INFLUENCE OF THE CHLOROPHYLLS ON THE ELECTROCHROMISM OF CAROTENOIDS IN THE MEMBRANES OF PHOTOSYNTHESIS

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1. Introduction

It was shown in a preceding paper by electrochromic measurements on photosynthetic pigments in thin capacitors that the carotenoid lutein forms specific complexes with chlorophylls [1]. By this complex formation, the quadratic rise of the electrochromic absorption-change of lutein with the external electric field strength is changed into an approximately linear rise. This effect is coupled with an amplification of the electrochromism of lutein, and with a shift to longer wavelengths (as compared with lutein molecules in liquid solution). By such a complex formation, several features of field-indicating absorption-change spectra of photosynthesis research could be explained, and some conclusions on the asymmetrical arrangement of the different pigments in the membrane could be drawn [1].

These results shed new light also on various further observations of other authors, some of which will be discussed in the following.

2. Results and discussion

2.1. *Absorption-changes of carotenoids induced by oxidation of bacteriochlorophyll*

Okada and Takamiya [2] have found that the absorption spectrum of a carotenoid (spheroidene) in chromatophores of *Rhodopseudomonas spheroides* is shifted by 2–2.5 nm towards longer wavelengths, when the bulk bacteriochlorophyll BChl 885 is oxidized in the dark, e.g., by addition of ferricyanide at sufficiently high concentrations. About 1 molecule of the carotenoid is converted to a form absorbing at longer wavelengths in step with the oxidation of 1.1 molecules of BChl 885. This oxidation and the coupled wavelength shift of the carotenoid can be reversed by addition of reducing agents. The coupling between oxidation of BChl 885 and absorption shift of carotenoid is abolished by addition of the detergent Triton X-100, which is known to loosen molecular complexes. On the other hand, the coupling is not affected by mere electron transport inhibitors or by uncouplers of photophosphorylation. A solution of the isolated carotenoid, extracted from chromatophores, does not show a reversible absorption shift upon addition of ferricyanide. However, a similar shift of the carotenoid absorption as with ferricyanide is caused by addition of Mg^{2+} to the chromatophores, and this shift is not accompanied by any absorption-change of BChl 885.

In view of the results in ref. [1], the observations of Okada and Takamiya [2] can be explained by a carotenoid–BChl complex, in which electrons are drawn from the carotenoid to the Mg-atom of a BChl molecule. This effect is equivalent to the action of a local electric field [3], which shifts the absorption spectrum of the carotenoid to longer wavelengths. When the BChl molecule is oxidized, the local field, and hence the absorption shift, are increased. Similarly, it was observed in ref. [1] that the main maximum of the electrochromic spectrum of lutein in complex with chlorophyll *b* (Chl *b*) is located at longer wavelengths (517 nm) than in complex with chlorophyll *a* (Chl *a*) (512 nm). (For reasons of analogy, note that Chl *b* is an oxidized form of Chl *a*, and the electron affinity of Chl *b* is greater than that of Chl *a* by about 0.13 eV [4].)

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2.2. *Light-induced absorption-changes of carotenoids*

A light-induced absorption-change spectrum of *Rhodopseudomonas spheroides* was also measured by Okada and Takamiya [2]; this was similar to the ferricyanide-induced one, but still more shifted towards longer wavelengths by about 3 nm. Probably, this difference spectrum is not due to the same bulk carotenoid as the ferricyanide-induced difference spectrum, but to some distinguished carotenoid molecules that are bound in other complexes, so that their absorption maxima are located at longer wavelengths in the dark already.

A light-induced absorption-change spectrum of a special mutant of *Rhodopseudomonas spheroides* (GIC mutant), which has only one single major carotenoid (neurosporene), was published by Crofts et al. [5]. This light-dark difference spectrum was very similar to the first derivative of the absorption spectrum, except that it was shifted by 3.5 nm to the red. For example, the long-waved maximum of the dark absorption spectrum was located at 492 nm, whereas the zero-crossing of the light-dark difference spectrum was located at 495.5 nm. The difference spectrum was interpreted [5] by the model that a small proportion of the pigment (about 10%) had been spectrally shifted by about 7 nm through the light (twice the wavelength difference between the difference spectrum and the first derivative of absorption). However, a shift of this order of magnitude cannot be caused by a homogeneous light-induced electric field across the membrane, which may be about 10^6V/cm for continuous light. Such a shift might only be due to some local molecular field, caused by some kind of chemical reaction.

However, if a new chemical species with a new absorption spectrum would be formed by the light-induced field, an increase of this field should alter only the amplitudes, but not the wavelength positions of the difference spectrum. This is in contradiction to flashlight-induced absorption-changes, which were also measured by Crofts et al. [5]. Each flash increases the electric field strength across the membrane. After four short, saturating flashes, about 80% of the steady state field strength (like that induced by continuous light) is reached, as judged from the absorption-changes at 503 nm [5]. After the first flash, the zero-crossing of the difference spectrum of the GIC mutant was about 1.75 nm away from the maximum of the absorption spectrum. This looks like as if the absorption spectrum had been shifted by about 3.5 nm. After the following flashes, there were *additional* apparent shifts, but these were only of the order of 0.6 nm. This discrepancy cannot be explained by a simple electrochromic shift of the dark absorption spectrum, nor by a light-induced chemical reaction.

However, in view of the results in ref. [1], the observations can be explained by the assumption that the dark absorption spectrum hides the contribution of a certain proportion of the carotenoid molecules, which are spectrally shifted in the dark already and which are made particularly field-sensitive by an oriented, asymmetrical complex formation with polarizing molecules, so that the electrochromic response of these distinguished molecules is much greater than that of the rest. For example, let the absorption maximum of these molecules be shifted by complex formation in the dark from about 492 nm to 493.6 nm*. The first flash produces an additional electrochromic shift, e.g., from 493.6 to 493.9 nm, so that the absorption-change, observed at 493.75 nm, is zero after the first flash. This is because in the time-interval, when the maximum of the absorption band is shifting through the fixed wavelength of observation, no absorption-change is observed, cf. fig.1. If the second flash causes an additional shift of 0.2 nm, i.e., from 493.9 to 494.1 nm, the absorption-change at 494 nm should be zero after the second flash, and so on. Just this is seen in fig.2, taken from Crofts et al. [5], which shows the flash-induced absorption-changes of the carotenoid as function of time at different wavelengths. The negative peaks in the upper curve indicate the moments when the flashes Nos. 1–4 are fired. At 494.2 nm, the absorption-change is zero after the third flash, i.e., the membrane potential that is reached by this flash has shifted the absorption maximum of the electrochromically sensitive species up to about 494.3 nm.

Theoretically, an electric field strength F causes an electrochromic frequency shift $\Delta\nu$ of the absorption band, given by the equation [6,7]

$$h\Delta\nu = -\Delta\mu F\cos\vartheta - \tfrac{1}{2}\,\Delta\alpha F^2 \qquad (1)$$

*The complexes must have a preferential orientation, so that the effective, local molecular fields, exerted on the distinguished carotenoids, have a mean component parallel to the light-induced field [3]

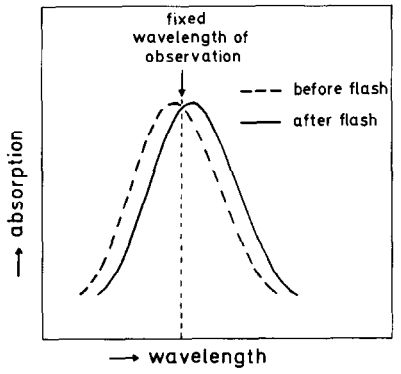

Fig.1. Electrochromic shift of an absorption band by a flash. In the case shown here, the observed absorption-change is zero. However, by a *further* flash, when the left flank of the shifting absorption band reaches the wavelength of observation, the absorption-change will be negative, i.e., de transmission-change will be positive.

where h is the Planck constant, and $\Delta\mu$ and $\Delta\alpha$ are the dipole moment difference and the polarizability difference, respectively, of the pigment molecule between the ground state and the excited state. ϑ is the angle between the vectors $\Delta\mu$ and $\boldsymbol{F}$, and $\Delta\alpha\boldsymbol{F}^2$ means the scalar product between the field strength $\boldsymbol{F}$ and the induced dipole moment difference $\Delta\alpha\boldsymbol{F}$. The frequency shift $\Delta\nu$ can be expressed as a wavelength shift $\Delta\lambda$ by the equation

$$\Delta\nu = -c\Delta\lambda/\bar{\lambda}^2 \qquad (2)$$

where c is the velocity of light.

If a polarizing complex partner is attached to one end of a symmetrical carotenoid**, the polarizing force can be formally described as a local molecular field $\boldsymbol{F}_1$ parallel to the long axis of the molecule [3]. If the polarizability component in this direction is denoted by $\alpha_{||}$, the wavelength shift due to the complex formation is given by the second term of eq.1 as

$$h\Delta\nu = -h\,c\Delta\lambda/\lambda^2 = -\tfrac{1}{2}\,\Delta\alpha_{||}\boldsymbol{F}_1^2 \qquad (3)$$

For the present carotenoid (neurosporene, having

**A symmetrical carotenoid in its all-trans conformation has no permanent dipole moment. The structure of neurosporene is practically symmetrical

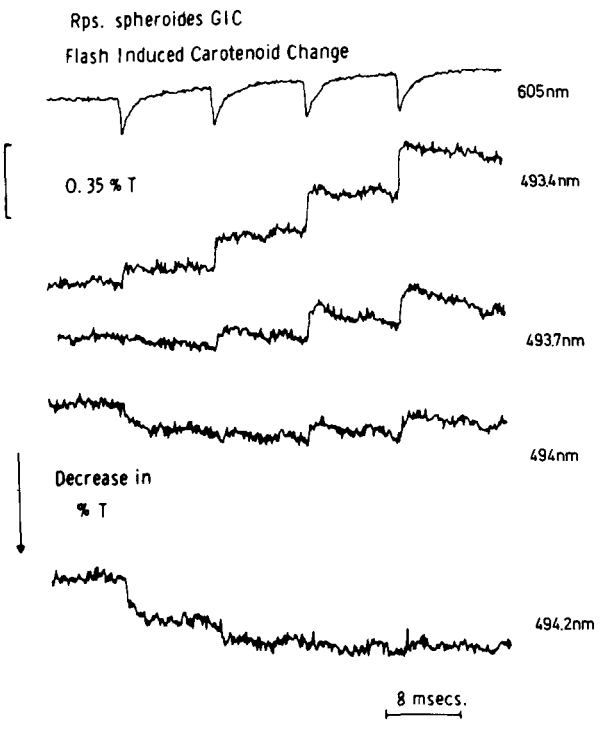

Fig.2. Transmission-changes of *Rhodopseudomonas spheroides* GIC mutant as function of time, at different wavelengths, induced by four successive flashes, from Crofts et al. [5]. The four lower traces are corrected, to show only the kinetics of the carotenoid absorption changes. The flash-induced transmission-change is positive or negative, depending on whether the fixed wavelength of observation is located behind or in front of the shifting absorption maximum of the field-sensitive distinguished carotenoid molecules.

9 conjugated double bonds), a polarizability difference of $\Delta\alpha_{||} = 8.7 \cdot 10^{-38}$ C m^2 V^{-1} may be assumed [8]. With $\lambda = 492$ nm and a shift of $\Delta\lambda = 1.6$ nm by complex formation in the dark (as suggested above in view of fig.2), the local molecular field can be estimated from eq.3:

$$\boldsymbol{F}_1 = \left(\frac{2\,h\,c\,\Delta\lambda}{\lambda^2\,\Delta\alpha_{||}}\right)^{1/2} = 1.7 \cdot 10^6 \text{ V/cm} \qquad (4)$$

Such a value seems not unlikely to be caused by asymmetrical complex formation.

If the carotenoid molecule is additionally exposed to a light-induced external field $\boldsymbol{F}_a$, the dipole moment difference

$$\Delta\mu_{ind} = \Delta\alpha_{||} F_l \quad (5)$$

which is induced by the local molecular field F_l, can be regarded like a *permanent* dipole moment difference $\Delta\mu$. Thus, we get for the additional, light-induced band shift from eqs.1,2, and 5:

$$-h\Delta\nu = h\,c\,\Delta\lambda/\lambda^2 = \Delta\alpha_{||} F_l F_a \cos\vartheta + \tfrac{1}{2}\,\Delta\alpha_{||} F_a^2 \cos^2\vartheta \quad (6)$$

if the components of $\Delta\alpha$ perpendicular to the long axis of the carotenoid molecule are neglected (cf. eq. 14 in ref. [3]).

The field strength across the chromatophore membrane, induced by a short saturating flash, may be of the same order of magnitude as in the thylakoid membrane: $F_a = 2 \cdot 10^5$ V/cm [9–11]. For the angle ϑ between the long molecular axis and the normal of the membrane, a mean value of 45°C is assumed [12]. Since $\frac{1}{2}F_a\cos\vartheta$ is much smaller than the above value of F_l, the second term in eq.6 can be neglected as compared to the first one, so that the band shift approximately becomes a linear function of the light-induced field F_a:

$$\Delta\lambda = \frac{\lambda^2}{h\,c}\,\Delta\alpha_{||}\cos\vartheta\, F_l F_a \quad (7)$$

Inserting the above mentioned values, we get for the additional band shift that is induced by the first flash: $\Delta\lambda = 0.26$ nm. This is in good agreement with the value of 0.3 nm, suggested above from fig.2. So far, the model to explain the observations of Crofts et al. [5] is rather consistent.

The model can be refined, if the contribution of the distinguished, field-sensitive carotenoids to the dark absorption spectrum is taken into account, which has been neglected in the above calculation. (Indeed, the fraction of the distinguished carotenoids may be about 30% of the total amount, as judged from the flash-induced absorption-change and the first derivative of absorption, calculating with a shift of about 0.3 nm.) Then, the maximum of the not distinguished carotenoids should be assumed at a wavelength a little shorter than 492 nm (which was the averaged maximum of the total amount). Hence, the wavelength difference, induced by complex formation, becomes a little greater than 1.6 nm, and the electrochromic shift, resulting from eq.7, becomes a little greater than 0.26 nm, but it remains in agreement, within the limits of error, with the observed value of about 0.3 nm.

The essential point in this model is that the most field-sensitive carotenoid molecules have already been shifted in the dark by complex formation, so that the remaining flash-light-induced shifts are small enough to be interpreted as electrochromism. In the case of the continuous light-dark difference spectrum, the remaining shift is still rather strong, if the wavelength scales are comparable, i.e., if the absorption maximum of the complex in the dark is assumed at 493.6 nm. However, it is known that, e.g., in chloroplasts, the light-dark difference spectra contain additional, slow absorption-changes due to structural changes [13], which are not 'field-indicating'. Similar phenomena might also in the present case give rise to additional solvatochromic shifts. The lower trace in fig.2 looks like as if the maximum of the electrochromical sensitive species cannot be shifted very far beyond 494.3 nm, since the third flash does no more cause a step in this trace, and the fourth flash does not cause a step *either*. However, the monotonous slight transmission decrease suggests that any slow reaction is superimposed.

2.3. *Location of field-indicating molecules in the membrane*

In chloroplasts, the light-induced field-indicating absorption-change at 520 nm (ΔA_{520}) can be attributed mainly to a complex of lutein with Chl *b* [1]. However, Chl *b* is located in the regions of the membrane that belong to photosystem II (PS II) [14,15]. Thus, the main part of ΔA_{520} can only be observed, if an electric displacement exists in the regions of PS II. If photoreaction II is blocked (which can be achieved by addition of DCMU and hydroxylamine, and preillumination [16]), ΔA_{520} indicates only the electric displacement that is still generated in PS I and then spread all over the membrane, including the regions of PS II. However, if this spreading of the electric displacement is hindered (e.g., by cooling down), a breakdown of ΔA_{520} can be expected.

Conjeaud et al. [17] have measured the amplitude of ΔA_{520} in a chloroplast suspension with 65% glycerol as function of temperature, with photoreaction II being blocked. On cooling the suspension down to −80°C, the amplitude of ΔA_{520} is nearly constant, but below this temperature, the amplitude decreases, reaching a constant low value below −125°C of only about 15% of the value at higher temperatures. There is no breakdown of the photoactivity of PS I around −95°C, as judged from absorption-changes of *P* 700. So, the breakdown of ΔA_{520} around −95°C seems to be due to a freezing and localization of the electric displacement in the regions of PS I, where the electric field is not 'noticed' by the field-indicating molecules, since these are located in the regions of PS II. (This

explanation has already been proposed by Conjeaud et al. [17] as one of two possible hypotheses.) Only some less field-sensitive carotenoids may then be exposed to the electric field, thus accounting for the 15% rest absorption change, whose maximum is located around 530 nm [18]. If both photoreactions are operating, there is no breakdown of ΔA_{520} around $-95°C$ [18], because the electric displacement, generated in PS II, is directly indicated by the lutein-Chl-*b* complexes. Similar results as for ΔA_{520} should be expected for the field-indicating absorption-decrease at 480 nm, which is due to Chl *b* [19,8,10].

If it is possible by any technique to resolve the kinetics of the rise of the field-indicating absorption-change at 480 nm and of the main part of the absorption-change at 520 nm, it might be expected to see that the rise due to photoreaction II is faster than the rise due to photoreaction I, because, in the latter case, the electric displacement, generated in PS I, should need some time to reach the regions of PS II. This model agrees with the concept of Joliot et al. based on experiments on *Chlorella* at room temperature [20].

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