EVIDENCE FOR A LIGHT-INDUCED BLUE BAND SHIFT OF PART OF THE P515 PIGMENT POOL IN INTACT CHLOROPLASTS

W. J. VREDENBERG and A. H. C. M. SCHAPENDONK
Centre for Agrobiological Research, PO Box 14, 6700 AA Wageningen, The Netherlands

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1. Introduction

The difference spectrum of light-induced absorbance changes in the 460–540 nm wavelength region, measured in algae and chloroplasts, is characterized by a maximum around 518 nm, a minimum at about 480 nm and an inflection in the 490–500 nm wavelength region [1–4]. Evidence has been given that this spectrum is the result of an electrochromic response of chlorophyll *b* and carotenoid pigments to the electric field generated by the primary light-induced charge separation in the membrane [5,6]. It has been suggested that, at least in bacterial chromatophores, the pool of carotenoids that shows electrochromism may not be completely homogeneous and consists of molecules with slightly different peak wavelengths and bandshifts depending on the strength and orientation of local electric fields [7].

In this paper we discuss an effect of cations on the light-induced difference spectrum of P515 in chloroplasts. It is shown that in intact chloroplasts as well as in broken chloroplasts in the presence of cations part of the pigments in the pool respond in a way that upon flash illumination their absorption band is shifted towards a shorter wavelength. This blue wavelength shift, which gives rise to the inflection around 500 nm in the overall difference spectrum, is interpreted in terms of a cation-dependent change in the orientation of the pigment complex with respect to an induced photoelectric field, or of a cation-induced migration of the primary acceptor of PS II into the hydrophobic region of the membrane. This would suggest that a small part of the pigments might become exposed in the light to an oppositely oriented field between the primary acceptor and the other surface of the membrane.

2. Material and methods

Intact chloroplasts from fresh grown spinach routinely were isolated according to the method in [8]. Usually 75–90% of these chloroplasts retains intact envelopes as determined by ferricyanide reduction [9], or by phase contrast microscopy [10]. The incubation medium of intact chloroplasts contained 330 mM sorbitol and 30 mM tricine, pH 8.0. Broken chloroplasts were obtained by brief osmotic shock either in water, or salt solution containing 10 mM $MgCl_2$ and 15 mM KCl and subsequent washing in incubation medium containing, in addition to 330 mM sorbitol and 30 mM tricine, no salt or 10 mM $MgCl_2$ and 15 mM KCl, respectively. Stock suspensions of chloroplasts were diluted 6–10-fold in assay medium, similar to the incubation medium. Final chloroplast concentrations were equivalent to ~ 100 μg chlorophyll.ml^{-1}.

Absorbance changes, induced by single turnover light flashes (half-life 8 μs) of wavelengths above 665 nm, were measured in a modified Aminco Chance difference spectrophotometer [11]. The single beam mode of operation was used. A 3×3 mm sample cell was inserted into a special holder which maintained the temperature of the sample at ~ 4°C. Flash illumination reached the sample via a flexible light guide. Flashes were fired at a dark interval of 5 s. Usually the absorbance response of 128 flashes was sampled and averaged using a DL 102A signal averager.

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3. Results and interpretation

Figure 1a shows the spectra of flash-induced absorbance changes in intact and broken chloroplasts in ion-free medium. The two spectra appear to be distinctly different. The ratio of the absorbance changes at the maximum 518 and the minimum 480 nm in intact chloroplasts is higher than in broken chloroplasts. In addition the spectrum of the intact chloroplasts shows an inflection in the 490–500 nm region.

Induction and recovery of the flash-induced absorbance changes for each preparation had the same kinetics at all wavelengths studied. The magnitudes were found to vary linearly with chlorophyll concentration. The relaxation time of the dark recovery in intact chloroplasts as a routine was about 500 ms (fig.2a). In broken chloroplasts this relaxation time was found to be dependent on the presence of cations during the breaking procedure and incubation. The decay rate in broken chloroplasts, isolated and incubated in the presence of salts, was found to be about 2-times faster than the one observed in chloroplasts, prepared and incubated, in the absence of salts. In these chloroplasts the relaxation time was about 44 ms (fig.2b). However, subsequent addition of salts causes an immediate 100-fold decrease in the dark relaxation (fig.2c). Prolonged incubation in the presence of salts was found to cause a reversion of the fast decay towards a decay which resembled the one observed in chloroplasts isolated in the presence of

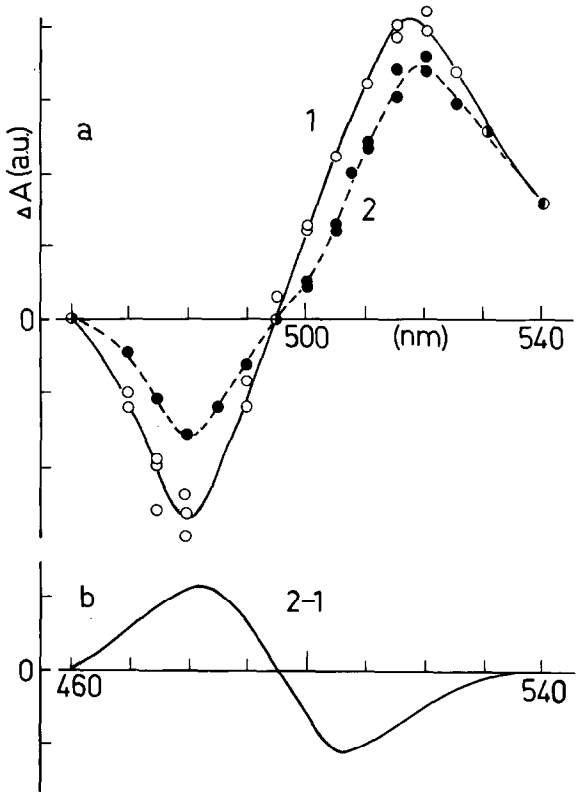

Fig.1a. Spectra of flash-induced absorbance changes in intact and broken chloroplasts, (curves 2 and 1, respectively). Intact chloroplasts suspensions contained in addition to sorbitol and tricine, 10 mM $MgCl_2$ and 30 mM KCl. Broken chloroplasts were prepared, incubated and measured in an ion-free sorbitol + trycine medium. 1b. (curve 2–1) Spectrum obtained by subtraction of the two spectra in a.

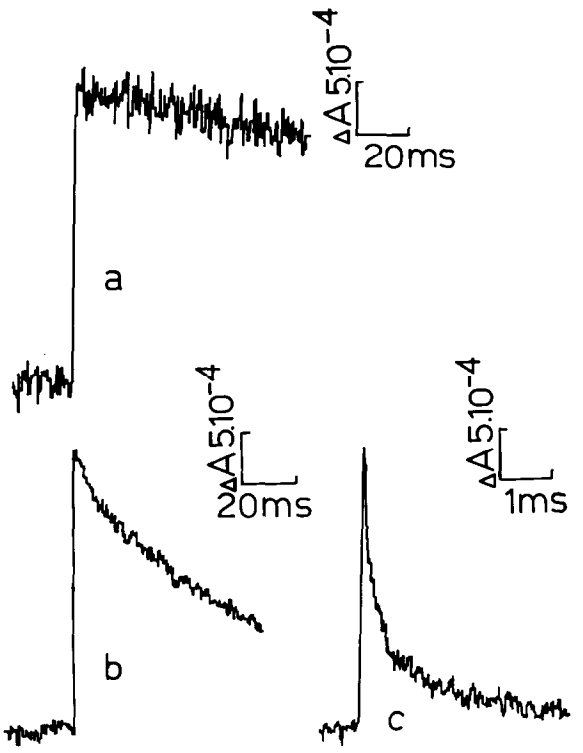

Fig.2. Absorbance changes upon a single turnover saturating light flash, in intact chloroplasts (a), and broken chloroplasts (b,c). Broken chloroplasts were isolated and incubated in ion-free medium. Flash-induced absorbance changes were measured in the absence (b) and the presence (c) of 10 mM $MgCl_2$ and 15 mM KCl.

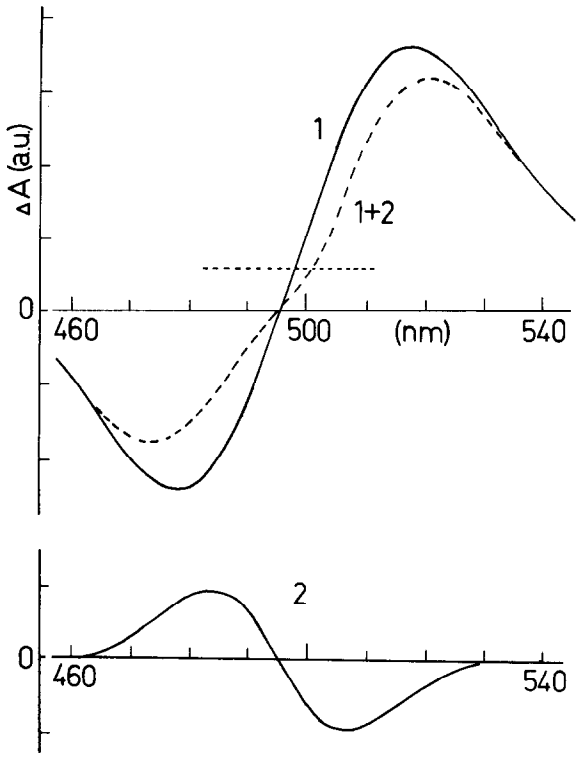

Fig.3. Computed difference spectrum (curve 1+2) composed of a 1 nm red bandshift (curve 1) of a single Gaussian band A and a 1 nm blue shift (curve 2) of a Gaussian band B (B = 0.18 A). The broken horizontal line is the original zero line of the spectra. Further details are in the text.

salts. The difference spectrum measured in chloroplasts broken and incubated in the presence of salts, was found to be similar, although not identical, to the spectrum of intact chloroplasts.

As shown in fig.1b, subtraction of the two spectra of fig.1a gives a spectrum with a minimum around 505 nm, a maximum at 483 nm and zero points at 460, 495 and 535 nm. According to this it appears that the spectrum of intact chloroplasts is composed of two different spectra. One similar to the spectrum observed in broken chloroplasts (fig.1a, see also ref. 11), indicative for a red-band shift, and one (fig.1b) which is indicative for a blue-band shift.

Figure 3 (curve 1+2) shows a computed difference spectrum composed of a 1 nm red bandshift of a single Gaussian band A (maximum at 497 nm, half width 44 nm) and a 1 nm blue shift of a Gaussian band B (B = 0.18 A, maximum at 495.5 nm, half-width 25 nm). For comparison the spectra of bandshifts A (curve 1) and B (curve 2) are given. It appears that the computed difference spectra 1 and 1+2 of fig.3 are a reasonable good simulation of the spectra 1 and 2 measured in the chloroplasts. Obviously, the width of the negative band of the experimental spectra (fig.1a) is smaller than that of the computed spectra. Moreover the latter ones would need a small baseline shift to match the asymetry observed in the experimental spectra.

These results lead us to suggest that in intact chloroplasts and in broken chloroplasts, in the presence of salts, part of the carotenoids are so oriented in the membrane with respect to the light-generated electric field that they manifest a blue electrochromic band shift. A small blue shift has been observed in chloroplasts from *b*-lacking mutants of barley [12]. Exposure of chloroplasts to an ion-free environment will amongst others lead to unstacking [13,14], changes in the membrane surface charge (potential) [15] and to structural changes in the membrane [13,16]. The structural changes might affect the mutual orientation of pigments and consequently the interaction of local electric fields of permanent and light-generated dipoles with the carotenoid molecules in the membrane [17], in a manner dependent on the hydrophobicity of the inner membrane phase. Thus in the presence of high concentrations of Mg and K, which for instance are known to exist in the intact chloroplasts [18,19], the carotenoids in the membrane might become exposed to electric fields which are altered in orientation and magnitude with respect to the situation in an ion-free environment. If so, our results can be interpreted in terms of two alternative models:

1. In stacked chloroplasts in the presence of mono- and divalent cations, part of the electrochromically responding carotenoids have a dipole orientation which, with respect to the light-generated electric field, is opposite to the one of the bulk of the carotenoids. This orientation would become reversed in the absence of cations.

2. All carotenoids have the same dipole orientation, but a small part of them, notably those located in the vicinity of the primary acceptor at the outer

core of the membrane, is assumed to become exposed to an oppositely oriented light-generated electric field between the charged primary acceptor of the reaction centre(s) and the outer membrane surface.

This will occur if the acceptor has migrated more into the hydrophobic region of the membrane under high salt conditions. The postulated migration would become reversed in the absence of ions. Although we have not been in the position to discriminate between the two alternatives we judge the second one as the most probable one. According to an interpretative model proposed [20] the migration of the primary acceptor Q of photosystem II including its proteinaceous shielding complex, could take place in the presence of surface reactants. A field-driven movement of Q in the membranal plane has been suggested by others [21].

The fraction of pigments that shows a blue bandshift cannot be estimated with precision as yet. Analyses of the data of fig.1,3 indicate that when the extent of the blue wavelength shift is assumed to be equal to that of the red bandshift of the bulk pigments, the former one is caused by an 18% fraction of the bulk pigments. The absorption band of the blue-shifting fraction would have a somewhat smaller halfwidth (25 nm) than that of the bulk (44 nm) and presumably is located at a slightly lower wavelength. These differences suggest a different chemical environment which would be consistent with the models discussed.

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