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Equilibration between cytochrome *f* and P700 in intact leaves

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Abstract

Electron transport between the two photosynthetic reaction centres of high plants is mediated by plastoquinone, a rieske iron–sulfur centre, cytochrome *f* and plastocyanin. Measurements of redox equilibration amongst these have produced confusing results, with apparent equilibrium constants being estimated that are inconsistent with in vitro measurements of redox midpoint potentials of the components concerned.

We have critically reexamined methods for deconvoluting cytochrome *f* absorbance signals in intact leaves. We have determined the decay of cytochrome f^+ following light to dark transitions from steady state and compared this with the decay of the oxidised photosystem I primary donor, $P700^+$. Measurements across a wide range of different irradiances and CO_2 concentrations were all consistent with cyt *f* and P700 existing in redox equilibrium, with a potential difference of around 117 mV. These results are discussed in relation to our understanding of the organisation of the photosynthetic electron transport. They also have implications for measurements of PSI electron flux—provided more than about 20% of $P700^+$ is oxidised in the light, then the initial decay in the concentration of $P700^+$ following a light to dark transition provides a good estimate of electron flux through PSI. Where P700 is largely reduced in the light, net reduction of cyt f^+ might need to be corrected for.

Keywords: Equilibration; Cytochrome *f*; P700

1. Introduction

It has long been recognised that there are two pathways of photosynthetic electron transport (ET) possible in higher plants—linear ET through both photosystem I and II, and a cyclic pathway incorporating only PSI. Both pathways involve the transfer of electrons through the rieske Fe–S and cyt *f* in the cytochrome b_6f complex and plastocyanin (PC) to the PSI primary donor, P700 (the high potential chain, HPC). In linear ET, electrons are passed from PSI to NADPH for use in photosynthetic metabolism. Cyclic ET involves electrons being passed back into the intersystem electron transport chain, via a poorly defined route, possibly involving a ferredoxin quinone oxidoreductase (FQR) and/or NAD(P)H dehydrogenase (reviewed in Refs. [1,2]). Both pathways generate a trans-thylakoid pH gradient (ΔpH). Only linear ET results in the net production of reducant.

Although the electron carriers involved in ET from PSII to PSI are well characterised, many aspects of the organisation and regulation of this pathway remain obscure. In particular, little is known about how the relative fluxes through the linear and cyclic pathways are controlled. Various arguments lead to the suggestion that the fluxes through the two pathways should be segregated in some way (reviewed in Ref. [2]). Joliot and Joliot [3] suggested that super-complexes of PSI and cyt b_6f might be involved in such segregation, however, there is no biochemical evidence to support this. Alternatively, segregation might be between the granal stacks (linear) and the stromal lamellae (cyclic) of the thylakoid membrane [4].

Recent evidence has identified a range of conditions under which cyclic ET can be dominant. Joliot and Joliot [3]

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have presented evidence for cyclic ET occurring during the first few seconds of illumination in dark-adapted leaves. Golding and Johnson [5] showed that the proportion of cyclic ET rises in leaves exposed to low CO_2. Under both of these conditions, linear ET is largely suppressed—due to the inactivation of the Calvin cycle either in the dark or through lack of its main substrate. If the two pathways were not at least partly segregated, then, under such conditions, electron donation from the cyclic pathway would have to compete with efficient donation by PSII into a largely reduced plastoquinone pool. Various lines of evidence suggest, however, that PQ diffusion in the membrane is limited and that only PQ located close to a PSII centre would take part in linear ET [6–8]. Thus, effective segregation of the linear and cyclic pathways could be easily achieved at the level of separate PQ pools.

If diffusion of plastoquinone in the membrane is highly restricted, then any long-range diffusion of electrons from PSII located in the grana to PSI in the stromal lamellae must be mediated mainly by PC. Such diffusion would allow interchange of electrons between cyclic and linear ET. Electron transfer from cyt *f* to PC and from PC to P700 is rapid, considerably faster than the rate of transfer from PQ to cyt *f*. Flow of electrons into this system is regulated in vivo at the level of PQH_2 oxidation [9–11]. This leads to the expectation that the components of the HPC will exist in redox equilibrium, however, a number of measurements have cast doubt on whether this is the case.

If the components of the HPC exist in equilibrium, the relative concentrations of oxidised and reduced forms of each of these components should correspond with values predicted from their redox midpoint potentials. The redox state of two electron carriers, P700 and cyt *f*, are readily measured spectroscopically and various workers have attempted to measure the apparent equilibrium between these. Based on published midpoint redox potentials, a K_{eq} between these components of around 90 is expected; however, estimates of this have varied considerably with values of the order of 1–25 being common. Measurements indicating a low apparent K_{eq} are typically taken under conditions where electron transport is not at a steady state, i.e., immediately following a dark–light transition [12–15]. Such observations have lead to the suggestion that K_{eq} for the HPC might be variable; however, they are probably more readily explained by assuming that distinct pools of electron carriers exist, having varying stoichiometries. The existence of such pools would have profound implications for our understanding of how electron transport is regulated.

Recently, Sacksteder and Kramer [16] published a theoretical and experimental assessment of the flow through the HPC. They noted that, following a light to dark transition, P700 reduction proceeds rapidly, whereas there is a distinct lag in the reduction of cyt *f*, consistent with an apparent equilibrium constant greater than 1. Sacksteder and Kramer [16] did not estimate the apparent K_{eq} but a replotting of their data suggests this to be substantially lower than the value expected if P700 and cyt *f* are in equilibrium (not shown). By contrast, a study by Oja et al. [17], analysing the decay of 830-nm absorbance, a measure of $P700^+$, indicated a much higher value, consistent with the in vitro midpoint potentials.

In the present study, we have reevaluated the equilibration between P700 and cyt *f*. In particular, we have critically examined methods for the deconvolution of absorbance signals associated with cyt *f*. We conclude that, under conditions of steady state photosynthesis, across a wide range of irradiances and CO_2 concentrations, cyt *f* and P700 exist at thermodynamic equilibrium. We discuss these results in relation to our understanding of the regulation of electron transport and also consider their implication for estimates of electron flux through PSI.

2. Materials and methods

2.1. Plants

Barley seeds (*Hordeum vulgare* L. cv Chariot) were supplied by PBI Cambridge (UK). Seeds were planted in 3-in. diameter pots containing multipurpose compost. Plants were grown in a growth room with a photon flux density (PFD) of 100 μmol m^{-2} s^{-1} provided by high frequency fluorescent strip lights, on a 12-h light/12-h dark cycle at 25 °C. Measurements were made on the first leaf of intact plants aged between 13 and 16 days old.

2.2. Cytochrome *f* and P700 measurements

Cytochrome *f* measurements were carried out on a spectrophotometer similar to that described previously [18,19]. Light sources were as stated in Joliot and Joliot [3]. P700 was measured as the difference between the signal obtained at 820 and 870 nm, using a modified version of the Joliot spectrophotometer to be described elsewhere. Measurement at these two wavelengths largely eliminates contribution to the signal from PC [20].

In order to model the equilibrium between cyt *f* and P700 for a given equilibrium constant between these two components, the relative concentrations of oxidised cyt *f* (f) and P700 (p) were calculated as:

$$p = \left[1 + K\left(\frac{1}{f} - 1\right)\right]^{-1}$$

The CO_2 concentration supplied to the leaf was controlled using a CIRAS1 infrared gas analyser (PP Systems, Hertfordshire, UK).

3. Results and discussion

When a leaf, illuminated to steady state, was transferred to darkness there was an apparent absorbance decline in the

region 540–580 nm (Fig. 1). This is due to the relaxation of an electrochromic shift, peaking around 515 nm, induced in the presence of an electrochemical potential gradient ($\Delta\Psi$) across the thylakoid membrane (see discussion in Ref. [21]). Superimposed on this signal decrease was a positive absorbance change around 554 nm (Fig. 1). This positive signal can largely be attributed to the reduction of cytochrome *f*. However, a number of absorbance signals have been recorded in this spectral region and, as such, it is necessary to deconvolute the cytochrome *f* signal to remove other possible contributory factors. These include cyt *b* and C550, which can be largely eliminated by selection of appropriate wavelengths and from P700 and PC, which have rather flat absorption spectra in the region of 554 nm and whose contributions can be eliminated using a three-wavelength deconvolution [15,19]. Most published deconvolutions have been developed in the presence of uncoupler of the trans-membrane electrochemical potential and have been shown to correct well for contaminating signals under such conditions [15]. To date, only a few studies have considered cyt *f* kinetics in plants in vivo [3,16]. The main problem with applying this method to intact plants is that the contribution of the 515-nm electrochromic shift to the corrected cyt *f* signal at 554 nm has not been determined. In order to establish the extent of any such contribution, we have attempted to measure a spectrum of this signal uncontaminated by the signal from cyt *f*. Applying a single turnover flash to a dark adapted leaf results in rapid generation of an electrical field and the oxidation of cytochrome *f* (Fig. 2). Whilst the field relaxes rapidly (within a few seconds) the oxidised cyt *f* produced is observed to be very stable ($t_{1/2}$ for reduction of several tens of seconds). By measuring the difference between the spectrum measured at 1 and 1000 ms after the flash, we obtain a spectrum that is largely uncontaminated by a signal from cyt *f*, as indicated by the absence of a peak at 554 nm in the difference spectrum (Fig. 2, black circles). Deconvolutions based on different time windows gave similar results. From this difference spectrum, it is possible to calculate the contribution of the field. The difference between the chord between 545 and 573 nm and the signal at 554 nm can be normalised to the maximum signal generated by the field, which is calculated as the signal change at 515 minus 480 nm. Using this method, we find the contribution of the field at 554 nm to account for ~5% of the total field signal.

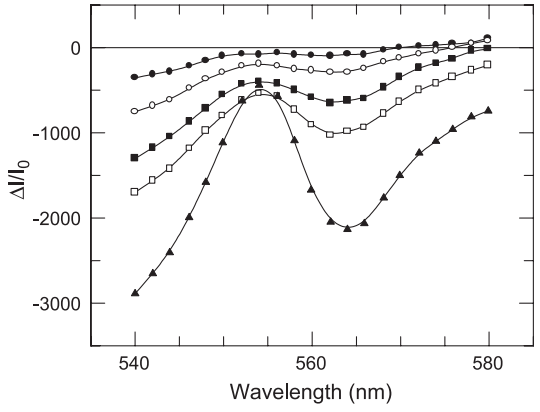

Fig. 1. Absorbance changes following a light to dark transition in leaves of barley illuminated to steady state in air. Black circles, 1 ms; open circles, 2.5 ms; black squares, 5 ms; open squares, 7.5 ms; black triangles, 10 ms.

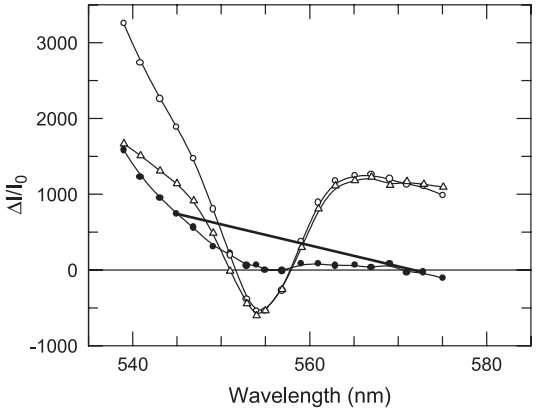

Fig. 2. Spectrum of electrochromic shift in the cytochrome region of the spectrum (540–580 nm). Leaves of barley were given saturaing single turnover flashes at 30-s intervals. Spectra shown were recorded 1 ms (open circles) and 1004 ms (triangles) after the flash. The difference between these two (closed circles) provides a spectrum of the electrochromic shift, largely uncontaminated by any contribution from cytochrome *f*.

The requirement to correct for the field can be seen in Fig. 3. In Fig. 3A, the black circles show the kinetic of the reduction at 554 nm without correcting for the field. The open circles show the signal generated by the field at 554 nm under the same conditions (i.e., 5% of the signal 515–480 nm). It can be seen that the uncorrected signal has two clearly defined overlapping kinetic components—one complete within 10 ms, the other taking 50–100 ms. Overlying this kinetic with that estimated for field at the same wavelengths shows that the fast component in the apparent cyt *f* kinetic matches this well. In Fig. 3B the cyt *f* kinetic is corrected to take account of this contribution. This gives rise to a single kinetic component with a pronounced lag. When calculating initial rates of cyt *f* reduction, correcting for the field makes a substantial difference. Examining the initial rates of cyt *f* reduction across a wide range of light intensities (140–1900 μmol m^{-2} s^{-1}) and CO_2 (2000–25 ppm) concentrations showed that no significant net reduction of cyt *f* occurred in the first 2–3 ms of the dark period (Fig. 3B shows an example plot, not all data shown). Discrepancies in published data have been evident when considering the apparent equilibrium constant between cyt *f* and P700. A wide range of values have been reported and the conditions under which the measurements are made seem to have a significant impact [12–15]. We measured both P700 and cyt *f* reduction kinetics over a wider range of conditions (light 25–2500 μmol m^{-2} s^{-1} and 2000–25 ppm CO_2) to determine whether equilibration of P700 and cyt *f* occurred. Fig. 4 shows an example plot of P700 (A) and cyt

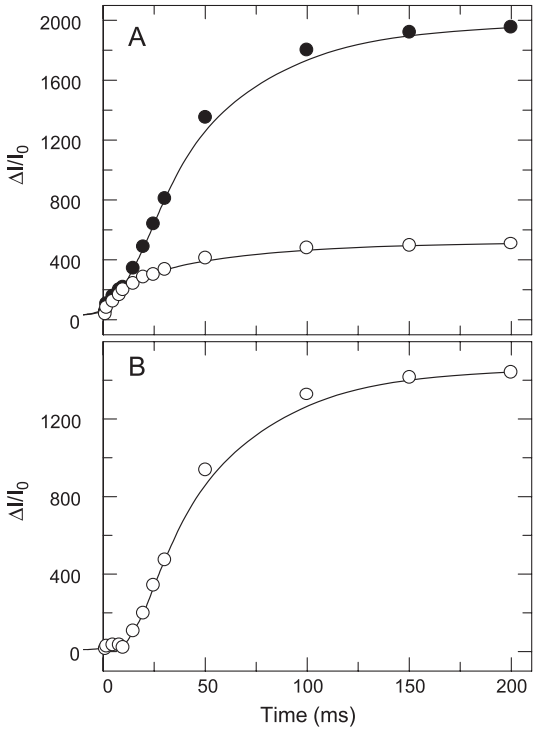

Fig. 3. The reduction of cytochrome *f* during a transition from light to dark. Black circles (A) represents the raw signal from the cytochrome *f* calculation prior to correction for the field. Open circles (A) represent the signal from the field. Open circles (B) represent the cytochrome *f* signal after correction for the field.

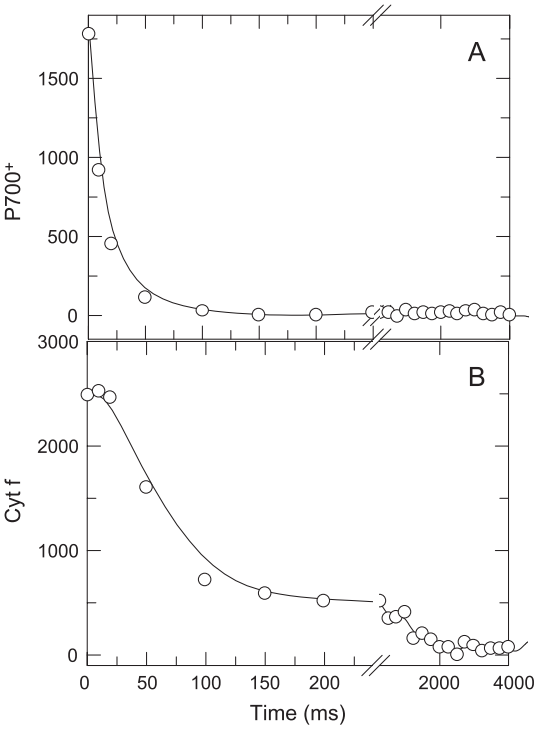

Fig. 4. Example signal showing the decay of $P700^+$ (A) and cyt f^+ (B) following a light to dark transition in a leaf illuminated at 1000 μmol m^{-2} s^{-1} in the presence of atmospheric CO_2.

f reduction (B). Whilst P700 is very rapidly reduced, there is a lag in the initial reduction of cyt *f*. This lag was apparent under all conditions measured (data not shown), even at light intensities as low as 25 μmol m^{-2} s^{-1}. When cyt f^+ is plotted against $P700^+$ (Fig. 5), it is evident that, above a certain level of P700 oxidation, cyt *f* redox state remains constant (i.e., fully oxidised). Based on redox potentials values of +447 mV for P700 [22] and +330 mV for cyt *f* [23], we have calculated the relationship expected between $P700^+$ and cyt f^+ (Fig. 5). This theoretical curve fits closely to our data. Estimates based on other published redox midpoint potentials gave similar results. Thus, our data show that under conditions of steady state illumination, cytochrome *f* and P700 are in redox equilibrium with one another. Thus, our data are consistent with the results of modelling studies by Oja et al. [17] but give an apparent K_{eq} higher than that estimated by performing a similar analysis on data published by Sacksteder and Kramer [16]. The lower apparent K_{eq} in the latter case can be explained by their not correcting contribution of the field.

The above observations have important implications for measurements of PSI electron flux. Under conditions where PSI turnover is limited by electron donation, it should be possible to use the decay kinetics of $P700^+$ to estimate electron flux through the reaction centre. Sacksteder and Kramer [16] pointed out, however, that net reduction of cyt f^+ during the first few milliseconds of darkness, following a period of illumination, needed to be included in estimates of electron flux. Our results show that this contribution will only be measurable under conditions where P700 is less than approximately 20% oxidised. Thus, $P700^+$ decay curves provide a good estimate of the rate of PSI electron

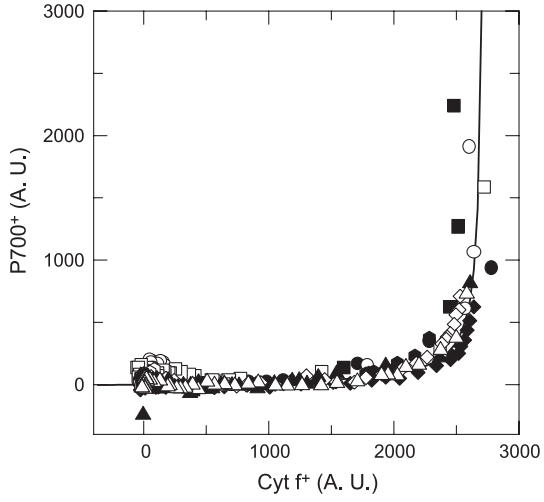

Fig. 5. Relationship between P700 and cytochrome *f*. Filled circles: 1000 μmol m^{-2} s^{-1}−CO_2; open circles: 1000 μmol m^{-2} s^{-1}+CO_2; filled squares: 2000 μmol m^{-2} s^{-1}−CO_2; open squares: 2000 μmol m^{-2} s^{-1}+CO_2; filled triangles: 1000 μmol m^{-2} s^{-1}; open triangles: 200 μmol m^{-2} s^{-1}; filled diamonds: 45 μmol m^{-2} s^{-1}; open diamonds: 25 μmol m^{-2} s^{-1}; filled hexagons: 100 μmol m^{-2} s^{-1} red light; open hexagons: 50 μmol m^{-2} s^{-1} red light. Black line, equilibrium constant of 96. Maximum P700 estimated as 3000 using far-red light as described previously [5].

flux over most physiological conditions. An alternative approach to measuring P700 flux, originally proposed by Harbinson and Woodward [24], uses P700 redox poise as an estimate of PSI quantum efficiency. However, observations of heterogeneity in the antenna size of PSI in different regions of the thylakoid membrane [4] render this approach invalid. Hence, measurements of P700 decay provide the best estimate of PSI turnover currently available.

The measurements described here were performed across a wide range of irradiances and CO_2 concentrations. We also compared the effects of different qualities of light, with varying excitation of PSI and PSII; however, under all conditions, the decay of cyt f^+ and $P700^+$ were consistent with a high K_{eq}. Across these different conditions, we expect to see variation in the extent of cyclic ET. Previously, Golding and Johnson [5] presented evidence for a pool of PSI centres that are specifically activated to support cyclic ET, however, the nature of this activation step is unclear—it may involve inactive centres being provided with an electron donor (donor-side activation) or an electron acceptor (acceptor-side activation). Whichever of these two models is correct, results presented here indicate that activation of these centres produces a pool of PSI centres that are kinetically indistinguishable from centres involved in linear ET. This suggests equilibration of the total PC pool, supporting the idea of acceptor side activation; however, it cannot be ruled out that regulation occurs directly in the PSI centres, e.g., with docking of PC being inhibited in 'inactive' centres.

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