ORIGIN OF THE SLOW COMPONENT OF THE ELECTROCHROMIC SHIFT:

A charge delocalisation model

L. F. OLSEN* and J. BARBER

ARC Photosynthesis Research Group, Botany Department, Imperial College, Prince Consort Road, London SW7 2BB, England

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1. Introduction

The absorbance change at 518 nm induced by illuminating chloroplasts with single turnover flashes has been interpreted as being due to the formation of an electric field across the thylakoid membrane [1,2]. The initial rise (phase *a*) of this electrochromic shift is very fast ($t_{1/2} < 20$ ns) and can be ascribed to the field created by the primary charge separations in the two photosystems [1,2]. Phase *a* is followed by a slower rise with a half-time in the ms region, often referred to as phase *b*. This slow component was first observed in whole algal cells [3] and later in intact and broken chloroplasts performing either linear or cyclic electron flow [4–7]. Several recent reports have concluded that phase *b* is due to a third electrogenic reaction and identified this with a protonmotive Q cycle [4,8–10]. The operation of such a Q cycle predicts an H^+/e^- ratio of 2 for the redox reactions involving plastoquinone [11].

We have determined the H^+/e^- ratio for plastoquinol oxidation under conditions where we also observed phase *b* of the electrochromic shift [12,13]. These results suggested an H^+/e^- ratio of 1 for this site and it was concluded that phase *b* cannot be taken as evidence for the electrogenic transfer of an additional proton. A similar result was obtained in [14]. Here, we have studied the effect of cations on the flash-induced electrochromic shift associated with photosystem I (PSI) only. The response of the fast and slow rise to various concentrations of mono- and divalent salts in the suspending medium suggest that:

(i) The fast component responds to a localized electric field; while

(ii) The slow component appears as a consequence of the delocalisation of charge due to the electron-transport linked release of H^+ and OH^- into the aqueous phases.

Abbreviations: chl, chlorophyll; DBMIB, 2,5 dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethyl urea; PSI (PSII), photosystem I (photosystem II); tricine, *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)-glycine

* Permanent address: Institute of Biochemistry, Odense University, Campusvej 55, 5230 M, Denmark

2. Materials and methods

A stock of intact chloroplasts from peas was prepared as in [15] and resuspended in a medium containing 0.33 M sorbitol and 1 mM $MgCl_2$ with the pH adjusted to 7.6 using Tris base. For experiments the chloroplasts were osmotically shocked in distilled water at 0°C for 30 s followed by addition of ice-cold double strength medium to obtain a final solution containing 0.33 M sorbitol, 1 mM tricine–KOH (pH 8.1), chloroplasts corresponding to 50–60 μM chl and salts as indicated. Further additions were 10 μM DCMU and 1 mM sodium dithionite. The addition of dithionite resulted in a change of pH to 7.0. This drop in pH is caused by formation of HSO_3^- when dithionite is oxidised by the O_2 in the solution. The buffering of the reaction medium is thus achieved by the combined buffering capacities of tricine (p*K* 8.1) and HSO_3^- (p*K* 6.9). Flash-induced absorbance changes at 518 nm were performed in a single beam flash spectrophotometer (Applied Photophysics Ltd., London) using a 10 × 10 mm cuvette. The flash which had a lifetime of 10 μs (width at half height)

was filtered through a 2 mm Schott RG 665 filter and the photomultiplier was protected by a 4 mm Corning 4-96 filter. The signal from the photomultiplier was passed to a Nuclear Measurements Model 546 signal averager. The time constant of the apparatus was normally 0.3 ms. The apparatus is described in [12]. The traces shown represent the average of 4 single sweeps (repetition rate 0.1 Hz) except when recording the spectra of phase *a* and *b* below 500 nm where 8 single sweeps were averaged. The temperature was 20°C ± 1°C. Chlorophyll was measured as in [16].

3. Results and discussion

Inhibition of chloroplast electron transport by DCMU results in an almost complete disappearance of the absorbance change at 518 nm induced by a single turnover flash following a few preilluminating flashes. Part of the signal can be restored by addition of reductants which will reduce the electron carriers on the oxidising side of PSI. It had been shown that by adding either dithionite [17] or a combination of NADPH plus ferredoxin [12] to DCMU-treated chloroplasts ~1/2 of phase *a* (due to PSI primary charge separation) and phase *b* of the electrochromic shift can be observed. Addition of DBMIB which inhibits oxidation of plastoquinol and the accompanying release of protons into the intrathylakoid space [18] results in a loss of phase *b*. Fig.1 shows the flash-induced spectra of the two phases measured with broken chloroplasts inhibited by DCMU and supplemented with dithionite. The spectrum due to phase *b* represents the amplitudes of the changes obtained by subtraction of the signals in the presence and absence of DBMIB. The two spectra are superimposable following multiplication of the spectrum of phase *b* by 1.2. This suggests that phase *b*, like phase *a*, is indeed an electrochromic shift in agreement with [3,4,10,19].

By changing the cation composition of the basic low salt suspending medium we observed changes in the amplitudes of phases *a* and *b*. Fig.2 shows the flash-induced electrochromic shifts associated with PSI when either 10 mM KCl or 3 mM $MgCl_2$ were added to the medium. We consistently observed that in the presence of 10 mM KCl the amplitude of the fast phase was ~25% larger than in the presence of 3 mM $MgCl_2$. As for phase *b* this relationship was reversed so that the amplitude was larger with $MgCl_2$ than with KCl present in the medium. It should be

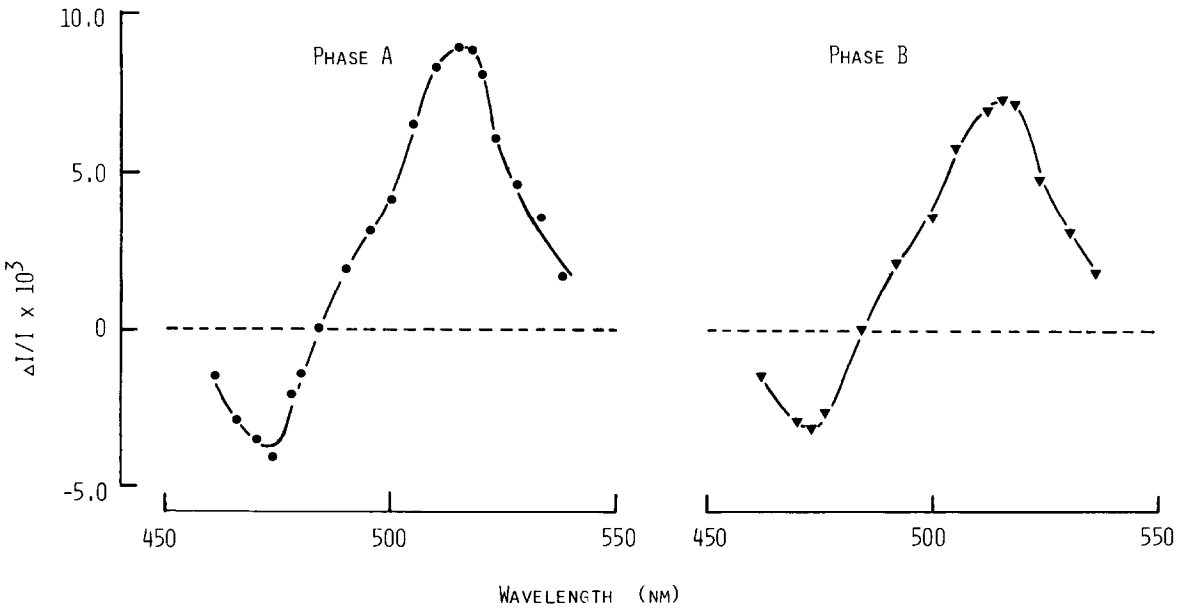

Fig.1. Spectra of the fast (phase *a*) and slow (phase *b*) rise of the electrochromic shift in DCMU-treated chloroplasts supplemented with 1 mM dithionite. Phase *b* was obtained by subtraction of the signal measured in the presence of 10 μM DBMIB from the control signal (minus DBMIB). Experimental conditions were as in section 2 except that 10 mM KCl was added to the basic reaction medium. Chlorophyll was 60 μM.

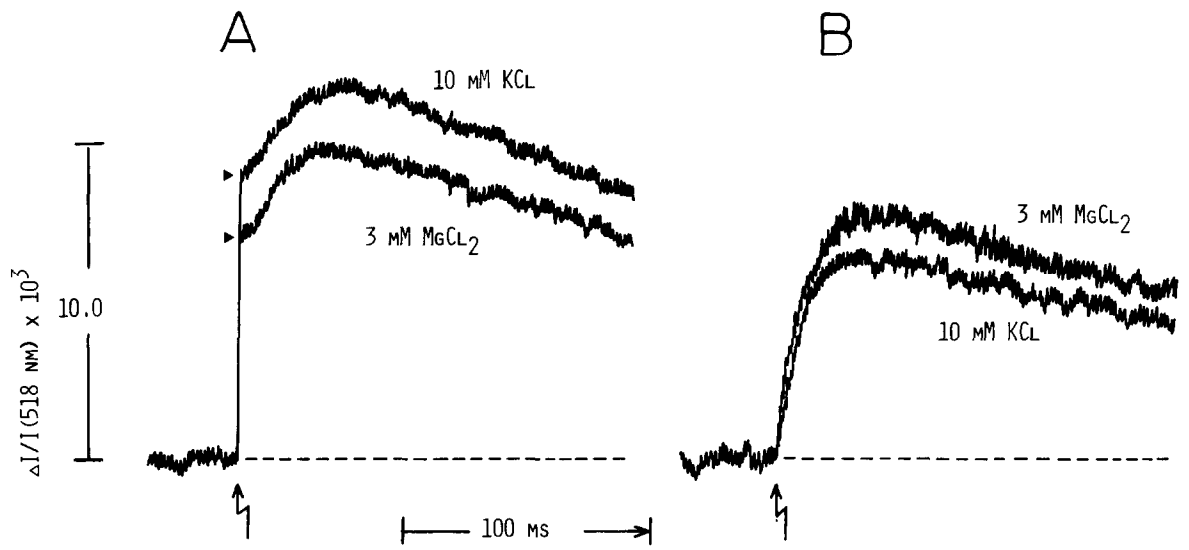

Fig.2. Flash-induced absorbance changes at 518 nm in DCMU-treated chloroplasts supplemented with dithionite when either 3 mM $MgCl_2$ or 10 mM KCl was added to the basic reaction medium. (A) Phase *a* plus phase *b*; (B) phase *b* obtained as the difference of the signals in (A) minus the corresponding signals measured in the presence of 10 μM DBMIB. The arrow heads indicate the amplitudes of phase *a*. Chlorophyll was 60 μM.

noted that this difference in the size of phase *b* is probably not due to an increase in the membrane conductivity when K^+ is present in the medium since we also observed that the decay of phase *a* (+ DBMIB) was slightly faster in the presence of 3 mM Mg^{2+} as compared with that in the presence of 10 mM K^+.

The two salt conditions used in the above experiments correspond to conditions where the thylakoids are stacked (3 mM $MgCl_2$) or unstacked (10 mM KCl) [20]. To investigate the relationship further experiments were conducted with a variety of mono- and divalent cation concentrations and the results are shown in fig.3 for phase *a*. As the concentration of monovalent cations increases from the low background

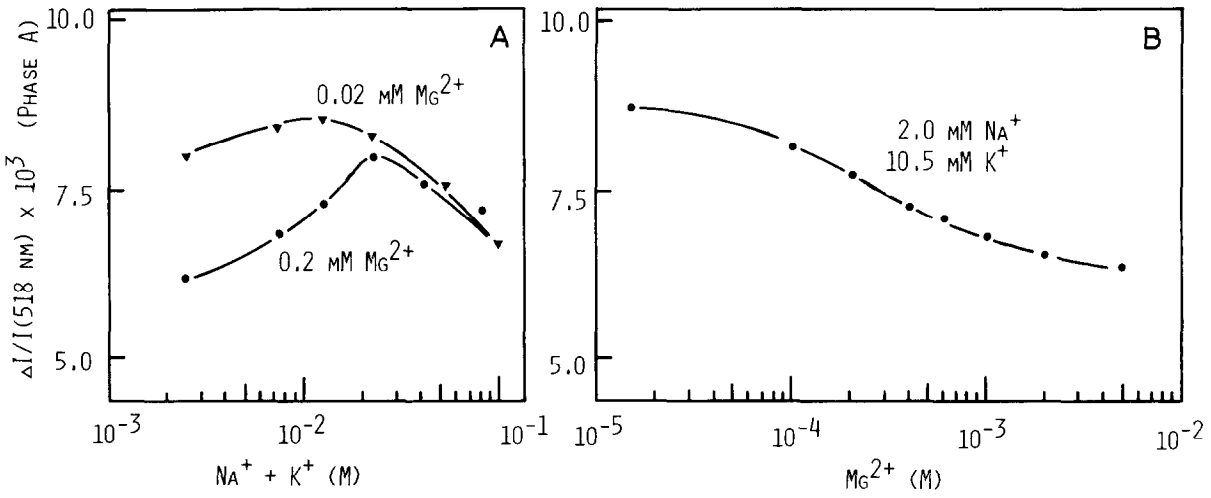

Fig.3. Effect of changes in the concentrations of mono- or divalent cations in the reaction medium on the amplitude of phase *a*. (A) Addition of monovalent cations ($Na^+ + K^+$) on constant backgrounds of either 20 μM or 200 μM Mg^{2+}; (B) Addition of Mg^{2+} on a constant background of 12.5 mM monovalent cations. Chlorophyll was 55 μM.

level of 2.5 mM the amplitude of phase *a* goes through a maximum before approaching a minimum level (fig.3a). The magnitude of phase *a* and the $[K^+]$ at which it has its maximum depends on the $[Mg^{2+}]$ present in medium. When the $[Mg^{2+}]$ is increased (with a constant background level of monovalent cations of ~12.5 mM) the amplitude of phase *a* decreases monotonically towards a minimum level (fig.3b).

The cation-induced changes in phase *a* shown in fig.3 are very similar to those observed for chlorophyll fluorescence in the presence of DCMU and for thylakoid stacking [20–25]. This similarity and the apparent antagonistic behaviour of phase *a* and phase *b* to variations of mono- and divalent cations shown in fig.2 leads us to propose a model to explain the cation effects on the two phases of the electrochromic shift. The model is based on the following assumptions:

(i) The flash-induced electrochromic shift involves pigments which are not associated with the PSI complex.

(ii) The electric field across the thylakoid membrane created by the primary charge separations in PSI declines rapidly with distance along the membrane/aqueous interface, that is to say the dipoles created by charge separation are discrete rather than uniform [26,27].

(iii) Phase *b* is associated with proton uptake/release due to plastoquinone oxidation/reduction reactions.

The model is diagrammatically illustrated in fig.4. The initial charge separation, which here is due to PSI only, creates an electric field, the magnitude of which declines with distance from the reaction centre. The field indicating pigment complex will therefore be exposed to an electric field smaller than that at the reaction centre (fig.4a). The neutralisation of the reaction centre dipole due to electron transport and associated proton translocation (fig.4b) will result in a release of diffusible charges in the form of H^+ and OH^- into the aqueous phases and hence a transformation of the initially non-uniform field into a uniform field (fig.4c). This charge delocalisation process results in an exposure of the field indicating pigment complex to an increased electric field. An increase in the distance between PSI and the field indicating complex (illustrated by the dotted arrow in fig.4) results in a decrease in the initial rapidly formed field sensed by the pigments whereas this would have no effect on the delocalised field. As a consequence of this phase *a* would be smaller whereas phase *b* would increase.

There is already a substantial amount of evidence in the literature for the model presented here:

(1) The flash-induced electrochromic shift can be ascribed to a fraction of carotenoids and chlorophyll *b* forming a complex. This complex is mainly associated with PSII [28,29].

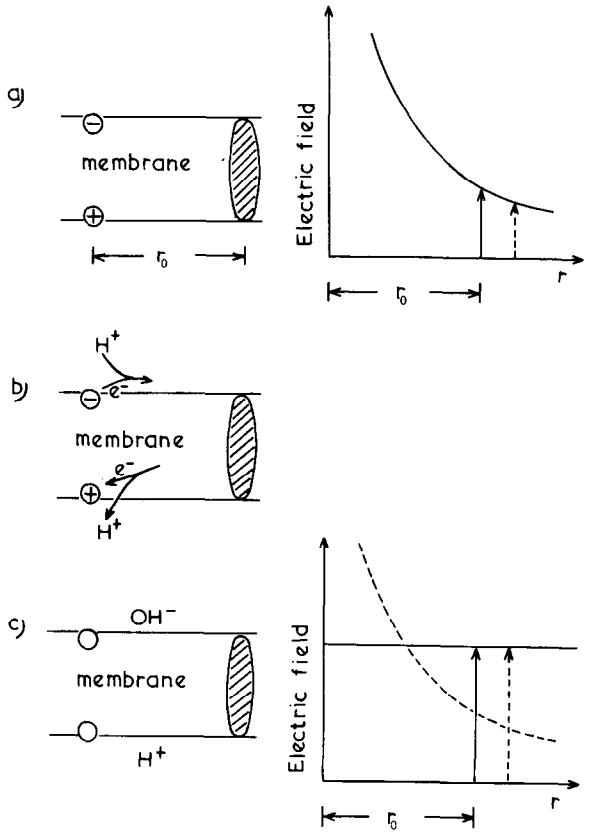

Fig.4. Model to explain the salt effects on the two components of the electrochromic shift. (a) The initial charge separation in PS I creates an electric field across the thylakoid membrane, the size of which declines along the membrane/aqueous interface. The field indicating pigment complex (shaded), which is situated at a distance r_0 from the PS I reaction centre dipole, senses a field (solid arrow) which is smaller than the field at the dipole itself. An increase in r_0, illustrated by the dashed arrow, results in a further decrease in the size of the field sensed by the pigment complex. Neutralisation of the reaction centre dipole due to electron transport is associated with proton uptake/release at the two membrane/aqueous interfaces (b). This release of diffusible charges results in a transformation of the initially non-uniform electric field into a uniform field (c), causing an increase in the field sensed by the pigment complex. This increase will be somewhat reduced because of the overlapping decay of the electric field due to passive diffusion of ions across the membrane.

(2) A large number of independent studies of phase *b* conclude that this is due to translocation of protons across the thylakoid membrane [4,6,9,10, 12–14,30].

(3) Changes in chlorophyll fluorescence induced by changes in the cation composition of the suspending medium can be ascribed to changes in spillover of energy from PSII of PSI which again can be interpreted as significant changes in distance between the two photosystems [20,24,25]. Similarly, salt-induced stacking of thylakoids seems to be associated with an increased segregation of PSI and PSII complexes in the lateral plane of the membrane [24,25,31]. These salt-induced changes in distance between the two photosystems correlate with the changes in the amplitude of phase *a*.

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