GENERATION OF THE 518 nm ABSORBANCE CHANGE IN CHLOROPLASTS BY AN EXTERNALLY APPLIED ELECTRICAL FIELD

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Received 20 February 1980

1. Introduction

The well-known ΔA_{518} caused by a light-induced membrane potential in chloroplasts has been ascribed to electrochromism of photosynthetic pigments, especially carotenoids [1,2]. This assignment requires some ad hoc assumptions in order to explain the linear field dependence and the spectrum of the ΔA: the carotenoids involved would have to be a minor fraction with a red-shifted absorption spectrum and a special molecular environment which causes a permanent polarization of the molecules [3–5]. In [5] we have shown that ΔA induced by applying an electrical field to osmotically swollen chloroplasts can be explained quantitatively in terms of the electrochromic theory. The spectrum was quite different from that of the light-induced ΔA, and the ΔA showed a quadratic rather than a linear field dependence. It was assumed that the linear ΔA did occur but could not be seen because their sign was opposite in the 2 halves of each membrane vesicle. We have now verified this assumption and found that the difference spectrum of linear electrochromism induced by an external field is indeed nearly identical to that of uncoupler-sensitive ΔA induced by a single light flash.

2. Materials and methods

Spinach chloroplasts were prepared as in [5]. Just before measurement a sample of the suspension was diluted to final concentrations of ~2 μM chlorophyll, 2 mM sucrose, 25 μM $MgCl_2$ and 50 μM KCl. The $MgCl_2$ and KCl content was usually somewhat increased, up to 3-fold, to accelerate the ΔA (see below). ΔA were measured with a single beam spectrophotometer. The signal was averaged (Datalab 102 S) and stored in a microcomputer. The sample was contained in a cubical plexiglass vessel of 10 mm with 2 vertical platinum sides, connected to a home-made high-voltage pulse generator. The electrical pulses had a rise and decay time of 1 μs (90% completion) and were limited to <800 V in order to avoid the saturation phenomenon reported [5]. The time between successive measurements was 30 ms. After 200–500 pulses the sample was renewed by a flow system.

3. Results and discussion

When chloroplasts are suspended in a strongly hypotonic medium the thylakoid systems unfold and form large spherical vesicles called blebs. The bleb wall consists of only a single membrane, while part of the thylakoid system remains concentrated in a few patches of the bleb surface [5]. If the inside of the bleb corresponds to the inside of the thylakoids the light-induced membrane potential is positive inside and negative outside (fig.1a). An externally applied field induces a membrane potential with opposite polarity in half of each bleb (fig.1b). Therefore, ΔA with a linear field dependence in one half of the bleb are cancelled by those occurring in the other half. However, measuring light passing the 2 halves of the bleb can be separated if the refractive index of the external medium is lower (fig.1c) or higher (fig.1d) than that of the internal medium. This was done by placing a diaphragm slightly to the right or left of an image of the monochromator slit, formed by a lens just behind the cuvette.

Electrical pulses of the shape shown in fig.2 were applied alternatingly in order to minimize artefacts due to movement of particles and ions in the cuvette.

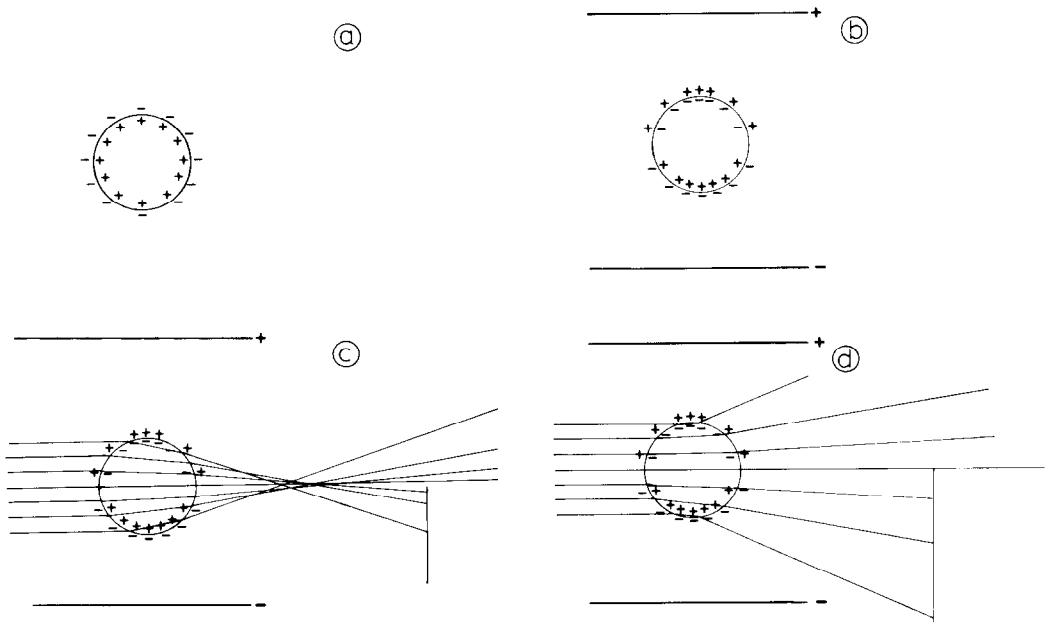

Fig.1. Schematic representation of the electric field in an osmotically swollen chloroplast (bleb) for a light-induced field (a) and a field induced by external electrodes (b). In (c) and (d) the deflection of a measuring beam is indicated in the case that the index of refraction of the inner medium is larger (c) or smaller (d) than that of the outer medium.

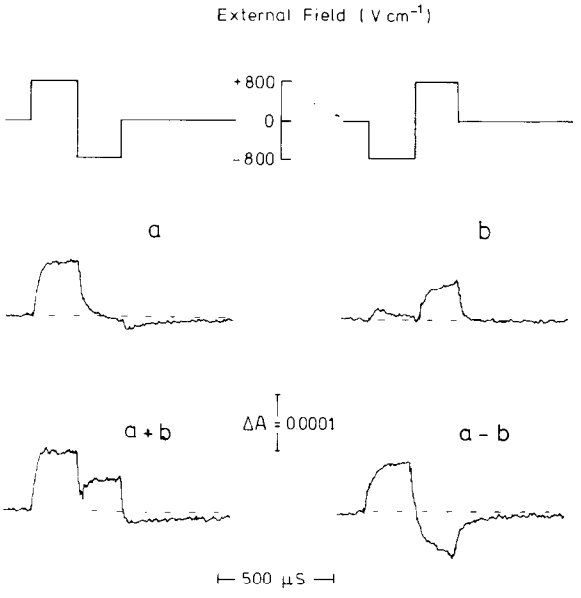

Fig.2. Kinetics of the ΔA_{520} measured in scattered light (a,b) induced by external field pulses shown in the upper traces. The ΔA represent the average of 4096 measurements; ΔA_{570} values were subtracted. The lower traces show the quadratic and linear components of the field-induced ΔA obtained by addition or subtraction of (a) and (b).

Some slow and apparently wavelength-independent changes nevertheless usually occurred and were subtracted. ΔA in scattered light induced by these pulses (fig.2a,b) were not exclusively caused by linear electrochromism, but also in part by quadratic electrochromism. These 2 components can be separated by addition or subtraction of 2 signals induced by pulses of opposite sign. Addition (fig. 2a + b) cancels the linear component and yields the same ΔA discussed in [5]. Subtraction (fig.2(a–b)) cancels the quadratic terms. Variation of the applied field strength confirmed the linear field dependence of the resultant signal (fig.2(a–b)).

The detection of quadratic electrochromism (fig.2 (a + b)) is mainly due to multiple scattering and imperfect optics. In order to obtain a sufficient signal-to-noise ratio a bleb concentration was used at which some multiple scattering occurred; the relative amplitude of the quadratic changes at a given field strength was much less at lower bleb concentrations. This means that even at the high membrane potentials generated here (see below), the linear ΔA are much larger than the quadratic ones.

It should be noted that the amplitude of the linear electrochromic changes is actually much larger than

indicated in the figure. Most of the light detected by the photomultiplier was scattered by the patches on the bleb surface and other impurities. The light intensity due to the deflection mechanism illustrated in fig.1, and also the absorbance of this light by the sample must be very small and could not be determined. In fact very large ΔA may be expected since the membrane potential generated by the applied field reached up to 600 mV for a bleb with 5 μM radius. This can be calculated from the relation [6]:

$$V = \tfrac{3}{2} F r \cos\theta \qquad (1)$$

where V is the membrane potential, F is the field strength applied, r is the bleb radius and θ is the angle subtended by the direction of the applied field and the normal to the membrane.

The difference spectrum of the linear electrochromic changes, measured as the peak-to-peak amplitude in recorder traces like the one shown in fig.2 (a–b) is shown in fig.3. The spectrum is very similar to that of P518 in chloroplasts [2,7,8] if the different particle flattening [9] effect in the 2 preparations is taken into account: as shown in [5], the electrochromic ΔA induced in a suspension of blebs by an externally applied field are not significantly affected by particle flattening, in contrast to light-induced electrochromic changes in chloroplasts.

The sign of the ΔA could be inverted by increasing the refractive index of the external medium with added serum albumin (fig.4), showing that without this addition the refractive index of the internal medium is slightly higher than that of the external medium. The sign of the changes was consistent with the hypothesis that the inside of the bleb corresponds to the inside of the thylakoids.

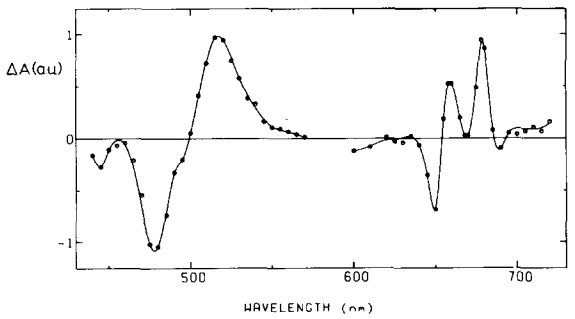

Fig.3. Spectrum of the linear ΔA in scattered light induced by electrical field pulses as in fig.2. The ΔA_{750} have been subtracted. Each point in the spectrum is a result of 8192 individual measurements. The sign of the changes shown corresponds to that half of the bleb where the potential is positive inside and negative outside.

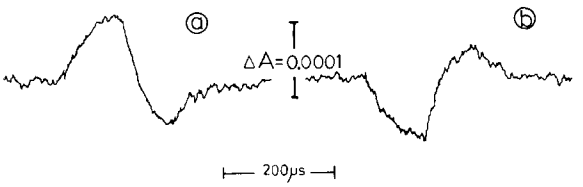

Fig.4. Kinetics of the linear ΔA_{520} measured in scattered light induced by external field pulses as in fig.2. (a) control: (b) with 3×10^{-4} M bovine serum albumin. ΔA_{570} values were subtracted.

From the refractive index increase by the concentration of serum albumin required to obtain zero signal, it was concluded that the refractive index of the internal medium was 0.23% higher than that of the external medium. For the geometry of field and optics used the effective average deflection in the absence of serum albumin was estimated at 0.15°, corresponding to a displacement of 0.2 mm at the position of the slit image. Since the edge of the slit image was sharp enough to place the diaphragm even closer than this, reasonably efficient detection of the signal may indeed be expected.

As discussed in [5], the enhanced local field in a bleb membrane is built up with a time constant τ determined by the specific resistance ρ_s of the internal and external media (assumed to be equal), the specific capacitance of the thylakoid membrane c_s and the radius of the bleb r according to [10]:

$$\tau(r) = \tfrac{3}{2} \rho_s c_s r \qquad (2)$$

Taking into account that the field in the membrane is proportional to the radius of a bleb (eq. (1)) and the number of pigments proportional to r^2 we obtain for the kinetic behavior of the absorbance changes:

$$\Delta A = k \sum_r n(r) r^3 \left(1 - e^{-\frac{t}{\tau(r)}}\right) \qquad (3)$$

where k is a constant, $n(r)$ the number of blebs with radius r obtained from the size distribution, measured with phase-contrast microscopy (for a typical example

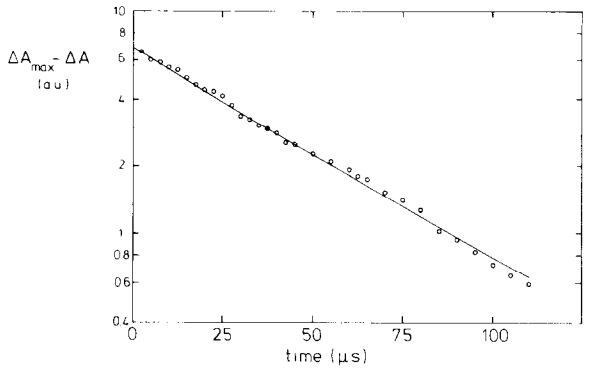

Fig.5. Semilog plot of the kinetics of the field-induced linear ΔA_{520} measured in scattered light. Circles, measurements; line, theoretical fit (see text).

see [5]) t is the time after the onset of the external field pulse, and $\tau(r)$ is given by eq. (2). The value of ρ_s was determined at $3.1 \times 10^4\ \Omega$. cm. In fig.5 a semilog plot of the experimental ΔA is given (circles) using eq. (3). A good fit of the experimental data was obtained with a specific capacitance of $1.7 \pm 0.2\ \mu F\ .\ cm^{-2}$ (solid line) in agreement with the value of $2.0 \pm 0.2\ \mu F\ .\ cm^{-2}$ obtained from the quadratic electrochromic changes [5].

3. Conclusions

Employing the lens effect of the blebs by measurements of small angle scattered light, it was shown that ΔA with a linear dependence on the external applied field strength do occur. Their absolute amplitude could not be determined but the linear changes are indeed much larger than the quadratic ones, even at the high membrane potentials used here. The kinetics of the linear ΔA, like those of the quadratic changes, seem to be determined by the kinetics of the enhanced electrical field in the membrane. The difference spectrum of the linear electrochromic ΔA induced by an externally applied field and that of the light-induced P518 changes are nearly the same.

These results provide strong evidence for the hypothesis [1] that the P518 difference spectrum is due to electrochromism induced by the membrane potential.

Acknowledgements

We wish to thank Dr J. Amesz for his continuous interest and critical reading of the manuscript. This investigation was supported by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

[1] Junge, W. and Witt, H. T. (1969) Z. Naturforsch. 23b, 244–254.
[2] Emrich, H. M., Junge, W. and Witt, H. T. (1969) Z. Naturforsch. 24b, 144–146.
[3] Schmidt, S., Reich, R. and Witt, H. T. (1971) Naturwissenschaften 58, 414.
[4] Sewe, K.-U. and Reich, R. (1977) Z. Naturforsch. 32c, 161–171.
[5] De Grooth, B. G., Van Gorkom, H. J. and Meiburg, R. F. (1980) Biochim. Biophys. Acta in press.
[6] Zimmermann, U., Pilwat, G. and Riemann, F. (1974) Biophys. J. 14, 881–899.
[7] Amesz, J. and De Grooth, B. G. (1976) Biochim. Biophys. Acta 440, 301–313.
[8] Breton, J. and Paillotin, G. (1977) Biochim. Biophys. Acta 459, 58–65.
[9] Duysens, L. N. M. (1956) Biochim. Biophys. Acta 19, 1–12.
[10] Pauly, H., Packer, L. and Schwan, H. P. (1960) J. Biophys. Biochem. Cytol. 7, 589–601.