Kinetic Behavior of Cytochrome *f* in Cyclic and Noncyclic Electron Transport in *Porphyridium cruentum*†

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ABSTRACT: The turnover of cytochrome *f* in the red alga *Porphyridium cruentum* was measured spectrophotometrically in modulated light of low frequency. Kinetic analysis of the postillumination reduction reveals that the total pool of cytochrome *f* reacts in two pathways depending upon excitation conditions. After oxidation by wavelengths longer than 680 nm, which excite photosystem 1, the reduction of cytochrome *f* is monophasic and can be accounted for by a first-order reaction with a half-time of about 150 msec at room temperature. The dark reduction was unaffected by 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea but was accelerated twofold by 20 μM carbonyl cyanide *m*-chlorophenylhydrazone. The inclusion of shorter wavelengths, which excite photosystem 2, either in the modulated actinic beam or as a continuous background supplementary to the modulated photosystem 1 light, resulted in a biphasic reduction. The reduction can be accounted for by two first-order reactions, one with a half-time of about 150 msec as was observed in photosystem 1 light alone, and one with a half-time of about 25 msec. The faster reduction associated with the activation of photosystem 2 was also accelerated by carbonyl cyanide *m*-chlorophenylhydrazone but was abolished by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. These data indicate that the cytochrome *f* pool reacts in both cyclic and noncyclic electron transport and this kinetic analysis permits a direct assessment of the control and the activities of these two pathways *in vivo*. The relative concentrations of the two kinetic forms in the total reactive cytochrome *f* pool and, therefore, the activities of cyclic and noncyclic electron transport were found to be a function of the spectral composition and intensity of the actinic flash. Cyclic transport saturates at low light intensities regardless of wavelength and is the major pathway when cellular maintenance rather than growth prevails. The noncyclic pathway is the predominant pathway at high intensities. An estimate of the contribution of cyclic transport to the total electron transport flux in saturating white light suggests that this pathway is insignificant during CO_2 assimilation. The interaction between photosystem 1 and photosystem 2 as measured by the turnover of cytochrome *f* is consistent with the Hill–Bendall model for noncyclic electron transport.

The general features of the electron transport system of oxygen-evolving photosynthesis whereby ATP[1] and NADPH are generated on a noncyclic pathway driven by two pigment systems are now well established (Bishop, 1971; Vernon and Avron, 1965). Although never explicitly proposed it is now generally accepted that cyclic electron transport and the associated phosphorylation is a short circuit of the long-wavelength photosystem 1. It is assumed to involve the same pigment system, trapping center and many of the intermediary electron transport components active in the noncyclic pathway plus some additional components for the short circuit itself. Since its discovery by Arnon *et al.* (1954), it has been repeatedly suggested that cyclic photophosphorylation must be engaged during carbon dioxide assimilation to supplement the cellular demand for ATP even though high P/e_2 measurements for noncyclic photophosphorylation have been reported (Izawa and Good, 1968). Although the important and exclusive role of cyclic photophosphorylation in the photoassimilation of substrates such as glucose (Kandler, 1954) and acetate (Wiessner, 1965) has been established for algae under conditions where photosystem 2 is inactive, it has thus far not been experimentally feasible to assess the contribution of the cyclic pathway to overall electron transport during carbon dioxide photosynthesis in natural conditions of white light excitation.

Despite the lack of information concerning the role of cyclic electron transport during the assimilation of carbon dioxide, suggestions have been made based on ultrastructural evidence that discrete regions on the free thylakoids of higher plant chloroplasts are solely dedicated to photosystem 1 activity whereas the appressed, or grana, regions are functional in both photosystems (Jacobi and Lehmann, 1969; Sane *et al.* 1970). These claims imply a definite gross morphological separation of a fraction of the cyclic pathway from the noncyclic pathway. The viability of these suggestions is made more appealing by the report that the ratio of the activities of the two photosystems is not constant throughout algal life cycles (Senger, 1970) indicating that the level of cyclic and noncyclic electron transport function may well be dictated by developmental status.

This report presents evidence that in a mixed population of exponential cells of *Porphyridium cruentum*, cytochrome *f* is distinguishable kinetically into that participating in the cyclic and noncyclic electron transport pathways. This has made possible an assessment of the contribution of cyclic transport to the total electron transport flux during carbon dioxide photosynthesis and the regulation of the two electron transport pathways. The role of cytochrome *f* with regard to the Hill and Bendall (1960) and Knaff and Arnon (1969) models for electron transport is discussed.

Materials and Methods

Biological. Porphyridium cruentum was obtained from the Culture Collection of Algae, Indiana University, Blooming-

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[1] Abbreviations used are: ATP, adenosine 5′-triphosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

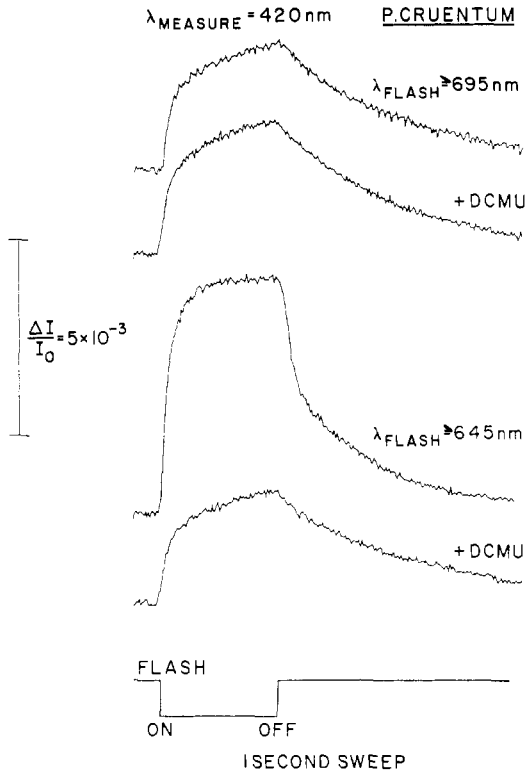

FIGURE 1: Photoinduced absorption transients at 420 nm in *P. cruentum*. The wavelengths of the excitation flashes were isolated with Schott RG 695 and Schott RG 645 color glasses providing wavelengths longer than 695 nm (photosystem 1) and longer than 645 nm (photosystem 1 plus 2), respectively. DCMU when added was 10 μM and the overall cycle time for the flash and dark recovery was 2 sec.

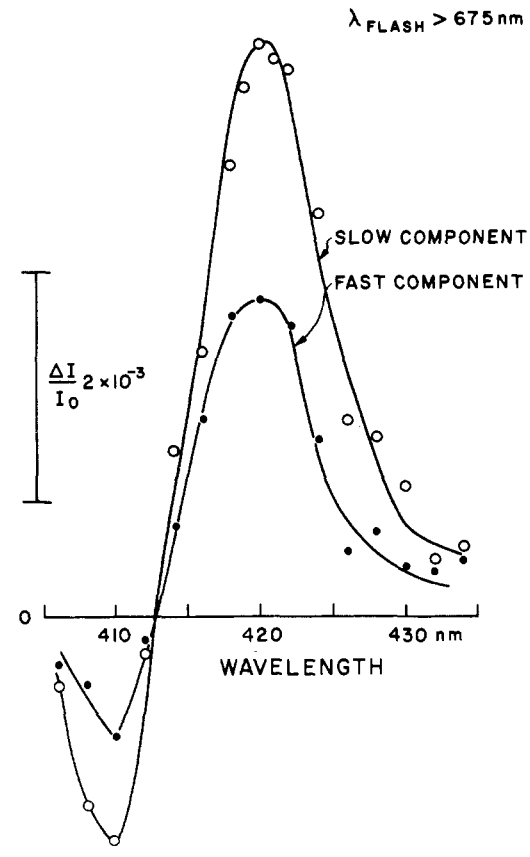

FIGURE 2: Absorption spectra for the components with fast (28 sec^{-1}) and slow (4.6 sec^{-1}) reduction subsequent to an excitation flash of wavelengths longer than 675 nm. Flash and modulation conditions as in Figure 1.

ton, and grown in batch culture on the enriched seawater medium F/2 (Guillard and Ryther, 1962) at 2.8% salinity. Cells were harvested during the exponential phase of growth with a chlorophyll concentration of about 5 μg/ml and used at 5–10 μg of chlorophyll/ml after washing and resuspension in fresh F/2 medium. Chlorophyll concentration was estimated spectrophotometrically in cold 80% acetone extracts of the cells after mild heat treatment (Gimmler and Avron, 1971).

Spectrophotometry. Photoinduced absorption transients for kinetic analysis were detected in whole cells by a single-beam spectrophotometer which employed a Bausch and Lomb 250-mm grating monochromator and S13 photomultiplier (RCA 6903). Measuring light bandwidth at half-height was 3.3 nm and the sample path length was 10 mm. Pulsed actinic illumination was provided by modulating a continuous beam with a shutter (Uniblitz Model 225, Vincent Associates, Rochester, N. Y.) operated by a locally fabricated pulse generator and driving circuit yielding flash half rise times of 2 msec.

The bandwidth of the detection system was 1 kHz and signal/noise improvement was accomplished by using a 256 data point Fabritek Model 1010 digital signal averager which was triggered by the actinic pulse timing circuitry. Usually 128 or 256 sweeps were sufficient to improve the signal/noise to better than 15. Sweep times of 1 sec and overall cycle times of about 2 sec were long enough for full recovery of the absorption transients induced by flashes of about 250-msec duration. Measurements were made at room temperature.

Pulsed and continuous background actinic wavelengths were isolated from 500-W tungsten iodide lamps by means of Baird-Atomic interference filters (100-Å bandwidth at half-height), various Corning and Schott long-pass color glasses as specified and 10 cm of water. The beams were attenuated by Corion Instrument Corp. neutral density filters. The actinic path was 5 mm long at the specimen and was 90° to the measure beam. The photomultiplier was blocked with two Optics Technology 600 nm short-pass cutoff filters plus a Corning 5-60 color glass.

Kinetic data were evaluated from individual data points recorded from the signal averager by a Hewlett-Packard Model 7004B X–Y plotter. The analysis was accomplished by constructing semilogarithmic plots of the absorption transients and estimating the rate constants from the slopes and the initial concentrations from the intercepts.

Slow changes and absorption amplitudes were recorded by means of an Aminco-Chance dual wavelength spectrophotometer utilizing a Princeton Applied Research Model 126 lock-in amplifier for signal recovery. The measuring beam bandwidth, blocking filters, and illumination sources were identical with those used on the kinetic instrument. Light intensities were measured with a YSI Model 65 radiometer (Yellow Springs Instrument Co., Inc., Ohio).

Results

The choice of *P. cruentum* as experimental material for this program was dictated by the clear spectral separation of the

two photosystems in red algae (Duysens and Amesz, 1962). The primary objective in the measurement of photoinduced absorption changes was the extraction of kinetic information particularly from the postillumination recoveries. Figure 1 illustrates some typical data for the absorption change at 420 nm in *P. cruentum*. It has been shown previously that this can be attributed exclusively to cytochrome *f* (Duysens *et al.*, 1961, 1962; Nishimura, 1966, 1968; Rurainski *et al.*, 1970). As shown in the upper two traces, wavelengths of 695 nm and longer, which only excite photosystem 1, lead to a net oxidation of the cytochrome. The postillumination reduction is monophasic and can be accounted for by a first-order reaction with a half-time of about 150 msec. A variation of about 10% was found in this rate constant (4.6 sec^{-1}) over a large sample range but much longer half-times in excess of 200 msec were observed in stationary cells. The steady state level of photooxidation and the rate constant were not affected by DCMU, indicating that the cytochrome turnover is driven solely by photosystem 1.

Inclusion of wavelengths in the flash which excite both photosystems, for example 645 nm and longer as shown in the lower two traces of Figure 1, results in a biphasic recovery comprising two first-order reactions. Analysis of the decay data revealed the presence of a component with a half-time of about 150 msec, as was found in photosystem 1 light alone, and an additional faster component with a half-time of about 25 msec. Addition of 10 μM DCMU to cells under such excitation conditions (lowest trace) resulted in a loss of the fast component whereas the rate constant of the component with the slow decay was unaffected. In both conditions of excitation, *i.e.*, photosystem 1 only and photosystem 1 plus 2, 20 μM CCCP led to a decrease in signal amplitude and acceleration of the decays by a factor of two, implying the association of rate limiting energy conservation steps with all pathways.

Spectra of the two kinetic species observed in short excitation wavelengths were obtained by measuring absorption transients at different wavelengths and estimating the concentration of each component from the decay data. Figure 2 is an example for algae sensitized by broad band excitation wavelengths of 675 nm and longer and shows that the two components have indistinguishable absorption spectra. Similar spectra were obtained for the biphasic decays induced by superimposition of a photosystem 1 flash on a continuous monochromatic photosystem 2 beam (see Figures 4 and 5 below). At flash wavelengths of 680 nm and longer where the component with the slow reduction is the only reactive species, the light minus dark difference spectrum was also identical with a maximum at 421 nm. This suggests that the total pool of cytochrome *f* is capable of reacting in two kinetically distinguishable pathways in this alga.

Figure 3 shows flash saturation curves for cytochrome *f* photooxidation by a variety of wavelengths. It should be emphasized that the actinic illumination in this experiment has a broad bandwidth as isolated by various long-pass cutoff filters and the large infrared component may account for the high saturation intensities. Consequently these saturation curves cannot be compared to those for the overall photosynthesis of thin algal films excited by narrow bandwidth illumination. The extent to which the cytochrome is oxidized is similar for all excitation conditions irrespective of whether photosystem 1 alone or both photosystems are excited. For each data point in the light intensity curve, the dark recovery was analyzed and estimates of the fraction of the reactive cytochrome with the fast reduction is illustrated in the graph on the right. As expected from the data above, after excitation

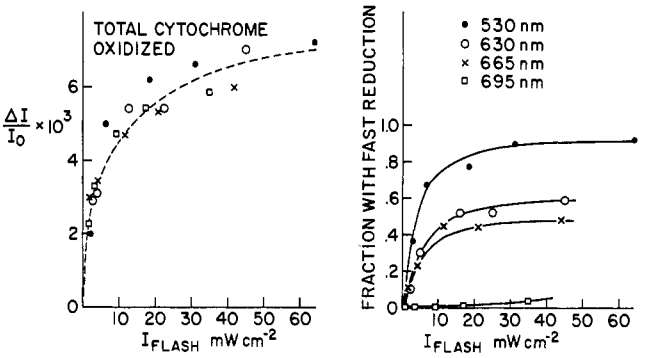

FIGURE 3: Flash saturation curve for cytochrome *f* photooxidation as measured at 420 nm. Broad band excitation wavelengths were isolated by Schott RG long-pass color glasses. For each wavelength the fraction of the total change associated with the fast dark reduction is illustrated on the right. Flash and modulation frequency as in Figure 1.

by wavelengths longer than 695 nm, the cytochrome is reduced with a low rate constant. With the inclusion of shorter wavelengths in the flash and consequent activation of photosystem 2 an increasing proportion of the active pool of cytochrome *f* is reduced with the higher rate constant. Thus the kinetic behavior of cytochrome *f* is a function not only of spectral composition but also of the intensity of the exciting flash.

These data suggest that the cytochrome *f* with the DCMU-insensitive slow reduction ($t_{1/2}$ = 150 msec) is a component of the cyclic turnover of photosystem 1 and the cytochrome with the fast reduction ($t_{1/2}$ = 25 msec) is a component of noncyclic electron transport driven by two photosystems. The bulk of the cytochrome *f* appears to be reactive in both pathways and the proportion of the total pool reacting in each pathway depends on the intensity and spectral composition of the incident excitation. Thus, the analysis of cytochrome *f* reduction kinetics affords a direct, simultaneous, nondestructive and quantitative measure of the activities of cyclic and noncyclic electron transport *in vivo*.

To investigate further the involvement of cytochrome *f* with the fast reduction in interaction between the two photosystems, the effects of continuous supplementary photosystem 2 wavelengths on the response elicited by photosystem 1 flashes were explored. The effect of a continuous superimposed monochromatic beam of 550 nm for activation of photosystem 2 on a photosystem 1 mediated transient is shown in Figure 4. The data show that as the intensity of the supplementary background beam is increased and interaction between the two photosystems is made possible, a fraction of cytochrome associated with the fast reduction appears at the expense of cytochrome with the slow reduction. At high intensities of the supplementary beam the overall signal amplitude is lower because the continuous green light also photooxidizes the cytochrome (Kuntz, 1965) and therefore the residual concentration available for the modulated response is lower. This pattern was also observed in the presence of 20 μM CCCP indicating that interaction of the two photosystems occurs in the absence of high-energy intermediates. Figure 5 shows that the fast decay which appears as a result of the supplementary photosystem 2 beam is abolished by DCMU.

Other possible combinations of supplementary beam wavelengths on absorption transients mediated by photosystem 1 on photosystem 2 flashes confirmed the interaction between

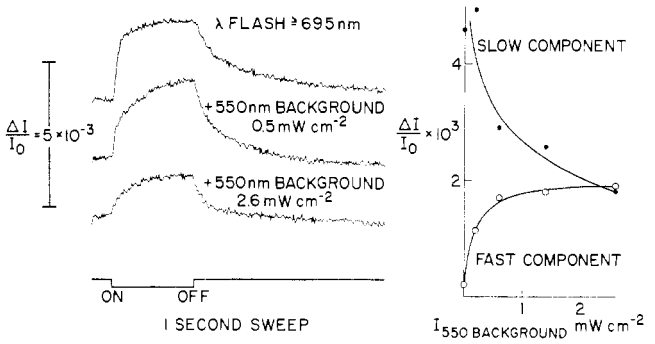

FIGURE 4: Effect of a continuous background beam of photosystem 2 light (Baird Atomic 550-nm interference filter plus Corning C3.70) on the 420-nm absorption change induced by a photosystem 1 flash in *P. cruentum*. The photosystem 1 flash intensity was 10 mW cm^{-2}. The graph illustrates the fraction of the total cytochrome change associated with the fast and slow rates of reduction. Overall cycle time was 1.5 sec.

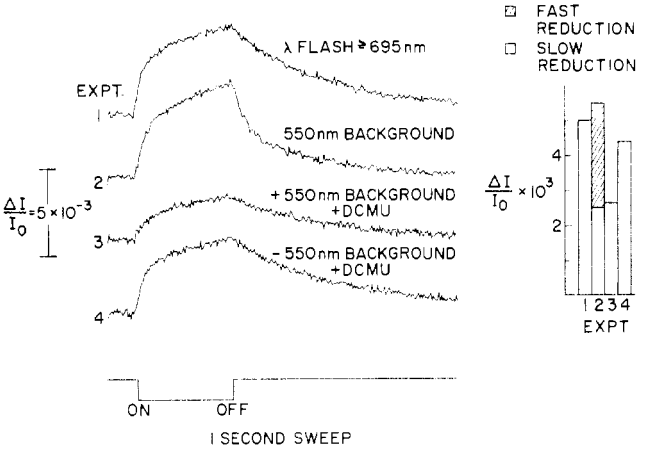

FIGURE 5: Effect of DCMU on cytochrome *f* kinetics during interaction between photosystem 1 and photosystem 2. Conditions as in Figure 4. The photosystem 1 (695 nm and longer) light intensity was 10 mW cm^{-2} and the continuous photosystem 2 (550 nm) light intensity in expt 2 and 3 was 2.6 mW cm^{-2}. DCMU was added in expt 3 and 4 at a concentration of 10 μM. The histogram shows the amplitude of the modulated photosystem 1 response for each treatment.

the two photosystems (not illustrated). Background illumination of wavelengths identical with the flash wavelength resulted in a decreased level of oxidation of cytochrome *f* only whereas a photosystem 2 flash superimposed on a photosystem 1 background beam led to a net reduction. This latter experiment is directly analagous to that reported by Duysens *et al.* (1961) who first showed the antagonistic effect of short wavelengths on the level of oxidation of cytochrome *f* in photosystem 1 light. This particular absorption change is very complex and the analysis will be reported in detail elsewhere.

Discussion

The photooxidation of cytochrome *f* in red algae was first reported by Duysens *et al.* (1961). We confirm this classical work and numerous subsequent reports (Amesz and Fork, 1967; Amesz *et al.*, 1972a,b; Hoch and Randles, 1971; Rurainski *et al.*, 1970) that excitation wavelengths longer than 680 nm result in cytochrome turnover *via* a DCMU-insensitive short circuit of photosystem 1, or cyclic electron transport. Measurements of the light minus dark difference spectrum in the cytochrome Soret region show the photoinduced redox change of a single cytochrome species and in all cases the postillumination recovery (reduction) is monophasic with a half-time of about 150 msec at room temperature. This rate constant is in excellent agreement with similar measurements made by Kuntz and Calvin (1965) who report a half-time for cytochrome *f* reduction of 160 msec in *P. cruentum*. Much slower decays may be calculated from the data of Amesz and Fork (1967) for a marine algal thallus, *Iridaea splendens*, and our preliminary studies on *Porphyra umbilicalis* collected from Narragansett Bay, R. I., also show much slower decays. This difference may prove to be a distinctive character between unicellular and multicellular red algae. Rurainski *et al.* (1970), measuring signals generated from *P. cruentum* excited by modulated photosystem 1 light of 13 Hz by steady-state relaxation spectrophotometry, report relaxation times of 16 msec for cytochrome *f*. This relaxation time, as computed from phase-shift measurements by means of a lock-in amplifier, corresponds to a half-time for an assumed first order reaction of 11 msec. This large discrepancy is most likely accounted for by the fact that a component with a half-time of 150 msec would be considerably attenuated at this frequency and these investigators would have had difficulty in measuring such a slow component. In agreement with the observations of this group and others (Amesz and Fork, 1967; Nishimura, 1966), we found that DCMU has no effect on the signal amplitude or the kinetics of the cytochrome change in photosystem 1 light. Although overall carbon dioxide assimilation is inhibited under these conditions, substantial endogenous activity remains *via* this cyclic pathway which can be utilized for synthetic purposes (Kandler, 1954; Wiesner, 1965). The possible significance of this cyclic pathway during excitation by white light will be elaborated upon below.

The data reveal that with the activation of photosystem 2 by the inclusion of shorter excitation wavelengths in the flash, a fraction of the cytochrome *f* pool is reduced with a high rate constant (28 sec^{-1}) and is abolished by DCMU. It is suggested that this fraction of the cytochrome *f* is reacting in the noncyclic pathway driven by two photosystems. Our measured rate constant for the reduction of cytochrome *f* in noncyclic electron transport is intermediate between the values obtained by other investigators including those who have made similar measurements by different experimental techniques. Both Nishimura (1968) and Kuntz and Calvin (1965) report half-times for cytochrome *f* reduction of about 10 msec in *P. cruentum*. Kelly and Sauer (1968) in a study of functional unit size for electron transport reactions in spinach chloroplasts report about 30 msec for interaction between the two photosystems. Thus, the turnover of cytochrome *f* in the noncyclic pathway is about six times faster than the cytochrome *f* in the cyclic pathway.

The first demonstration of the interaction between two spectrally dissimilar photosystems was originally demonstrated by monitoring the redox state of cytochrome *f* in *P. cruentum* under various illumination regimes. Duysens *et al.* (1961) showed that 562 nm illumination when supplied simultaneously to cells in the steady state in photosystem 1 light (680 nm) resulted in an amplitude decrease or reduction which was reversible and abolished by DCMU. This experiment has been repeated exactly by Nishimura (1966) and Kuntz (1965). A substantial number of investigators have

demonstrated the antagonistic effect of supplementary photosystem 2 illumination on the cytochrome *f* redox state by sequential rather than simultaneous presentation of the beams (Vernon and Avron, 1965). In the only kinetic analysis of this phenomenon Kuntz and Calvin (1965) showed that for activation by pulsed photosystem 1 on a continuous photosystem 2 beam, the decay half-time of cytochrome *f* was inversely proportional to the incident intensity of the supplementary short-wavelength beam. The data presented here (Figures 4 and 5) extend and amplify this work and show that the acceleration of the decay by inclusion of the supplementary beam is due to the participation of an increasing amount of the cytochrome *f* pool in a faster reaction as noncyclic transport is activated.

The demonstration of the existence of two kinetically distinguishable forms of cytochrome *f* associated with cyclic and noncyclic electron transport now makes it possible to assess the contribution of the two pathways to overall photosynthesis. The photosystem 1 regeneration half time as assayed by cytochrome *f* turnover in this study is about 150 msec which is substantially slower than the recovery time of 40 msec for complete photosynthesis (Emerson and Arnold, 1932). Our measurements in broad band light of high intensity ($\geq$530 nm in Figure 3) where both photosystems are saturated indicate that only 10% of the observable cytochrome reacts in the cyclic pathway under these conditions and thus the contribution of cyclic photophosphorylation in saturating white light must be very small. Velocity calculations on the electron transport flux indicate that no more than 2% of the total transport is *via* the cyclic pathway in this red alga in these conditions. At low intensities, however, regardless of excitation wavelength, the cyclic pathway becomes increasingly significant such that it must be a major contributor to the cellular energetic demand particularly in maintenance conditions below the compensation point. Similar conclusions have been reached by Tanner, Loffler, and Kandler (1969) based on a study of the anaerobic photoassimilation of glucose by green algae.

Hoch and associates (Rurainski *et al.*, 1971; Hoch and Randles, 1971) have suggested that the interaction of photosystem 1 and 2 is through the mediation of a high-energy intermediate and not by electron transport as originally postulated and generally accepted. The data presented here do not support this hypothesis because the interaction of the two photosystems as measured by cytochrome *f* turnover is observed to occur in the presence of uncoupler which should prevent the formation of any high-energy compounds.

An alternate proposal to the Hill and Bendall (1960) formulation for electron transport was reported by Knaff and Arnon (1969) whereby separate pathways were postulated for cyclic and noncyclic electron transport. In particular they proposed that cytochrome *f* reacts only in a cyclic pathway driven by a long-wavelength phtotosystem 1 and the noncyclic pathway is driven by two short-wavelength photosystems 2a and 2b. The data reported here are at variance with these suggestions for they demonstrate that cytochrome *f* reacts in both pathways and the noncyclic pathway is driven by long- and short-wavelength pigment systems. The data presented are consistent with the Hill and Bendall postulates and suggest that the bulk of the apparatus associated with photosystem 1 interacts with photosystem 2. At low light intensities, or in excitation conditions which limit photosystem 2, all of the photosystem 1 is capable of the cyclic short circuit. As the experiments reported here were conducted on a mixed population of exponential cells the possibility of some modification of this mechanism at certain developmental stages is not ruled out and clearly similar studies on a synchronous population of algae would be desirable.

The data presented reaffirm that the primary control of the interaction between the photosystems is spectral composition and intensity of the exciting light (Avron and Ben-Hayyim, 1969; Murata, 1969, 1970). A complete understanding of the detailed mechanism whereby these parameters are translated into terms of membrane biochemistry must await further study. The analysis of the kinetics of cytochrome *f* turnover as described in this report may prove useful in assessing the significance of ion movements and membrane conformation (Izawa and Good, 1966) in the regulation of the two electron transport pathways.

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Properties of the Fluorescence Probe Response Associated with the Transmission Mechanism of Colicin E1†

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ABSTRACT: Addition of colicin E1 to sensitive *Escherichia coli* in the presence of the fluorescence probe 8-anilino-1-naphthalenesulfonate (ANS) causes a blue shift of approximately 8 nm in the fluorescence emission spectrum of the bound dye. The colicin-induced fluorescence increase of the more lipophilic probe *N*-phenyl-1-naphthylamine (NPN) is very similar to that of ANS, is independent of potassium and sodium concentration from 0 to approximately 100 mM, and is not affected by preincubation in *N,N'*-dicyclohexylcarbodiimide which prevents the colicin-induced decrease in the intracellular ATP level. The decrease in ATP caused by colicin E1 is found to be unaffected by an increase in the potassium concentration of the medium. The decay of trypsin reversal of colicin lethal effects is slightly faster than the colicin-induced ATP decrease at 35°, and much faster than the rate of the colicin-induced increase of NPN fluorescence at 13°. The rate of the increase in probe fluorescence caused by colicin E1 is unaffected by a decrease of at least 30-fold in ambient oxygen concentration. It is concluded that the fluorescence probe response caused by colicin is an indicator of oxygen-independent structural or conformational changes in the cell envelope which are responsible for permeability changes and possible activation of ATPase. These structural changes occur at the same time or after the colicin becomes inaccessible or insensitive to trypsin.

Recent studies on the mode of action of colicin E3 have shown that the colicin itself has enzymatic properties as a specific ribosomal nuclease (Boon, 1971, 1972; Bowman *et al.*, 1971). These experiments imply that colicin E3 may exert an effect directly on its biochemical target, and to do so may penetrate the cell envelope at least to the level of the cytoplasmic membrane. These experiments imply that a long-range transmission mechanism previously hypothesized to operate between the colicin at a receptor site on the surface of the cell and target sites on the internal membrane (Nomura, 1964; Luria, 1964) may not be necessary to explain the mode of action of colicin E3.

Attempts to find an intrinsic DNase activity associated with colicin E2 have thus far been negative (Nomura, 1964; Almendinger and Hager, 1972), although this colicin at high concentrations can modify the DNA melting curve (Ringrose, 1972). It has been proposed that DNA degradation associated with the action of colicin E2 *in vivo* is caused by colicin binding to surface receptors and subsequently initiating an increase in the accessibility of periplasmic endonuclease I to the bacterial chromosome (Almendinger and Hager, 1972). This mechanism for colicin E2 could involve colicin-induced structural changes in the cell envelope or membrane.

In the case of colicins like E1 and K it seems likely that the mode of action involves perturbing the cell envelope in some way. This is simply because cells treated with colicin E1 rapidly lose potassium (Nomura, 1963; Wendt, 1970) and the potassium loss can occur in the absence of any decrease in intracellular ATP (Feingold, 1970). It is possible that envelope structural changes associated with colicin E1 could be the consequence of phospholipase activity associated with or induced by colicin E1 (Cavard *et al.*, 1968).

We have previously used the dye 8-anilino-1-naphthalenesulfonate (ANS)[1] as a probe for colicin-induced structural changes in the envelope of sensitive cells (Cramer and Phillips, 1970). Dyes such as ANS are useful as indicators of local changes in conformation or structure because their fluorescence is very sensitive to solvent parameters, in particular to solvent polarity, viscosity, and rigidity (references to the original work on this subject and the most recent general reviews are Weber and Laurence, 1954; McClure and Edelman, 1966; Turner and Brand, 1968; Stryer, 1968; Radda, 1971a,b; and Brand and Gohlke, 1972). Colicin E1, but not colicins E2 or E3, caused a large increase in the fluorescence of ANS bound to sensitive cells. That the ANS might be an indicator of structural changes caused by colicin E1 was indicated by the absence of any fluorescence change in cells colicinogenic for E1 and the qualitative similarity of the colicin-induced changes in fluorescence and intracellular ATP level (Cramer and Phillips, 1970). It was not possible to decide from this work whether the probe fluorescence response was (a) a primary

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[1] Abbreviations used are: ANS, 8-anilino-1-naphthalenesulfonate; NPN, *N*-phenyl-1-naphthylamine; DCC, *N,N'*-dicyclohexylcarbodiimide.