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ENERGY TRANSFER FROM CAROTENOIDS TO CHLOROPHYLL IN BLUE-GREEN, RED AND GREEN ALGAE AND GREENING BEAN LEAVES

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SUMMARY

From fluorescence action spectra, fluorescence spectra and absorption spectra measured at room temperature and at 77 °K of light petroleum (b.p. 40–60°)-treated and normal chloroplasts, it is concluded that:

1. In blue-green and red algae energy transfer from β-carotene to chlorophyll occurs in Photosystem I exclusively.

2. In green algae and greening bean leaves energy transfer from β-carotene to chlorophyll occurs in both Photosystem I and II.

3. Light absorbed by β-carotene is transferred to chlorophyll with nearly 100 % efficiency.

4. Light energy absorbed by xanthophylls is not transferred to chlorophyll.

INTRODUCTION

As indicated by fluorescence excitation spectra of green algae, light absorbed by carotenoids in these algae is transferred to chlorophyll *a* with an efficiency of about 40–50% (*cf.* ref. 1). This relatively low efficiency—as compared to the efficiency of transfer from chlorophyll *b* to *a*—can be brought about either by 100% efficiency of 40–50% of all carotenoid molecules and the absence of transfer from the remaining fraction, or by a 40–50% transfer of all carotenoid molecules present in the photosynthetic organelles.

With blue-green and red algae the activity of carotenoids with respect to energy transfer to chlorophyll *a*, as determined by fluorescence action spectra at room temperature, is even lower. With intact cells, however, the presence of phycobilin pigments, highly active in energy transfer, prevents an accurate determination of efficiency of energy transfer from carotenoids to chlorophyll. If phycobilin pigments are removed from the chloroplasts and thylakoids by centrifugation, the fluorescence action spectrum of the phycobilin-free suspension shows only bands at wavelengths corresponding to the chlorophyll maxima, but not at those corresponding to maxima of carotenoid absorption[2].

Different fluorescence action spectra are measured for cells at the temperature of liquid N_2. At this temperature the fluorescence spectrum of chlorophyll in photosynthesizing cells *in vivo* or chloroplasts prepared from these cells is changed from

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the one measured at room temperature into a spectrum usually consisting of a system of three bands[3,4], of which the maxima are designated F685, F695 and F720 (*cf.* ref. 5). The exact location of these maxima may vary from species to species and within the species depending on the condition of the cells. In the 77 °K fluorescence action spectrum of intact blue-green algae, two shoulders are detected at about 470 and 510 nm on the phycocyanin band, while in the fluorescence action spectra of phycobilin-free suspensions of thylakoids, two marked bands at the wavelengths mentioned are measured[2].

From fluorescence action spectra determined with the aid of interference filters (which transmitted either the F685, F695 or F720 fluorescence bands) and from action spectra determined for algal species with a different ratio of the fluorescence bands mentioned, it was concluded that the 471- and 506-nm bands in the fluorescence action spectra of blue-green algae measured at 77 °K are correlated with the F720 maximum and not with the F685 and F695 ones (*cf.* ref. 6). Since the fluorescence band F720 at 77 °K is assumed to represent emission by a chlorophyll form of System I of photosynthesis, while the F685 and F695 ones are assumed to represent emission by chlorophyll forms of System II (refs. 7–9), identification of the bands in the fluorescence action spectrum can give information about pigments energetically connected to each system. In the case of blue-green algae, the fluorescence action spectra at 300 and 77 °K suggest that energy is transferred from a carotenoid pigment to chlorophyll of System I, while no energy transfer occurs from carotenoids to chlorophyll of System II. In the case of green algae and higher plants, it seems likely that energy transfer from carotenoids to chlorophyll of both System I and II occurs, as a contribution of carotenoids is indicated in the fluorescence action spectra at both 300 and 77 °K.

To investigate the identity of transferring and possibly nontransferring carotenoids, chloroplasts of various blue-green, red and green algae were lyophilized and extracted with light petroleum (b.p. 40–60°).

Experiments by LYNCH AND FRENCH[10] and MILNER, FRENCH AND MILNER[11] with lyophilized chloroplasts of swiss chard (*Beta vulgaris*) and pokeweed (*Phytolacca americana L.*) showed that the pigment removed by light petroleum is almost exclusively β-carotene, while chlorophyll and xanthophylls are retained in the chloroplasts. After such treatment with light petroleum the chloroplasts were not capable of performing the Hill reaction, as measured by dye decolorization, but readdition of the supernatant resulted in restoration of the capacity to undergo the Hill reaction. This indicates that such treatment does not destroy the structure around the active pigment molecules to such an extent that photosynthetic activity is impossible.

Therefore fluorescence action spectra, fluorescence spectra and absorption spectra measured at 300 as well as at 77 °K of light petroleum-extracted and non-treated samples were compared.

METHODS

Absorption spectra were recorded with a Cary 14R spectrophotometer, provided with a cooling attachment. Fluorescence and fluorescence action spectra were recorded as described earlier[2,6].

To obtain a transparent sample at 77 °K the chloroplasts were suspended in

a mixture containing 40% phosphate buffer (0.02 M, pH 7.3) and 60% glycerol, and immediately cooled. Preparations of chloroplasts absorbed on filter paper and immediately frozen gave results similar to those obtained in the glycerol–buffer mixture but with broader bands.

The algae were grown in a light cabinet (Psychroterm, 14 h light *vs.* 10 h dark, at 27°, in air enriched with 5% CO_2). Synechococcus and Chlorella were harvested after 7 days, Porphyridium and Tribonema after 11 days. Beans (*Phaseolus vulgaris*) were soaked 2 h before planting in vermiculite, then stored overnight in an illuminated room and grown further in complete darkness. As it was found that blue light or green "safe light" affected the time lag of greening (*cf.* ref. 12), watering of the plants also was done in darkness.

Chloroplasts of leaves, green and red algae and thylakoids from blue-green algae were obtained by grinding with carborundum (type F) at 4°, in phosphate buffer (pH 7.3). Carborundum was removed by low-speed centrifugation. Chloroplasts or thylakoids were precipitated by 40 min centrifugation at 20000 × *g*. The supernatant, containing phycobilins in the case of blue-green and red algae, was discarded, and the chloroplasts were taken up in phosphate buffer. For effective extraction with light petroleum (b.p. 40–60°) the chloroplasts of red and green algae were broken by 3 min sonication in a Lehfeld sonicator operated at full power. The preparations were cooled during this treatment after every 30 sec of sonication. A suspension of small fragments of chloroplasts was prepared by removing larger particles by centrifugation of the sonicated suspension for 30 min at 10000 × *g*. These smaller fragments were lyophilized and extracted with light petroleum (b.p. 40–60°) in a Potter–Elvehjem homogenizer. After extraction they were precipitated by centrifugation and immediately taken up in phosphate buffer. Good extraction could be obtained only with completely dry preparations.

RESULTS

Blue-green algae

Blue-green algae are known to contain a special carotenoid, myxoxanthophyll[13]. The absorption bands of this pigment in organic solvents are located at longer wavelengths than those of other algal carotenoids in similar solvents. It is possible that the bands at 506 and 471 nm in the fluorescence action spectra of blue-green algae could be ascribed to myxoxanthophyll. Besides this pigment, the other major carotenoids of blue-green algae are β-carotene and lutein[14].

Myxoxanthophyll and lutein are nearly insoluble in light petroleum but have a fairly good solubility in methanol. Thus, if the bands in the fluorescence action spectrum at 506 and 471 nm are to be ascribed to myxoxanthophyll absorption, it is unlikely that they should disappear as a result of light petroleum treatment, while if they are to be ascribed to β-carotene, the bands should be absent in the treated samples.

If these bands do not belong to any accessory pigment (*c.q.* carotenoid) but to the chlorophyll form responsible for F720 emission, it is also unlikely that they should disappear as a result of light petroleum treatment, provided that such treatment does not affect the shape of the low-temperature fluorescence spectrum.

In Fig. 1a the absorption spectrum of a light petroleum-extracted and a non-

extracted suspension of thylakoids from Synechococcus is given, measured at 77 °K. The corresponding fluorescence action spectra in the blue and green part of the spectrum are given in Fig. 1b, while Fig. 1c gives the fluorescence spectra of these samples, measured at 77 °K and excited with light of 436 nm. In this figure the spectra are normalized at 725 nm. In Fig. 1d both the absorption spectrum of the light petroleum extract and the absorption spectrum of a methanol extract prepared from thylakoids pretreated with light petroleum is given.

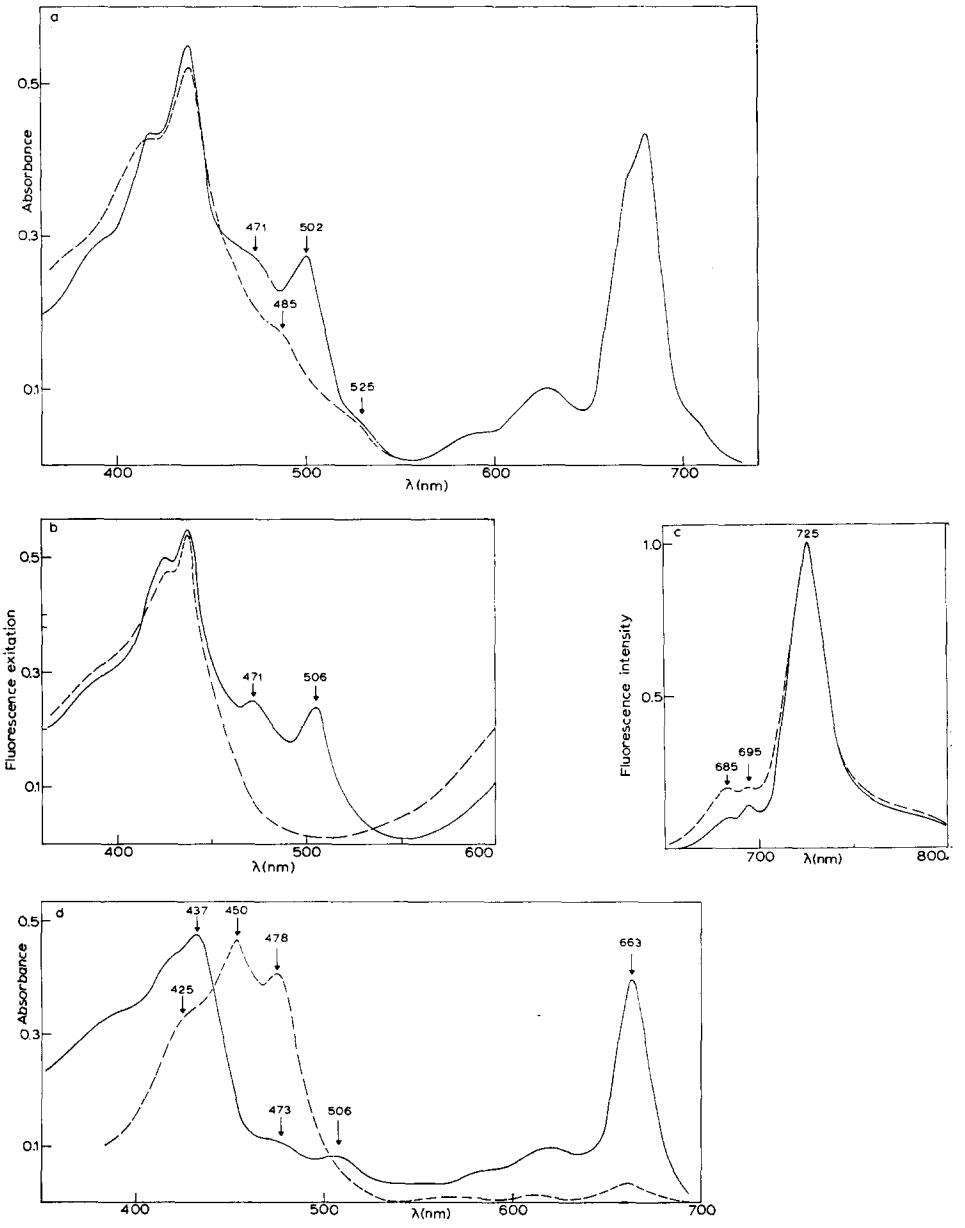

Fig. 1. For legend see next page.

It follows from Fig. 1b that the bands at 506 and 471 nm in the fluorescence action spectrum of the light petroleum-treated sample have completely disappeared. Fig. 1a shows that in the absorption spectrum, the main carotenoid maximum at 502 nm and the shoulder at about 470 nm have disappeared, while two weak bands are visible at 525 and 485 nm. Comparison with the room-temperature absorption spectrum of the same sample indicates that the carotenoid maximum at 495 nm shifted to 502 nm due to cooling. Consideration of the fluorescence spectra (Fig. 1c) shows that the F685 and F695 bands are somewhat increased relative to the band at 725 nm as a result of light petroleum treatment, but this does not change the shape of the spectrum markedly. In the absorption spectrum of the light petroleum fraction a pigment spectroscopically similar to β-carotene occurs, while a small fraction of chlorophyll is extracted. The ratio carotenoid absorption/chlorophyll-*a* absorption with respect to the long-wavelength carotenoid and chlorophyll bands is about 30 in this preparation. In preparations which were not completely dry, this ratio is considerably lower. Absorption difference spectra of extracted *vs.* nonextracted suspensions of thylakoids indicate that this chlorophyll *a* is derived mainly from the 683-nm form *in vivo*. The major fraction of chlorophyll is present in the methanol extract of the treated thylakoids. According to the absorption spectrum, this solution also contains myxoxanthophyll (with maxima at 506 and 473 nm) and other xanthophylls.

An acetone extract of pigments from Synechococcus was chromatographed following a procedure given by HAGER AND MEYER-BERTENRATH[15]. The absorption spectra of β-carotene and myxoxanthophyll obtained in this way are given in Fig. 1e. If the acetone extract was made of a light petroleum-treated sample, the β-carotene band in the chromatogram disappeared.

These spectral data render it unlikely that the 506- and 471-nm bands in the

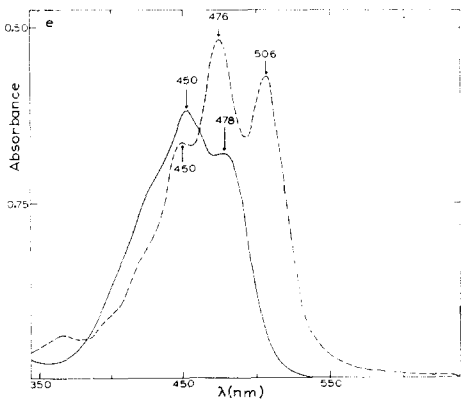

Fig. 1. a. Absorption spectrum of a suspension of thylakoids prepared from the blue-green alga *S. cedrorum*, measured at 77 °K. Nonextracted thylakoids (———) and thylakoids after extraction with light petroleum (— — —). b. Fluorescence action spectrum of light petroleum-treated (— — —) and nontreated (———) thylakoids at 77 °K measured with a filter transmitting beyond 700 nm. c. Fluorescence spectrum of light petroleum-treated (— — —) and nontreated (———) thylakoids measured at 77 °K and resulting from excitation with light of 436 nm. d. Absorption spectrum of a light petroleum extract (— — —) and of a methanol extract (———) prepared from thylakoids pretreated with light petroleum. e. Absorption spectrum of β-carotene (in light petroleum–acetone) (———) and myxoxanthophyll (in ethanol) (— — —) obtained by chromatography.

fluorescence action spectrum of F720 are due either to myxoxanthophyll or to fluorescing F720 itself. The only pigment which is quantitatively extracted with light petroleum and which absorbs in this wavelength region is β-carotene.

Similar results were obtained with suspensions of thylakoids from some other blue-green algae: *Oscillatoria amoena*, *Nostoc calcicola* and *Anabaena catinata*, and, though less markedly, with *Anacystis nidulans*. In one experiment β-carotene, obtained by thin-layer chromatography of a pigment extract from intact cells, was readded to the lyophilized and light petroleum-extracted thylakoids. After evaporation of the solvent and resuspension of the thylakoids in phosphate buffer (0.02 M, pH 7.3), the action spectrum of F720 fluorescence was determined. The 504- and 471-nm bands were not restored.

Readdition of the concentrated but not purified extract resulted in a slight restoration of the bands mentioned (10–15%). These results indicate that the β-carotene chlorophyll "complex" needed for energy transfer between these pigments is of a specific nature and is not likely to be restored by mere readdition.

In intact cells the bands at 506 and 471 nm are usually seen as shoulders on the phycocyanin band in the low-temperature fluorescence action spectrum. Heating the cells for 30 sec at 75° prior to freezing results in a complete destruction of phycocyanin, though the chlorophyll fluorescence spectrum, if excited with light absorbed by chlorophyll, does not change markedly. In the fluorescence action spectrum of such heated cells the bands at 506 and 471 nm are clearly visible, while absorption and fluorescence are similar to those of a suspension of thylakoids.

Red algae

Besides phycocyanin, red algae contain phycoerythrin, which also transfers light energy to chlorophyll *a* with a high efficiency[1]. The absorption spectrum of phycoerythrin is such that no conclusion can be drawn about energy transfer from carotenoids to chlorophyll in intact cells, due to an overlapping of pigment absorption. However, phycobilins can be washed out of the chloroplasts. In the absorption spectrum of the washed chloroplast suspensions only chlorophyll and carotenoids are present. In Fig. 2a the absorption spectra for intact cells and washed chloroplast suspensions of *Porphyridium cruentum* measured at 77 °K are given.

In contrast to blue-green algae, the photosynthetic apparatus of red algae consists of chloroplasts instead of "free thylakoids". A high percentage of carotenoids in these chloroplasts consist of β-carotene, while in some species the fraction of α-carotene is appreciable. They do not contain myxoxanthophyll, but generally lutein as a major xanthophyll[16].

The fluorescence action spectrum of chloroplasts of Porphyridium indicates that carotenoids are ineffective in producing room-temperature chlorophyll fluorescence[2]. It proved to be difficult to remove phycoerythrin from the chloroplasts to such an extent that the band of phycoerythrin was completely absent in the action spectrum. Reduction of the size of the chloroplast fragments by sonication and subsequent washing results in a marked decrease of this band relative to the chlorophyll bands.

As is shown in Fig. 2b, bands at 504 and 471 nm were found at 77 °K in the fluorescence action spectrum of F720. As the low-temperature absorption bands in the carotenoid region are located at 503 and 471 nm, there is a good agreement between fluorescence action spectrum and absorption spectrum in this spectral region.

This indicates that the major fraction of carotenoids transfers absorbed light energy to chlorophyll *a* responsible for F720 emission.

Extraction of lyophilized chloroplast fragments with light petroleum results in a nearly complete removal of the 504- and 471-nm bands of the action spectrum if sonicated fragments are used, while the extraction in nonsonicated chloroplasts is much less pronounced. It is assumed that this is due to a disruption of the chloroplast structure which makes the fragments more susceptible to extraction. The absorption spectrum of the light petroleum extract is similar to that obtained by extraction of Synechococcus (*cf.* Fig. 1d). The absorption spectrum of a methanol extract of the light petroleum-treated fragments showed that little xanthophyll is present in the Porphyridium samples used.

The same experiments were performed with *Porphyra lacineata*. The absorption spectrum measured at 77 °K was in the carotenoid region similar to that of Synechococcus. This was probably due to a higher amount of xanthophyll being present in

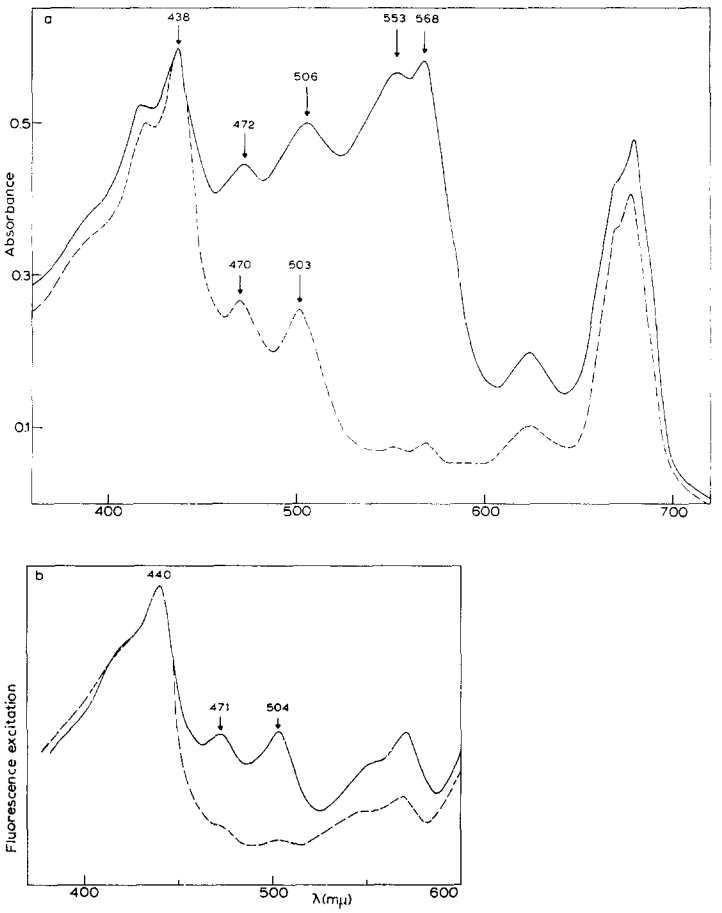

Fig. 2. a. Absorption spectrum of intact cells and washed chloroplasts from the red alga *P. cruentum*, measured at 77 °K. b. Fluorescence action spectrum of chloroplast fragments from Porphyridium before (———) and after (— — —) light petroleum treatment, measured at 77 °K with a filter transmitting fluorescence beyond 700 nm.

the chloroplasts than was the case with Porphyridium. As the carotenoid content was found to depend on growth conditions, this difference is related to environmental conditions rather than to species.

Green algae

With green algae the absorption bands due to carotenoids are generally overlapped by the Soret band of chlorophyll *b*, which *in vivo* is located at about 472 nm. Some species, *e.g.*, *Tribonema equale*, do not contain chlorophyll *b*. Absorption bands in the spectral region from 520 to 460 nm are due to carotenoids only.

In the room-temperature fluorescence action spectrum of this species two bands at 495 and 470 nm are observed in the region of carotenoid absorption, while the Soret band of chlorophyll *a* at 438 nm is dominant (Fig. 3a). Thus, in contrast to blue-green and red algae, light absorbed by carotenoids in Tribonema is transferred

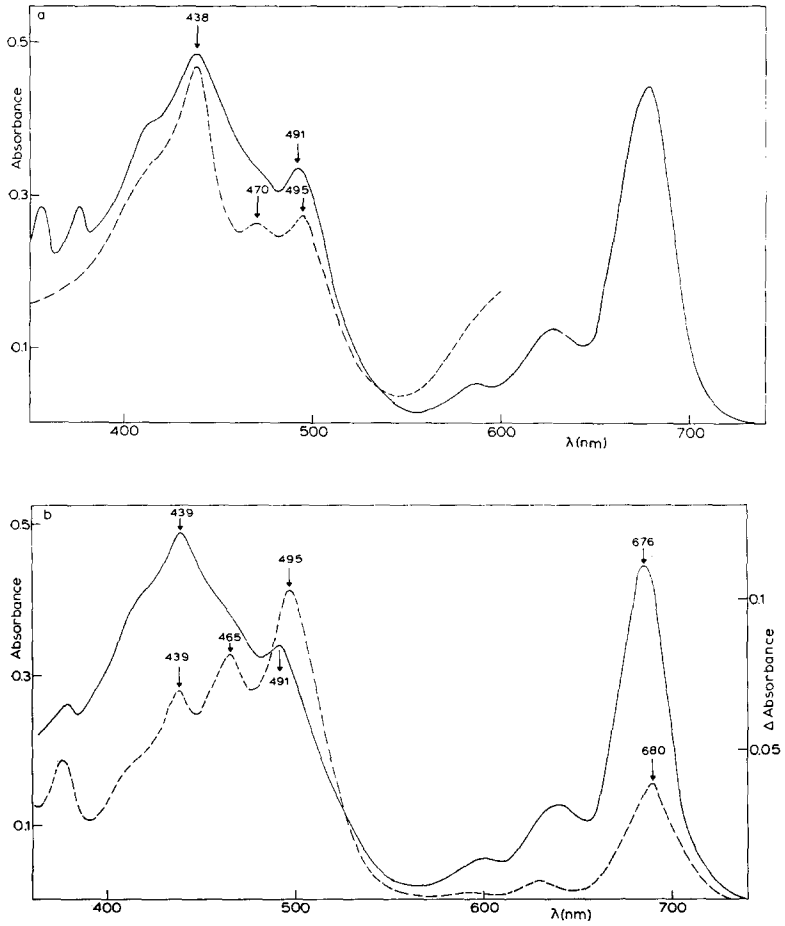

Fig. 3. a. Fluorescence action spectrum (— — —) and absorption spectrum (———) of the green alga *T. equale*, measured at 300 °K. b. Absorption difference spectrum (— — —) between light petroleum-treated and nontreated samples of Tribonema, and absorption spectrum (———) of the former.

at room temperature to chlorophyll *a*. The fluorescence action spectrum in the carotenoid region does not coincide with the absorption spectrum, but shows more pronounced bands which are shifted to longer wavelengths with respect to the absorption bands.

Treatment of the lyophilized cells or chloroplasts with light petroleum results in extraction of carotene—probably mainly β-carotene—and some chlorophyll *a*. After such treatment the bands in the fluorescence action spectrum at 495 and 470 nm are markedly decreased, the amount depending on the size of the fragments used. The long-wavelength maximum of the carotenoids remaining in the chloroplasts is shifted towards the short-wave side. This suggests that these remaining carotenoids (mainly xanthophylls) are not capable of transferring absorbed light energy effectively to chlorophyll. In Fig. 3b the absorption difference spectrum between a treated and a nontreated sample, together with the absorption spectrum of a light petroleum-treated one, are given. The latter picture shows that the location *in vivo* of the absorption maxima of the carotenes nearly coincides with those in the fluorescence action spectrum.

Cooling the cells to liquid N_2 temperature does not change the shape of the fluorescence action spectrum. This also holds if the action spectrum of F720 only is considered. Extraction with light petroleum decreases the bands in the carotenoid region. Due to a decrease of F720 the 77 °K fluorescence sample of the treated cells is somewhat changed. However, the major fraction of fluorescence beyond 710 nm, transmitted by the filter used, can still be ascribed to F720.

A consideration of fluorescence action spectra determined with Chlorella chloroplasts shows that at room temperature, high activity occurs around 470–480 nm. Cooling to 77 °K does not change the F720 action spectrum markedly, though more activity is present around 495 nm in the cooled sample (Fig. 4).

Treatment with light petroleum results in the extraction of carotenes with an

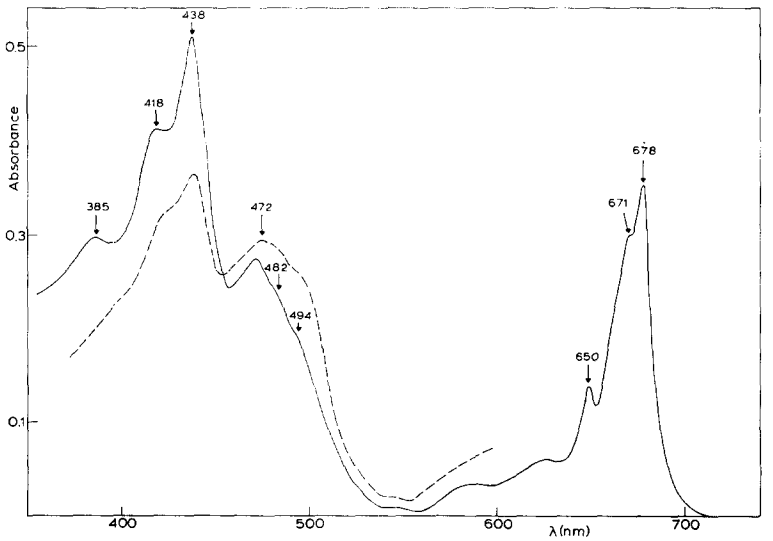

Fig. 4. Fluorescence action spectrum (— — —) and absorption spectrum (———) of the green alga *C. pyrenoidosa*, measured at 77 °K.

absorption spectrum similar to that given for Tribonema, but less chlorophyll is extracted. In the fluorescence action spectrum of the treated samples the activity between 480 and 610 nm is decreased relative to the nontreated samples. This is assumed to be due to a decrease in β-carotene content.

Etiolated bean leaves

The absorption, fluorescence and fluorescence action spectra of a bean leaf in the "post-etiolated state"—that is, after 10 min of illumination but before onset of photosynthetic capacity (*cf.* ref. 17)—and measured at 77 °K, are given in Fig. 5. These spectra show that, although carotenoids dominate the absorption spectrum, no energy transfer from carotenoids to chlorophyll occurs. After 10 min of irradiation the fluorescence action spectrum shows only bands due to chlorophyll *a*. A similar result was found by BUTLER[18]. The same holds if these spectra are measured at room temperature. As no new bands at 695 and 720 nm are formed due to cooling, the

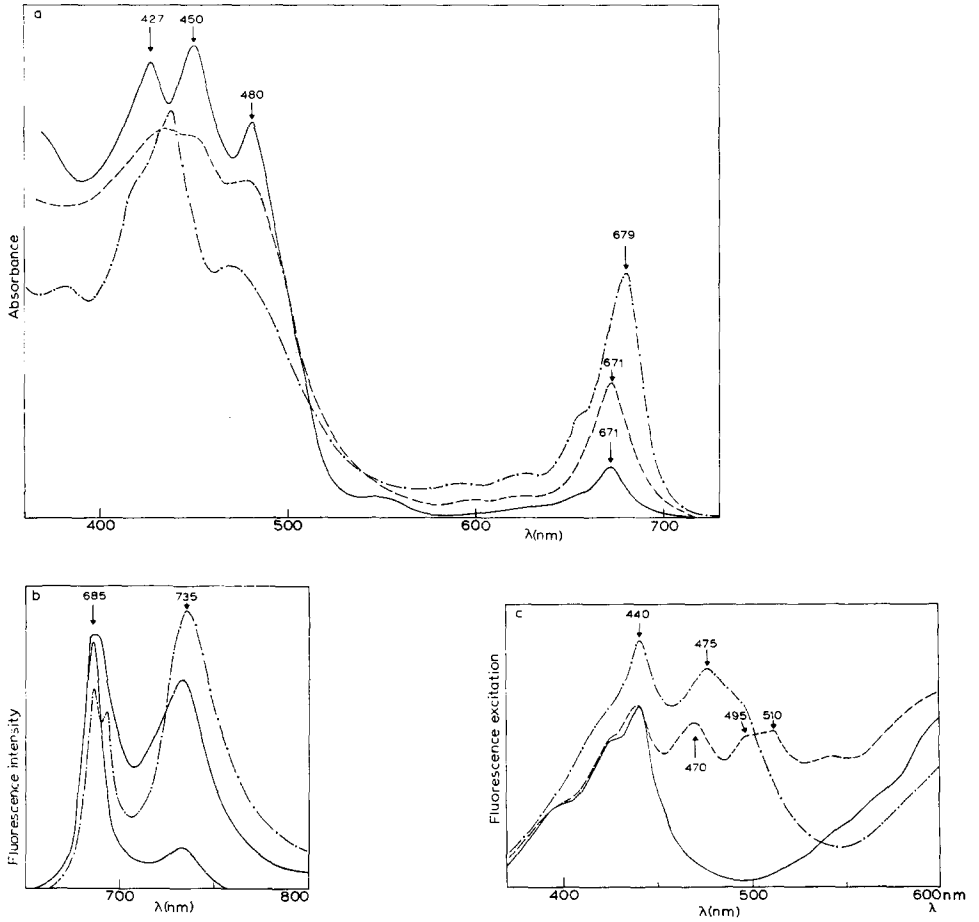

Fig. 5. a. Absorption spectra of etiolated bean leaves. b. Fluorescence spectra of the same leaves. c. Fluorescence action spectra of the same leaves. The various spectra are measured at 77 °K and after 10 min (———), 3.5 h (— — —) and 24 h (-·—·-) of illumination.

fluorescence spectrum at 77 °K is similar in shape to that measured at room temperature[19]. The carotenoids in the "post-etiolated state" are to a large extent lutein[16], while little or no β-carotene is present. After 3.5 h of illumination (under our growth conditions) two bands appear in the fluorescence action spectrum in the region of carotenoid absorption: at 470 and 495 nm (Fig. 5c). Cooling to 77 °K results in a marked broadening of the 495-nm band, while the 470-nm band is unaffected. During further greening the 470-nm band increases relatively faster than the 495-nm band, until the two combine with a maximum at 475 and a shoulder at about 490 nm. As chlorophyll *b* is also formed during greening, the band at 470 nm is ascribed to this pigment, while the 495-nm band is ascribed to β-carotene. In accordance with this suggestion it was found that the 495-nm band is largely removed by light petroleum treatment, while the 470-nm band is largely retained.

The increase in amount of chlorophyll *a*, chlorophyll *b* and β-carotene relative to that of xanthophylls[20] is assumed to result in the disappearance of the marked bands at 480, 450 and 427 nm in the low-temperature absorption spectrum.

DISCUSSION

The fluorescence action spectra of blue-green and red algae indicate that at room temperature no energy transfer occurs from carotenoids to chlorophyll. In the fluorescence action spectra measured at 77 °K, the two bands at 506 and 471 nm occur only in the action spectrum of F720 but not in the ones of F685 and F695. As both room-temperature fluorescence and the F685 and F695 bands in the 77 °K spectrum are ascribed to emission by chlorophyll of System II of photosynthesis, this implies that the carotenoids present in the photosynthetic organelles do not transfer absorbed light energy to chlorophyll of System II either at room temperature or at 77 °K. As the F720 band at 77 °K is ascribed to emission of a chlorophyll form of System I of photosynthesis, the 506- and 471-nm bands in the action spectrum of this emission should be ascribed either to accessory pigments of Photosystem I or to a chlorophyll form of Photosystem I. The second possibility is unlikely. These bands disappeared after light petroleum treatment, although very little chlorophyll is extracted, and the fluorescence spectrum itself is nearly unchanged. It thus is more probable that the bands belong to an accessory pigment which is extracted by the treatment mentioned. According to the absorption spectrum of the extract this pigment is likely to be β-carotene.

From these results it is concluded that in blue-green and red algae, light energy absorbed by carotenoids is not transferred to chlorophyll of Photosystem II at all, while only light absorbed by β-carotene (the concentration of other carotenes was too low to be considered) is transferred to chlorophyll of Photosystem I. In view of the height of the bands in the fluorescence action spectrum of F720, this energy transfer occurs with an efficiency approaching 100%. Although according to the absorption spectra xanthophylls are present in the photosynthetic organelles used, they do not transfer light energy to chlorophyll of either photosystem.

The absorption spectra of light petroleum-treated and nontreated blue-green and red algae demonstrated that β-carotene generally dominates xanthophylls in the measured samples.

More evidence for the identification of the 506- and 471-nm bands in the fluorescence action spectra of F720 with β-carotene is obtained from consideration of absorption difference spectra between treated and nontreated samples. At room temperature the difference spectra show bands at 495 and 465 nm which, since β-carotene is the only pigment quantitatively extracted, are presumably due to β-carotene absorption *in vivo*. Cooling to 77 °K results in a shift of these bands to 506 and 471 nm.

Whether in blue-green and red algae energy transfer from β-carotene occurs directly to the chlorophyll form responsible for F720 emission or indirectly *via* another nonfluorescing chlorophyll-*a* form present in a higher concentration cannot be decided from fluorescence measurements described in this paper. In Fig. 1 it is seen that a shoulder is present at about 705 nm in the 77 °K absorption spectrum of Synechococcus. This shoulder is supposed to represent the pigment responsible for F720 emission. The fluorescence action spectrum at this temperature shows a maximum in the chlorophyll band at about 680 nm (*cf.* ref. 2), indicating an efficient energy transfer from a 680-nm chlorophyll-*a* form to the one responsible for the 705-nm band. Therefore, of the two mentioned transfer possibilities,

$$h\nu \longrightarrow \beta\text{-carotene} \longrightarrow C_a705 \nearrow^{F720} \tag{1}$$

and

$$h\nu \longrightarrow \beta\text{-carotene} \longrightarrow C_a680 \longrightarrow C_a705 \nearrow^{F720} \tag{2}$$

the second one seems better.

The fluorescence action spectra of various species of blue-green algae are not always identical. With *A. nidulans* the F720 band is less pronounced than in other blue-green algae. Consequently fluorescence at wavelengths longer than 710 nm is partly due to vibrational levels of F685 and F695. As a result the bands at 506 and 471 nm are less marked in the fluorescence action spectra.

In green algae and higher plants the energy-transfer phenomenon from carotenoids to chlorophyll of both photosystems appears to be more complex than in blue-green and red algae. The green alga *Tribonema equale*, which lacks chlorophyll *b*, shows bands at 495 and 465 nm in the room-temperature fluorescence action spectrum. These bands correspond to the absorption bands of β-carotene *in vivo*, as determined from absorption difference spectra between light petroleum-treated and nontreated algae. Hence, light absorbed by β-carotene here is most probably transferred to chlorophyll of Photosystem II, fluorescing mainly at room temperature. Treatment with light petroleum results in a marked decrease of the bands in the fluorescence action spectrum, especially with chloroplast fragments. The results suggest that xanthophylls with absorption maxima on the short-wave side of those of β-carotene do not transfer energy to chlorophyll of Photosystem II.

Cooling to 77 °K does not change the shape of the fluorescence action spectrum markedly if the spectrum for F720 emission is compared to that for room temperature emission. This may indicate that in Tribonema, light absorbed by β-carotene is also transferred to chlorophyll *a* of Photosystem I, responsible for the F720 emission. Consequently in Tribonema one fraction of β-carotene should be intimately connected to chlorophyll of System II, and another fraction to chlorophyll of System I.

If at low temperature an effective energy transfer occurs from System II to System I, bands of the accessory pigments of System II should also be present in the action spectrum of System I. In this case the ratio of carotenoid bands/chlorophyll bands may be expected to drop if β-carotene is ineffective in System I, as chlorophyll *a* is divided over both systems. On the other hand, if this ratio does not decrease, β-carotene should be active in both systems. The action spectra indicate that the latter situation holds. Thus, even if effective energy transfer occurs at 77 °K between both photosystems, the results suggest that β-carotene transfers absorbed light energy to chlorophyll *a* of both photosystems in Tribonema.

Similar results were obtained with the green alga *Chlorella pyrenoidosa* and with spinach chloroplasts. The picture with these organisms is complicated by the presence of a strong Soret band due to chlorophyll *b* at about 475 nm. This pigment is present in both photosystems but in a different ratio from chlorophyll *a* (*cf.* ref. 21). Here a decrease in effectivity in the action spectrum around 490 nm due to light petroleum extraction is ascribed to removal of β-carotene. No definite conclusion about the inactivity of xanthophylls can be drawn from studies of these organisms, though it might be assumed that, similar to Tribonema and blue-green and red algae, no energy transfer to chlorophyll occurs from these pigments.

This hypothesis is supported by the results with etiolated bean leaves. In the "post-etiolated state", xanthophylls, and of these mainly lutein, are present in a high carotenoid/chlorophyll ratio, although no trace of carotenoid bands is seen in the action spectrum of chlorophyll fluorescence at any temperature measured. After 3.5 h of illumination bands appear at 475 and 495 nm in the fluorescence action spectrum, of which the former is thought to represent mainly chlorophyll *b*, and the latter the long-wave band of β-carotene. Both these pigments are absent in the "post-etiolated state", and formed only after several hours of illumination. In the absorption spectrum, lutein with its absorption maxima *in vivo* at 480, 450 and 426 nm is still the dominant carotenoid. As the bean leaves after 3–4 h of illumination are fully active in photosynthesis, show the general type of fluorescence spectrum and have chloroplasts which contain lamellae, these results suggest that no energy transfer occurs from xanthophylls to chlorophyll *a*.

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