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INTERACTION BETWEEN ELECTRON TRANSPORT COMPONENTS IN CHLOROPLASTS

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SUMMARY

The relative concentrations of the electron carriers between the two photosystems and their interactions were studied in isolated chloroplasts.

1. By monitoring the redox states of P700 and cytochrome *f* we determined relative concentrations of 12:1:1 for the Photosystem II electron acceptor pool (Q + A), cytochrome *f* and P700, respectively. In addition we accounted for a third, unidentified component ("Pc"), associated with Photosystem I, possibly plastocyanin, of approximately the same pool size as P700. We thus observed 15 electron equivalents in the electron transport chain between the photoacts, 12 on the System-II and 3 on the System-I side of the rate-limiting step.

2. Apparent equilibrium constants were determined from observations of the redox states of cytochrome *f* and P700 ($K_{\mathrm{Cyt},f\text{-P700}}$) and of "Pc" and P700 ($K_{\text{"Pc"-P700}}$) under various conditions. The behavior of the "Pc"–P700 system was symmetrical during reduction in darkness and photo-oxidation by far-red light suggesting a true equilibrium $K_{\text{"Pc"-P700}} \simeq 20$ ($\Delta E_0' \simeq 80$ mV). The interaction between cytochrome *f* and P700 proved complex, suggesting a high equilibrium constant in dark and low values in the light ($K_{\mathrm{Cyt}.f\text{-P700}} \simeq 5$–10).

3. The cytochrome *f*–P700 data were compatible with non-interacting three-component reaction chains: cytochrome *f* → "Pc" → P700 or cytochrome *f* → P700 ← "Pc". This model, however, did not fit the observed interactions between "Pc" and P700. These results, like earlier observations of the overall system (Q → P)[4] suggest a light-induced change in the properties of the reaction chain.

INTRODUCTION

One of the dark reactions in the electron transport chain which connects the two photochemical light reactions of photosynthesis is rate-limiting in strong light. In bright light the primary traps (Q) and secondary reactants (A) of the O_2-evolving System II accumulate in the reduced form, while the traps of System I (P700) and

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Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea.

components on the System-I side of the limiting step (*e.g.* cytochrome *f*), accumulate in their oxidized state. Upon darkening, the reducing equivalents formed by System II are rapidly transferred to $P700^+$ and its associated photooxidized components[1]. It is possible to determine the size of the pools (reducing equivalents) associated with System II (Q + A) relative to the traps of System I (P700) either by monitoring rates of viologen reduction with the modulated electrode system or by spectroscopically monitoring P700 (ref. 1). A (Q + A)/P700 ratio of about (10–12)/1 was estimated. A similar ratio, in terms of electron equivalents, was also reported by SCHMIDT-MENDE AND RUMBERG[2] for plastoquinone relative to P700. MALKIN[3], however, has reported maximum ratios of about (5–6)/1 and suggested that P700 may interact with only part of the pool associated with System II.

Using the modulated polarograph, JOLIOT *et al.*[4] measured the degree of oxidation of the photochemical traps of System II (Q) and System I (P) during steady-state illumination with weak light of varying wavelength. From these measurements, they computed a very low equilibrium constant ($K = 3$–8); *i.e.* a small loss of free energy, for the overall dark reaction sequence $Q \rightleftharpoons P$. Similar values were observed by DELRIEU[5] with Chlorella. These results appeared inconsistent with the high equilibrium constant suggested by: (1) The observations that in darkness both trapping centers revert completely to their active forms ($Q \rightarrow Q^+$, $P^+ \rightarrow P$), and (2) the midpoint potentials generally assigned to the various components of the chain.

To further analyze this reaction system we have studied the interactions of some of its components: P700, cytochrome *f* and a third component, presumably plastocyanin. We have reexamined pool sizes and apparent equilibrium constants by monitoring the redox states of P700 and cytochrome *f* during both transient and steady-state conditions.

METHODS AND MATERIALS

Spinach chloroplasts were prepared as described by SCHWARTZ[6] and diluted in a medium containing 0.16 M sucrose, 40 mM Tricine, 5 mM phosphate, 2 mM $MgCl_2$ and 0.05 mM methyl viologen at pH 7.6.

Absorption changes were measured with a dual-wavelength spectrophotometer using 554 *vs.* 571 nm to monitor cytochrome *f* and 700 *vs.* 715 nm or 720 nm for P700. Difference spectra verified that we were viewing cytochrome *f* (α bands, 554 nm) and P700. The modulated measuring beams were detected with an S-20 photomultiplier and changes in absorption recorded with a Moseley x–y recorder. Time response of the apparatus was $\leqslant 1$ sec. Actinic light, obtained from a 28-V microscope projection lamp, was passed through 2 cm of water, a heat filter and appropriate color filters. A 725-nm interference filter (half-width, 12 nm) and wire screens were inserted for varied intensities of far-red actinic light. In some experiments, narrow-band interference filters (Thin Film Products, half-width 5 nm) were used to obtain different wavelengths of actinic light. The actinic beam was blocked from the phototube by a rotating disk which enabled alternate actinic illumination and measurement of the sample absorbance (60 cycles/sec). Chloroplasts containing about 150 μg chlorophyll per ml were stirred continuously in a cell having a 3-mm optical path. All experiments were done at 12°.

RESULTS AND INTERPRETATION

Induction effects with P700 and cytochrome f

In the presence of a low-potential acceptor such as methyl viologen, strong white or 650-nm light reduces the large pool of System II reductants (pools Q + A) and oxidizes cytochrome *f* and P700 (ref. 1). Upon cessation of the light, the latter components become reduced in a few msec. As shown in Fig. 1, if a weak far-red beam is subsequently presented, a marked lag precedes the photo-oxidation of P700. The response time of the instrument did not permit observation of the rapid initial reduction of $P700^+$. The lag, observed in algae[7,8] and in isolated chloroplasts[1,3], has been attributed to the pool of System-II reductants formed during the pre-illumination, which maintains P700 in the reduced state until all intermediates in the chain are eventually oxidized by System I. Repeating the experiment while monitoring the redox state of cytochrome *f* reveals a similar lag preceding the oxidation of this intermediate. To estimate relative pool sizes from the above type of measurement, it was assumed that the effectiveness or rate of electron removal by the far-red beam is proportional to $P700/P700_{\text{total}}$ ($P700_{\text{total}} = P700 + P700^+$) and that $P700\Delta t$ is proportional to the number of electron equivalents removed *via* System I in the time Δt. Therefore, the area under the P700 curve in Fig. 1 should be proportional to the total number of electron equivalents removed from the systems by turnover of P700. The areas bounded by the cytochrome *f* and P700 transients in Fig. 1 were found to be inversely proportional to the intensity of the far-red beam presented. This $I \times t$ relationship shows that these areas reflect a fixed amount of reductant accumulated in bright white light and reoxidized by the far-red light.

It was further assumed that during the transients, equilibrium conditions between the reaction partners were maintained since variation of intensity varied the time scale of the transient but not its shape. Obviously, within the range of

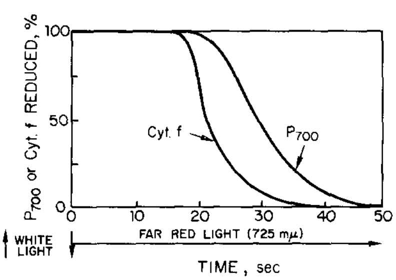


Fig. 1. Time-course of P700 and cytochrome *f* oxidation in far-red light following pre-illumination with 10 sec bright white light.

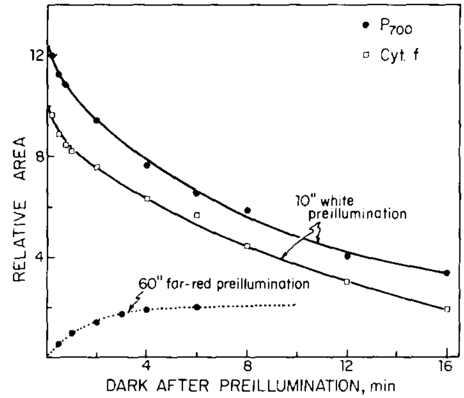


Fig. 2. Dark restoration of System II pools as measured *via* cytochrome *f* and P700. Solid curves, areas bounded by curves in Fig. 1 as a function of the dark period between 10 sec white pre-illumination and subsequent far-red light. Broken curve, areas bounded by P700 oxidation curves as a function of the dark interval between exposures to far-red light (see Curves A, B and C of Fig. 3).

intensities used, the dark reactions between these components are considerably more rapid than the rate of electron removal by System I. The oxidation of cytochrome *f* previous to the oxidation of P700 suggests that cytochrome *f* has a lower midpoint potential than P700 and precedes it in the chain of electron carriers between System II and System I. The initial flat portions of the curves (100 % reduced) in Fig. 1 show that P700 and cytochrome *f* do not become oxidized until the pool (Q + A) is largely depleted and this suggests that the midpoint potential of (A) is considerably higher than that of P700 and cytochrome *f*.

The data of Fig. 1 also imply that P700 and cytochrome *f* interact with the same pool of System II reductants (Q + A). Additional support for this hypothesis was obtained by determining the rate of oxidation of the pool Q + A in darkness, following its reduction by white light. This reoxidation, presumably by O_2, was reflected by a decrease of the relative areas bounded by the P700 and cytochrome *f* transients. In Fig. 2 these areas were plotted as a function of the dark interval separating the white pre-illumination and the subsequent presentation of the 725-nm actinic beam. The similar time-courses of the dark oxidation of (Q + A) measured either *via* cytochrome *f* or *via* P700, suggest that both components react with the same pool of reductant. As shown in Figs. 1 and 2 the P700 transients bound larger areas than the cytochrome *f* transients. This is expected since one views, *via* P700, a slightly larger pool of reductant than *via* cytochrome *f*. As discussed later, this extra pool appears to consist of reduced P700 and one other component.

Relative pool size of Q + A, cytochrome f, "Pc", and P700

Comparison of the observed maximal absorption changes at 554 *minus* 571 nm and 700 *minus* 715 nm (assuming difference molar extinction coefficients of $8 \cdot 10^4$ $cm^{-1} \cdot M^{-1}$ for P700 and $2.5 \cdot 10^4$ $cm^{-1} \cdot M^{-1}$ for cytochrome *f*)[9] indicated a cytochrome *f*/P700 ratio of about 1:1 (however, see ref. 2). The ratio of the concentration of pools Q + A and P700 was estimated as follows: in dark, after complete photo-oxidation of P700 and cytochrome *f* by the 725-nm actinic beam, no detectable reduction of cytochrome *f* occurs in the first 8–10 min. During this time, however, P700 is slowly re-reduced, presumably by some endogenous reductant present in the chloroplasts. This slow re-reduction of P700 after far-red light, which was not affected by 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), could be seen by the reappearance of the P700 transient in subsequent illumination with 725-nm light. The area bounded by these transients, as a function of the dark interval separating the 725-nm illuminations, is shown as a dashed curve in Fig. 2. After some 6 min in the dark, P700 has become totally reduced and no further significant increase in area occurs. This final area equals about 1/6 of the area observed immediately after white pre-illumination. This would agree with the estimate of MALKIN[3] of a (Q + A)/P700 ratio of about 6:1, if only P700 became re-reduced in dark after far-red light. However, the data of Fig. 3 show that in the dark, not only P700 is reduced but another component of approximately equal pool size (which is not cytochrome *f*) is also reduced. The main curve in Fig. 3 shows the time-course of P700 reduction in darkness following a 725-nm pre-illumination. In a second experiment (Curve A) the far-red beam was switched on again after a dark time of approx. 20 sec. At this moment P700 was for about 25 % reduced and far-red light induced a simple, first-order photooxidation transient. The area under the transient may be assumed to be equivalent to approx.

25 % of the area which would have been seen if P700 had been fully reduced. However, if the far-red beam is given after total reduction of P700 (Curve C), a transient is observed which bounds an area 8 times higher than Curve A; *i.e.* twice (actually 2.1 ×) the predicted value. This suggests that besides P700 another intermediate of about equal pool size is involved. This suggestion is further substantiated by the kinetics of the P700 oxidation in Curve C. The photochemical removal of only P700 should yield a first-order transient. Instead, the curve shows an initial delay which precedes a final exponential phase. Obviously, in the dark an additional component is reduced which has a lower midpoint potential then P700 and which initially keeps the pigment reduced. The areas bounded by transients in Fig. 3 were proportionally altered by increasing or decreasing the intensity of the far-red beam ($I \times t$ relation), indicating we were viewing true concentrations.

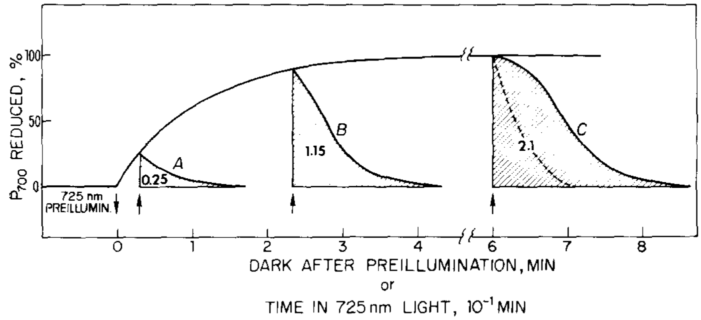


Fig. 3. Time-courses of the reduction of P700 in darkness and its re-oxidation in far-red light. Curves A, B and C represent P700 oxidation transients obtained following different dark periods. Dashed curve, computed time-course of "Pc" oxidation (see text). Reaction mixture contained $1 \cdot 10^{-5}$ M DCMU. Far-red light on ↑ and off ↓.

We arbitrarily demoted the observed intermediate "Pc". Conceivably, it may be plastocyanin, which is known to occur in about equal concentrations as P700 and which has a lower midpoint potential than P700 (refs. 10, 11). Taking this additional secondary oxidant into account, we estimate from Fig. 2 a ratio $(Q + A)/P \simeq 12:1$, and $(Q + A)$: cytochrome *f*: "Pc": P700 = 12:1:1:1.

The above pool size (Q + A) estimates were all made in the presence of methyl viologen. This acceptor rapidly re-oxidizes the reductant (X) of System I and the rate-limiting step in the chain is between the photoacts:

$$H_2O \xrightarrow{h\nu\,II} \underbrace{Q,\ A}_{12\ \text{equiv}} \rightarrow\rightarrow \underbrace{\text{cytochrome } f,\ \text{“Pc”},\ \text{P700}}_{3\ \text{equiv}} \xrightarrow{h\nu\,I} X \dashrightarrow \text{methyl viologen} \quad (I)$$

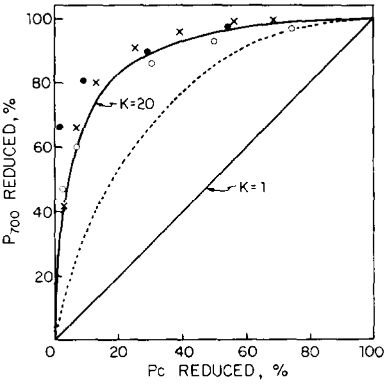
Consequently, in bright light, (Q + A) are reduced and P700, cytochrome *f* and "Pc" are oxidized. We expect that upon darkening, 3 of the 12 electrons in the (QA) pool rapidly reduce P700, "Pc" and cytochrome *f* and that subsequent far-red light oxidizes 12 equiv (see Eqn. I). Without an acceptor the overall rate is limited by the re-oxidation of (X^-) and now bright light reduces all members of the chain. After darkening, and certainly when viologen is added, (X^-) is re-oxidized. We now expect that subsequent far-red light oxidizes 15 equiv. Indeed, when samples were preilluminated in the absence of an acceptor (and methyl viologen was added before the illumination with far-red light) the P700 oxidation curves (Fig. 1) showed about 20 % more area than those pre-illuminated in the presence of methyl viologen. This percentage was independent of the dark spacing (15–60 sec) between white and far-red light indicating that, as expected, an additional pool of about three P700 area units was reduced in the absence of an acceptor.

Interaction between P700 and "Pc"

Assuming we view a two-component system, as discussed above, the dark-reduction curve and the far-red-light transients in Fig. 3 can be analyzed in terms of the relative concentrations of P700/P700$^+$ and "Pc"/"Pc$^+$": At any time t, the signal height represents the (relative) amount of P700$_t$ (P700$_t^+ = 1 - $P700$_t$). The area bounded by a rate transient observed after dark time t, when the fraction of P700$_{\text{total}}$ in the reduced state equals P700$_t$, represents P700$_t$ + "Pc$_t$". Thus, "Pc$_t$" (the fraction of "Pc$_{\text{total}}$" reduced at time t) can be computed.

The results of such computations, plotted as solid circles in Fig. 4, show the relation between the redox states of P700 and "Pc" during the dark reduction of both components. In a similar fashion one can analyze the far-red light-induced transients and thus obtain the time-course of the two components during their photo-oxidation (*e.g.* the dashed curve in transient C, Fig. 3 shows the computed time-course of "Pc"). The respective redox states of "Pc" and P700 during their re-oxidation by far-red light are plotted as open circles in Fig. 4.

In still another approach to view the individual time-courses of P700 and "Pc" during their reduction in dark, experiments similar to those of Fig. 3 were done except that at various moments during the dark reduction a brief ($t_{1/2}$ approx. 5 μsec), saturating flash was given to convert all P700 to P700$^+$. DCMU was added to prevent the flash from adding reducing equivalents *via* System II. We assumed that the



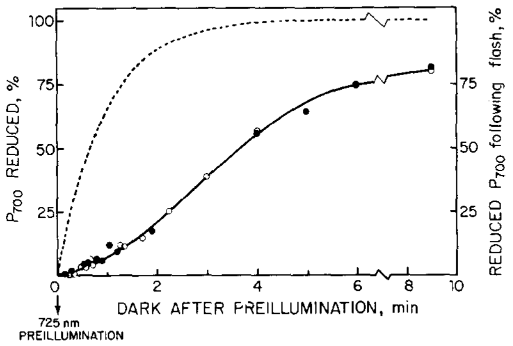


Fig. 4. Corresponding redox states of "Pc" and P700 determined during their dark reduction (solid circles) or (open circles) during their oxidation (see text and Fig. 3). Crosses were obtained from flash experiments (see text and Fig. 5). Solid curves, theoretical plots for equilibrium constants K"$_{\text{Pc}"-\text{P700}}$ of 1 and 20. Dashed curve, theoretical plot for separate chain model (see text).

Fig. 5. Effect of a brief flash given at various dark intervals following far-red pre-illumination. Dashed curve, left ordinate: time-course of the reduction of P700 in darkness after far-red pre-illumination. Experimental points obtained from two different chloroplast preparations indicate the percentage of reduced P700 observed shortly after a single flash. Reaction mixture contained $1 \cdot 10^{-5}$ M DCMU. Blue flash (Corning 4-97 filter) obtained from an argon flash lamp (G.E. FT 230), 24 J/flash.

electron transfer "Pc" → P700 was slow compared to the duration of the flash but fast compared to the endogenous dark-reduction rate. After the flash, any reduced "Pc" which had accumulated in the preceding dark period rapidly equilibrated with $P700^+$, so that shortly after the flash, the signal reflects the amount of $P700^+$ re-reduced by "Pc". In Fig. 5 the dashed line shows the percentage of reduced P700 seen before each flash, the full line (open and solid circles) shows the corresponding percentages seen after each flash, both plotted as a function of the dark interval between far-red pre-illumination and flash. In first approximation (see below), the full line in Fig. 5 reflects the time-course of "Pc" reduction in dark. The momentary redox states of "Pc" and P700 from these experiments are plotted in Fig. 4 (crosses). They show a similar relationship to those computed from Fig. 3, where "Pc" was computed from areas. It appears that both during the reduction of "Pc" and P700 in dark and their oxidation by far-red light, equilibrium between the components is maintained. The reasonable fit with the computed solid line suggests an equilibrium constant $K_{\text{"Pc"-P700}} \simeq 20$ ($\Delta E_0' \simeq 80$ mV).

It is apparent in Fig. 5 that P700 was not totally re-reduced after a single flash following long dark periods (6–8 min). This is consistent with the previous estimate $[\text{"Pc"}]_{\text{total}}/[\text{P700}]_{\text{total}} = 1$, and an equilibrium constant $K \simeq 20$. A flash, given when both "Pc" and P700 are totally reduced (6–8 min dark), would result in approx. 82 % re-reduction of P700 ("Pc" approx. 18 % reduced) after equilibrium with a $K = 20$. About 75–80 % reduced P700 was observed after a single flash following a 6–8-min dark period (Fig. 5).

Interaction between P700 and cytochrome f

We initially suspected that the component which became slowly reduced in darkness after far-red light (Fig. 3), along with P700, was cytochrome *f*. Direct spectral observation of cytochrome *f*, however, showed that it remained essentially oxidized during the first ~8 min of darkness. Only after much longer periods could we see some cytochrome *f* reduction and a corresponding increase of the transient area for the photooxidation of P700. For instance, in Fig. 3, after a 15-min dark period the area rose to approx. 2.5 $[\text{P700}]_{\text{total}}$ and approx. 40 % of the cytochrome *f* was reduced. These observations suggest that cytochrome *f* has a lower midpoint potential than "Pc". Consequently, the electron transport sequence "Pc" → cytochrome *f* → P700 seems unlikely. The data do not allow a decision between the series sequence cytochrome *f* → "Pc" → P700 suggested by GORMAN AND LEVINE[12] or a parallel connection of the two donors cytochrome *f* → P700 → "Pc" (ref. 13).

The time-courses of P700 and cytochrome *f* reduction could be viewed directly when we added small amounts of ascorbate to speed up the sluggish endogenous reduction. In Fig. 6 (triangles) we plotted the momentary redox states of the two pigments observed during such time-courses. Assuming that equilibrium was maintained, these data suggest a value for $K_{\text{Cyt.}f\text{-P700}}$ of 50–100; *i.e.* a difference of 100–120 mV between the midpoint potentials.

The above evaluation of $K_{\text{Cyt.}f\text{-P700}}$ in darkness disagreed with observations made during weak illumination, which indicated a much smaller difference in midpoint potentials. We exposed chloroplast samples containing viologen to low intensities of monochromatic light of various wavelengths (630, 680, 690 nm) and recorded the steady-state redox levels of P700 and cytochrome *f* attained after 1–2 min. The steady-state level of both components varied with wavelength, depending upon the ratio of System II/System I activity; at any particular wavelength of light it was constant over at least a 4–5-fold intensity range. Some variation was observed among different chloroplast preparations. For instance, at 680 nm 64–79 % of the cytochrome was oxidized, while correspondingly, 17–34 % of P700 was oxidized. Apparently the ratio of System II/System I activity with 680-nm light can vary slightly. The results of these steady-state experiments are plotted in solid squares in Fig. 6. To facilitate an evaluation we calculated theoretical curves (solid lines) for various K values. The steady-state light data suggest low K values, between 3 and 10.

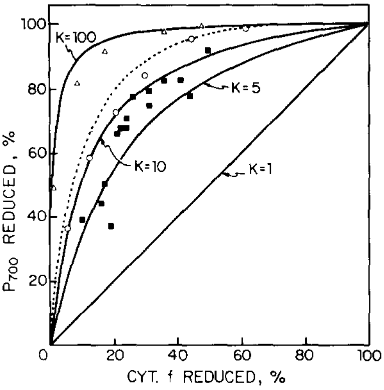


Fig. 6. Corresponding redox states of cytochrome f and P700 in transient and steady-state conditions. Solid squares, steady-state redox levels attained in different chloroplast preparations at low intensities of 630-, 680-, and 690-nm light. Open circles, plotted from curves of Fig. 1. Open triangles, reduction of P700 and cytochrome f in dark following pre-illumination with far-red light in the presence of $5 \cdot 10^{-5}$ M ascorbate. Solid curves, theoretical plots for equilibrium constants $K_{\text{Cyt.-P700}}$ of 1, 5, 10 and 100. Dashed curve, theoretical plot for separate chain model (see text).

A third set of data in Fig. 6 (open circles) shows the momentary concentrations of P700 and cytochrome f computed from curves similar to those in Fig. 1, the oxidation of the two components in far-red light. In this case the data seem to show a value of $K \sim 10$, but like steady-state data, they show no true fit with any of the predicted curves.

In summary, the above observations fail to reveal a true equilibrium between cytochrome f and P700. Instead we find apparent $K_{\text{Cyt.}f\text{-P700}}$ values which vary with the conditions of measurement.

Considered by themselves, the cytochrome f–P700 results can be explained by assuming that the reactants are structure-bound, each molecule with one reaction partner and unable to communicate with others (*i.e.* there is no true equilibrium). MALKIN[14] recognized that at steady-state in weak light, a system comprising independent chains of components with largely different midpoint potentials could reveal low apparent K values. The low values would likewise be unaffected by phosphorylation uncouplers.

We have evaluated a three-component independent chain sequence $\xrightarrow{\text{System II}}$ cytochrome $f \rightarrow$ "Pc" $\rightarrow$ P700 $\xrightarrow{\text{System I}}$ assuming with MALKIN[14] large differences in midpoint potentials and rapid transfer rates. In agreement with the observations, the model predicts an apparent K value of $\simeq 6$ at steady-state in weak 680-nm light where the rates of the two photosystems are about equal. Including an (A) pool in this model would lower the predicted apparent K value slightly. The model also predicts the redox states of cytochrome *f* and P700 observed during the slow transient oxidation of the system in far-red light (Fig. 1): The time-courses for the following redox states were considered:

$$\text{cytochrome } f\text{–“Pc”–P700} \xrightarrow{k_{L1}} \text{cytochrome } f^{+}\text{–“Pc”–P700} \xrightarrow{k_{L2}} \text{cytochrome } f^{+}\text{–“Pc}^{+}\text{”–P700} \xrightarrow{k_{L3}} \text{cytochrome } f^{+}\text{–“Pc}^{+}\text{”–P700}^{+} \quad (1)$$

Since the intermediate conversions such as cytochrome f–"Pc"–P700^{+} $\rightarrow$ cytochrome f^{+}–"Pc"–P700 are assumed to be rapid compared to the rate of electron removal from the system, they are not considered. We assume that $k_{L1} = k_{L2} = k_{L3} = I\Phi$ P700 where I is the light intensity absorbed in System I and B the quantum yield for the primary photooxidation. For a large collection of independent chains we can determine the average population of the four states at any moment of time by assuming values for the rate constants k_{L1}, k_{L2} and k_{L3}. As illustrated in Fig. 6, the model (broken line) adequately predicts the experimental observations (open circles).

Reaction sequence 2 should describe the reverse sequence of events, when the system, oxidized by far-red light, is being reduced, *e.g.* by ascorbate:

$$\text{cytochrome } f^{+}\text{–“Pc}^{+}\text{”–P700}^{+} \xrightarrow{k_{D1}} \text{cytochrome } f^{+}\text{–“Pc}^{+}\text{”–P700} \xrightarrow{k_{D2}} \text{cytochrome } f^{+}\text{–“Pc”–P700} \xrightarrow{k_{D3}} \text{cytochrome } f\text{–“Pc”–P700} \quad (2)$$

k_{D1}, k_{D2} and k_{D3} are the respective rates with which reducing equivalents react with the system in its respective redox states. The observation that "Pc" and P700 become reduced in darkness while cytochrome *f* remains virtually oxidized suggests that the endogenous reductant or added ascorbate react much more rapidly with "Pc" or P700 than with cytochrome *f*, *i.e.* k_{D1} and $k_{D2} \gg k_{D3}$.

DISCUSSION

Our estimate of 12 electron equivalents for the electron acceptor pool on the reducing side of System II measured *via* P700 agrees with those based on plastoquinone absorbance-change measurements for spinach chloroplasts[2, 15]. On the oxidizing side of System I, we viewed a pool of only 3 electron equivalents, while SCHMIDT-MENDE AND WITT[16] report an electron acceptor ("U") pool of 5 equivalents. It is uncertain whether this difference is trivial or oxidants other than the ones viewed by us (P700, "Pc", cytochrome *f*) are reduced by plastoquinone.

One difficulty in evaluating the (QA) pool is the incongruence between measurements *via* System I or plastoquinone on the one hand and O_2 gushes and fluorescence rises on the other hand. In the latter case, one views a pool approximately twice as large (expressed either in terms of pool/traps or total chlorophyll[1]). The reaction of only part of the (A) pool with P700 (ref. 1) and the possibility that P700 may not be the exclusive reaction center of Photosystem I (ref. 3) have been considered. MALKIN[3], however, reported identical kinetics of reduction or oxidation of the pool whether measured *via* fluorescence or P700. He concluded that the same pool is measured in both experiments.

Besides such uncertainties as the relative abundancies of the traps of Systems I and II and the extinction coefficient for P700, a notable difference is that pool estimates based on observations of P700 are made in the presence of an electron acceptor, while those based upon the O_2 gush or the fluorescence rise are made without an acceptor. Conceivably, the latter experiments could reflect not only the pool of electron carriers between the photosystems, but an additional pool on the reducing end of System I. The existence of such a pool has been suggested[17].

Reaction sequence, equilibrium constants

According to our best evaluation, the "Pc"–P700 system behaved symmetrically in dark reduction and photooxidation by far-red light, *i.e.* the two components appeared to be in true equilibrium—where all molecules in the system can react with each other. The computed equilibrium constant $K \simeq 20$ ($\Delta E_0' \sim 80$ mV) also seems in agreement with the published midpoint potentials for plastocyanin and P700. The interplay between cytochromes *f* and P700, however, proved incompatible with equilibrium and suggested a three-component independent chain sequence. This situation is analogous to measurements of the overall system ($Q \rightarrow P$) where in weak light an apparent $K < 10$ was observed while in dark K appeared to be infinite[4].

We encounter the difficulty that true equilibrium of "Pc" and P700 is incompatible with independent chains of cytochrome *f*–P700 since all chains would equilibrate *via* "Pc". In Fig. 4 (dashed curve), we plotted the relationship between "Pc" and P700 as predicted by the independent chain model (Eqn. 1). Even if we allow considerable experimental uncertainty, due to the indirect method for measuring "Pc", the difference between observation and prediction remains obvious. Consequently, we have considered alternative models such as: (1) The molar extinction for P700 is twice the value we assumed (see ref. 2) so that there are two P700 molecules per cytochrome *f* molecule. This would allow separate P700 fractions: one equilibrating with "Pc" and one cooperating with cytochrome *f* as independent pairs. The fact that we see complete oxidation and reduction of P700 and "Pc" under certain conditions where cytochrome *f* remains oxidized argues against this hypothesis. In addition, the kinetics of P700 oxidation and reduction would differ from those observed. (2) P700 is not the exclusive reaction center of Photosystem I such that we have parallel reaction chains, one involving "Pc" and P700, the other cytochrome *f* and some unknown reaction center[3,18]. The data of Figs. 1 and 2 argue against this: cytochrome *f* and P700 appear to interact with the same (A) pool, which would require the unlikely assumption that both System I's have nearly identical efficiencies and absorption. Also, quantum yields of about 1 have been measured for P700 photooxidation[19]. (3) Interacting electron transport chains which undergo some change of state (apparent K value) in the light, possibly related to membrane phenomena. Although presently we cannot conceive a detailed explanation, we favor this third alternative, because of the analogy with the behavior of the entire $Q \rightarrow P$ system. As described earlier[4], this system showed similar low apparent K values in light and high values in the dark. The independent chain model cannot explain the kinetic behavior of the multi-component $Q \rightarrow P$ system. In addition, there appears to be at least partial communication between (A) pools (P. JOLIOT, personal communication; B. RUMBERG, personal communication).

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