

The Effect of Molecular Polarization on the Electrochromism of Carotenoids*

II. Lutein-Chlorophyll Complexes: The Origin of the Field-Indicating Absorption-Change at 520 nm in the Membranes of Photosynthesis

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Electrochromic spectra of monolayers of carotenoids (lutein and β -carotene) in contact with monolayers of chlorophylls and of pheophytin a are measured in thin capacitors. A specific interaction of one of the OH-groups of lutein with the Mg-atom of chlorophyll is found. The formation of this oriented complex accounts for the fact that a part of the electrochromic absorption-change of lutein depends linearly on the electric field strength, whereas for lutein alone only a smaller, quadratic electrochromism is found. In the preparation with chlorophyll a, the maximum of this linear electrochromism is located at shorter wavelengths (512 nm) than in the preparation with chlorophyll b (517 nm).

The permanent field that has been postulated in photosynthetic membranes (to explain the linear dependence of the field-indicating absorption-changes of the carotenoids) may also be attributed to such a complex formation with chlorophylls. Especially, the field-indicating absorption-change at 520 nm can now be attributed mainly to a lutein-chlorophyll b complex. The absorption-change at 520 nm, calculated according to this model from the present experiments *in vitro*, is of the same order of magnitude as observed *in vivo*. Furthermore, this model agrees with the hitherto unexplained observation that in chlorophyll-b-lacking mutants the absorption-change at 520 nm is smaller than in normal plants, and the maximum is located at shorter wavelengths. Besides, it is concluded that lutein is mainly located in the regions of photosystem II. The contributions of the other carotenoids (especially of neoxanthin) to the spectrum of the field-indicating absorption-changes are also discussed.

From the above model, some conclusions are drawn on the asymmetrical arrangement of the different pigments in the membrane of photosynthesis: The bulk chlorophyll molecules that serve as complex partners for the carotenoids should be located near to the inner surface of the thylakoid membrane, and the carotenoids attached to these chlorophylls should be located more to the outside. The phytol chain of a chlorophyll molecule should form an acute angle with the plane of the porphyrin ring.

1. Introduction

Comparing the spectrum of the light-induced field-indicating absorption-changes of chloroplasts as measured by Emrich *et al.*² with a superposition of electrochromic spectra of the isolated dyes *in vitro*, it has been concluded that the light-induced absorption-increase at $\lambda = 520$ nm is due to the carotenoids^{3–5}. In order to explain the discrepancy that the absorption-change *in vivo* is a linear function of the electric field-change across the membrane whereas the electrochromism of symmetrical carotenoids *in vitro* is a quadratic function of the electric field strength, it was assumed that the dyes are exposed to an effective permanent field perpendicular

to the membrane, which is much stronger than the light-induced field-changes^{3, 4, 6}.

Now it has been found that such a permanent field need not be postulated for the chlorophylls, but only for the carotenoids⁵; hence it is probably due to some asymmetrical complex formation of the carotenoids with electron-attracting or electron-repelling molecules. As a model for such a complex, it has been shown in part I that a molecular polarization by a carboxylic group that is inserted asymmetrically into the molecule acts like a permanent field, producing a linear electrochromism from the quadratic one¹. The effective permanent field being equivalent to the influence of this carboxylic group was even about six times greater than the permanent field postulated in photosynthesis research¹.

Two questions have remained open in this concept: 1) What are the polarizing complex partners of the carotenoids? 2) What is the kind of their

* As to Part I, see Ref. 1.

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molecular interaction? First of all, the chlorophylls have to be examined as possible complex partners, since they are available in great amounts in the vicinity of the carotenoids. Besides, a special hint on the chlorophylls is given by an old contradiction, based on experiments with chlorophyll-b-lacking mutants: The absorption-change at 520 nm *in vivo* that is attributed to the carotenoids is smaller and the maximum is shifted to shorter wavelengths, if chlorophyll-b-lacking algae⁷ or chlorophyll-b-lacking mutants of barley⁸ or of pea⁹ instead of the normal

plants are considered. Originally, this findings had led to the conclusion that the absorption-change at 520 nm was due to chlorophyll b (Chl b)¹⁰.

To answer these questions, the electrochromic spectra of asymmetrical assemblies of monomolecular layers of chlorophylls and carotenoids are investigated in thin capacitors. The methods of the experiments and of their evaluation are described in former publications^{1, 4, 11, 12}.

2. Results and Discussion

The spectra of the linear and the quadratic electrochromism of a layer-system of lutein + chlorophyll a (Chl a) are shown in Fig. 1. The Chl a monolayers (with an area per molecule of $A = 107 \text{ \AA}^2$ at a surface-pressure of $\pi = 10 \text{ dyn/cm}$, pH 7.6) have been transferred onto the slide during the dipping process, the lutein monolayers (with $A = 205 \text{ \AA}^2$ at $\pi = 10 \text{ dyn/cm}$) during the withdrawing process, so that the chromophore of Chl a is in contact with the lutein (*cf.* Fig. 5, left hand).

The electrochromisms of lutein alone and of Chl a alone were tested in similar preparations, each with tripalmitin instead of the other respective dye layers. The linear and the quadratic electrochromism of Chl a alone are much smaller than those in Fig. 1 and show no characteristic peaks in the wavelength region between 470 and 570 nm. The linear electrochromism of lutein alone was zero within the limits of error. Thus, it can be stated from Fig. 1 that a strong linear electrochromism is caused by the contact of lutein with Chl a.

The quadratic electrochromism of lutein alone was similar to Fig. 1 (bottom) in the wavelength region between 470 and 570 nm, except that the bands were a little broader and more fused (*cf.* text concerning the quadratic-electrochromism in Fig. 6). On the other hand, in this wavelength region, the quadratic electrochromism in Fig. 1 (bottom) has just the same shape as in a earlier preparation of lutein alone^{4, 13}, where the lutein molecules had got a steeper orientation by addition of Cd-arachidate and application of a higher surface-pressure. Perhaps, the vicinity of the chlorophyll layer induces a similar arrangement in the lutein layer as in that earlier preparation, but the absorption of Chl a is not directly involved in the electrochromic absorption change between 470 and 570 nm.

The similarity between the two spectra in Fig. 1 suggests that the linear electrochromism between 470 and 570 nm is produced from the quadratic electrochromism of lutein by some permanent field

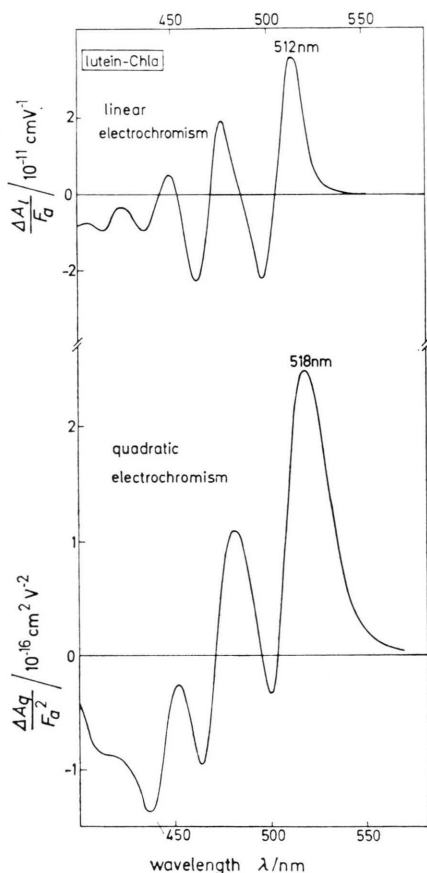


Fig. 1. Electrochromic spectra of lutein + chlorophyll a in contact. Top: Linear electrochromism. Bottom: Quadratic electrochromism. Arrangement of the layers on the microscope slide: Transparent aluminium film / 15 monolayers of Cd-arachidate / 4 monolayers of tripalmitin / 1 monolayer of Chl a / 1 monolayer of lutein / 4 monolayers of tripalmitin / 1 monolayer of Chl a / 1 monolayer of lutein / 4 monolayers of tripalmitin / 16 monolayers of Cd-arachidate / transparent aluminium film. Total amounts of the dyes per area of the light ray cross section: $\Gamma(\text{lutein}) = 1.6 \times 10^{-10} \text{ mol/cm}^2$; $\Gamma(\text{Chl a}) = 3.1 \times 10^{-10} \text{ mol/cm}^2$. Alternating voltage: 12 V_{rms} (results are independent of frequency between 60 Hz and 400 Hz).

according to the following equation (cf. Eqn (55) in ref. 6) :

$$\Delta A_l/F_a = 2(\Delta A_q/F_a^2)F_p. \quad (1)$$

Since corresponding peaks of $\Delta A_l/F_a$ and of $\Delta A_q/F_a^2$ in Fig. 1 have the same sign (*i. e.*, a maximum corresponds to a maximum, and a minimum corresponds to a minimum), it can be concluded from Eqn. (1) that the effective permanent field F_p must be positive, *i. e.*, directed away from the slide. Since the Chl a layer was deposited on the slide prior to the lutein layer, it follows that the field is directed from the Chl a to the lutein, *i. e.*, the force exerted on a *negative* charge in the lutein molecule is directed towards the Chl a.

Now the question arises if this effect is due to a specific interaction of one Chl a molecule with one

lutein molecule, or if it is due to a unspecific permanent field, exerted on the whole lutein layer by the neighbouring Chl a dipole layer? In the latter case, the spectrum of the linear electrochromism should have exactly the same shape as that of the quadratic electrochromism, as it was found *e. g.* for rhodamin b in Cd-arachidate layers^{6, 14}. However, although the two spectra in Fig. 1 are similar, they nevertheless show characteristic differences, *e. g.* for the band width and the wavelength of the main maximum (512 nm as compared to 518 nm), and in the magnitude of the minimum around 500 nm. Thus, the latter model cannot explain the observations, and a more specific interaction of lutein with Chl a must be assumed.

Some ideas for an interpretation of the differences between the two spectra in Fig. 1 are obtained, when the absorption spectrum of lutein in *liquid solution* and its first derivative (Fig. 2, top and middle) are compared with the absorption spectrum of lutein in *solid layers*, which is depicted (together with its first derivative and its quadratic electrochromism) in Fig. 5 of ref. 4 (see also Fig. 5 of ref. 13) :

In *solid layers*, the absorption spectrum of lutein is shifted to longer wavelengths (as compared to liquid solutions), and the absorption bands are broadened and fused, due to the exciton interaction between equal molecules⁵. Since there is no very distinct minimum between the middle and the right absorption band, the first derivative scarcely goes under the zero line around $\lambda = 500$ nm. The same is true for the quadratic electrochromism of the lutein layers, which has the same shape as the first derivative of the fused layer absorption spectrum on the one hand and as the quadratic electrochromism of the lutein-Chl-a preparation in Fig. 1 (bottom) on the other hand.

In contrast to these spectra, the absorption spectrum of lutein in *liquid solution* has rather sharp, separated absorption bands (Fig. 2, top). Consequently, the first derivative of absorption is strongly negative around 500 nm (Fig. 2, middle). This spectrum (which has been shifted by 470 cm^{-1} to smaller wavenumbers) agrees very well with the spectrum of the linear electrochromism of the lutein-Chl-a preparation from Fig. 1 (top), which has been depicted once more in Fig. 2 (bottom). The agreement suggests that this linear electrochromism is due to single lutein molecules with separated ab-

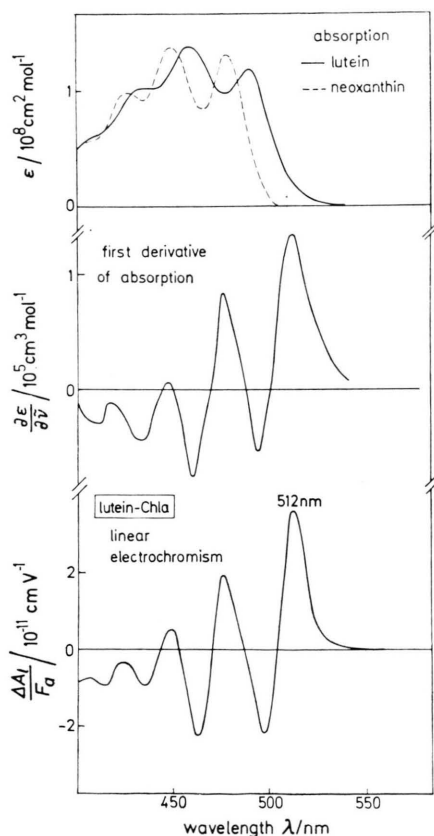


Fig. 2. Top: Absorption of lutein (solid line) and of neoxanthin (dashed line, from ref. ²⁵, with wavelengths of the maxima conformed to the quotations of ref. ²⁶) in liquid solution, solvent: benzene. Middle: First derivative of absorption of lutein with respect to the wavenumber, but shifted to smaller wavenumbers by $\Delta\tilde{\nu} = -470\text{ cm}^{-1}$. Bottom: Linear electrochromism of lutein + Chl a from Fig. 1.

sorption bands like those of lutein in liquid solution. These single lutein molecules may be distinguished from the bulk lutein in the layer by a specific interaction with Chl a.

Thus, we are led to the supposition that the two spectra in Fig. 1 are not due to quite the same species: The quadratic electrochromism is caused by the bulk of the lutein molecules, which seem to be in a similar aggregational state as those in refs 4 and 13, whereas the linear electrochromism in Fig. 1 seems to be due to a small fraction of the lutein molecules, which are distinguished from the bulk by a complex formation with Chl a, and whose contribution to the quadratic electrochromism is hidden by that of the bulk lutein.

The fraction y of distinguished lutein molecules can be estimated from the supposition that the

maxima of their (hidden) quadratic electrochromism would have the same wavelength positions as the maxima of the linear electrochromism in Fig. 1 (top). On the other hand, the maxima of the quadratic electrochromism in Fig. 1 (bottom) have the same wavelength positions as the maxima of the quadratic electrochromism of lutein alone within the limits of error. From this, it can be estimated that only a fraction of $y < 0.1$ of distinguished lutein molecules might be hidden in the spectrum in Fig. 1 (bottom).

Electrochromic spectra of lutein + chlorophyll b (Chl b) in an analogous preparation as in Fig. 1 are shown in Fig. 3. The interpretation of these spectra is more difficult than in the case of lutein + Chl a, since the blue absorption band of Chl b partly overlaps with that of lutein, and moreover it seems to be shifted to longer wavelengths by the interaction with lutein (see below), so that the absorption of Chl b yields a direct contribution to the absorption-change even at $\lambda = 500$ nm. However, at $\lambda = 517$ nm, the contribution of Chl b to the linear electrochromism may be considered as an indirect one only, since the linear and the quadratic electrochromism of Chl b alone at this wavelength are small compared to those in Fig. 3¹⁵. Thus, we can state that a strong linear electrochromism of lutein is produced from the quadratic one also by a contact with Chl b (similar to the influence of Chl a).

Besides, it should be mentioned that the linear electrochromisms $\Delta A_1/F_a$ was independent of the sign of the applied field strength F_a not at every wavelength: The spectrum shown in Fig. 3 (top) was measured with F_a directed from chlorophyll to lutein. In the opposite case, an additional negative peak around $\lambda = 522$ nm was superimposed, which seems to be due to a light-induced charge-transfer from Chl b to lutein. A similar effect was found in the preparation of lutein + Chl a around $\lambda = 528$ nm. These effects are excluded in the present discussion and will be studied in a following paper (Sewe and Reich, in preparation).

Comparing the influences of Chl a and of Chl b on the linear electrochromism of lutein (Fig. 1, top, and Fig. 3, top), the question arises why the main maximum of the lutein-Chl-b complexes appears at longer wavelengths (517 nm) than that of the lutein-Chl-a complexes (512 nm)? It seems that the inherent absorption band of lutein is shifted to longer wavelengths (compared to the spectrum in liquid solution) by Chl b more strongly than by Chl a.

Two causes might contribute to such a result: 1. The partly overlapping of the absorption bands of

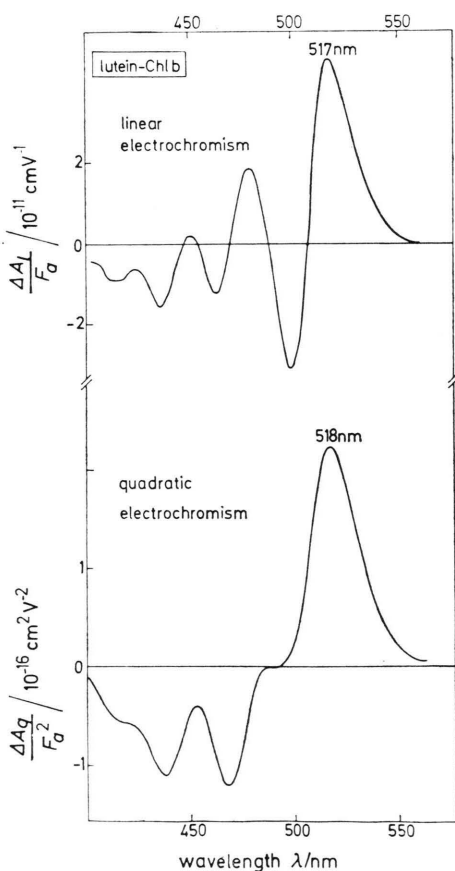


Fig. 3. Electrochromic spectra of lutein + chlorophyll b in contact. Top: Linear electrochromism. Bottom: Quadratic electrochromism. Arrangement of the layers analogous to Fig. 1 $\Gamma(\text{Chl b}) = 2.9 \times 10^{-10} \text{ mol/cm}^2$.

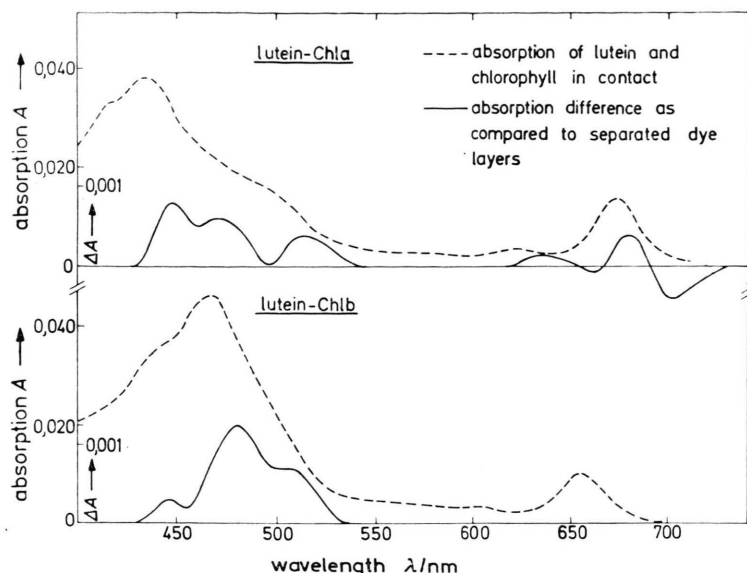


Fig. 4. Top: Total absorption A of a monolayer of lutein and a monolayer of chlorophyll a in contact (dashed line) and absorption difference ΔA between dye layers in contact and dye layers separated (solid line). Bottom: Analogous measurements as above, but with chlorophyll b instead of chlorophyll a. Arrangement of the layers on the slide: 7 monolayers of Cd-arachidate / 4 monolayers of tripalmitin / 1 monolayer of chlorophyll / 1 monolayer of lutein / 4 monolayers of tripalmitin. Cf. Fig. 5.

lutein and Chl b yields a stronger dispersion interaction of lutein with Chl b than with Chl a. 2. The electron affinity of Chl b is greater than that of Chl a by about 0.13 eV¹⁶, thus Chl b may exert a stronger local field on lutein, which should also contribute to the stronger solvatochromic shift of the absorption band.

The hypothesis that only small fractions of the lutein molecules and of the chlorophyll molecules are distinguished by a complex formation with each other is further tested by the absorption spectra in Fig. 4. The dashed lines show the total absorption A of lutein + chlorophyll in contact. The total absorption of the separated dyes differs only very slightly from these spectra: This can be seen from the absorption difference ΔA between dye layers in contact and dye layers separated, which is given in the same figure, but in a scale magnified by a factor of 16 (solid lines). To measure these difference spectra ΔA , a slide, whose layer assembly is shown schematically in Fig. 5, has been moved to an fro in the light ray.

The interpretation of Fig. 4 is easier in the red spectral region, where the chlorophylls are the only absorbing species. At $\lambda = 700$ nm, ΔA in Fig. 4 (top) is negative, *i.e.*, the absorption of Chl a is decreased by the contact with lutein. The absorption at this wavelength may be due to certain hydrated Chl a dimers as discussed by Katz and Norris¹⁷ and by Fong and Koester¹⁸, which seem to dissociate by the action of lutein. (Chl b does not form such

hydrated dimers; this explains why the absorption difference of lutein + Chl b in Fig. 4, bottom, is zero in the red spectra region). The Chl-a-lutein aggregates that are formed instead of the hydrated Chl a dimers are assumed to absorb around $\lambda = 680$ nm, leading to the positive ΔA at this wavelength. If for these aggregates at 680 nm approximately the same molar absorption coefficient as for the bulk Chl a at 675 nm is assumed, the fraction of the Chl a molecules that are involved in the complex formation with lutein is about 0.028. Since the amount of lutein is about half the amount of

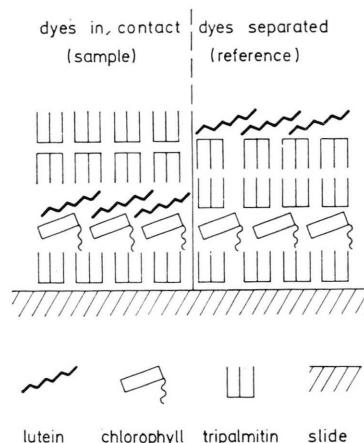


Fig. 5. Schematic picture of the layer assembly to measure the absorption difference spectra in Fig. 4. (Specific complex formation is not indicated in this picture; for this, cf. Fig. 10.)

Chl a in the layers, the fraction of lutein molecules that are distinguished by complex formation with Chl a should be $y \approx 0.056$ (*cf.* estimation from Fig. 1).

In the blue spectral region, the absorption difference ΔA in Fig. 4 has no negative values, *i. e.*, the absorption on the whole is increased by the contact of the dyes. The absorption maxima can be mainly attributed to the chlorophylls according to their wavelength positions (434 nm for Chl a and 465 nm for Chl b). The main maxima of the ΔA -spectra might also be tentatively attributed to special chlorophyll molecules, whose absorption bands are increased and shifted to longer wavelengths by about 14 nm through the interaction with lutein. However, if the absorption band is broadened and shifted so strongly, it must be concluded once again from the small ratio of $\Delta A/A$ that only a small fraction of the chlorophyll molecules are involved in this complex formation.

The absorption differences in the region from 510 to 530 nm in Fig. 4 will be discussed in a following paper in connection with the above mentioned charge-transfer effect (Sewe and Reich, in preparation).

The observations of Fig. 4 suit well with the above model that only a small fraction ($y < 0.1$) of

the lutein molecules display a specific interaction with chlorophyll molecules, which were shown to exert attraction forces on negative charges in the lutein. In order to test, which part of the chlorophyll molecules accounts for these attraction forces, analogous measurements as in Fig. 1 were performed on a preparation of lutein with pheophytin a instead of Chl a. (Pheophytin a was produced from Chl a by shuttling the benzene solution with diluted aqueous HCl.) Fig. 6 shows the absorption spectrum of this preparation, together with the linear and the quadratic electrochromism. In the wavelength region from 470 to 570 nm, the quadratic electrochromism agrees exactly with that of an analogous preparation of lutein alone, except that the shoulder around 540 nm is missing in the absence of pheophytin. In the same wavelength region, the linear electrochromism is practically zero.

So, pheophytin does not produce a linear electrochromism of lutein from the quadratic one, in contrast to Chl a. Since the only difference between

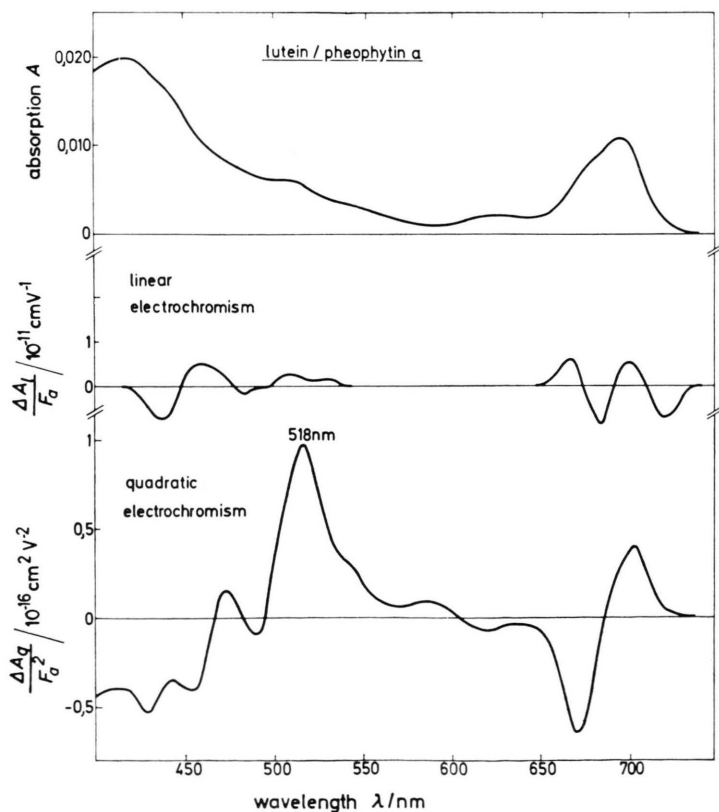


Fig. 6. Spectra of lutein + pheophytin a in contact. Top: Absorption. Middle: Linear electrochromism. Bottom: Quadratic electrochromism. Arrangement of the layers analogous to Fig. 1.

Chl a and pheophytin a is given by the central magnesium atom of Chl a, the magnesium atom must account for the attraction forces exerted on the electrons in the lutein.

It is now the question if similar forces are also exerted on other carotenoids existing in the membrane of photosynthesis, or if the interaction is restricted to the characteristic groups of lutein? The structure formulae of the four main carotenoids occurring in chloroplast are depicted in Fig. 7.

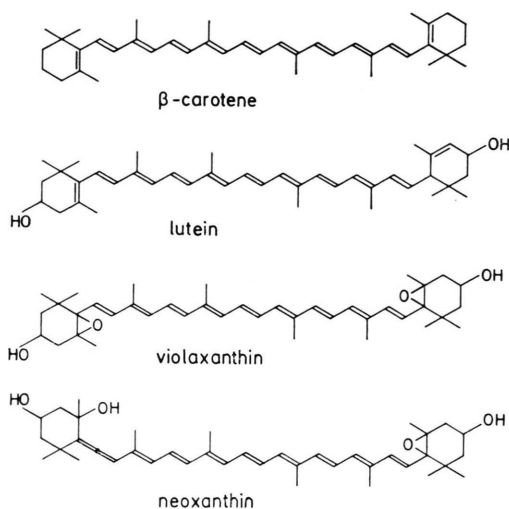


Fig. 7. Structure formulae of the four main carotenoids occurring in chloroplasts.

Assemblies with pure monolayers of β -carotene cannot be prepared in the same way as with lutein, since β -carotene has no hydrophilic groups and so does not form stable monomolecular films on a watersurface. However, stable monolayers are formed, e. g., of a mixture of β -carotene + Chl a in the molecular proportion 1 : 3. In such a film, the porphyrin rings lie on the water surface, and the β -carotene molecules are located together with the phytol chains on the air side of the film, as can be concluded from surface-pressure/area diagram. Depositing this film onto a slide during the withdrawing process, alternating with monolayers of tripalmitin, a similar asymmetrical arrangement as with lutein + Chl a was built up.

The quadratic electrochromism of β -carotene + Chl a is shown in Fig. 8 (thin line). For comparison, the quadratic electrochromism of Chl a alone is also shown (dashed line). The difference between both spectra corresponds to the quadratic electrochromism of β -carotene alone (fat line). The

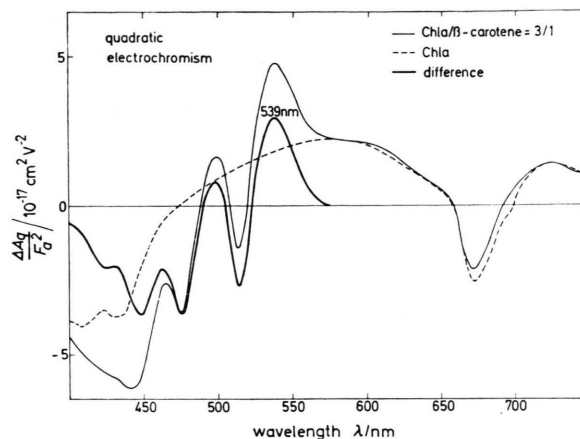


Fig. 8. Quadratic electrochromism of β -carotene + chlorophyll a in contact (thin solid line), of Chl a alone (dashed line), and of the difference between these two spectra (fat solid line). The mixed monolayers of β -carotene + Chl a (1 : 3) at $\pi = 10$ dyn/cm were deposited on the slide during the withdrawing process. Arrangement of the layers on the slide: Transparent aluminium film / 5 monolayers of Cd-arachidate / 21 monolayers of tripalmitin / 1 monolayer of β -carotene + Chl a (1 : 3) / 5 monolayers of tripalmitin / 1 monolayer of β -carotene + Chl a (1 : 3) / 20 monolayers of tripalmitin / 6 monolayers of Cd-arachidate / transparent aluminium film. $I'(\text{Chl a}) = 3 \times 10^{-10}$ mol/cm²; $I'(\beta\text{-car}) = 1 \times 10^{-10}$ mol/cm². The linear electrochromism between $\lambda = 470$ nm and $\lambda = 570$ nm of this preparation was zero.

linear electrochromism of β -carotene + Chl a was zero in the wavelength region from 470 to 570 nm. So there is no asymmetrical complex formation between β -carotene and Chl a. Thus, for the complex between lutein and Chl a, it can be concluded that one of the OH-groups of the lutein molecule accounts for the interaction with the magnesium atom of chlorophyll.

According to Katz *et al.*¹⁹, such an interaction can be explained by the fact that in chlorophyll the magnesium atom with coordination number 4 is coordinatively unsaturated, and the electron gap can be occupied by nucleophilic electron donor groups of polar molecules, such as water, alcohols, ethers, ketones, or of such groups from other chlorophyll molecules. Obviously, by such an interaction, negative charges are drawn from the lutein to the chlorophyll, in agreement with our observations. For coordination to the magnesium atom of a chlorophyll, the lutein has to compete with water and with the keto oxygen of other chlorophylls. This explains that only a small fraction of the lutein molecules are distinguished by a complex formation with chlorophyll.

Is there any difference between the two OH-groups of lutein with regard to the interaction with chlorophyll? It should be expected that the allyl-OH-group (on the right hand in the structure formula of lutein in Fig. 7) is more favourable than the other OH-group, since in case of a partial transfer of negative charge to the chlorophyll, the remaining positive partial charge can be stabilized by delocalization over the neighbouring allyl double bond. Such a mesomeric effect is not possible with the other OH-group of the lutein molecule, nor with the OH-group of violaxanthin, but equally well with one of the OH-groups of neoxanthin (*cf.* Fig. 7). In the latter case, the positive partial charge is shifted even nearer to the conjugated π -electron chain of the carotenoid molecule, thus it should cause a stronger effective permanent field.

3. Conclusions on Biological Systems

The present results *in vitro* suggest that the effective permanent field, which is imposed on the carotenoids in the membranes of photosynthesis, is also due to a complex formation of carotenoids with chlorophylls. In this case, the field-indicating absorption-change of chloroplasts around $\lambda = 520$ nm should be mainly due to the linear electrochromism of the complex of lutein + Chl b, which is depicted in Fig. 3 (top). For a quantitative comparison, the absorption-change at 520 nm *in vivo* can be approximately calculated by the equation

$$\Delta A_{\text{calc}}^{520} = \left(\frac{\Delta A_1}{F_a} \right)_{\text{vitro}} \cdot F_{\text{vivo}} \cdot \frac{A_{\text{vivo}}}{A_{\text{vitro}}}, \quad (2)$$

if possible differences between *in vivo* and *in vitro* with regard to the orientation of the distinguished lutein molecules in the electric field are neglected. In this equation, A_{vivo} and A_{vitro} denote the absorptions in the maximum of the most long-waved band of the lutein molecules that are distinguished by complex formation with Chl b.

If the total amount of lutein *in vivo* is tentatively assumed to be involved in this complex formation, A_{vivo} can be calculated from the equation

$$A_{\text{vivo}} = 2.3 \varepsilon c_{\text{car}} x_{\text{lut}} l \quad (3)$$

where $c_{\text{car}} = 2 \times 10^{-6} \text{ M}$ is the total concentration of carotenoids⁵ in the experiments of Emrich *et al.*², $x_{\text{lut}} = 0.333$ is the relative mole fraction of lutein, $l = 2 \text{ cm}$ is the length of the cuvette⁵, and ε is approximately equal to the molar decadic absorption coefficient of lutein in benzene solution in the maximum at $\lambda = 491 \text{ nm}$ ($\varepsilon = 1.19 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$).

A_{vitro} can be estimated from the spectrum in Fig. 4 (top), where the absorption $A = 0.014$ at $\lambda = 500 \text{ nm}$ is mainly due to lutein. This value has to be multiplied with a factor of 2 (since the preparation of Fig. 4 contained only half the dye layer of Fig. 3), and with the fraction y of distinguished lutein molecules *in vitro*. Calculating with the value $y = 0.056$, which was estimated for the lutein-Chl-a complexes, we get: $A_{\text{vitro}} = 0.0016$. The field strength across the membrane induced by a short flash is $F_{\text{vivo}} = 2 \times 10^5 \text{ V/cm}$ ^{15, 20, 21}. From the maximum in Fig. 3 (top) at 517 nm, we read: $(\Delta A_1/F_a)_{\text{vitro}} = 4.6 \times 10^{-11} \text{ cm/V}$.

Thus, it follows from Eqn. (2): $\Delta A_{\text{calc}}^{520} = 2.1 \times 10^{-3}$. This result agrees with the experimental absorption-change at 520 nm in chloroplasts from ref. 2: $\Delta A_{\text{exp}}^{520} = 1.7 \times 10^{-3}$. In view of the neglects and simplifications of the calculation, this agreement should not be overrated, but it shows that the amount of lutein in chloroplasts would be sufficient to account for the field-indicating absorption-change at 520 nm by complex formation of the lutein with Chl b. (If some fraction of the lutein is bound to Chl a instead of Chl b, $\Delta A_{\text{calc}}^{520}$ becomes a little smaller, but the agreement with $\Delta A_{\text{exp}}^{520}$ still remains preserved within the uncertainty of the approximation.)

On the basis of this model, it should be expected that the absorption-change at 520 nm becomes much smaller, if Chl-b-lacking mutants instead of normal plants are considered. In Fig. 9 (top), the light-dark difference spectra of normal pea leaves and of Chl-b-lacking pea mutant leaves as measured by Heber⁹ are compared. One might argue that these measurements also contain slow absorption-changes (1 s), which have nothing to do with the breakdown of the light-induced electric field across the membrane; however, in the wavelength region between 470 and 530 nm, the essential differences between the two spectra could be qualitatively confirmed also in rapid flash-photometric control measurements (20 ms) by Ch. Wolff (1972, unpublished). These differences are the following: In the mutant, as compared to the normal pea, the absorption-change at 520 nm is substantially diminished, although the carotenoid content is even a little larger*. A smaller maximum appears at 512 nm. In the zero-crossing of the normal form at 495 nm, the mutant has a minimum, and at the minimum of the normal form at 480 nm, the mutant has a maxi-

* Per 100 molecules of Chl a, the normal pea contains 25.4 Chl b and 47.7 carotenoids, and the pea mutant 1206 A contains no Chl b and 53.5 carotenoids.

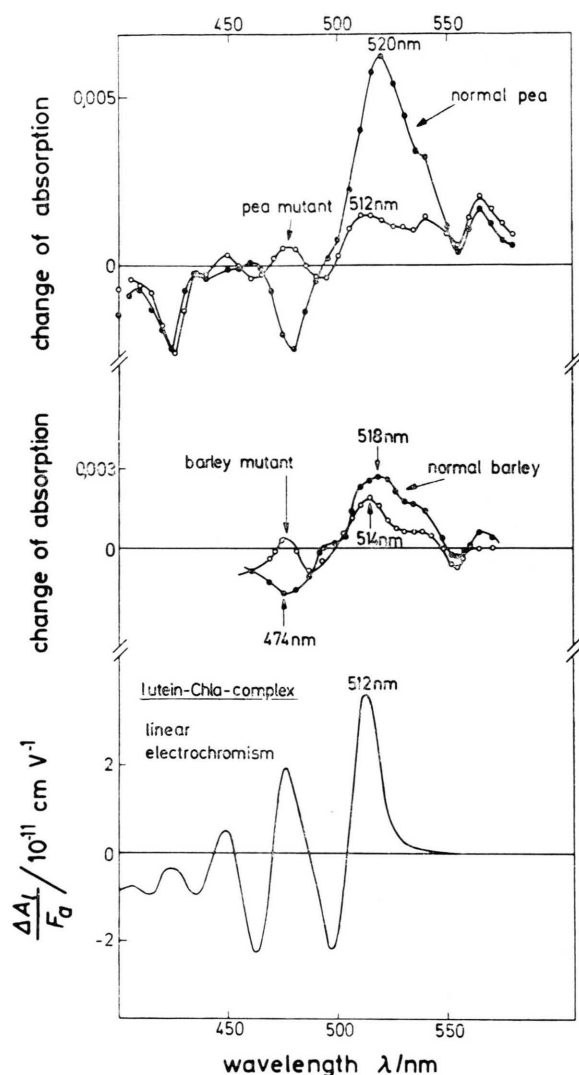


Fig. 9. Top: Light-dark difference spectra of a normal pea leaf and of a Chl-b-lacking pea mutant leaf (1206 A), measured by Heber⁹. Decrease of absorption within 1 s after illumination for 5 s with red light (1.09×10^{-2} W/cm²; filter combination: RG 630 Schott + Calflex C + 6 cm water). Middle: Light-induced absorption-change spectra of normal barley leaf and of Chl-b-lacking barley mutant leaf, measured by Hildreth⁸ (intermediate phase, 100 μ s after laser flash). Bottom: Linear electrochromism of lutein + Chl a in contact, from Fig. 1.

mum. Similar differences between a Chl-b-lacking mutant and the normal form were found by Hildreth⁸ in flash-photometric measurements on barley leaves. These absorption-change spectra are depicted in Fig. 9 (middle).

The lack of Chl b in the mutants is indicated by the absence of the minimum around 478 nm, which

has been attributed to Chl b since a long time already²². However, the interesting point is that the lack of Chl b also diminishes the "carotenoid-signal" around 520 nm, which (according to our new model) is mainly due to lutein-Chl-b complexes. The corresponding absorption-change of lutein-Chl-a complexes, which are still present in the mutants, is located at shorter wavelengths (512 nm).

The whole spectrum of the linear electrochromism of lutein + Chl a is once more shown in Fig. 9 (bottom). From this comparison, the absorption-change of the mutant spectra around 512 nm can be attributed to the electrochromism of lutein-Chl-a complexes. However, it is rather surprising that the spectrum in Fig. 9 (bottom) agrees with the mutant spectra in Fig. 9 (middle and top) not only in the wavelength of this maximum, but also approximately in the wavelengths of the other minima and maxima. So, the contributions of the other carotenoids to the electrochromic spectrum of the mutants seem to be not very essential.

The minimum of the mutant spectra around 493 nm in Fig. 9 can be explained by the corresponding minimum of the lutein-Chl-a complex; however, the question arises why the *normal* spectra in Fig. 9 have a shoulder at this wavelength, although the lutein-Chl-b complex has also a minimum here (*cf.* Fig. 3)? This suggests that an electrochromic maximum of any other species is superimposed around 493 nm, and the amount of this species should be strongly diminished in the Chl-b-lacking mutants.

To answer this question, the possible contributions of the other carotenoids have to be considered. The relative mole fractions of the four main carotenoids in normal barley and in the barley mutant from measurements of Thornber and Highkin²³ are given in the following Table:

	normal barley	barley mutant
β -carotene	0.165	0.155
lutein	0.44	0.475
violaxanthin	0.24	0.29
neoxanthin	0.15	0.075

The only carotenoid, whose mole fraction is substantially diminished in the mutant (by a factor of 2), is neoxanthin. It is true that the absolute content of neoxanthin is not very high, but, on the

other hand, neoxanthin is expected to give a particularly strong linear electrochromism by complex formation with chlorophylls (*cf.* last section). From a comparison of the absorption spectra of lutein and neoxanthin (*cf.* Fig. 2), the linear electrochromism of neoxanthin-Chl-b complexes may be expected around 493 nm. So, these complexes should account for the shoulder of the normal spectrum in Fig. 9 (middle) at this wavelength.

From these results, the carotenoid violaxanthin seems to play a minor role in the field-indicating absorption-change spectrum of photosynthesis, at least in the case of barley. Perhaps, violaxanthin is not located in the hydrophobic region of the membrane (where the light-induced electric field strength is maximal), since it was found by Hager²⁴ that both epoxid groups are accessible from the hydrophilic inner space of the thylakoid.

In the spectral region beyond 530 nm, the absorption-changes in Fig. 9 (top and middle) are partly due to some reactions of the electron transport chain (cytochromes) which have not been separated from the field-indicating absorption-changes in these measurements. Additionally, around 539 nm, a small quadratic electrochromism of β -carotene should be expected from the results

The previous attribution of Chl a, Chl b, and the carotenoids to the different maxima and minima of the field-indicating absorption-change spectrum of spinach chloroplasts⁵ is more specified by the new results, with regard to the individual carotenoids. It should be assumed that a similar electrochromic spectrum of the summarized carotenoids as in that paper can also be constructed without violaxanthin, but with a stronger weight on neoxanthin, as a superposition of the electrochromic spectra of lutein- and neoxanthin-complexes with the different chlorophylls, and further contributions of β -carotene. If all lutein molecules *in vivo* are assumed to be exposed to a permanent field by complex formation, the absorption spectrum of these lutein molecules would have nearly the same gradient around 520 nm as the spectrum of the weighted and summarized carotenoids in ref. 5. From this, by Eqn. (6) in ref. 5, the mean angle between the electric field and the long axis of the molecules was approximately calculated to be $\vartheta \approx 74^\circ$, if the values $F_a = 2 \times 10^5$ V/cm for the light-induced field-change and $F_p = 2 \times 10^6$ V/cm for the permanent field were inserted⁵. (Since the values of F_p and F_a both may be uncertain about by a factor of 2, the mean value of ϑ resulting from Eqn. (6) in ref. 5 varies between 56° and 82° .) From the values of ϑ and F_p , a local molecular field of $F_l = 5.5 \times 10^5$ V/cm parallel to the long axis of the carotenoid molecule was calculated (by Eqn. (26) in ref. 1). Now we can say that this local field is caused by the electron-attracting forces of the magnesium atom of chlorophyll.

of Fig. 8, which may contribute to the spectra of Fig. 9 at this wavelength.

Since in normal plants the field-indicating absorption-change at 520 nm is much greater than at 512 nm, we can conclude that lutein is preferably bound to Chl b rather than to Chl a, although in chloroplasts the total amount of Chl a is greater than that of Chl b about by a factor of 2. However, it is known that Chl b is located mainly (or even exclusively) in the regions of the membrane that belong to photosystem II^{27, 28}, and here the concentration of Chl b is higher than that of Chl a. Hence, lutein should also be restricted mainly to photosystem II. This would agree with the finding of Radunz and Schmid²⁹ that an antiserum to lutein inhibits some reactions in photosystem II, but none in photosystem I.

From the model that the bulk chlorophylls account for the effective permanent field that is imposed on the carotenoid molecules *in vivo*, some conclusions on the asymmetrical arrangement of the dye molecules in the membrane of photosynthesis can be drawn: Since the electric field is directed from the chlorophyll to the carotenoid

orientation of the chlorophylls and carotenoids

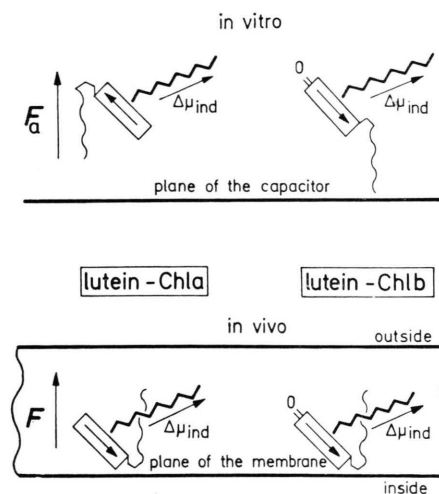


Fig. 10. Asymmetrical arrangement of the lutein-chlorophyll complexes *in vitro* (in the capacitor) and *in vivo* (in the thylakoid membrane). The cross sections through the porphyrin rings are symbolized by rectangles, the characteristic CHO-group of Chl b by an O-atom, the phytol chains by thin zigzag lines, and the lutein molecules by fat zigzag lines. The arrows indicate the orientation of the permanent dipole moment difference of the blue absorption band of the chlorophylls⁵, and the orientation of the induced dipole moment difference of the lutein molecules, respectively, relative to the applied external field F_a in the capacitor or to the light-induced field F in the membrane.

(cf. text following Eqn. (1) in the last section), and since the permanent field is directed from inside to outside of the thylakoid¹, the chlorophylls must be located more to the inside and the carotenoids more to the outside of the thylakoid. The resulting molecular arrangements *in vitro* and *in vivo* are depicted schematically in Fig. 10. The orientation of the chlorophyll molecules *in vitro* is opposite to that in ref. 5, since they had been transferred onto the slide during the dipping process in the present work, but during the withdrawing process in ref. 5. *In vivo*, the porphyrin ring is oriented with the corner bearing the phytol chain towards the inside of the thylakoid¹⁵. Since the carotenoids must be located more to the outside of the thylakoid, and since the hydrophobic phytol chain will point to the hydro-

phobic carotenoids, it will form an acute angle with the porphyrin plane. One end of the carotenoid is attached to the magnesium atom of a chlorophyll, and the dipole moment difference that is induced in the carotenoid by this complex formation has a component from inside to outside of the thylakoid.

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