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LIGHT-INDUCED SHIFTS IN PIGMENT ABSORPTION IN GREEN, RED AND BLUE-GREEN ALGAE

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SUMMARY

1. Light-induced absorption changes in the region 640–730 nm which were not associated with the oxidation of the primary electron donor of system 1, P700, were observed in green, red and blue-green algae. The shape of the difference spectra, showing alternating negative and positive bands, suggested shifts in the absorption bands of chlorophyll *a* types and of chlorophyll *b*.

2. The chlorophyll absorption changes were correlated with changes in the blue spectral region, due to carotenoids. Absorption shifts of phycobilins were not observed. The uncoupler carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) at low concentration stimulated the carotenoid and chlorophyll changes and inhibited at higher concentration.

3. Difference spectra of the changes brought about by Photosystems 1 and 2 in *Scenedesmus obliquus* were approximately proportional to each other, indicating that shifts of chlorophyll *a* and *b* and carotenoid occur in the same relative proportion, independent of the activating photosystem.

4. The results, together with older evidence, indicate that shifts in absorption spectra of photosynthetic pigments are universal phenomena, probably correlated with an energized state of the photosynthetic membrane. Evidence that the well-known change at 515 nm in green algae and higher plants is due to a carotenoid rather than to chlorophyll *b* is discussed.

INTRODUCTION

During recent years many studies have been made of changes in pigment absorption which occur in photosynthetic material upon illumination. Some of these changes are due to the oxidation–reduction reaction of reaction-center chlorophyll or bacteriochlorophyll, but there exists also a second type of absorption changes which is probably not caused by a chemical reaction, but by a physicochemical change in the environment of the molecule. In photosynthetic bacteria these changes consist of red shifts of carotenoid bands and of infrared absorption bands of bacteriochlorophyll[1–3]. In algae and higher plants band shifts of carotenoids have been observed[4,5]

Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; PMS, *N*-methylphenazonium methosulphate.

(which are probably at least in part responsible for the well-known absorption increase at 515 nm, discovered already 17 years ago[6] in green algae), as well as absorption changes attributed to chlorophyll *b* (ref. 7). Recent evidence also indicated the participation of chlorophyll *a* in spinach chloroplasts[8] and perhaps also in blue-green algae[9].

This paper gives the results of a comparative study of these phenomena in several species of green, red and blue-green algae. The results indicate that in all species tested, light-induced band shifts of chlorophylls occur, which are, except in blue-green algae, accompanied by changes in carotenoid absorption, and which are probably all caused by the same effect, presumably a physicochemical change in the photosynthetic membrane.

MATERIAL AND METHODS

The algae were grown at 25° in liquid culture medium as described elsewhere[10]. *Scenedesmus obliquus*, *Chlorella vulgaris* and *Porphyridium cruentum* were grown in the media given by refs. 11, 12 (M. C. medium) and 13, respectively; the growth media for *P. aerugineum* and *Anacystis nidulans* are given in ref. 10.

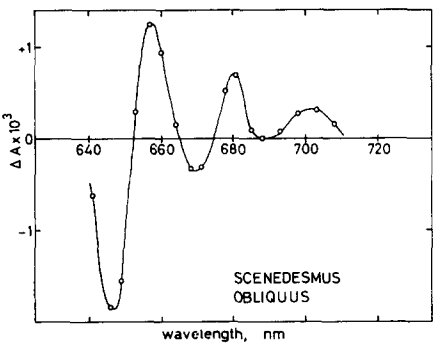
Light-induced absorption changes were measured with an apparatus described earlier[14]. The algae, suspended in fresh growth medium, were contained in a 1-mm vessel. The absorbance at 680 nm, corrected for scattering was 0.6. The intensities of actinic light given were those at the place of the vessel; the intensities "seen" by the cells in the suspension were at least 50% lower. The measurements were done at room temperature (about 22°).

RESULTS

Fig. 1 shows a difference spectrum of the green alga *S. obliquus*, obtained upon illumination with light of moderate intensity. The spectrum shows alternating negative and positive bands, which can be analysed as red shifts of a chlorophyll *b* band with a maximum at or close to 652 nm and of a chlorophyll *a* band at about 674 nm. The small band at about 700 nm, suggesting a red shift of a chlorophyll *a* band at 690–695 nm, was variable in size and absent in some samples. *C. vulgaris* gave a similar difference spectrum with relatively smaller chlorophyll *a* bands, however. Under our conditions, no shift in the oxidation state of P700 was observed; when 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was added, accumulation of oxidized P700 occurred upon illumination. A negative band at about 650 nm in Chlorella has been observed already by KOK[15] and RUMBERG[7], but at longer wavelengths their spectra were different from those presented here, partly because of additional absorption changes due to P700 and associated band shifts[15,16]; partly for reasons which are not clear[7]. A recent report of EMRICH *et al.*[8] presents a difference spectrum of spinach chloroplasts obtained by kinetic analysis of fast absorption changes which, except for a different ratio of the heights of the various bands, is similar to that of Fig. 1.

Absorption difference spectra obtained with red and blue-green algae were more difficult to analyse. Normally with these algae the difference spectra in the red region were dominated by the bleaching of P700 (ref. 15) and its associated bandshift[16]

at about 685 nm. However, addition of a redox catalyst such as *N*-methylphenazonium methosulphate (PMS), 2,3,5,6-tetramethyl-*p*-phenylenediamine or 1,4-naphthoquinone eliminated or strongly diminished these absorption changes, presumably because of a fast re-reduction of P700 catalysed by these substances, and under these conditions spectra of the type shown in Fig. 2 were obtained. The spectra of the red algae *P. cruentum* and *P. aerugineum* are almost identical, showing positive bands at



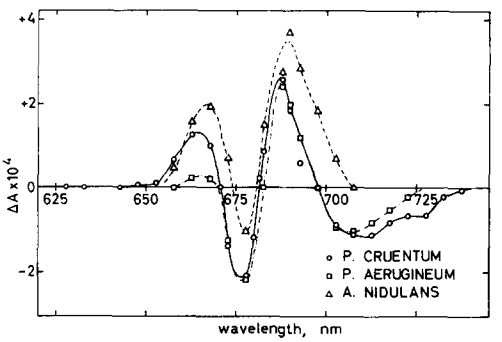


Fig. 1. Absorption difference spectrum (light *minus* dark) of *S. obliquus*. Illumination was by a band at 540 nm, half-width 40 nm, intensity 4 nEinstein·cm^{-2}·sec^{-1}. Light regime: 30 sec dark, 3 sec light.

Fig. 2. Adsorption difference spectra (light *minus* dark) of red and blue-green algae. The spectra of *P. cruentum* and *P. aerugineum* were measured in the presence of $1 \cdot 10^{-4}$ M PMS. Illumination was by a band at 425 nm, half-width 45 nm, intensity 3.0 nEinstein·cm^{-2}·sec^{-1}. Light regime: 15 sec dark, 5 sec light. For *A. nidulans* the conditions were: $1 \cdot 10^{-5}$ M PMS, illumination by a band at 530 nm, half-width 45 nm, intensity 1.0 nEinstein·cm^{-2}·sec^{-1}, light regime: 20 sec dark, 6 sec light.

687 nm and 665 nm, and negative bands at 676 and above 700 nm. The main difference was in the height of the band of 665 nm. The shape of the spectra suggest that both blue and red shifts of chlorophyll *a* absorption bands occur in these species. The spectra could be described as a red shift of a band at about 680, and blue shifts of bands at about 697 and 670 nm. The spectrum of *A. nidulans* was similar, but the total area above was much larger than that below the zero line, indicating that the spectrum cannot be analysed by bandshifts alone in this species. No absorption changes in the region of phycocyanin absorption were observed in *P. aerugineum* and *P. cruentum*.

The relation between chlorophyll and carotenoid shifts; effect of various additions

The characteristics of the absorption changes in the red were compared with those of carotenoids in the blue-green region. Fig. 3a shows the intensity dependence of the absorption changes at 680, 658–648 and 516 nm in *S. obliquus*. The light curves, especially of the last two phenomena, are seen to be very similar. This was also true in the presence of DCMU and 25 μM PMS. As will de discussed below, the absorption increase at 516 nm is probably due, at least in part, to a red shift of the absorption spectrum of a carotenoid; for sake of simplicity we shall call it, like the corresponding changes in other algae, "carotenoid shift".

DCMU gave a partial inhibition of the carotenoid shift (as observed earlier, *e.g.* ref. 17) and of the chlorophyll shifts; subsequent addition of PMS gave a stimulation. PMS alone had little effect.

Corresponding results obtained with *P. aerugineum* are shown in Fig. 3b. In the presence of PMS with *P. aerugineum* and *P. cruentum* difference spectra were observed with maxima at 452, 482 and 518, and minima at 467 and 498 nm, similar to those observed with other red algae[4] and indicating the red shift of a carotenoid. As Fig. 3b shows, the light curves at 520 and 690 nm were similar; the one at 484 nm showed a deviation at low intensities.

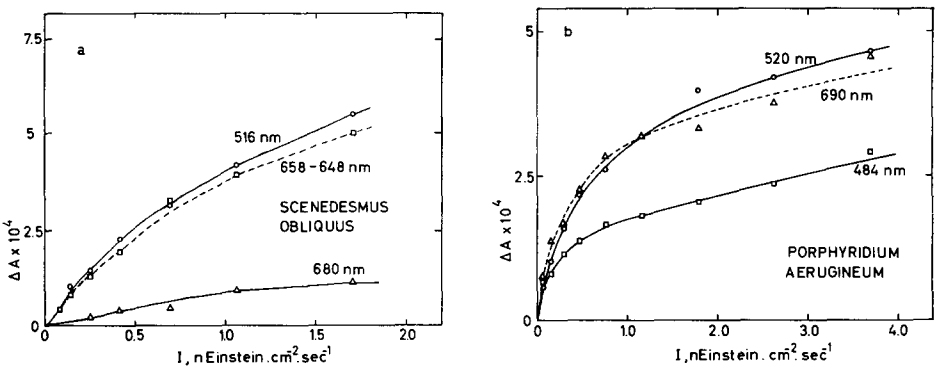


Fig. 3. (a) Intensity dependence of the absorption increase at 516 and 680 nm and of the absorption increase at 658 *minus* the decrease at 648 nm in *S. obliquus*. Illumination was by a band at 425 nm, half-width 45 nm, light regime: 10 sec dark, 5 sec light. (b) Intensity curves for *P. aerugineum* in the presence of $1 \cdot 10^{-4}$ M PMS. Illumination: 618 nm, half-width 15 nm, light regime: 20 sec dark, 3 sec light. Since part of the change,especially at 520 nm, did not or slowly reverse in the dark, the rapidly (within 1 sec) reversible part only was plotted.

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF FCCP UPON THE ABSORPTION CHANGES IN *S. obliquus*

Illumination as for Fig. 3a, intensity 3.0 nEinstein·cm^{-2}·sec^{-1}. Light regime: 15 sec dark, 5 sec light. The absorbance changes are measured in $\Delta A \times 10^4$.

Wavelength (nm)	$\Delta A \times 10^4$			
	FCCP concn. (μM): 0	0.2	1	2
515	8.6	19.5	13.3	2.9
658–648	8.4	18.5	11.5	3.4
680	3.0	5.7	3.7	1.1

The carotenoid and chlorophyll absorption shifts were similarly affected by carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) both in *S. obliquus* and *P. aerugineum* (Table I and Fig. 4). Low concentrations gave a stimulation, higher concentrations inhibited the absorption changes. The concentration for 50% inhibition was about 1.5 μM; stimulation occurred at around 0.1 μM. In *P. aerugineum*, the band at 452 nm was only partially inhibited by FCCP, indicating that it was caused at least in part, by a different component. This also applied to a negative band at 430 nm, observed in the presence of PMS, and which may have been due to a *b*-type cytochrome (*cf.* ref. 18). Gramicidin D did not inhibit the chlorophyll and carotenoid shifts in *S. obliquus* and *C. vulgaris*.

As with other blue-green algae[4], absorption changes due to a carotenoid were not observed in *A. nidulans*, either with or without PMS. In the blue region, with PMS, a negative band at 432 and a positive band at about 412 nm was formed upon illumination, probably due, like in *P. aerugineum*, to oxidation of cytochrome *b*. The usual bands of cytochrome *f* were absent with PMS in red and blue-green algae.

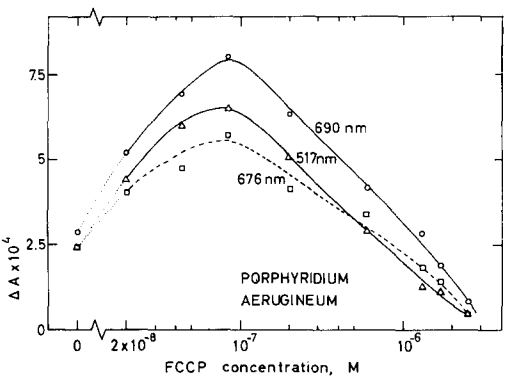


Fig. 4. Effect of FCCP upon the absorption change at three different wavelengths in *P. aerugineum*. Other conditions as for Fig. 2.

Action of Systems 1 and 2

Earlier experiments have shown that the carotenoid changes in various types of algae[4,5,17,19] and in spinach chloroplasts[20] are caused by both Photosystem 1 and 2, and that the action of the photosystems can be separated by choosing appropriate conditions.

In order to determine whether the shifts of chlorophyll *a* and *b* absorption in *S. obliquus* were brought about by both photosystems, and in what proportion, difference spectra were measured of the absorption changes brought about by each photosystem. The results are given in Fig. 5. The spectra shown here were measured (a) with DCMU and PMS and (b) with a continuous background of far-red light without

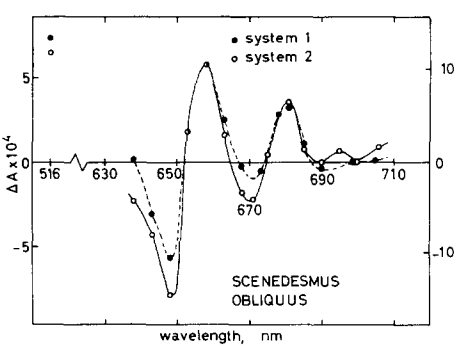


Fig. 5. Absorption difference spectra of *S. obliquus* brought about by System 1 (●---●) and System 2 (○—○), respectively (see text). Illumination as for Fig. 3a, intensity 1.1 nEinstein·cm^{-2}·sec^{-1} for both spectra. The left-hand ordinate scale applies to the first, the right-hand scale to the second spectrum; the deflections at 516 nm have been reduced by a factor of two. The first spectrum was obtained with a light regime of 1 min dark and 1 sec light in the presence of 25 μM of DCMU and PMS. The second spectrum was obtained without additions, but with a continuous background of 698 nm (0.4 nEinstein·cm^{-2}·sec^{-1}) and 2 sec additional illumination per min.

added substances. In order to check whether these conditions were suitable to distinguish the actions of Systems 1 and 2, we measured the effectivities of far-red (692 nm) and blue light (485 nm) to bring about the absorption decrease at 648 nm. The activity of far-red compared to blue light was found to be 2.5 times higher with DCMU and PMS than with far-red background without these compounds. This indicates that the corresponding difference spectra of Fig. 5 represent the action of System 1 and System 2 mainly; the result agrees with action spectra for the absorption changes at 515 and 482 nm of *Ulva lobata*[17] and *Botrydiopsis alpina*[5], respectively measured under similar conditions. It can be seen that the two difference spectra are very similar, indicating that both photosystems bring about a shift in the absorption of chlorophyll *a* and *b* and carotenoid (516 nm) in the same proportion.

DISCUSSION

The data given in this paper indicate that light-induced shifts in pigment absorption are universal phenomena in photosynthetic organisms. In the various types of algae tested and in spinach chloroplasts, absorption shifts of chlorophyll have been observed; carotenoid shifts have been seen in higher plants and eucaryotic algae and in purple bacteria both carotenoid and bacteriochlorophyll shifts have been recorded. The experiments reported here indicate a correlation between chlorophyll and carotenoid shifts in green and red algae under various conditions. This is in agreement with data of EMRICH *et al.*[8] for spinach chloroplasts, who reported a correlation of the decay rates with various concentrations of gramicidin. The lack of correlation between the absorption changes at 515 and 648 nm reported by HILLER[21] stands in contrast to these conclusions. However, no corresponding spectra were given, so it is difficult to conclude whether other phenomena complicated his results.

Consideration of all evidence available now (see also refs. 22, 23) leads to the conclusion that the absorption change at 515 nm in green algae and higher plants, which has been attributed to a variety of compounds (see ref. 24 for a review) is for the main part due to the red shift of a carotenoid. The main reason to assume that it is due to chlorophyll *b* (ref. 7), *viz.* its correlation with absorption changes at about 475 and 650 nm is of doubtful value now, since there is also a correlation with absorption changes at 670 and 680 nm, which are probably due to chlorophyll *a*. Moreover, in other algae and in a barley mutant without chlorophyll *b*, the bands at 515–520 nm are clearly due to red shifts of carotenoids[4,5], as indicated by the difference spectrum. The experiments reported here indicate that, at least in red algae, these bands too are correlated with chlorophyll absorption shifts. Therefore, the most logical conclusion appears to be that in chlorophyll *b* containing organisms the band at 515 nm is also due to a carotenoid, and that the carotenoid bands at shorter wavelengths are obscured by chlorphyll *b* absorption changes in these species.

The wavelengths of the band shifts produced by light are in good agreement with the wavelengths of chlorophyll *a* bands that have been observed by absorption spectroscopy[25]. In *S. obliquus* the band shift centered at 674 nm corresponds with the chlorophyll *a* component absorbing at about 673 nm. The chlorophyll *a* type absorbing at about 683 nm shows no significant band shift; the band at 690–695 nm may correspond to the band at 695 nm deduced from absorption and fluorescence spectra.

The spectra of *P. aerugineum*, *P. cruentum* and *A. nidulans* suggest band shifts of all three types of chlorophyll.

The difference spectra induced in *S. obliquus* by the action of System 1 and System 2 were found to be nearly proportional to each other. Action spectra of reactions driven by Systems 1 and 2, and the composition of sub-chloroplast particles prepared by means of detergents[26] indicate a quite different pigment composition of the two pigment systems. This does not exclude the possibility that each photosystem may contain sufficient amounts of chlorophyll *b*, carotenoid and the chlorophyll *a* type absorbing at 673 nm to account for the light-induced shifts observed. However, the similarity of the spectra, with the same relative size of chlorophyll *a* and *b* and carotenoid shifts, strongly suggests that the action of either photosystem is not confined to its own pigments alone and that excitation of both systems gives rise to the phenomenon which causes the pigment shifts. Such a phenomenon may be a potential generated over the photosynthetic membrane[20,27] or some other form of energized state of the membrane. This is also in line with the inhibition of the chlorophyll and carotenoid signals by FCCP. The absence of shifts in phycocyanin absorption (Fig. 2) is in agreement with evidence[28] that the phycobilins are located outside the photosynthetic membrane.

The stimulation by low concentrations of FCCP, which was also observed for the carotenoid shift in *Rhodopseudomonas spheroides*[3] and *Chlamydomonas reinhardi*[19] might be due to a specific increase in membrane permeability of one cation (*e.g.* H^+) only at these concentrations (*cf.* ref. 29). This then might result in an increase in membrane potential. Increased permeability, also for other ions, at higher concentrations then would result in a breakdown of the membrane potential by exchange diffusion.

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