Localization of a Site of Energy Coupling between Plastoquinone and Cytochrome *f* in the Electron-Transport Chain of Spinach Chloroplasts†

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ABSTRACT: The effect of ADP, NH_4Cl, and energy-transfer inhibitors on the steady-state oxidation level of cytochrome *f* was determined under conditions where the phosphorylation step is rate limiting. The existence of energy coupling on the system II side of cytochrome *f* is confirmed. An oxidation of cytochrome *f* seen upon ATP addition is also consistent with this conclusion. The difference spectra for addition of ADP and NH_4Cl, energy-transfer inhibitors, and ATP do not provide any evidence for phosphorylation linked to either the oxidation or the reduction of cytochrome *b*-559. Spectral changes which occur in the region of the *b*-559 absorption upon addition of energy-transfer inhibitors and ATP appear to be due only to light scattering, and any spectral changes associated with cytochrome *b*-559 would have to be smaller than those for cytochrome *f*. Similar experiments were performed in the ultraviolet region of the spectrum where plastoquinone is expected to absorb. A 254-nm-absorbing component which is most likely plastoquinone was found to be initially completely oxidized in the dark, reduced by red actinic light, and reoxidized in the dark and by far-red light. The extent of the dark and far-red light oxidation was increased by NH_4Cl and ADP. There was no effect of ADP and NH_4Cl on the extent of the reduction by red actinic light. It is concluded that ATP synthesis is coupled to electron transport from plastoquinone to cytochrome *f*.

An understanding of the mechanism by which light energy trapped in reducing equivalents is converted to ATP[1] in chloroplasts will very likely depend on the identification of the site or sites in the electron transport chain which are coupled to ATP synthesis. One approach to the localization of discrete phosphorylation sites is to study the effect of agents known to affect phosphorylation on the steady-state oxidation level of the electron-transport carriers. These ideas have been extensively applied to oxidative phosphorylation (Chance and Williams, 1956; Muraoka and Slater, 1969). When the rate of electron transport in a linear chain is limited by the absence of ADP (state 4 in the nomenclature of Chance and Williams, 1956), addition of ADP (state 3) or of uncouplers will in general change the steady-state redox levels of the electron carriers which equilibrate with the phosphorylation site or sites. The observable effects are expected to be simple in the case of a single phosphorylation site or for the carriers on either side of a series of sites. The effects will be more complex when a carrier is situated between discrete phosphorylation sites and will depend on the relative changes in the rate constants for electron transfer at the adjacent sites (Muraoka and Slater, 1969). For a single site the state 4 to state 3 transition caused by ADP addition is expected to cause oxidative and reductive changes, respectively, in the rapidly equilibrating carriers on the reducing and oxidizing sides of the site. Uncouplers added in state 4 will induce oxidative and reductive effects similar to those of ADP, whereas addition of energy-transfer inhibitors in state 3 will cause redox changes opposite in direction to those caused by ADP and uncouplers.

The term "crossover" has been applied to the general class of experiments where an agent causes oppositely directed changes in oxidation state on the donor and acceptor sides surrounding its site of action (Chance and Williams, 1956).

In a spectrophotometric study of the effect of ADP and the uncoupler NH_4Cl on the oxidation state of cytochrome *f* when phosphorylation is rate limiting, it has been shown that the noncyclic phosphorylation site(s) are most likely on the system II side of cytochrome *f* (Avron and Chance, 1966). Further application of this approach seemed to indicate that a phosphorylation site exists on the system II side of cytochrome *b*-559 (Ben Hayyim and Avron, 1970).

"Crossover" experiments reported in this paper confirm that a coupling site precedes cytochrome *f*. It has not been possible to show that cytochrome *b*-559 is directly involved at the coupling site. By studying the plastoquinone associated absorbance changes, it is demonstrated that the amplitude of plastoquinone oxidation, but not reduction, is increased by ADP and NH_4Cl. It is concluded that ATP synthesis is very likely coupled to electron transfer between plastoquinone and cytochrome *f*.

Methods

Class II chloroplasts with photosynthetic control ratios around 3 were prepared as described previously (Böhme and Cramer, 1971) from spinach grown at 22° in a controlled climate facility. The reaction medium for all spectrophotometric assays consisted of 25 mM Tricine–NaOH (pH 7.8), 5 mM $MgCl_2$, 5 mM K_2HPO_4, unless otherwise indicated, and chloroplasts at a concentration of approximately 70 μg/ml of chlorophyll for cytochrome and approximately 20 μg/ml for plastoquinone measurements. Methylviologen (0.1 mM) was added for the cytochrome experiments. No electron acceptor was present in the plastoquinone measurements. ADP and

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[1] Abbreviations used are: ADP, adenosine 5′-diphosphate; ATP, adenosine 5′-triphosphate; Cyt *b*-559; cytochrome *b*-559; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

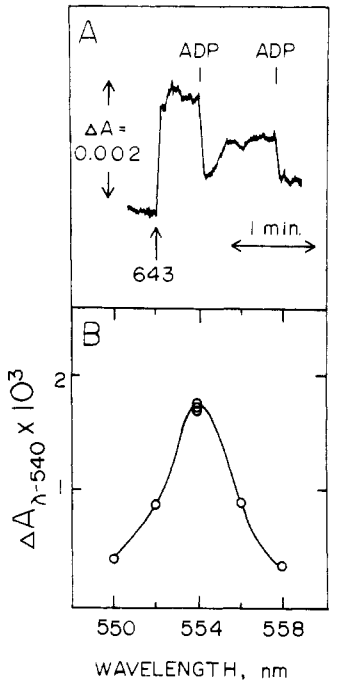

FIGURE 1: (A) Effect of ADP on the light-induced absorbance changes of cytochrome *f*. Cytochrome *f* changes measured at 554 nm with 540 nm as reference. The reaction mixture at pH 7.5 contained in mM: sorbitol, 100; Tricine–NaOH, 25; $MgCl_2$, 5; K_2HPO_4, 5; methylviologen, 0.1; chlorophyll concentration, 70 μg/ml; first addition of ADP, 3 μM; second ADP addition, 6 μM. Upward deflection indicates oxidative change. (B) Difference spectrum for the ADP-induced absorbance change of Figure 1A.

ATP were purchased from Calbiochem (Cat. No. 117325) and Sigma (Cat. No. A3127), respectively. Phlorizin was obtained from Calbiochem. A sample of Dio-9 was very kindly donated by Mr. Jack Wilson.

Cytochrome oxidation–reduction was assayed in the α-band region using a dual-wavelength spectrophotometer as described (Böhme and Cramer, 1971). The actinic light intensity was about 6×10^4 ergs/(cm² sec) for continuous 713- and 643-nm illumination. Plastoquinone absorbance changes were also followed with a dual-wavelength spectrophotometer, using the Sylvania DE50A deuterium source supplied with the Amino-Chance instrument operated between 20 and 30 W, and an EMI 6255B photomultiplier tube blocked with an ultraviolet inference filter. The bandwidth of the measuring light in the ultraviolet region was 1.4 nm, with the reference wavelength set at 276 nm, the isosbestic wavelength for extracted plastoquinone A (Crane and Dilley, 1963). The wavelength calibration was checked using the 253.7-nm Hg line. The intensities of continuous actinic illumination at 713 and 643 nm were approximately 8×10^3 ergs/(cm² sec).

Results and Discussion

It is well known that in the absence of an electron-acceptor cytochrome *f* can be almost completely oxidized by far-red light and then almost completely reduced by red light (Avron and Chance, 1966; Cramer and Butler, 1967). In the presence of an electron acceptor and in the absence of a phosphate acceptor, phosphorylation is rate limiting. Under these conditions red light causes a larger oxidation of cytochrome *f* indicative of a slow step in electron transport on the system II side of cytochrome *f*. Addition of ADP partially, and of NH_4Cl more completely, reversed the oxidative effect of red light (Avron and Chance, 1966). This result is confirmed in Figure 1 where it is shown that addition of ADP reversed by 70% the oxidative effect of red light on cytochrome *f* in the absence of a phosphate acceptor. Addition of a small amount of ADP in the presence of 643-nm light and methylviologen as electron acceptor induces a large transient reductive change at 554 nm, indicating enhanced electron flow from photosystem II to cytochrome *f*. During the course of illumination the ADP is used up and the system returns to a controlled state with cytochrome *f* at a more oxidized level. A second addition of ADP produces another reductive transition (Figure 1A). The spectrum for the ADP-induced cytochrome *f* reduction has a peak at 554 nm and is symmetric as far as it goes, up to 558 nm on the long-wavelength side of the spectrum (Figure 1B). The spectrum for the ADP-induced absorbance change should either change sign between 554 and 559 nm if there was a phosphorylation site between cytochrome *b*-559 and cytochrome *f*, or it should show a shoulder and a shift of the peak away from 554 nm toward longer wavelength if there is a phosphorylation site on the system II side of cytochrome *b*-559 (Ben Hayyim and Avron, 1970). The limited spectrum of Figure 1 provides a preliminary indication, supported by the data of Figures 2 and 3, that it is not possible to show by "crossover" experiments that cytochrome *b*-559 is linked to phosphorylation.

The experiment of Figure 1A would more easily detect an ADP-induced oxidation of the *b*-559 than an ADP-induced reduction, the change reported by Ben Hayyim and Avron (1970) in lettuce chloroplasts. This is because the cytochrome *b*-559 in coupled spinach chloroplasts is already mostly reduced under the illumination conditions of Figure 1A before ADP is added (Cramer and Butler, 1967). For this reason a reductive change caused by ADP addition might not be as easily detected in spinach as in lettuce chloroplasts. The effect of energy transfer inhibitors such as phlorizin and Dio-9 on the electron transport carriers linked to a phosphorylation site should be opposite in sign from those of ADP. Thus, it should be possible with the energy transfer inhibitors to detect a coupling site on the PS II side of cytochrome *b*-559 in spinach chloroplasts, since the energy-transfer inhibitors would cause an oxidative change. The experiments shown in Figure 2 show that phlorizin and Dio-9 reverse the effect of added ADP on the absorbance changes measured at 554 nm. The effect of the energy-transfer inhibitors can in turn be reversed by NH_4Cl (Figure 2A). The difference spectra of Figure 2B show the following. (1) The initial oxidative change caused by red light in the presence of an electron acceptor is exclusively that of cytochrome *f* and little or no *b*-559 is in oxidized under these conditions, as stated above. (2) The difference spectra for the reductive absorbance changes caused by addition of ADP and NH_4Cl show, as did the more limited spectrum of Figure 1A, that there is no indication of an oxidative or reductive change of cytochrome *b*-559 along with cytochrome *f*. This is shown more conclusively in the ADP spectrum than in that for NH_4Cl addition, as the latter shows a larger amplitude at longer wavelength. Because the amplitude of the spectrum for the ADP change is almost zero at 565 nm, it is thought that the additional amplitude at the longer wavelengths in the NH_4Cl spectrum is caused by a scattering change induced by NH_4Cl, whose amplitude would increase as the wavelength separation between the sample and 540-nm reference beams is increased; (3) the difference spectrum for the phlorizin-induced absorbance change shown in Figure 2A is given by the closed squares and continuous trace

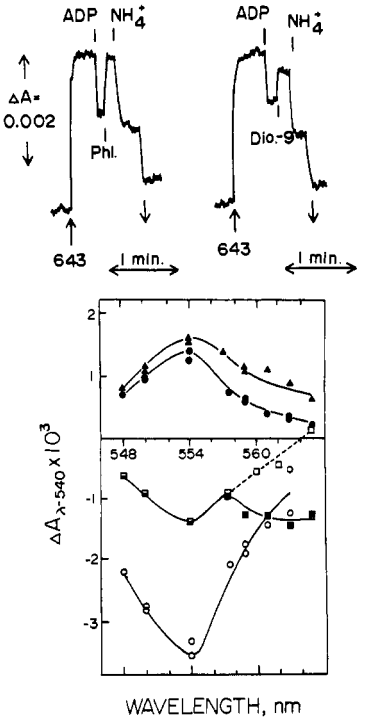

FIGURE 2: (A, top) Effect of ADP, phlorizin or Dio-9, and NH_4Cl on the light-induced absorbance change of cytochrome *f*. Conditions as described in the Methods section; addition during 643-nm illumination as indicated, in mM; ADP, 0.06, phlorizin, 0.5; NH_4Cl, 2; Dio-9, 10 μg/ml; upward arrows, 643 nm light on, downward arrows, light off. Upward deflection indicates oxidative change. (B, bottom) Difference spectra for the absorbance changes induced by ADP, phlorizin, and NH_4Cl. Conditions as in Figure 2A. Initial 643-nm light-induced change (○); subsequent additions of ADP (●) phlorizin, initial change (■); phlorizin, steady-state level (□, dashed line); NH_4Cl (▲).

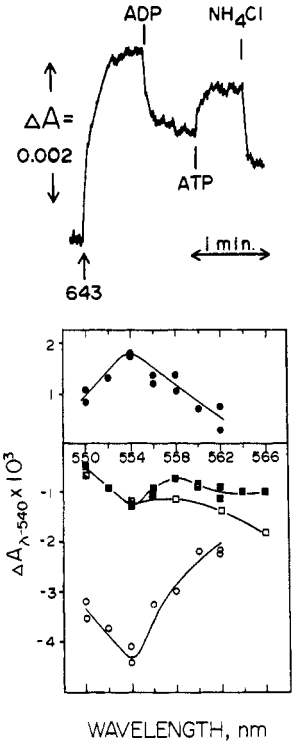

FIGURE 3: (A, top) Effect of ADP, ATP, and NH_4Cl on the light-induced absorbance change of cytochrome *f*. Conditions as described in Methods; additions during 643-nm illumination, in mM: ADP, 0.12; ATP, 1; NH_4Cl, 2. (B, bottom) Difference spectra for the absorbance changes induced by ADP and ATP. Conditions as in part A; 643-nm illumination (○); ADP (●); ATP, 1 mM (■); ATP, 2 mM (□).

of Figure 2B. The spectrum marked by open squares and the dashed lines refers to experiments (recorder trace not shown) where the phlorizin change shown in Figure 2A was only transient at the longer wavelengths and returned to a steady state level of zero oxidative change at 564 nm. The spectra for both the initial and the steady-state phlorizin-induced oxidative absorbance changes show clearly that cytochrome *f* is oxidized upon phlorizin addition. In the case of the steady-state change (dashed line) the spectrum shows a monotonic decrease in amplitude above 554 nm and there is no evidence for a phlorizin-induced oxidation of cytochrome *b*-559. The spectrum for the transient phlorizin change is flat from 559 to 565 nm, indicating that most of the change in this region of the spectrum is probably caused by scattering effects. We tend not to expect any contribution of the *b*-563 to these spectra since it is fully oxidized initially (Cramer and Butler, 1967), but we note that in the crossover experiments of Ben Hayyim and Avron (1970) the peaks of the difference spectra for the cytochrome reduction caused by gramicidin addition and the oxidation caused by Dio-9 addition indicate an appreciable contribution of the *b*-563 as well as the *b*-559. Our spectra for Dio-9, however, are very similar to those for phlorizin addition shown in Figure 2A, though the Dio-9 data are not shown here. Thus, the ADP and NH_4Cl data of Figures 1 and 2 show that phosphorylation is probably not linked to oxidation of Cyt *b*-559 by PS I. The experiments with the energy-transfer inhibitors in Figure 2 do not provide evidence for phosphorylation linked to reduction of *b*-559 by PS II. We think that the reductive change caused by ADP at 560 nm in lettuce chloroplasts is residual cytochrome *f* change, and not due to reduction of cytochrome *b*-559 as concluded by Ben Hayyim and Avron (1970).

ATP should act like an energy-transfer inhibitor in its effect on the electron-transport chain. Concentrations of 1–3 mM ATP have been found to inhibit noncyclic electron transport and phosphorylation by 50% (Shavit and Herscovici, 1970). If these inhibitory effects of ATP are due to a reversal of the normal phosphorylation reaction, then ATP should affect the electron-transport carriers adjacent to the phosphorylation site as do phlorizin and Dio-9 (this experiment was suggested to us by Dr. N. Shavit). Figure 3A shows that the reductive absorbance change at 554 nm caused by ADP addition is reversed by addition of 1 mM ATP. The difference spectra for the oxidative change caused by ATP added at concentrations of 1 and 2 mM are shown in Figure 3B. The oxidative effect of ATP on cytochrome *f* is established by the spectrum. If there is any effect of added ATP on the cytochrome *b*-559, the *b*-559 oxidation is again small compared to similarly directed scattering changes, as indicated by the flatness of the spectrum between 560 and 566 nm for the 1 mM ATP addition. The spectrum for the changes caused by addition of 2 mM ATP is even more indicative of scattering effects. The cytochrome *f* oxidation caused by ATP becomes too small to measure for ATP additions much below 1 mM. The ATP experiments, like those with the energy-transfer inhibitors, indicate that if there is any "crossover" effect associated with an energy-coupling site linked to the photoreduction of cytochrome *b*-559, it is smaller than the cytochrome *f* "crossover" effect and too small to be seen in the presence of the scattering changes.

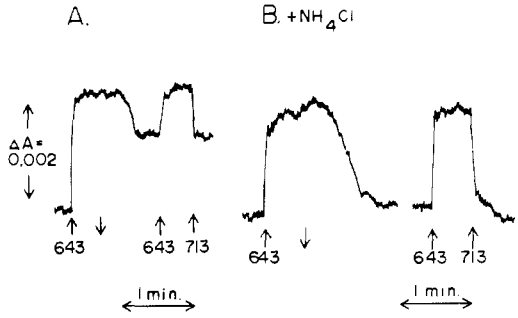

FIGURE 4: Light induced absorbance changes of plastoquinone in the absence and presence of the uncoupler NH_4Cl. Plastoquinone absorbance changes were measured at 254 nm with 276 nm as reference wavelength; conditions described in the Methods section; 1 mM NH_4Cl added in trace B prior to illumination. Upward arrows, either 643 or 713 nm light on; downward arrows, light off; when 713 nm followed the 643-nm illumination, there was no dark period in between. Upward deflection indicates reductive change.

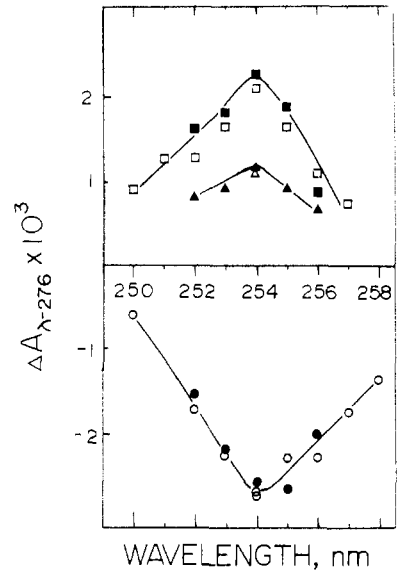

FIGURE 6: Difference spectra for the NH_4Cl-induced absorbance change with NH_4Cl added either during 713-nm illumination (Figure 5A) or before 643-nm illumination (Figure 4B). Compare to trace of Figure 5A: initial reduction by 643-nm light (●); subsequent oxidation by 713-nm light (▲), and by 1 mM NH_4Cl added during 713-nm illumination (■). Compare to trace of Figure 4B: initial reduction by 643-nm light (○); subsequent oxidation by 713 nm light (□); control value for oxidation by 713 nm light in the absence of NH_4Cl (△).

The experiments were extended to measurements of plastoquinone in the ultraviolet region of the spectrum. Plastoquinone is required for noncyclic electron transport and phosphorylation (Bishop, 1959; Crane *et al.*, 1960; Krogmann, 1961; Trebst *et al.*, 1963). In chloroplasts (Friend and Redfearn, 1963) and the blue-green alga *Anacystis nidulans* (Amesz, 1964) the plastoquinone pool appears to be on the system I side of the DCMU block. More recently, it has been suggested that PQ semiquinone may be the primary acceptor of PS II (Stiehl and Witt, 1969). Experiments with the quinone analog DBMIB introduced into photosynthetic research by Trebst and coworkers (Trebst *et al.*, 1970; Böhme *et al.*, 1971) indicate that it inhibits the photooxidation of Cyt *b*-559 by photosystem I but not its reduction by PS II, and that the inhibition can be relieved by added plastoquinone (Böhme and Cramer, 1971; Cramer *et al.*, 1972). The DBMIB experiments suggested that part of the plastoquinone pool might be the electron donor for cytochrome *f*. Since the redox potential of isolated plastoquinone A is around +100 mV (Carrier, 1966), and that of cytochrome *f* around +350 mV (Davenport and Hill, 1952), electron transport between plastoquinone and cytochrome *f* would seem energetically favorable for coupled phosphorylation. A phosphorylation site between plastoquinone and cytochrome *f* is also suggested by kinetic experiments which indicate that the rate limiting step in noncyclic electron transport is the oxidation of plastoquinone (Rumberg *et al.*, 1963; Witt, 1967). It has also been shown that ammonia increases the oxidation level of plastoquinone extracted from chloroplasts (Friend and Redfearn, 1963).

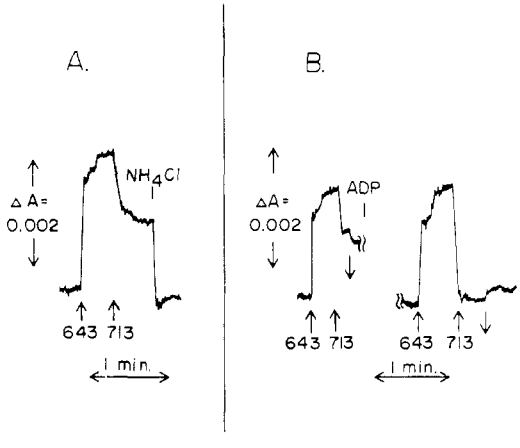

FIGURE 5: (A) Effect of the uncoupler NH_4Cl on the light-induced absorbance change of plastoquinone. Conditions as in the Methods section; 0.5 mM NH_4Cl was added during 713-nm illumination. Upward deflection in traces A and B indicates reductive change. (B) Effect of ADP on the light-induced absorbance change of plastoquinone. Conditions as in the Methods section; chlorophyll concentration, 18 μg/ml; 6 μM ADP was added to the same reaction mixture after the light was turned off.

Figure 4 shows that in the absence of an electron-acceptor red actinic light causes a decrease in absorbance at 254 nm relative to a 276-nm reference beam, which is partly reversed in the subsequent dark period and completely reversed in the dark in the presence of NH_4Cl. The increase in absorbance caused by far-red illumination shows the same dependence on NH_4Cl. If these changes are caused by plastoquinone, the decrease and increase in absorbance at 254 nm relative to 276 nm correspond, respectively, to reduction and oxidation. Figure 5A shows the effect on the far-red light-induced change when NH_4Cl is added during the illumination. ADP cannot be added to the cuvet in this way because of its ultraviolet absorption, but Figure 5B shows the increase in the absorbance change caused by far-red light when ADP is present in the cuvet during the course of illumination. Difference spectra for the far-red light-induced oxidation in the presence and absence of NH_4Cl are shown in Figure 6. The peak is at 254 nm. The spectrum also shows the increase in amplitude in the presence of NH_4Cl. Figure 6 shows also that the amplitude of the reduction by red actinic light is the same in the presence or absence of NH_4Cl and also has a maximum at 254 nm. The spectra for the red light reduction and far-red light-induced oxidation in the presence and absence of ADP again peak at 254 nm (Figure 7), and the results for the absorption changes measured in the presence of ADP are very similar to those for addition of NH_4Cl.

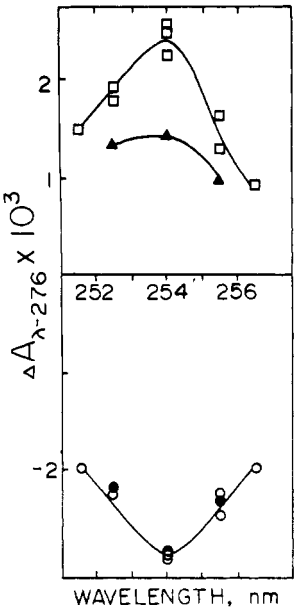

FIGURE 7: Difference spectra for the light-induced absorbance changes before and after addition of ADP. Conditions as in Figure 5B. Absorbance changes in the absence of ADP induced by light of wavelength 643 (●) and 713 nm (▲), and in the presence of 6 μM ADP by light of wavelength 643 nm (○) and 713 nm (□).

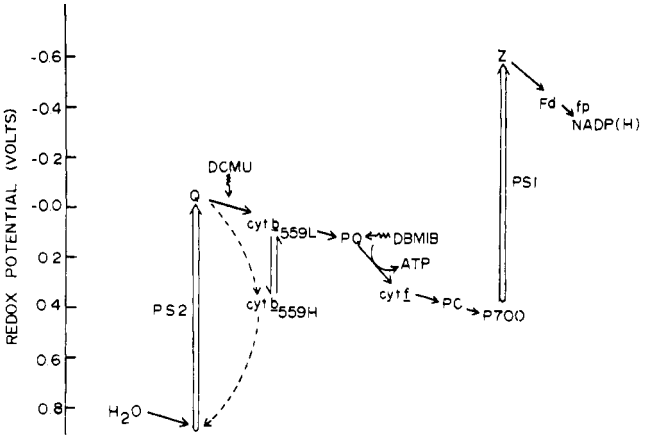

FIGURE 8: Current view of noncyclic photosynthetic electron transport coupled to ATP formation.

Because the difference spectra show a maximum at 254 nm which is close to the oxidized-reduced peak of extracted plastoquinone at 255 nm (Crane and Dilley, 1963; Crane, 1968), we tentatively conclude that these absorbance changes can be attributed to plastoquinone. In terms of relative concentrations and molar extinction coefficients (Henninger and Crane, 1963; Crane, 1968), the specific extinction coefficient on a chlorophyll basis of plastoquinone A in chloroplasts is 25–30 times that of plastoquinone B or C. However, the amplitude of the plastoquinone absorbance changes seen in Figures 4–7 is small enough so that it could be caused by one of these minor components. The far-red, red actinic light-induced difference spectrum for plastoquinone A in *A. nidulans* has a peak between 255 and 260 nm, although the isosbestic point varies between 273 and 280 nm depending on whether the sample was illuminated with red light continuously, or alternately with red and far-red light (Amesz, 1964). The peaks of our spectra shift somewhat toward longer wavelength if the reference wavelength is moved to 280 nm. It appears to be difficult to find a completely stable reference wavelength in this region of the spectrum where there is so much absorption by the aromatic amino acids. Chloroplast quinone absorbance changes induced by high-intensity flash illumination superimposed on a steady far-red illumination of intensity ~1–8 × 10^3 ergs/(cm² sec) in single-beam measurements give spectra with the main peak at 263 nm and a pronounced shoulder at 254 nm (Witt, 1967) or with a single peak at 260 nm (Keck *et al.*, 1970). The difference between the peak values reported here and those of the single-beam measurements may be due either to our use of a reference wavelength, or to the plastoquinone spectrum being affected by the different illumination regimes. We also note in this respect that the reason the difference spectra of Figures 6 and 7 were not extended over a longer wavelength range is because of slow absorbance changes of large amplitude which occurred at the longer wavelengths during red illumination and at the shorter wavelengths during far-red illumination. Thus, there are experimental problems which make the value of the wavelength maximum of the plastoquinone changes *in situ* somewhat uncertain. The main point in Figures 4–7 is that the system I photooxidation of plastoquinone is stimulated by ADP and NH_4Cl.

Figures 4–7 also provide data to estimate the size of the plastoquinone pool which turns over during illumination. Assuming an oxidized-reduced extinction coefficient of 1.5 × 10^4 for plastoquinone (Crane and Dilley, 1963), the amplitude of the red light-induced plastoquinone reduction in Figure 4A is equivalent to the reduction of 1.8 × 10^{-7} M plastoquinone in a sample containing 2.2 × 10^{-5} M chlorophyll, or about 1 plastoquinone/120 chlorophylls. On the basis of a 500-chlorophyll unit, this would correspond to 4 plastoquinone molecules/unit, a value similar to that obtained by Amesz (1964) and Witt (1967). This similarity implies that the chloroplasts used in Figures 4–7 had an appreciable level of system I endogenous acceptor, as no exogenous electron acceptor was added because of the extra ultraviolet absorption and a signal:noise problem in the ultraviolet region. The ability of ADP and NH_4Cl to accelerate the far-red light-induced oxidation of plastoquinone also implies the existence of a pool of endogenous system I acceptor in these experiments.

Figures 4–7 show that there is a detectable rate-limiting step between plastoquinone and system I, but not between system II and plastoquinone, which is relieved by addition of the uncoupler NH_4Cl or by ADP. From these experiments and those on cytochrome *f* (Figures 1–3) we conclude that ATP synthesis is coupled to electron transfer from plastoquinone to cytochrome *f*, as shown in Figure 8. This figure incorporates these data and also our recent studies on cytochrome *b*-559 (Böhme and Cramer, 1971; Cramer *et al.*, 1972; Cramer and Böhme, 1972).

Measurements of the P:O ratios for noncyclic electron transport exceed 1.0 and approach 2.0 when corrected for basal electron transport (Izawa and Good, 1968), implying that two ATP molecules are synthesized for every pair of electrons transferred through the main chain. The simple nature of the crossover data shown above would suggest that either (1) all ATP synthesis in the main chain is coupled to electron transfer between plastoquinone and cytochrome *f* or (2) if there is a second site of ATP synthesis in another region of the main chain, it would be subject to much less photosynthetic control. The midpoint potential span between plastoquinone and cytochrome *f* is about 250 mV, or about 11.5 kcal/mole for the transfer of two electrons with perhaps an additional

3–4 kcal/mole available from poising of the carriers. The measurements of Kraayenhof (1971) indicate that approximately 15 kcal/mole is required for ATP synthesis in class II chloroplasts in the state 3–state 4 transition. These considerations indicate that there is just enough potential drop between plastoquinone and cytochrome *f* for synthesis of one ATP molecule. The potential requirements also make it difficult to place another discrete phosphorylation site in the main chain, and we have omitted any suggestions along this line from Figure 8. Because of the complexity of the cytochrome *b*-559 interactions, it seems very possible that this component is somehow coupled to phosphorylation, perhaps involving transitions between high and low potential states of the cytochrome (Cramer *et al.*, 1972). However, as discussed above we have not been able to find spectrophotometric evidence to support an involvement of the cytochrome *b*-559 in coupled phosphorylation.

Finally, these crossover experiments bear on the validity of the three photosystem model for photosynthetic electron transport which has been proposed as an alternative to the Z scheme (Arnon *et al.*, 1971). A distinguishing feature of the alternative electron transport scheme is that cytochrome *f* is not in the main chain coupled to photosystem II, but is restricted to the cyclic electron-transport pathway of photosystem I. The crossover experiments involving cytochrome *f* presented here, and originally in the work of Avron and Chance (1966), show that photosystem I oxidizes cytochrome *f* and photosystem II reduces oxidized Cyt *f* in a pathway coupled to phosphorylation.

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