OXIDATION–REDUCTION POTENTIAL DEPENDENCE OF THE FLASH-INDUCED 518 nm ABSORBANCE CHANGE IN CHLOROPLASTS

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1. Introduction

Absorbance changes in the 520 nm region, first observed in *Chlorella* cells [1], have been studied extensively in chloroplasts [2,3]. The interpretation of these absorbance changes is that they are the result of an electrochromic response of pigment molecules (chlorophyll *b* in the 480 nm region and carotenoids in the 520 nm region) to the electric field formed across the membrane by the primary charge separation of the two chloroplast light reactions, photosystem I and II (reviewed [4]). Both chloroplast photosystems have been reported to contribute equally to the electric potential difference across the membrane [5,6] and no evidence has been obtained which indicates there are electrogenic reactions in chloroplasts other than the primary photochemical events.

Electrochromic absorption shifts of carotenoids have also been reported in chromatophores from several photosynthetic bacteria [7,8]. By application of potentiometric techniques in conjunction with flash activation, it has been found in *Rhodopseudomonas sphaeroides* that this electrogenic response is associated with the primary charge separation but that secondary electron transfer reactions, such as cytochrome oxidation, contribute as well [9]. We have attempted here to define the electrogenic reactions in chloroplasts in greater detail by measuring the flash-induced ΔA_{518} as a function of oxidation–reduction potential. At least three components (two associated with photosystem II and one with photosystem I) have been identified as contributing to the electrogenic response and the possible identity of these components is discussed.

2. Materials and methods

Chloroplasts were isolated from pea leaves by homogenizing in a solution containing 0.33 M sorbitol, 50 mM Na_2HPO_4, 50 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1% NaCl, 0.2% sodium isoascorbate and 1 mg/ml bovine serum albumin. After filtering through a cheese-cloth–cotton wool pad, intact chloroplasts were isolated by centrifugation at 3000 × *g* for 1 min. The chloroplast pellet was resuspended in a medium containing 0.33 M sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 50 mM Hepes buffer (pH 7.6) at chlorophyll conc. 2–3 mg/ml.

Flash-induced absorbance changes were measured at 25°C in a single-beam unchopped Applied Photophysics (London) apparatus. The measuring beam intensity was 0.13 $W.m^{-2}$ and the flash duration was approx. 10 μs (half-width, half-height). The actinic flash was filtered through a Schott RG645 cut-off filter and the photomultiplier (EMI 9558B) was screened from the flash with Schott BG18 and BG38 filters. The output from the photomultiplier was fed into a Datalab DL 905 transient recorder and could then be plotted on an *X*–*Y* recorder for further analysis.

Flash-induced absorbance changes in chloroplasts at fixed oxidation–reduction potentials were

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measured in a cuvette described [10,11]. A combined platinum–calomel electrode (Radiometer PK149) was used to monitor the oxidation–reduction potential of the medium. The following procedure was followed: 4.0 ml medium containing 0.66 M sorbitol, 4 mM EDTA, 2 mM $MgCl_2$, 2 mM $MnCl_2$ and 100 mM Hepes buffer (pH 7.6) were placed in the cuvette with 3.0 ml distilled water and the following redox mediators were added: diaminodurene, phenazine methosulfate, phenazine ethosulfate, pyocyanine, and duroquinone (all at conc. 10 μM); anthraquinone disulfonate and 2-hydroxy-1,4-napthoquinone (both at conc. 15 μM). The solution was bubbled with deoxygenated nitrogen gas for 5 min. Intact chloroplasts were shocked by diluting a sufficient volume of chloroplasts in distilled water (final vol. 1.0 ml) to give a chlorophyll concentration of 50 μg/ml when added to the reaction mixture. The chloroplasts were injected into the reaction mixture through the gas outlet tube in the cuvette stopper while gas was passed over the surface of the solution. Titrations were performed under an anaerobic atmosphere with concentrated solutions of ferricyanide, ascorbate or dithionite (freshly prepared in degassed 0.5 M Tris–HCl buffer, pH 8.0) as respective oxidants and reductants. Measurements were done by adjusting the potential to a desired value, allowing for equilibration and flash-activating the sample. Each titration point represents the extent of the ΔA_{518} resulting from a single flash at the indicated potential. The system was considered to be close to equilibrium when the flash-induced absorbance change was similar if approached from either the high or low potential side; in addition, all titrations were fully reversible in the oxidative and reductive direction. A single titration took approx. 1–2 h; because of chloroplast instability a sample was used for only one of the two potential ranges being studied (+500– +150 mV or + 150 – –350 mV). Control experiments with the omission of mediators or changes in mediator concentration demonstrated there were no significant contributions of the mediators to the absorbance changes.

3. Results

Under the above described experimental conditions, the rise-time of the flash-induced ΔA_{518} was instrument limited and the decay showed a half-time of approx. 200 ms. In the following experiments no attempt was made to distinguish kinetically components contributing to the ΔA_{518} and the amplitude to the change was measured as a function of the redox poise of the suspension.

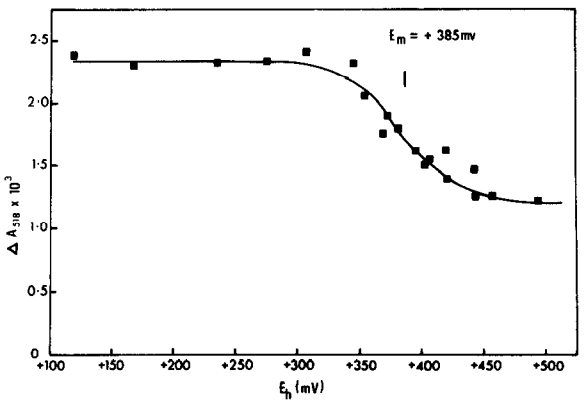

Fig.1. Dependence of the extent of the flash-induced ΔA_{518} in chloroplasts on redox potential (+150 – +500 mV). Conditions were as in section 2.

Figure 1 shows the dependence of the flash-induced ΔA_{518} on redox potential at pH 7.6 in the potential range from +150 – +500 mV. A single component, designated component 1, with a midpoint oxidation–reduction potential (E_m) of +385 mV, was observed. In the potential range from +150 – –350 mV (fig.2) two components were observed, one with E_m +35 mV (component 2) and the other (component 3) with E_m –235 mV. All three com-

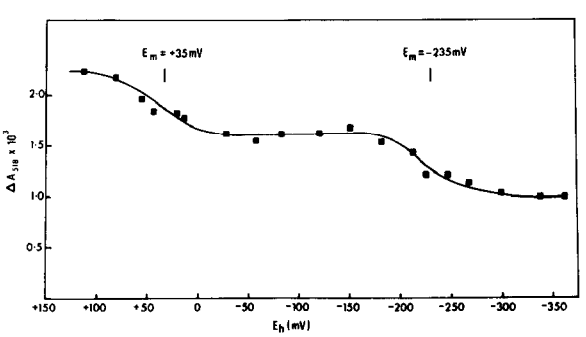

Fig.2. Dependence of the extent of the flash-induced ΔA_{518} in chloroplasts on redox potential (+150––350 mV). Conditions were as in section 2.

ponents show the titration behaviour expected for a one-electron transition.

The relative contribution of the three components to the total ΔA_{518} can be estimated from fig.1,2. Components 2 and 3 contribute equally to the absorbance change and the sum of these two components is approximately equal to the contribution of component 1.

Analysis in the 500–550 nm region did not indicate any absorbance differences when the suspension was poised at redox potentials of +450 mV, +150 mV, −50 mV and −300 mV. Technical limitations did not allow for a fuller spectral examination, but these findings indicate that the spectral properties of components 1,2 and 3 are similar.

It was possible to associate component 1 with the photosystem I light reaction and components 2 and 3 with the photosystem II light reaction. As shown in fig.3, the photosystem II inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), decreased the extent of the ΔA_{518} by half (compare with fig.1) with only a small effect on the E_m of component 1. In the presence of DCMU, component 1 contributed at least 80% of the ΔA_{518} while its contribution was only 50% in untreated chloroplasts. The remaining 20% contribution in the presence of DCMU came equally from components 2 and 3. Similar results were obtained with a second inhibitor of photosystem II, *o*-phenanthroline, which is known to function at or near the DCMU site in chloroplasts [12,13]. It was also possible to associate component 1 with photosystem I by flash activation of samples with light utilized primarily by photosystem I. In these experiments, far-red light (obtained with 2 RG700 Schott filters over the flash) gave results similar to those obtained by the addition of DCMU.

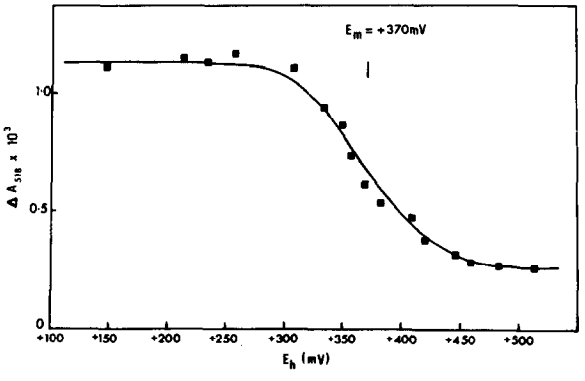

Fig.3. Dependence of the extent of the flash-induced ΔA_{518} in chloroplasts (in the presence of DCMU) on redox potential (+150 – +500 mV). Conditions were as in section 2 except for the addition of 1 μM DCMU.

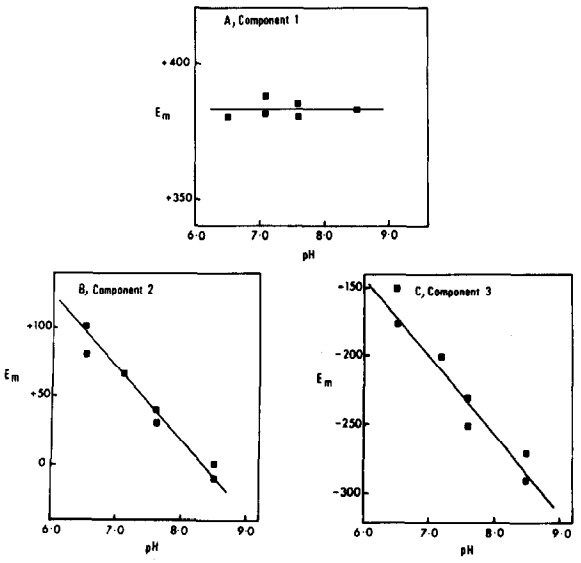

Fig.4. pH-dependence of the electrogenic components' E_m in the 518 nm response of chloroplasts. Conditions were as in section 2 except that 50 mM Hepes buffer at the indicated pH was used. (A) Component 1. (B) Component 2. (C) Component 3.

Further characterization of the three components was obtained in studies of the pH dependence of the E_m of the respective component. As shown in fig.4A, the E_m of component 1 was independent of pH from pH 6.5–8.5. Components 2 and 3 showed pH-dependent midpoint potentials (fig.4B,C). Both had a −60mV/pH unit dependence from pH 6.5–8.5, indicative of a component which takes up one H^+ as well as one electron. Because of the instability of the chloroplast samples in relation to the ΔA_{518}, it was not possible to carry out titrations at pH values more alkaline than 8.5.

4. Discussion

The extremely rapid rise-time of the ΔA_{518} measured [14] suggests the electric potential difference across the chloroplast membrane arises as a

consequence of the photochemical charge separation. In a study of the redox potential dependence of the electrogenic contribution of the two components in a single photoact, one might anticipate the presence of two redox transitions, one related to the P/P^+ couple (P being the reaction center chlorophyll) and the second related to the A/A^- couple (A being the primary electron acceptor). In the case of chloroplasts, contributions from P-680 and 'Q' as well as from P-700 and 'X' would be expected to be observed over the entire redox span. Two of these components (P-680 and X) have midpoint potentials outside the range studied in this work and, hence, would not be expected to affect the electrogenic response under our redox conditions.

On the basis of this reasoning, we would assign the transition associated with component 1 to P-700. The midpoint potential of P-700 is controversial and values from +375–+520 mV have been reported [15–18]). Our determination of an E_m +385 mV is similar to that reported [18] in photosystem I subchloroplast preparations. Consistent with this assignment is the association of component 1 with photosystem I based on far-red flash activation and the effect of DCMU and o-phenanthroline. The pH-independent midpoint potential of component 1 is the same as reported for P-700 [19].

The evidence which associates component 1 with photosystem I also relates components 2 and 3 with photosystem II. Both these components behave in a similar manner as regards the effects of far-red flash activation, DCMU and o-phenanthroline. We would suggest that component 2 is related to the primary electron acceptor of photosystem II on the basis of its E_m and the pH dependence of this potential. The flourescence quencher, Q, has a pH-dependent E_m [20] and the acceptor, based on measurements of the c-550 absorbance change [21], shows a similar dependence. Our values for the E_m of component 2 are similar to [20]. The identity of component 3 is unknown at this time. The only photosystem II component with properties similar to component 3 is a low-potential fluorescence quencher first observed in titrations [20]. The observed E_m and pH dependence in this work are similar to those of the component observed [20]. The function of this component in the photochemistry of photosystem II is not known and remains an area for future consideration.

Evidence for electrogenic reactions in chloroplasts other than those associated with the primary charge separation was not obtained in our studies. These results are to be contrasted with those in bacterial chromatophores [9]. These results do indicate the two chloroplast photoreactions contribute equally to the membrane electric potential, and that the photosystem II contribution is more complicated than predicted on the basis of its presently known photochemistry.

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