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# Role of Cyclic Electron Transport in Photosynthesis as Measured by the Photoinduced Turnover of $P_{700}$ in Vivo†

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ABSTRACT: The light-induced turnover of $P_{700}$ was measured spectrophotometrically in a wide variety of algae and some photosynthetic mutants. Analysis of the postillumination recovery of $P_{700}^+$ revealed that the apparent first-order rate constant for reduction via the cyclic pathway was much lower than that via the noncyclic pathway. After activation of photosystems 1 and 2 the half-time for reduction of $P_{700}^+$ was 5–20 ms, whereas after activation of primarily photosystem 1 a longer half-time of ca. 150 ms was observed. The extent of the photooxidation of $P_{700}$ was the same in both regimes of illumination. The longer half-time was also noted after inhibition of photosystem 2 by 3-(3,4-dichlorophenyl)-1,1-dimethylurea or mild heat shock and in mutant algae known to lack a functional photosystem 2. No signal was observed in mutants lacking $P_{700}$ itself but those strains lacking either plastocyanin or cytochrome *f* were capable of a very slow turnover (reduction $t_{1/2} > 500$ ms at room temperature). This very slow turnover was not affected by carbonyl cyanide *m*-chlorophenylhydrazone or the plastoquinone antagonist, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, indicating that the pathway for reduction of $P_{700}^+$ in these mutants is not energy linked and does not utilize the intersystem electron transport chain. The slow, 150 ms, reduction of $P_{700}^+$ due to cyclic flow was not observed when cells were engaged in photosynthesis at high-light intensities. The data are interpreted as evidence for the involvement of the total functional pool of $P_{700}$ in both electron transport pathways, and we suggest that cyclic electron transport does not contribute to photosynthesis in oxygen-evolving autotrophs.

The assimilation of carbon dioxide by oxygen-evolving photoautotrophs is driven by ATP and $NADPH_2$ that are generated as a consequence of photochemically-induced electron transport reactions by thylakoids. Two major pathways have been demonstrated in vitro: noncyclic photophosphorylation, which produces ATP and $NADPH_2$ via the interaction of two photochemical reactions, and cyclic photophosphorylation, which only produces ATP, in a reaction driven by the long wavelength PS1.[1]

Both pathways have been shown to operate in vivo. However, the experimental demonstration of the cyclic pathway in intact cells requires physiological conditions that may have limited relevance to those that exist during the photosynthesis of carbon dioxide. The possible contribution of the cyclic pathway to photosynthesis itself is uncertain, although it has been demonstrated that this pathway can be utilized for a wide variety of energy-linked functions (Simonis and Urbach, 1973).

A previous report from this laboratory described a kinetic analysis of the photo-induced turnover of cytochrome *f* in *Porphyridium cruentum* during cyclic and noncyclic electron transport (Biggins, 1973). It was concluded from direct spectrophotometric measurements on intact cells that cyclic electron transport does not contribute to the photoassimilation of carbon dioxide. However, the extensive overlap of the absorption bands in the cytochrome Soret and $\alpha$ regions precluded a similar spectrophotometric analysis in other species.

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[1] Abbreviations used are: PS1, photosystem 1; PS2, photosystem 2; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; ATP, adenosine 5′-triphosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

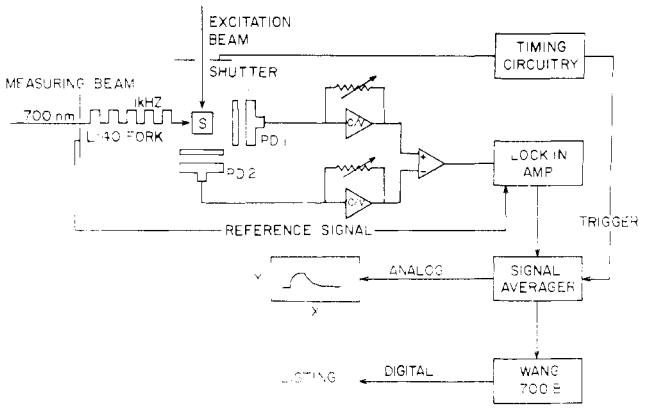


FIGURE 1: Block diagram of the spectrophotometer. In practice, the excitation beam was directed below the sample by means of a flexible light guide. See the text for details.

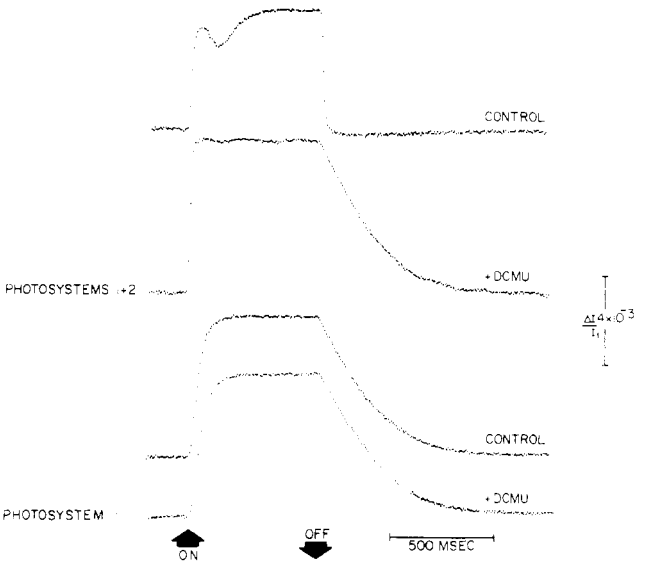


FIGURE 2: Light-induced turnover of $P_{700}$ in the blue-green alga *Aphanocapsa* 6714. Saturating white light for activation of photosystem 1 + 2 was provided by a tungsten-halide source filtered through heat glass (incident intensity of $10^5$ ergs/cm$^2$-s). Light for activation of primarily photosystem 1 was provided by filtering the same beam through a Schott RG 695 filter (incident intensity of $2 \times 10^4$ ergs/cm$^2$-s). The data are the average of 128 sweeps and the dark interval between flashes was 1.3 s. DCMU was used at a concentration of $10^{-6}$ M.

As a consequence, it was not possible to test the general applicability of the findings, particularly in the case of green algae and higher plants. The present report on the kinetics of $P_{700}$ turnover confirms and extends these earlier observations.

$P_{700}$ is the long wavelength primary photooxidant discovered by Kok (1957) and has been shown to be an essential component of cyclic and noncyclic electron transport. The ubiquity of $P_{700}$ in oxygen-evolving photoautotrophs (Kok and Hoch, 1961) has permitted this investigation of the kinetic behavior of the pigment in a wide variety of algae. The data are readily interpreted because of the complete absence of additional components undergoing absorption changes in the same spectral region.

The data presented are consistent for representatives drawn from several algal divisions and show that the functional pool of $P_{700}$ interacts in both electron transport pathways. The rate constant for the postillumination recovery of $P_{700}^+$ during cyclic transport is very different from that during noncyclic transport. This has made possible a critical determination of the activities of the two pathways in vivo that is corroborated by results of the investigation of certain mutant strains. The data reveal that the turnover of $P_{700}$ in the cyclic pathway does not occur at high-light intensities and we, therefore, suggest that cyclic photophosphorylation does not contribute to photosynthesis.

## Methods

*$P_{700}$ Turnover.* The photoinduced absorption change attributable to $P_{700}$ was monitored using the single-beam spectrophotometer illustrated in Figure 1. The 700-nm measuring beam of 5.5-nm bandwidth at half-height was modulated at 1 kHz using a tuning fork chopper (Model L-40, Bulova Watch Co., Flushing, N.Y.) that replaced the entrance slit of the monochromator (Aminco-Chance, American Instrument Co., Silver Springs, Md.). Signal detection was performed using two matched photodiodes of 1-in. diameter (United Detector Technology PIN-25) each blocked by a 700-nm narrow bandwidth interference filter and supplementary glass cut-off filter (Corning 2-64) to minimize chlorophyll fluorescence. One photodiode was placed in line with the modulated measuring beam and detected the transmitted light plus fluorescence from the sample. The second photodiode, at 90° to the modulated measuring beam, detected sample fluorescence only. After current to voltage conversion, the outputs of the two photodiodes were attenuated electronically (measuring beam "off" and excitation light "on") to compensate for the sample fluorescence. For absorption measurements the attenuated difference output of the preamplifier was demodulated and amplified further by means of a lock-in amplifier (Model 120, Princeton Applied Research, Princeton, N.J.) tuned to the 1 kHz reference signal from the tuning fork driver. The instrument had an overall constant of 3 ms.

The sample was excited from below by a beam of light that was modulated at low frequency by means of a shutter (Model 225, Uniblitz, Vincent Associates, Rochester, N.Y.). Typically, flash times of 200–400 ms and dark times longer than 1 s permitted full activation and recovery of electron transport components. Signal/noise improvement of the repetitive reversible absorption change at 700 nm was accomplished using a digital signal averager (Model 1010, Fabri-Tek Instruments, Inc., Madison, Wis.). The analog data were recorded directly from the averager using an X–Y plotter (Model 7004A, Hewlett-Packard Co., Palo Alto, Calif.) and digital data were listed using a programmable calculator (Model 700B, Wang Laboratories, Inc., Tewksbury, Mass.) that was interfaced to the signal averager.

Routine control experiments showed that the combination of the difference and phase-sensitive detection methods effectively eliminated the fluorescence artifacts inherent in absorption measurements in this wavelength region.

As pointed out by Kuntz and Calvin (1965), the initial velocity of the postillumination recovery of a redox component undergoing light-induced change is equivalent to the turnover of the component during the steady state in light where the rate of oxidation equals the rate of reduction. The kinetics of the postillumination recoveries were analyzed from the digitized data either by construction of semilogarithmic plots or by more stringent numerical analysis of multiple exponential decays by a method of moments developed by Isenberg et al., (1973).

*Biological. Scenedesmus obliquus, Chlorella pyrenoidosa, Anacystis nidulans,* and *Porphyridium cruentum* were obtained from the Culture Collection of Algae, Indiana Uni-

versity, Bloomington, Ind. *Aphanocapsa* 6714 was obtained from Dr. R. Pelroy, University of California, Berkeley, Calif., *Skeletonema costatum* from Dr. T. Smayda, Narragansett Marine Laboratory, University of Rhode Island, Kingston, R.I., *Chryptochrysis* sp. from Dr. A. W. Coleman, and *Euglena gracilis* from Dr. A. W. Holowinsky, both of this University. The algae were grown photoautotrophically in batch culture at 25 °C on the following media: *S. obliquus* on the medium of Kessler et al. (1957), *C. pyrenoidosa* on a modified Myer's medium (Krauss, 1953), *P. cruentum* and *S. costatum* on enriched sea water, F/2 (Guillard and Ryther, 1962), *A. nidulans* and *Aphanocapsa* on medium 11 (Hughes et al., 1958), *Chryptochrysis* on supplemented soil water (Starr, 1964), and *E. gracilis* on Hutner's medium (Hutner et al., 1966). *Ulva lactuca* and *Porphyra umbilicalis* were collected locally from Narragansett Bay, R.I., and maintained on F/2 at 5 °C.

The photosynthetic mutants of *S. obliquus* (numbers 8, 11, and 134) provided by Dr. N. I. Bishop, Oregon State University, Corvallis, Ore., were grown in darkness on the medium of Kessler et al. (1957) supplemented with 0.5% glucose and 0.25% yeast extract (w/v). Mutants of *Chlamydomonas reinhardi* (numbers f-1 and ac 208) were provided by Dr. R. Togasaki, Indiana University, Bloomington, Ind. and grown on a Tris-acetate–phosphate medium (Gorman and Levine, 1965) in darkness.

The unicellular algae were harvested in the mid-logarithmic phase of growth and resuspended in fresh growth medium buffered with 10 mM bicarbonate–carbonate (pH 9.0) at a chlorophyll concentration of 5 μg/ml for the blue-green algae and *P. cruentum* and at 10 μg/ml for the remaining species. The total chlorophyll concentrations were determined in 80% acetone after extraction with hot absolute methanol (Strain et al., 1971). For the spectrophotometric observation of the macro algae, thalli were supported by means of two pieces of plastic in the cuvette such that a single thickness of thallus lay in the path of the measuring beam.

## Results

*Kinetics of $P_{700}$ Turnover in Vivo.* The photoinduced absorption change attributable to $P_{700}$ was measured in samples of algae under excitation conditions that activated either both PS1 and PS2 or PS1 only. Figure 2 shows representative data for the blue-green alga *Aphanocapsa* 6714. The upper transient illustrates the reversible photooxidation of $P_{700}$ in response to a saturating flash of relatively long duration that excited both photosystems and could, therefore, drive cyclic and noncyclic electron transport. The postillumination reduction was observed to be first order and occurred with a half-time of ca. 5 ms at room temperature.

The activation of primarily PS1 by wavelengths longer than 695 nm or the inhibition of PS2 by DCMU would only allow cyclic electron transport. Such conditions resulted in slower first order reductions of ca. 150 ms half-time (2nd and 3rd transients). As expected, the addition of DCMU to samples activated by photosystem 1 light only (lower transient) did not affect the recovery. As was noted previously for cytochrome *f* turnover (Biggins, 1973), the rate-limiting step in the PS1 driven cyclic turnover of $P_{700}$ appears to be much slower than that in the noncyclic electron transport.

Generally, the extent of the photooxidation of the $P_{700}$ pool was the same regardless of whether PS1 alone or both photosystems were excited. Occasionally, however, the addition of DCMU resulted in an amplitude increase. These data confirm that $P_{700}$ is engaged in noncyclic electron flow (Kok and Hoch, 1961) and imply that the entire intracellular pool of $P_{700}$ is capable of interacting in both pathways in vivo.

As may also be noted in Figure 2, the photooxidation of $P_{700}$ showed a pronounced induction effect at high intensities of broad-band light where the initial oxidation was offset by a transient reduction. This effect was not observed when the cells were inhibited with DCMU or activated with PS1 light. The effect was found to be dependent on intensity and was especially prominent after long dark periods. This induction transient was not seen at all in the quasi-steady state that was obtained by utilizing short dark intervals (40 ms) in the modulated actinic beam. Such short dark intervals allowed the intermittent observation of the full recovery and subsequent reoxidation of $P_{700}$ during otherwise steady state illumination. Thus, it appears that the induction effect occurs after dark intervals in excess of the electron transport recovery time in contrast to the decay kinetics that were found to be independent of the repetition rate.

To ascertain that the results obtained above for *Aphanocapsa* are generally applicable in photosynthesis, a large number of species representing the major divisions of algae were investigated. The half-times for the postillumination reduction of $P_{700}^+$ resulting from activation by PS1 or by both photosystems, and the DCMU responses are summarized in Table I. The values are very similar for all species studied and consistent with the data shown above for *Aphanocapsa*. In general, the half-time for reduction of $P_{700}^+$ after activation of both PS1 and PS2 was between 5 and 20 ms, whereas the rate of reduction after activation of primarily PS1 or the addition of DCMU resulted in a much longer half-time. There was much more variability with regard to the slow turnover of $P_{700}$ and, occasionally, biphasic recoveries were found as indicated for some species when illuminated by wavelengths longer than 695 nm. However, in the presence of DCMU all the recoveries were monophasic. Shorter recovery times were usually observed with stationary cultures and aged samples suggesting that the stage of growth and physiological condition of the cells are significant.

The functional $P_{700}$ concentration was determined from the amplitude of the absorption transient and related to the total chlorophyll concentration in the samples. The values for the functional $P_{700}$/total chlorophyll ratio show that a large proportion of the $P_{700}$ pool was monitored.

In the absence of the terminal electron acceptor of photosynthesis, carbon dioxide, noncyclic electron transport is not expected to occur in vivo. After removal of carbon dioxide from a sample of *A. nidulans* by vigorous sparging with nitrogen, we observed that the fast $P_{700}$ turnover we attribute to noncyclic electron transport converted to the slower turnover even in high-intensity broad-band light. This conversion was reversed upon readdition of bicarbonate buffer to the sample but not completely.

A similar conversion of the functional $P_{700}$ pool engaged in noncyclic electron transport to one engaged in cyclic electron transport was also found upon mild heat treatment of a sample of cells at 50 °C for 10 min. This treatment is known to eliminate oxygen evolution by destruction of components on the oxidizing side of PS2 (Hinkson and Vernon, 1959). As shown in Figure 3, a sample of *C. pyrenoidosa* after such heat treatment exhibited the slow turnover that was not altered by the addition of DCMU.

*$P_{700}$ Turnover in Photosynthetic Mutants.* Investigations of mutant algae that lack specific components in the photosynthetic apparatus have provided valuable insight into the mechanism of photosynthesis (Levine, 1969). We therefore

TABLE I: Summary of Kinetic Data for $P_{700}^+$ Reduction in Vivo.

| Division | Species | Half-time of $P_{700}^+$ Reduction (ms) | | | | Total Chlorophyll/ Functional $P_{700}$[a] |
|---|---|---|---|---|---|---|
| | | Photosystems 1 + 2 | | Photosystem 1 only | | |
| | | Control | +DCMU | Control | +DCMU | |
| Cyanophyta | *Anacystis nidulans* | 6 | 140 | 257 | 178 | 147 |
| | *Aphanocapsa* 6714 | 10 | 145 | 164 | 175 | 157 |
| Rhodophyta | *Porphyridium cruentum* | 6 | 150 | 150 | 150 | 158 |
| | *Porphyra umbilicus* | 9 | 154 | 19 (49%)[b]<br>92 (51%) | nd[c] | nd[c] |
| Cryptophyta | *Cryptochrysis* sp. | 20 | 170 | nd[c] | nd[c] | 918 |
| Chrysophyta | *Skeletonema costatum* | 10 | 123 | 22 (39%)[b]<br>156 (61%) | nd[c] | 865 |
| Euglenophyta | *Euglena gracilis* | 7 | 249 | 7 | 227 | 646 |
| Chlorophyta | *Scenedesmus obliquus* $D_3$ | 4 | 98 | 26 (58%)[b]<br>77 (42%) | 109 | 689 |
| | *Ulva lactuca* | 10 | 152 | 107 | 117 | nd[c] |
| | *Chlorella pyrenoidosa* | 9 | 164 | 29 (63%)[b]<br>*163 (37%)* | 195 | 537 |

[a] An extinction coefficient of 70 mequiv$^{-1}$ cm$^{-1}$ was used for $P_{700}$ (Ke, 1973). [b] The initial concentrations of the two kinetic components in the recoveries are expressed as percentages of the transients. [c] Not determined.

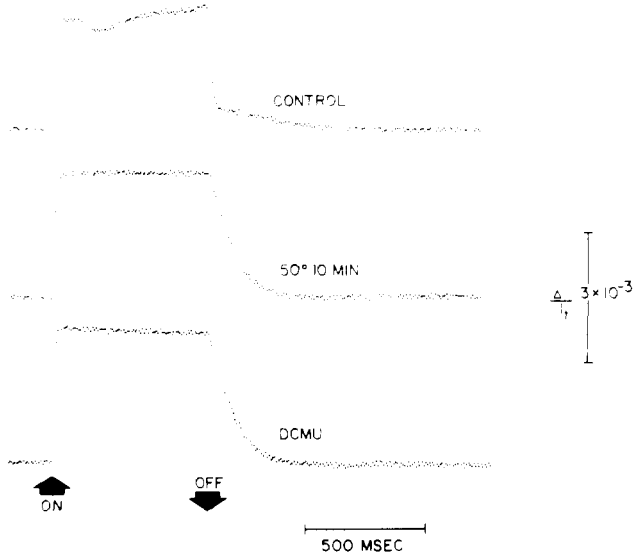


FIGURE 3: Effect of mild heat treatment on the light-induced turnover of $P_{700}$ in the green alga *C. pyrenoidosa*. White excitation light was used and other conditions are as in Figure 2.

examined the photoinduced $P_{700}$ absorption change in several mutants of the green alga *S. obliquus*, which were isolated by Bishop and of *C. reinhardi* which were isolated by Levine. All strains have been well characterized with respect to their partial photosynthetic capabilities (Levine, 1969; Powls et al., 1969; Bishop and Wong, 1971).

*Scenedesmus* 8, and *Chlamydomonas* f-1 both lack $P_{700}$ itself and did not elicit any absorption change at 700 nm under any conditions of excitation (not shown).

*Scenedesmus* 11 is blocked on the reducing side of PS2 but has a functional PS1 (Bishop and Wong, 1971). This mutant is capable of the anaerobic photoassimilation of glucose (Tanner and Kandler, 1969) indicating the presence of in vivo cyclic photophosphorylation. As shown in Figure 4, even though both photosystems were excited, the $P_{700}^+$ reduction in mutant 11 is slow (half-time of 87 ms) and is not modified by the addition of DCMU. This shows that the slow $P_{700}$ turnover is undoubtedly a result of cyclic transport via PS1 and not a result of modification of the photochemistry or electron transport by DCMU itself.

*Scenedesmus* 134 has a functional PS2 but lacks cytochrome *f* and is, therefore, incapable of both cyclic and noncyclic transport (Bishop, personal communication). This mutant did show a photoinduced turnover of $P_{700}$, but the rate of reduction was extremely slow (half-time of 575 ms) as can be seen in Figure 4 (note the change in time scale). A similar very slow turnover (not shown) was noted for *Chlamydomonas* ac-208 that lacks plastocyanin (Gorman and Levine, 1966) and is also effectively blocked on the oxidizing side of PS1. The rate of $P_{700}^+$ reduction in *Scenedesmus* 134 was not accelerated by addition of CCCP as was the case with the wild type, indicating that the very slow cyclic electron transport is not energy linked.

The substituted quinone, DBMIB, has been shown to inhibit both cyclic and noncyclic electron transport in chloroplasts (Trebst et al., 1970; Böhme et al., 1971) and the in vivo cyclic pathway in algae (Biggins, 1974) at the level of plastoquinone. The addition of DBMIB to mutant 134 did not alter the very slow (575 ms) reduction of $P_{700}^+$, indicating that the pathway of electrons from the primary photoreductant, $P_{430}^-$, to $P_{700}^+$ is not via plastoquinone or the intersystem electron transport chain. Reduction of $P_{700}^+$ in this strain is most probably a consequence of either a very slow back-reaction from $P_{430}^-$ or reduction by other compounds endogenous to the chloroplast.

## Discussion

*$P_{700}$ Turnover in Vivo.* The data above confirm that $P_{700}$ undergoes turnover in vivo during cyclic and noncyclic electron transport and our kinetic analyses of the dark recoveries reveal that the rate-limiting step for both pathways is sufficiently different to allow distinction between them. The observed half-time of ca. 5–20 ms for $P_{700}^+$ reduction is consistent with the previous measurements made in vivo by Kok (1957), Ke and Ngo (1965), Rurainski et al. (1970) and Hoch and Randles (1971). The variability between our data and these

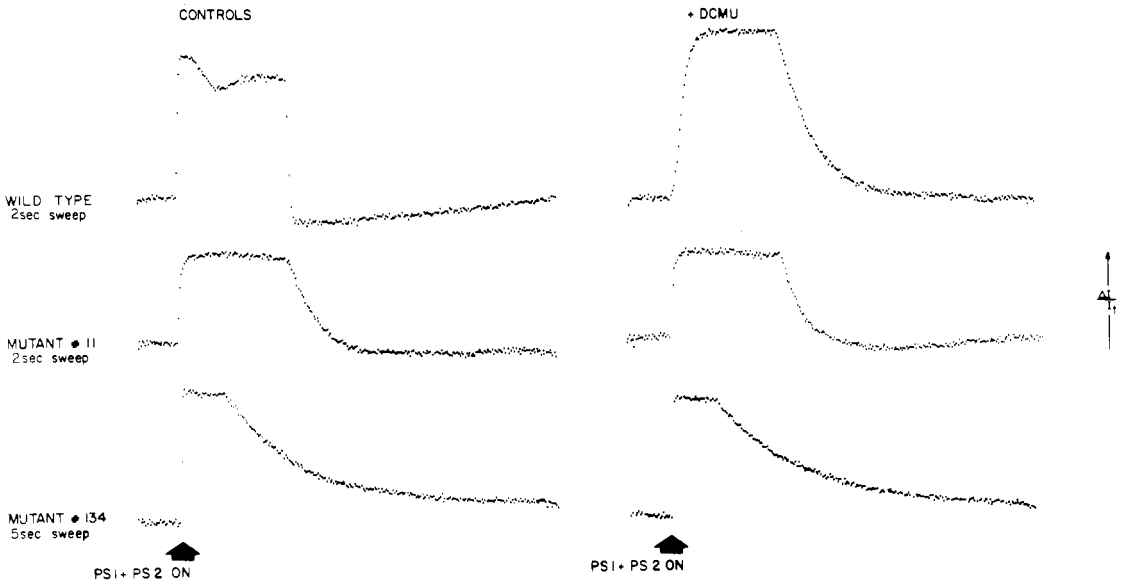


FIGURE 4: Light-induced turnover of $P_{700}$ in mutant strains of the green alga *S. obliquus*. The samples were all of the same chlorophyll concentration (10 μg/ml) and activation of both photosystems was provided by white light. The flash duration was 700 ms in all cases and the dark interval between flashes was 1.9 s for the wild type and mutant 11 and 4.3 s for mutant 134. The data are the average of 128 sweeps and DCMU was used at a concentration of $10^{-6}$ M.

published values is almost certainly related to the physiological state of the cells. Recently, Myers and Graham (1975) have shown by flashing light experiments in synchronous *S. obliquus* that the photosynthetic unit turnover time can vary considerably depending upon the stage of growth of the cells in the life cycle.

We showed that the inactivation of PS2 by inhibition with DCMU, mild heat treatment, mutation, illumination with far-infrared light (wavelengths longer than 695 nm), or removal of the terminal electron acceptor, carbon dioxide, resulted in a much slower turnover of $P_{700}$. In these instances, the half-time for $P_{700}^+$ reduction was generally ca. 150 ms and, undoubtedly, in response to the in vivo cyclic electron transport driven by PS1. This pathway has been shown to be energy linked (Simonis and Urbach, 1973) and to utilize the electron transport segment from plastoquinone to $P_{700}$ (Biggins, 1974). Thus, the rate-limiting step for this pathway must reside between $P_{430}^-$ and plastoquinone.

This result is in agreement with the slow turnover of the cyclic pathway as measured by following cytochrome *f* reduction in *P. cruentum* (Biggins, 1973), but there is a paucity of similar information for comparison on the PS1-induced turnover of $P_{700}$ in vivo. Kok and Hoch (1961) reported that $10^{-7}$ M DCMU inhibited the reduction of oxidized $P_{700}$ ("positive shift") by photosystem 2 and the "negative shift" was inhibited 50% at $10^{-4}$ M DCMU. Thus, a much reduced turnover of $P_{700}$ was observed in the presence of very high concentrations of inhibitor that we suggest was most probably a result of a longer half-time for reduction and, therefore, incomplete recovery of the $P_{700}^+$ pool in the 60 ms dark time they employed in the phosphoroscope. The transient absorption changes attributable to $P_{700}$ in the blue-green alga *Plectonema boryanum* in response to rapid flash excitation were reported by Ke and Ngo (1965). They observed that mild heat treatment and addition of DCMU affected a signal that had a decay half-time of 7 ms (R transient) and resulted in a much longer half-time. It is very likely that the R transient described by these investigators corresponds to the fast (5 ms) decay we generally observe. If this is the case then our results are consistent with their observations. However, since some of their measurements were recorded at 430 nm, it is conceivable that the increased complexity of their signals may be attributed to $P_{430}$ and cytochromes as well as $P_{700}$. More recently, Hoch and co-workers (Rurainski et al., 1970; Hoch and Randles, 1971) investigated $P_{700}$ turnover in intact algae by steady-state relaxation spectrophotometry. This technique relies upon time separation between signal detection and sample activation. The actinic light is modulated further at low frequency and the rate constant for the electron transport carrier under observation is estimated from the corresponding phase shift assuming a suitable kinetic model (Hoch, 1972). Although they observed an increase in relaxation time following addition of DCMU in *P. cruentum* and *A. nidulans*, the increase was never greater than a factor of two. It is possible that there may have been considerable attenuation of slowly decaying components at the 13-Hz frequency they used for modulation of the actinic beam.

It might be argued that the slow, 150 ms, $P_{700}^+$ reduction we observed after inhibition of PS2 may be a result of the severe metabolic restrictions placed on the cells by their inability to fix carbon dioxide. After PS2 inactivation, it is possible that the high intrachloroplast ATP concentration maintained by cyclic photophosphorylation could substantially reduce an otherwise rapid rate of electron transport and result in a slower turnover of the cyclic pathway. Such effects of ATP have been demonstrated in chloroplasts undergoing photophosphorylation (Avron et al., 1958) but even at very high concentrations of ATP (50 mM), Reeves and Hall (1973) showed only a 50% inhibition of state 3 electron transport. These authors also reported on the endogenous content of adenine nucleotides in type A plastids and concluded that the levels were too low to have a significant effect on coupled transport. In the experiments reported here, we observed that the slow $P_{700}$ turnover due to activation by PS1 persisted in green algae even in the presence of added glucose that would have acted as an energy sink by virtue of its active transport (Tanner and Kandler, 1967) and assimilation into carbohydrates (Tanner et al., 1966). Also, addition of CCCP never accelerated the decay time by more than a factor of three (to ca. 45 ms half-time at the fastest), showing that even in the absence of energy cou-

pling the in vivo cyclic electron transport is an order of magnitude slower than noncyclic transport. Such a slow turnover of the in vivo cyclic pathway is consistent with and satisfactorily explains the low-light intensity saturation of PS1-driven reactions in intact algae. Reactions such as the assimilation of substrates, ion uptake, and the synthesis of certain enzymes in algae have been shown to saturate at light intensities some tenfold lower than the saturation intensity for carbon dioxide assimilation (Simonis and Urbach, 1973). Our data suggest that the electron transport rate is the limiting factor in such reactions.

*Role of Cyclic Electron Transport in Vivo.* Our results confirm the existence of a cyclic electron transport in vivo and suggest that this pathway is not engaged during photosynthesis at high intensities. The slow, 150 ms, rate of $P_{700}^+$ reduction we attribute to turnover in this cyclic pathway is not observed in broad-band light that excites both photosystems and certainly cannot be of significance to an overall process that has a 30–40-ms relaxation time (Emerson and Arnold, 1932; Kok, 1956). Similar suggestions have been made previously based upon results concerning the differential sensitivity of photosynthesis and glucose assimilation to certain inhibitors (Tanner et al., 1969), studies on ion transport (Raven, 1970), and also consideration of the recently improved stoichiometry for noncyclic photophosphorylation (Reeves and Hall, 1973) by chloroplasts.

A consequence of our suggestion that cyclic photophosphorylation is not utilized during photosynthesis is that the noncyclic pathway must provide the entire metabolic demand of reductant and ATP for carbon dioxide assimilation. However, proposals have been made that considerably more ATP is required in the cell than is necessary to drive the Calvin cycle (Hoch, 1964). Cyclic photophosphorylation has usually been postulated as the pathway for the generation of such phosphate transfer potential that would be utilized for the purposes of transport and biosynthesis. A consideration of the quantum efficiency for carbon dioxide assimilation in vivo is pertinent in this respect. If an in vivo requirement of 9 quanta/carbon dioxide is accepted for photosynthesis (Ng and Bassham, 1968), then only two $NADPH_2$ may be generated by noncyclic transport. This would satisfy the metabolic demand for reductant for the Calvin cycle (2 $NADPH_2/CO_2$) but excludes further photochemistry that would be required to drive additional cyclic electron transport. Such postulated cyclic transport may only be conceivably accommodated if the reported in vivo quantum requirements are much too low. It should be noted, however, that the quantum efficiencies were measured at light intensities much lower than saturation, whereas our conclusions apply only to conditions at high intensities.

The induction effect we observe when both photosystems are activated by high-intensity light would not have been detected by previous investigators who have observed $P_{700}$. Kok (1957) and Rurainski et al. (1970), have utilized integrating signal recovery techniques, whereas Ke and Ngo (1965) and Witt (1967) have relied upon fast flash activation. Relatively long periods of illumination and dark recovery times considerably in excess of the photosynthetic unit turnover time are necessary. These conditions suggest the involvement of phenomena such as ion movements and gross membrane conformational changes that have much longer time constants. It is possible that the induction effect is correlated with the distribution of quanta between the two photosystems during the onset of photosynthesis and possibly the regulation of photosystem 2. A detailed analysis of this effect will be reported elsewhere (Maxwell and Biggins, in preparation).

The primary objective of the investigations reported here was to ascertain the role of cyclic photophosphorylation in oxygen-evolving photosynthesis. We have shown that the kinetic analysis of $P_{700}$ may be effectively used as an intrinsic probe of electron transport, permitting a direct observation of the rates and activities of cyclic and noncyclic electron transport in vivo. We suggest that the cyclic pathway does not contribute to the energetic demands of photosynthesis and that sufficient ATP and $NADPH_2$ must be generated for the carbon reduction cycle by noncyclic photophosphorylation.

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# Proton Magnetic Resonance Study of the Intramolecular Association and Conformation of the α and β Pyridine Mononucleotides and Nucleosides†

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ABSTRACT: The chemical shifts and coupling constants are reported for the proton nuclear magnetic resonance (NMR) spectra of the α and β anomers of the oxidized and reduced pyridine mononucleotides and nucleosides. The pseudorotational conformation analyses of the ribose coupling constants indicate that the ribose conformation for β-nicotinamide mononucleotide, βNMN, can best be described by a 3:1 mixture of interconverting 3′-exo (S) and 2′-exo (N) conformers. Reduction of βNMN to βNMNH results in phase angles consistent with interconverting 2′-endo (S) and 3′-endo (N) conformers without changes in the conformer populations. Cleavage of the 5′-phosphate from βNMN has a significant effect on the phase angles (becoming more like those for βNMNH), conformer population (the N and S conformers become nearly equal), and the distribution of the rotational isomers around the ribose 4′–5′ bond to the exocyclic methylene (the gauche–gauche population decreases by about 25%). In contrast, for βNMNH these parameters are all insensitive to dephosphorylation. The pseudorotational analysis has been extended to define the conformational parameters of α nucleotides. Analysis of the coupling constants for the α anomers indicates that the phase angles, conformer populations, and rotational isomers are generally insensitive to dephosphorylation, whereas both the phase angle and conformer populations are strongly dependent on the redox state of the base, αNMN being predominantly 2′-endo and αNMNH exclusively 2′-exo. The rotational isomers around the 4′–5′ and 5′–O bonds are found to be insensitive to the large changes in ribose conformation in the absence of any interaction with the base. The results are discussed in terms of relative contributions from base–ribose, ribose–side chain, and base–side chain interactions to the general conformational restraints imposed by the *cis*-2′,3′-hydroxyl interaction in β nucleotides and the additional *cis*-2′-hydroxyl–base interaction in α nucleotides. The significance of these interactions with respect to the enzymatic and nonenzymatic properties of the pyridine nucleotides is also considered.

Previous proton NMR[1] investigations of the pyridine mononucleotides (Oppenheimer et al., 1971; Birdsall and Feeney, 1972; Birdsall et al., 1975; Blumenstein and Raftery, 1972; Sarma and Mynott, 1973; Egan et al., 1975; Zens et al., 1975) have dealt exclusively with the β anomers because of their significance as the active moiety in βNAD(P) and βNAD(P)-H, the "natural" coenzymes for the pyridine nucleotide linked dehydrogenases.[1] In contrast, the α-pyridine nucleotides have not been investigated in detail despite their isolation from a number of tissue extracts and organisms (Kaplan et al., 1955; Ricci et al., 1965), the equilibration of α- and β-reduced

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[1] Abbreviations used are: NMN, nicotinamide mononucleotide; NMNH, reduced NMN; NR, nicotinamide ribonucleoside; NRN, reduced NR; TSP, 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$, sodium salt; DEAE, diethylaminoethyl; EDTA, (ethylenedinitrilo)tetraacetic acid; NMR, nuclear magnetic resonance; α and β refer to the anomeric configuration of the pyridine base to the ribose; *P* is the pseudorotational phase angle and $\tau_m$ the degree of puckering for the ribose ring as defined by Altona and Sundaralingam (1972, 1973).