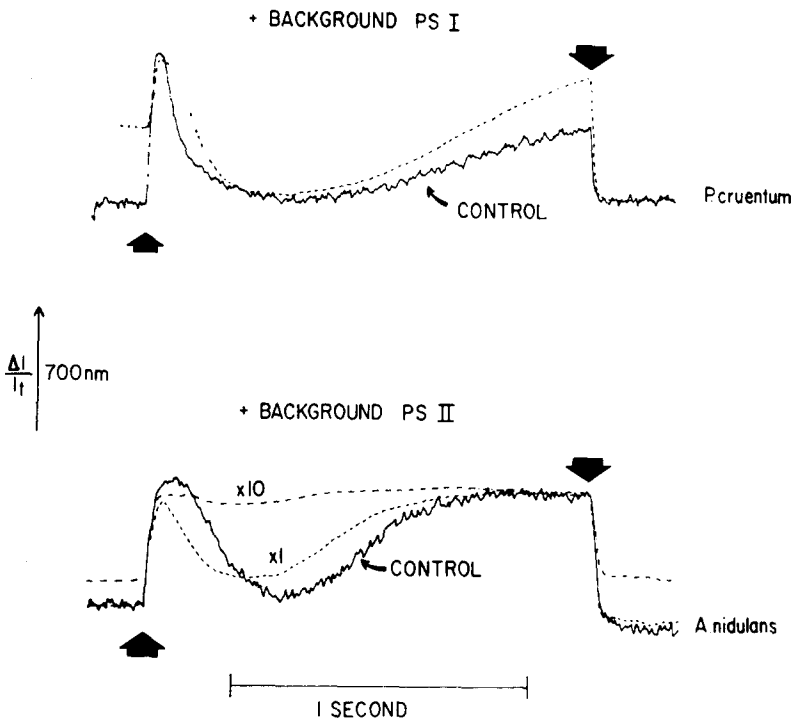

Fig. 4. Effect of pre-illumination and supplementary background Photosystem I light and Photosystem II light on the induction of *P*-700. The chlorophyll concentration in the upper traces using *Porphyridium cruentum* was 5 μg/ml. The chlorophyll concentration in the lower traces using *Anacystis nidulans* was 2.5 μg/ml. The white actinic flash was of 1.5 s duration and was presented every 63 s. The supplementary beams were continuous and Photosystem I light was of wavelengths $\geqq$ 695 nm and Photosystem II light was of wavelengths 540 to 600 nm (Optics Technology 600 nm short pass cut-off filter + Oriel Technology 540 nm long pass filter). The intensities were adjusted using neutral filters.

illumination ($\times$ 1) did not change the redox level of *P*-700 prior to the flash but markedly modified the absorption transient. The amplitude of the initial spike was lower and the rate of $P\text{-}700^+$ reduction was accelerated. The rate of the second oxidation was then slightly lowered. This is consistent with the data on Photosystem I background light described above and may be accounted for by the increased activation of Photosystem II and electron transport. It also suggests that the rapid $P\text{-}700^+$ reduction during the first spike is a consequence of reductant generated by Photosystem II and intersystem transfer to P via non-cyclic flow rather than by Photosystem I-induced cyclic transport. A higher intensity of background Photosystem II illumination ($\times$ 10) resulted in a greater level of $P\text{-}700^+$ prior to the flash. The flash induced maximal *P*-700 oxidation but the modulation was eliminated.

Although the effect of the low intensity Photosystem II background illumination is explicable in terms of increased activation of Photosystem II the higher intensity resulted in additional effects. Prior to the flash some 20 % of the *P*-700 pool was oxidized by the $\times$ 10 Photosystem II background light in turnover presumably due to noncyclic transport and resulting in a low rate of carbon dioxide reduction. This low

activity caused changes within the cells sufficient to light-adapt the entire population of units. This did not occur even after substantial Photosystem I-induced cyclic flow suggesting that factors at the level of carbon dioxide reduction must be implicated in the induction mechanism.

Effect of exogenous oxidant and reductant

The addition of methyl viologen to cells has been shown by Munday and Govindjee [7] to modify the fluorescence induction transient in *Chlorella* and eliminated ϕ_P in the fluorescence induction. They, therefore, suggested that ϕ_P is caused by a block in the oxidation of XH. The data presented in Fig. 5 are consistent with the proposal that the *P*-700 oscillation is related to the fluoresence transient and that the inability to oxidize XH is a primary cause of the modulation. As shown in the second trace, the incubation of cells with the low potential oxidant results in complete elimination of the oscillation of *P*-700. Thus, our results also suggest that there must be an exhaustion of Photosystem I acceptor during the first several hundred ms after illumination which contributes to the induction.

The effect of chemical reduction by 2,6-dichlorophenolindophenol (DCPIP) and ascorbate is shown in the lower trace. The effect was very similar to that induced by anaerobiosis (Fig. 3) in that the initial spike remained but was lower in amplitude and the second slow oxidation was much slower. In reducing conditions, as induced by

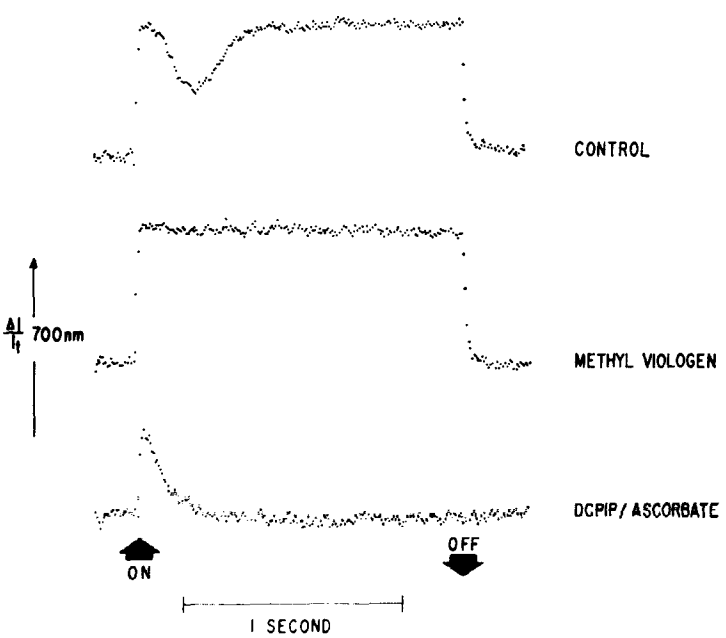

Fig. 5. Effect of exogenous oxidant and reductant on the *P*-700 induction in *Chlorella pyrenoidosa*. The chlorophyll concentration was 10.6 μg/ml. Saturating actinic flashes were of 1.5 s duration and the dark intervals between flashes were 63 s. The traces represent the average of 128 transients. Methyl viologen was used at a final concentration of 167 μM and the measurements were made after 15 min incubation. The lower trace was obtained after 10 min incubation of the cell suspension with 100 μm DCPIP and 10 mM sodium ascorbate.

anaerobiosis or $DCPIPH_2$, it is known that Q and the intersystem pool of plastoquinone are reduced thus leading to accelerated electron transport Q → P and, therefore, most likely accounting for the observed decrease in level of $P\text{-}700^+$.

DISCUSSION

The data presented above show that induction effects occur at the level of Photosystem I during the onset of photosynthesis. We propose that the modulation of *P*-700 during the induction as reported here is related to the events which occur in Photosystem II and the intersystem pool as visualized by the fast biphasic chlorophyll fluorescence transient (Kautsky effect, ref. 14). We suggest that the general interpretation of such events [1] that account for the Kautsky effect satisfactorily account for the *P*-700 induction as follows. Upon illumination *P*-700 is rapidly oxidized via Photosystem I followed by a reduction. The reduction may be complete provided that the preceding dark adaptation is sufficiently long. Our data show that the reduction utilizes electrons that are generated by Photosystem II rather than via a cyclic route around Photosystem I. This initial phase most likely corresponds to the ϕ_O–ϕ_I–ϕ_D portion of the fluorescence curve. After the reduction of Q during the ϕ_O–ϕ_I rise, secondary pools between Q and P are reduced thus regenerating oxidized Q at ϕ_D at about 20 ms. The ϕ_I–ϕ_D decline is sensitized by far red light and, therefore, Munday and Govindjee [7] suggested that the electron transfer from the secondary pool (AH_2) is to Photosystem I rather than to other acceptors. It is known that the rate constant for the intersystem electron transport chain, Q → *P*, is approx. $30\ s^{-1}$ [15, 16], and thus full reduction of *P*-700 is expected to occur between 20 and 100 ms. This was observed (see Flash No. 1, Fig. 2) confirming the suggestion of Munday and Govindjee [7]. At this point, *P*-700 remained fully reduced even in continuous bright light for several hundred ms. During this time there is generally an increase in fluorescence yield, ϕ_D–ϕ_P, denoting that Q is reduced faster than it is oxidized, and, therefore, consequent reduction of intersystem carriers [3, 7]. ϕ_P is reached at 200 ms to 1 s depending upon light intensity [6]. After ϕ_P, there is a slow decline in fluorescence yield to the level ϕ_S which has been interpreted as reflecting an increase in Photosystem I activity relative to Photosystem II. Accordingly, we rationalize that the point where *P*-700 is expected to be fully reduced is at ϕ_P and that the second slow oxidation of *P*-700 parallels the initial phase of the ϕ_P–ϕ_S decline.

After approx. 1 s *P*-700 remains fully oxidized, and we have not observed any further changes in redox state that would correspond to the slower events in the fluorescence curve. *P*-700 remains oxidized thereafter because the rate of reduction by the steps Q → *P*-700 are limiting in the overall electron transport chain [15, 16].

According to Munday and Govindjee [7], a block in the oxidation of XH results in an accumulation of reduced components between Q and P which cause ϕ_P. Evidence for their proposal resulted from their experiments that showed the elimination of ϕ_P by the addition of methyl viologen, a low potential acceptor capable of oxidizing XH but not components between Q and P. This has recently been confirmed by Lavergne [17]. Our data show that the addition of methyl viologen eliminates the *P*-700 oscillation as predicted from the fluorescence data and is consistent with the interpretation that an inability to oxidize XH is a factor in control of the induction. The fact that *P*-700 remains reduced in continuous high intensity light (Flash No. 1,

Fig. 2) indicates that a large pool of reductant exists between Q and *P*-700 during this period.

The effect of supplementary background illumination on the induction (Fig. 4) provides further evidence that limitations in the carbon reduction cycle are significant factors in the modulation of *P*-700 and the electron transport pathway. The low level of photosynthetic activity as induced by pre-illumination with Photosystem II is sufficient to light-adapt the cells. The light-adaptation process could include the activation of Calvin-cycle enzymes by localized changes in pH or Mg^{2+} concentration by transmembrane ion fluxes and also the establishment of threshold levels of cycle intermediates.

Pre-illumination with Photosystem I light is expected to induce significant changes in membrane potential, ΔH^+ and concentration of ATP as a result of in vivo cyclic electron transport [8]. We observed that such pre-illumination even to the extent of 66 % turnover of *P*-700 did not significantly modify the induction process thus excluding such phenomena as sole participants in control of the early stages of the induction mechanism. We do recognize, however, that ion movements and associated conformational changes in the thylakoid are implicated in the later stages of photosynthetic induction as the final steady state is attained [18, 19].

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