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FLUORESCENCE ACTION SPECTRA OF ALGAE AND BEAN LEAVES AT ROOM AND AT LIQUID NITROGEN TEMPERATURES

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SUMMARY

Fluorescence action spectra were determined, both at room temperature and at liquid nitrogen temperature, with various blue-green, red and green algae, and greening bean leaves.

The action spectra of algae were established with samples of low light absorption as well as dense samples.

Fluorescence at room temperature, with a maximum at about 685 mμ, was for the greater part emitted by a short wave "form" of chlorophyll *a*, possibly C_a670. To this form energy was transferred from β-carotene, chlorophyll *b* and phycobilins with an efficiency approaching 100 %. The action spectra of blue-green algae suggested the presence of a 650-mμ phycocyanin "form", which seemed bound more firmly to the photosynthetic apparatus than the bulk of phycocyanin absorbing around 620 mμ.

The absence of action in the carotenoid region in blue-green and red algae and in bean leaves shortly after transformation of protochlorophyll to chlorophyll is ascribed to the presence of a high percentage of xanthophyll, presumably inactive in transferring energy to chlorophyll.

All pigments active in producing the 685-mμ room temperature fluorescence band were found to be active in producing the 720-mμ low temperature fluorescence band. Additionally, the 720-mμ fluorescence band was excited by absorption in long wavelength chlorophyll *a* forms (*e.g.* C_a680 and C_a695) and, in blue-green and red algae, by one or more unidentified pigments with absorption maximum at 475 and 520 mμ.

Marked differences between "high density" action spectra of blue-green and green algae are described.

INTRODUCTION

The room temperature fluorescence spectrum of cells containing chlorophyll *a* shows usually only a single sharp maximum around 685 mμ and a lower, broad, maximum at around 730 mμ. Cooling the samples to liquid nitrogen temperature results in a change of the spectrum into a more complex structure. In algal cells the 685 mμband is sharpened and usually decreased in intensity, while two new

bands occur, one at about 696 mμ and one at 717–720 mμ (refs. 1 and 2). In green algae and higher plants the 720 mμ band (located in most green plants at 730–740 mμ) is by far dominating at low temperatures.

In blue-green and red algae, especially in young cultures, the 696 mμ low temperature band may be of a height equal to that of the 720 mμ one.

In the photosynthetic apparatus, chlorophyll *a* is present in several "forms" (for a survey of recent literature *cf.* SMITH AND FRENCH[3]). Also, according to present knowledge, in photosynthesis at least two pigment systems need to cooperate:

1. the "p" system (*cf.* GOEDHEER[4]) corresponding to "system II" of DUYSENS, AMESZ AND KAMP[5] and the "accessory pigment system" of FRENCH AND FORK[6]. This pigment system comprises one or more "short wave chlorophyll *a* forms" and some accessory pigments;

2. the "q" system, corresponding to "system I" or the "long wave chlorophyll *a*" system. This system comprises one or more "long wave chlorophyll *a* forms" and, possibly, also some accessory pigments.

In a former publication[2] the assumption was made that the three low-temperature fluorescence bands were correlated with three chlorophyll *a* forms in the following way: C_a670–F686, C_a680–F696, C_a695–F720. The chlorophyll *a* form C_a670 probably participates in the "p" system. This form may be responsible for the major fraction of fluorescence emission at room temperature. In addition this form is responsible for emission of afterglow in the "p" system.

The chlorophyll forms C_a680–F696 and C_a695–F720 might then be active in the "q" system. As an explanation for the appearance of a marked low temperature band at around 720 mμ it was assumed that a strong increase in energy transfer from C_a670 and C_a680 to C_a695 occurs as a result of structural shrinkage due to excessive cooling. If this is true, the action spectrum of the low temperature fluorescence at 720 mμ will include the absorption of all accessory pigments and chlorophyll forms of both "p" and "q" systems, while the room temperature action spectrum of fluorescence would comprise only those pigments participating in the "p" system. Fluorescence action spectra measured for different temperatures and different wavelengths of fluorescence thus will indicate the composition of the pigment systems with respect to chlorophyll forms and accessory pigments.

The complete pigment system necessary for photosynthesis appears to develop during greening of etiolated leaves. In bean leaves immediately after first illumination only one chlorophyll form seems to be present with an absorption maximum at around 683 mμ, which maximum shifts to about 672 mμ in a few minutes[7,8]. Little or no photosynthesis can be measured in the first period after protochlorophyll transformation[9] and no extra bands are present in the low temperature fluorescence spectrum[10]. Fluorescence yield and spectral behaviour after cooling are similar to those of chlorophyll in organic solution. Only after some hours of illumination are extra fluorescence bands formed upon cooling and onset of photosynthetic capacity is observed. A time lag is also required for the formation of chlorophyll *b* and carotenes[11,12] while, concomitantly, the absorption maximum shifts from 672 to 678 mμ (ref. 7), fluorescence yield drops and fluorescence induction phenomena can be measured[8]. All of these changes may be correlated with the building up of the pigment systems, necessary for the establishment of complete photosynthesis.

For further study of these pigment systems it is of interest to measure fluores-

cence action spectra of various objects with diverging pigment composition at room and at liquid nitrogen temperature. The results of such a study are given below.

METHODS

Monochromatic light for determining the fluorescence action spectra was obtained from a Bausch and Lomb grating monochromator (500 mm, blazed for 3500 Å). As a light source an incandescent lamp was used, operated slightly at over-voltage. Light intensity was measured with a thermopile. A correction factor was determined to obtain an equal number of light quanta with respect to a fixed wavelength, and applied afterwards. This procedure holds only provided fluorescence is directly proportional to the intensity of incident light. It was checked that, with the relatively weak monochromatic light, this condition holds; no deviations from linearity could be detected.

Inherent wavelength anomalies were checked with simple pigment systems. This was done by determining fluorescence action and absorption spectra for chlorophyll *a* dissolved in methanol or acetone and for rhodamine B and FMN dissolved in water.

The monochromator used showed three irregularities of about 10 % transmission change: at 720, 645 and about 630 mμ. Due corrections were applied.

For measuring low temperature action spectra the samples were placed in a three-walled Dewar vessel provided with a window. Cell suspensions and aqueous extracts ("lamellae" or chloroplast fragments) were brought on filter paper which was lightly pressed against the window.

Room temperature spectra were determined with cell suspensions or aqueous extracts in a 1 mm cuvette. Room temperature spectra were also determined with cells on filter paper. The action spectra were found to be similar to those obtained with cells in a cuvette. For the "low density" action spectra, absorption did not exceed 50 % in the peaks, and usually was appreciably below this value. "High density" action spectra were made with suspensions of very high absorption, approaching 100 % in the peaks.

Fluorescence was detected with a liquid nitrogen cooled Dumont 6911 multiplier. Fluorescence at room temperature was isolated from scattered incident actinic light with a Schott RG5 filter combined with a Kodak Wratten 88 filter. This combination transmits light with a wavelength longer than about 705 mμ. Fluorescence at liquid nitrogen temperature was isolated with a Wratten 88A filter, combined with a Schott RG8 filter, transmitting light of a wavelength longer than 720 mμ. Also fluorescence action spectra were determined when fluorescence was isolated with interference filters transmitting at 681, 695 or 713 mμ. Action spectra of samples with a very high pigment density were made with a Wratten 87 filter, transmitting light of a wavelength beyond 740 mμ.

Aqueous suspensions were prepared by grinding the cells with sand in a cooled mortar. Intact cells were removed by low speed centrifugation. The particles containing "native" chlorophyll and carotenoids (chloroplast fragments from green algae and "lamellae" from blue-green ones) were spun down by high speed centrifugation and taken up in phosphate buffer (pH 7.2).

Beans (*Pheaseolus vulgaris*) were germinated and grown in the dark on vermiculite at 22°.

They were harvested after 7 days. The blue-green algae *Synechococcus cedrorum* and *Anacystis nidulans* and the green alga *Chlorella pyrenoidosa* and *Tribonema equale* were harvested 3–4 days after inoculation. The red alga *Porphyridium cruentum* was harvested after 7–10 days, the green algae *Euglena gracilis* after 14–18 days. All cells were grown in a light cabinet (temp. about 20°, light intensity about 300 ft candles) and flushed with air enriched by 5 % CO_2.

RESULTS

The results are listed below according to the various organisms and the pigments studied.

Blue-green algae

Intact cells, dilute suspension

Room temperature fluorescence action spectrum. In Fig. 1A the room temperature fluorescence action spectrum for a dilute suspension of the blue-green alga *Synechococcus cedrorum* is given.

(1). Chlorophyll. The shape of the action spectrum differed from that of the absorption spectrum by the absence of a distinct band in the region of the red chlorophyll absorption. With *Anacystis nidulans*, the phycocyanin band was less broad and a shoulder was seen at about 670 mμ. In the blue region of the spectrum a weak Soret band of chlorophyll *a* was visible at about 440 mμ.

(2). Phycocyanin. Phycocyanin dominated the fluorescence action spectrum at room temperature. With Synechococcus, the maximum was at about 622 mμ, while a shoulder was present at about 645 mμ. With Anacystis no such shoulder was visible, while the maximum of the action spectrum was located close to 630 mμ. Also in the absorption spectra there was some difference between the two mentioned blue-green algae in the phycocyanin region (location of absorption maximum of phycocyanin and deepness of "trough" between phycocyanin and chlorophyll). It was noted that in actively growing young cells the phycocyanin and red chlorophyll peaks were of about equal height in the absorption spectrum.

Fig. 1A also shows the fluorescence action spectrum of extracted phycocyanin. This action spectrum followed, within close limits, the absorption spectrum of extracted phycocyanin. If buffered at pH 6, phycocyanin extracted from Synechococcus was not completely spectroscopically similar to phycocyanin extracted from Anacystis. The differences, however, were much smaller than the differences between fluorescence action spectra of phycocyanin in the cells.

(3). Carotenoids. The fluorescence action spectra of Synechococcus, Anacystis and Oscillatoria if measured at room temperature did not show any bands attributable to carotenoids.

Liquid nitrogen temperature fluorescence action spectrum. The fluorescence action spectrum of Synechococcus measured at liquid nitrogen temperature is given in Fig. 1B.

(1). Chlorophyll. A red chlorophyll band was present with maximum at about 678 mμ and a shoulder at about 670 mμ. No band or shoulder was visible on the long wavelength side of the red chlorophyll band. The Soret band of chlorophyll at about 440 mμ was more marked than at room temperature.

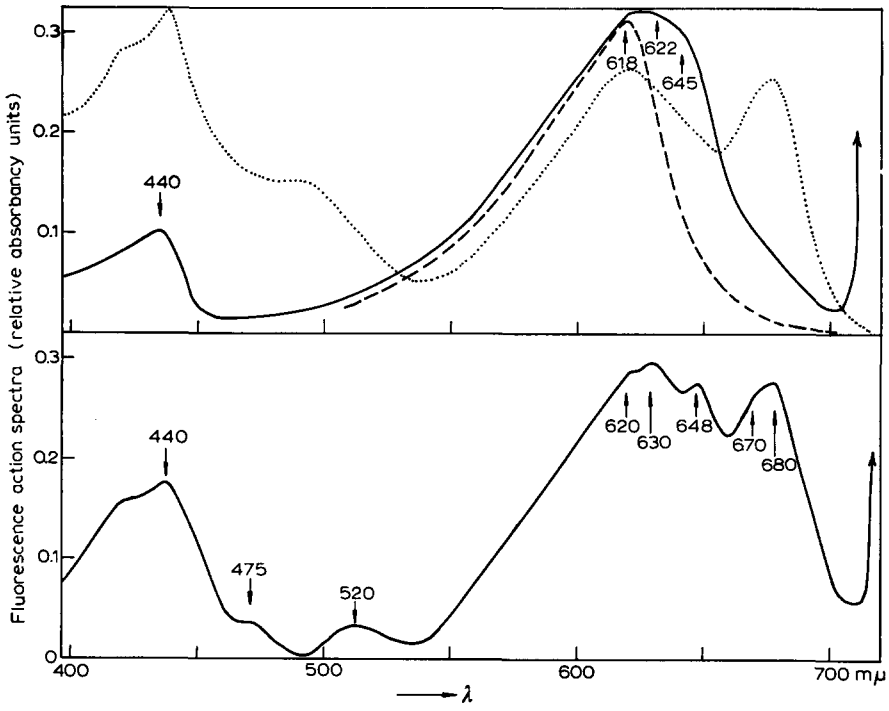

Fig. 1. (A), Room temperature fluorescence action spectrum (———) and absorption spectrum (·····) for the blue-green alga Synechococcus. The dashed line (– – – –) shows the fluorescence action spectrum for phycocyanin extracted from the cell. This action spectrum follows the absorption spectrum of extracted phycocyanin. (B), Liquid nitrogen temperature fluorescence action spectrum for Synechococcus.

(2). Phycocyanin. In the phycocyanin region, the shoulder in the room temperature spectrum at about 645 mμ was sharpened into a maximum, while other maxima were found at about 620 and 630 mμ. The ratio of values 645/620 did not differ much from that in the room temperature spectrum. Cooling of Anacystis cells resulted in the appearance of a chlorophyll *a* band of a shape similar to that of the band in Synechococcus. The phycocyanin band here also was split into three components, but the 645 mμ fraction showed up only as a shoulder.

In old cultures of Synechococcus the ratio phycocyanin/chlorophyll was lower than the one shown in Fig. 1, in the absorption spectrum as well as in the fluorescence action spectrum. In contrast to Synechococcus, the ratio phycocyanin/chlorophyll in Anacystis was higher in old cultures than it was in young ones.

(3). Carotenoids. Two bands were visible in the region of carotenoid absorption in all three blue-green algae investigated: one at about 475 mμ and one at about 520 mμ. These bands appeared at temperatures below —100°.

Intact cells, dense suspension

In a sufficiently dense suspension the absorption spectrum, expressed in % absorption, approaches a straight line over a wide range of wavelengths. If all pigments transfer their absorbed light energy with an efficiency of nearly 100 % to the fluores-

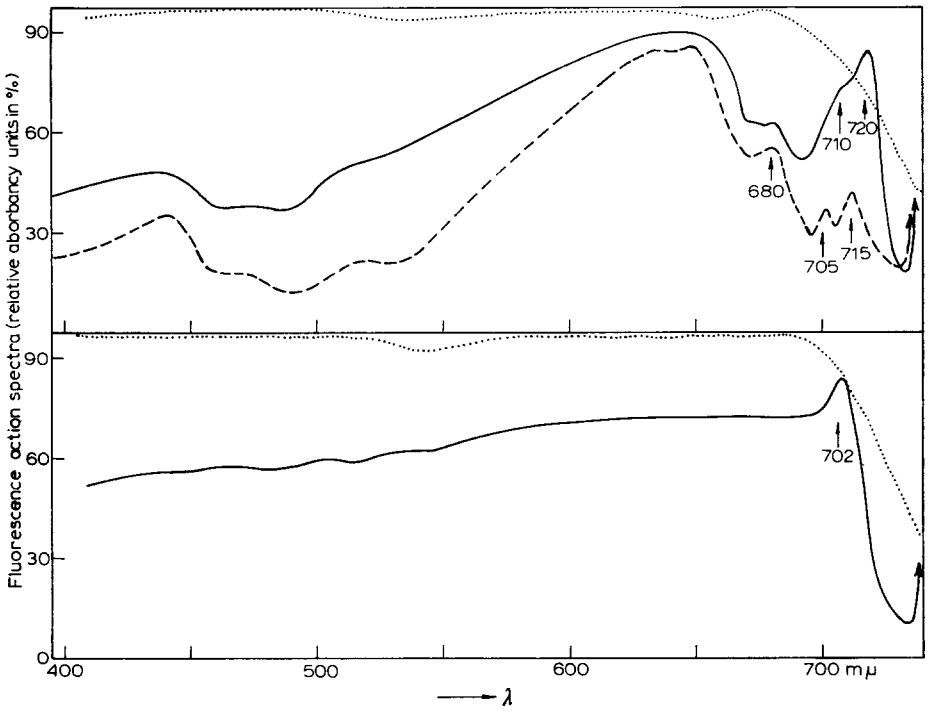

Fig. 2. (A), Liquid nitrogen temperature fluorescence action spectrum of dense preparations of Synechococcus. Absorption in the peaks 98% (———) and 85% (– – – –). (B), Liquid nitrogen temperature fluorescence action spectrum of a dense preparation (peak absorption 98%) of Chlorella. In both A and B the dotted curve (· · · · · ·) gives the absorption spectrum (98% absorption in the peaks) measured at room temperature.

cing pigments, and provided fluorescence is measured sufficiently far out in the red that reabsorption is avoided, fluorescence action spectra and absorption spectra are similar. If, however, an appreciable fraction of pigments do not transfer all their excitation energy to the fluorescing one, a "dip" in the fluorescence action spectrum due to absorption of these pigments will appear. "High density" action spectra thus may differ considerably from the "low density" ones.

"High density" spectra also show more detail in regions where absorption of "low density" ones approaches zero, such as in the long wavelength "tail" of the chlorophyll band.

"High density" action spectra of blue-green algae measured at room temperature did not give much structure in the red part of the spectrum. Fluorescence was high from about 580 to 650 mμ and then dropped gradually to zero. The spectra became more complicated upon cooling. A chlorophyll band was measured at 680 mμ, a "trough" in the spectrum occurred at about 690 mμ, while in the more dense sample a new band occurred at about 720 mμ, with a shoulder at 710 mμ. In the somewhat less dense sample two bands in the far red were seen at 705 and 715 mμ. The band at 705 mμ and the shoulder at 710 mμ in the more dense sample disappeared rather suddenly at temperatures higher than —100°, whereas the band at 715 mμ (720 mμ in the more dense sample) declined gradually when warming up.

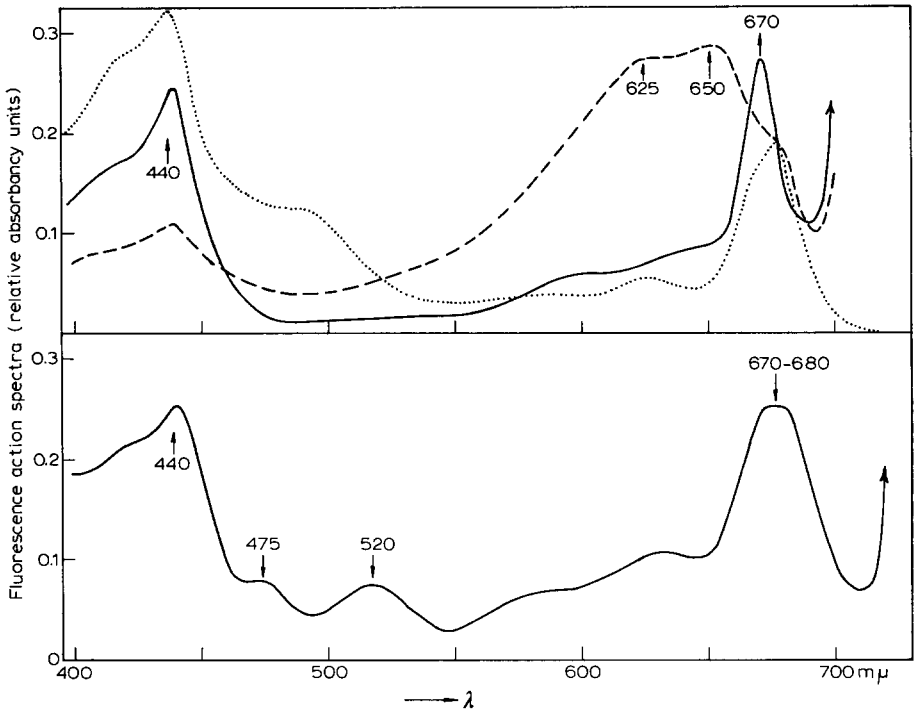

Fig. 3. (A), Room temperature absorption spectrum (·····) and fluorescence action spectrum of a suspension of "lamellae" of the blue-green alga Anacystis extracted once (– – – –) and twice (———) with 0.01 M phosphate buffer (pH 7.2). (B), Liquid nitrogen temperature fluorescence action spectrum of Anacystis lamellae

The blue part of the spectrum resembled that of dilute suspensions.

Fragmented cells and heated cells

If blue-green algae are ground and suspended in an aqueous medium, phycocyanin leaks out and the "lamellae", containing mainly native chlorophyll and carotenoids as pigments, can be obtained. Fig. 3A shows the absorption spectrum of preparations of *Anacystis nidulans* in 0.01 M phosphate buffer (pH 7.2).

If blue-green algae were heated 1 min at 65°, phycocyanin was destroyed to a large extent (about 85 %) without much change in the chlorophyll absorption band. A 1-min heating to 75° (fresh sample) removed more phycocyanin. The absorption spectrum after a heat treatment was generally similar to the spectrum of "lamellae" of blue-green algae given in Fig. 1A.

Room temperature fluorescence action spectrum. Fig. 3A gives a room temperature fluorescence action spectrum of "lamellae" of Anacystis.

(1, 2). Chlorophyll and phycocyanin. After the first "washing" a high percentage of phycocyanin was removed, as was evident from the absorption spectrum. The maximum in the action spectrum was located at about 650 mμ. After a second "washing" (separated from the first by a one night's storage at 4°) the absorption spectrum was nearly identical with that after the first washing, but the fluorescence action spectrum showed a maximum at about 673 mμ, close to the absorption maximum of chlorophyll.

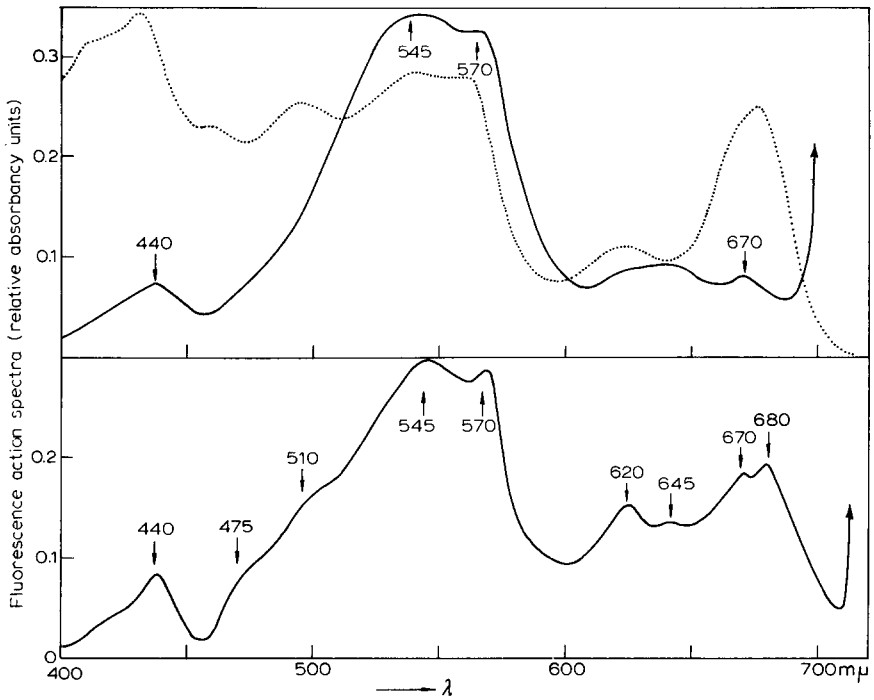

Fig. 4. (A), Room temperature fluorescence action spectrum (———) and absorption spectrum (·····) of the red alga Porphyridium. (B), Liquid nitrogen temperature fluorescence action spectrum of *Porphyridium cruentum*.

The maximum at 650 mμ in the fluorescence action spectrum of the once-washed sample was most probably not due to the presence of some extracted phycocyanin in the sample. For, if extracted phycocyanin was added to the twice washed sample, a spectrum with maxima at 620 and 670 mμ resulted, and not with a single maximum at 650 mμ.

A fluorescence action spectrum with a maximum at 650 mμ could also be obtained by heating samples of intact cells of blue-green algae. According to the absorption spectrum, a 1-min heating to 60° destroyed about 20 % of phycocyanin, but the fluorescence action spectrum was unaltered. A 1-min heating to 70° destroyed about 90 % of phycocyanin, and a fluorescence action spectrum with a maximum at 650 mμ was observed. A 1-min heating to 80° destroyed a further fraction of phycocyanin, while after this treatment the maximum of the fluorescence action spectrum was located at about 675 mμ.

(3). Carotenoids. Though the carotenoids were present in the absorption spectra of the "lamellae" of blue-green algae, no activity could be seen in the region around 480 mμ in the fluorescence action spectrum.

Liquid nitrogen fluorescence action spectrum

(1, 2). Chlorophyll and phycocyanin. The red chlorophyll band in the twice-washed Anacystis lamellae was markedly broadened after cooling (Fig. 3B). In the once-washed preparation the band was shifted to about 678 mμ, while a shoulder was present at 650 mμ.

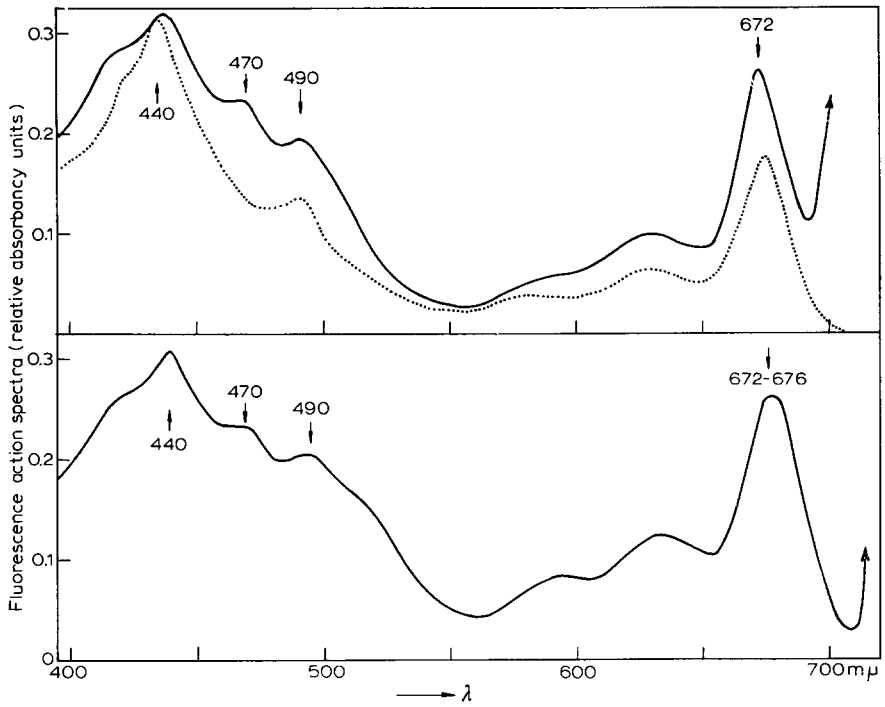

Fig. 5. (A), Fluorescence action spectrum of a cell suspensions (———) and absorption spectrum of a chloroplast suspension (·····) of the chlorophyll *b* deficient green alga *Tribonema equale*. (B), Liquid nitrogen temperature fluorescence action spectrum of Tribonema.

In the fluorescence action spectrum of the samples heated to 70° a shoulder appeared at about 680 mμ. The red chlorophyll band in the samples heated to 80° was not broadened, but consisted of a rather sharp peak at about 682 mμ.

(3). Carotenoids. In Anacystis preparations or heated Anacystis cells bands appeared at about 475 and 520 mμ as a result of cooling (Fig. 3B). These bands were also found to occur with Synechococcus and Oscillatoria.

Red algae

Intact cells, dilute suspension

Room temperature fluorescence action spectrum. Fig. 4A gives the room temperature fluorescence action spectrum of the red alga *Porphyridium cruentum*. This figure also gives the absorption spectrum of a cell suspension of these algae.

(1). Chlorophyll. A weak red chlorophyll *a* band at about 670 mμ was present in the action spectrum, while a Soret band of chlorophyll occurred at about 440 mμ.

(2). Phycobilins. The phycocyanin band had a broad maximum ranging from 620 to 645 mμ. The major contribution was due to phycoerithrin, with peaks at 545 and 570 mμ.

(3). Carotenoids. Although the presence of the phycoerithrin band prevented an accurate estimation of carotenoid efficiency, the activity of carotenoids seems, like that in blue-green algae, very weak.

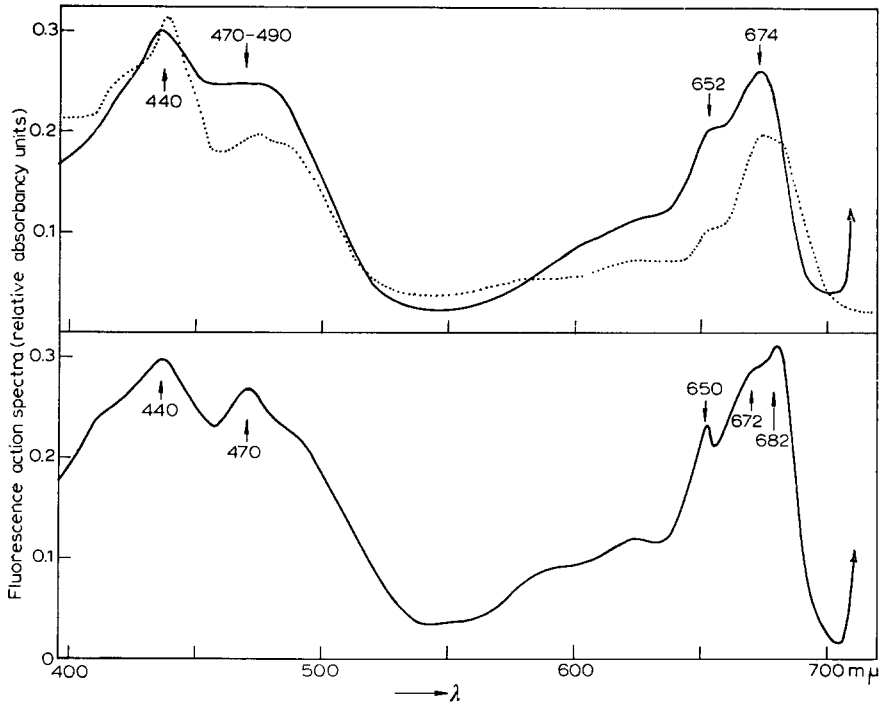

Fig. 6. (A), Room temperature fluorescence action spectrum (———) of a cell suspension and room temperature absorption spectrum (·····) of a chloroplast suspension of the green alga *Chlorella pyrenoidosa*. (B), Liquid nitrogen temperature fluorescence action spectrum of Chlorella.

Liquid nitrogen fluorescence action spectrum. Fig. 4B gives the low temperature fluorescence action spectra of Porphyridium.

(1). Chlorophyll. The red chlorophyll band was doubled (maxima at about 670 and 680 mμ) and considerably increased in intensity relative to the phycobilin bands.

(2). Phycobilins. Phycocyanin showed two maxima, one at about 645 mμ and another at 620 mμ. The band at 620 mμ was increased relative to the band at 645 mμ as a result of cooling. In the phycoerithrin region two maxima were seen, at 545 and 570 mμ.

(3). Carotenoids. In the region of carotenoid absorption, on the short wave side of the phycoerithrin bands, two weak bumps were present.

Intact cells, dense suspension

The fluorescence action spectrum of a dense suspension of Porphyridium cells was similar to that of blue-green algae. After cooling to liquid nitrogen temperature two bands also appeared here in the far red, their magnitude and ratio depending on the concentration of cells.

Fragmented cells and heated cells

Suspensions of chloroplasts of red algae lack phycobilins and show an absorption spectrum similar to the spectrum of "lamellae" of blue-green algae. The fluorescence action spectrum showed only a red chlorophyll band at 670 mμ, which band was broadened upon cooling, and a chlorophyll Soret band at about 440 mμ.

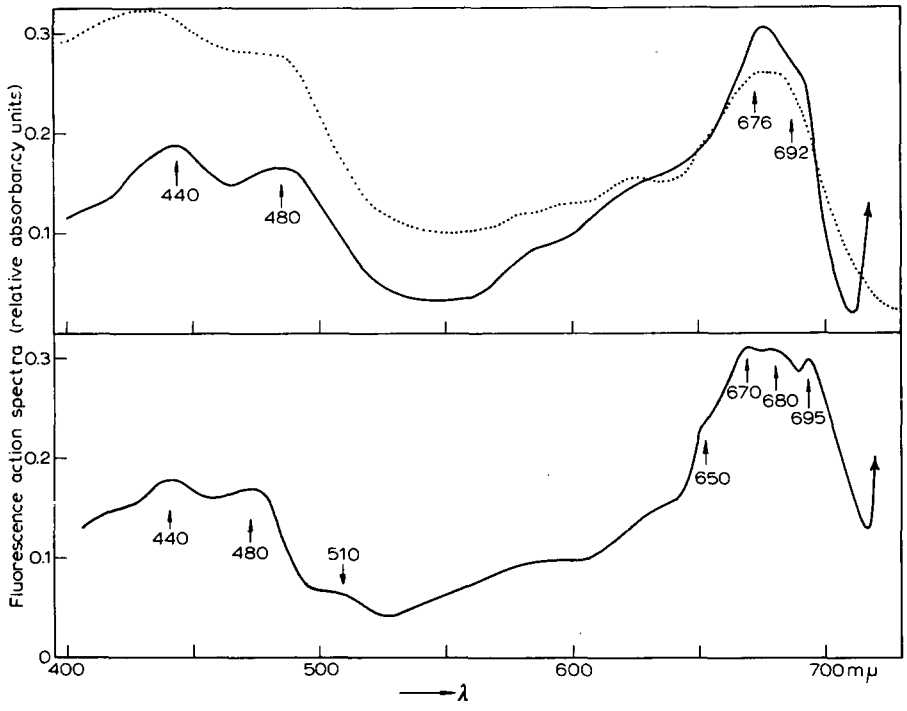

Fig. 7. (A), Room temperature fluorescence action spectrum (———) and absorption spectrum (·····) of a cell suspension of a 14 days old culture of *Euglena gracilis*. The fluorescence emission spectrum of these algae shows a maximum at about 705 mμ. (B), Liquid nitrogen temperature fluorescence action spectrum of a 14 days old Euglena culture.

In the carotenoid region two bands appeared at about 475 and 520 mμ as a result of cooling.

Green algae

Intact cells, dilute suspension

Figs. 5–7 show the absorption and fluorescence action spectra of the green algae *Tribonema equale, Chlorella pyrenoidosa* and *Euglena gracilis.*

Room temperature fluorescence action spectra. (1). Chlorophyll. In the chlorophyll *b* deficient alga *Tribonema equale* two marked chlorophyll *a* maxima stood out in the fluorescence action spectrum: one at about 672 and the other at about 440 mμ.

In the green alga *Chlorella pyrenoidosa* also, a marked band ascribed to chlorophyll *b* was present at about 652 mμ. A broad band occurred in the spectral region around 480 mμ, which was partly ascribed to the Soret band of chlorophyll *b*. The red chlorophyll *a* maximum was located at 674 mμ.

In the green alga *Euglena gracilis* the red chlorophyll *a* maximum was located at about 676 mμ, while a shoulder occurred on the long wave side at about 692 mμ. The samples used were taken from an aged culture of this alga. In the fluorescence emission spectrum of such a sample a marked maximum occurred around 705 mμ, while the usual 685 mμ fluorescence band was seen merely as a shoulder on the short

wave side of the 705 band in the fluorescence emission spectrum (*cf.* GOEDHEER[2], FRENCH AND BROWN[13]).

The fluorescence band at 705 mμ is assumed to be emitted by a chlorophyll *a* form with a maximum absorption at about 695 mμ. However, according to the fluorescence action spectrum, absorption by the other chlorophyll *a* forms also resulted in emission of the 705 mμ band.

The fluorescence action spectrum did not show a marked contribution of chlorophyll *b*.

(2). Carotenoids. Two bands, at 470 and 490 mμ approximately, showed up in the fluorescence action spectrum of Tribonema.

With Chlorella a high broad band occurred round 480 mμ, which was ascribed to the combined action of carotenoids and chlorophyll *b*. With Euglena also a marked broad band occurred in the action spectrum in this region.

Liquid nitrogen fluorescence action spectrum. (1). Chlorophyll. The fluorescence action spectrum of Tribonema did not show new bands at low temperatures. The red chlorophyll *a* maximum was broadened, but not doubled.

The action spectra of Chlorella showed that the red chlorophyll *a* band at low temperatures was composed of two parts: a maximum at 682 mμ and a shoulder at about 672 mμ. The intensity of fluorescence was sufficiently high to use a band width of 4 mμ, and to obtain a curve with a high signal/noise ratio. The curve thus obtained indicated, that the component of the red chlorophyll band which produced the shoulder at 672 mμ was appreciably broader at low temperature than the component of the red chlorophyll band which produced the peak at 682 mμ. The band present at about 650 mμ and ascribed to chlorophyll *b*, was also sharpened as a result of cooling.

The low temperature fluorescence action spectrum of Euglena showed a marked band at 695 mμ. Other maxima in the red chlorophyll band were seen at about 670 and 680 mμ, while a shoulder at 653 mμ indicated action of chlorophyll *b*.

(2). Carotenoids. The bands in the carotenoid region of the measured green algae did not change markedly as a result of cooling. The bands of Tribonema and Chlorella were not sharpened as might have been expected, but were somewhat broadened. With Euglena the band at 480 mμ was somewhat sharpened. A new weak band at about 510 mμ was seen in some cultures at low temperature.

Intact cells, dense suspension

As is seen in Fig. 2B, the fluorescence action spectrum of a dense suspension of Chlorella differed markedly at low temperature from the spectrum of blue-green algae. No marked "trough" was present at 690 mμ, a slight peak was observed around 700 mμ, but there were no bands further in the red. A similar "high density" action spectrum was found with samples of Tribonema and Euglena.

Greening bean leaves

The absorption spectrum of an etiolated bean leaf, measured 15 min after first illumination, is given in Fig. 8.

Room temperature fluorescence action spectrum. Fig. 8A also gives the room temperature fluorescence action spectrum of this bean leaf.

(1). Chlorophyll. Only chlorophyll *a* was found to be active in this leaf, which pigment showed bands at 670 and about 440 mμ.

(2). Carotenoids. Although the absorption spectrum was characterised by its high carotenoid absorption, no activity of carotenoids was found in the fluorescence action spectrum.

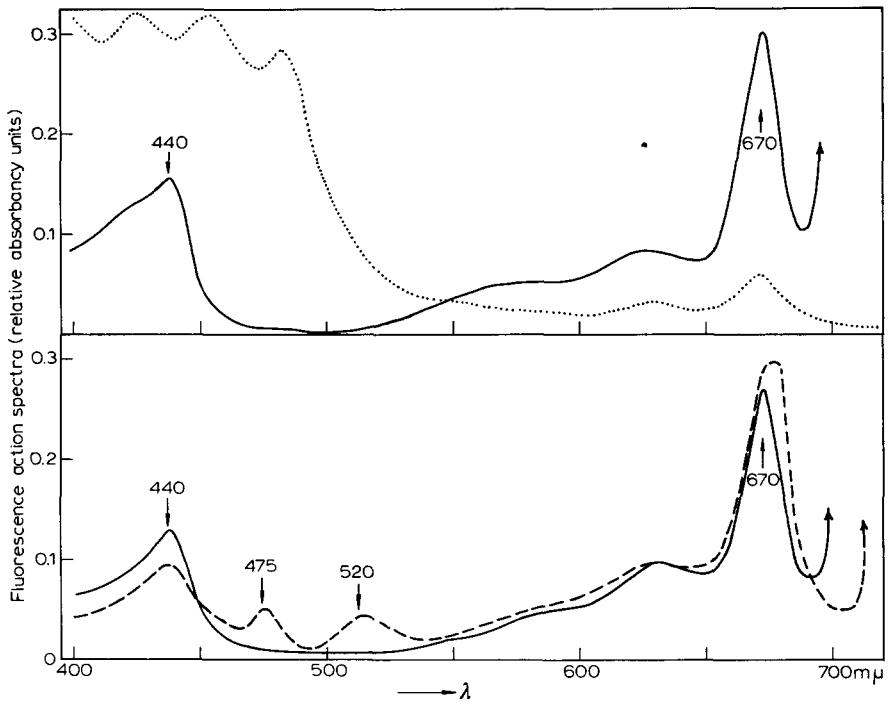

Fig. 8. (A), Room temperature fluorescence action spectrum (———) and absorption spectrum (·····) of a greening bean leaf measured 15 min after start of illumination. (B), Liquid nitrogen temperature fluorescence action spectrum of a greening bean leaf determined after the leaf has been illuminated for 15 min (———) and 4 h (– – – –).

Liquid nitrogen fluorescence action spectrum. (1). Chlorophyll. The red chlorophyll band of the "15-min" sample was sharpened as a result of cooling, while its maximum remained at 670 mμ. A fluorescence action spectrum measured after 1 h of illumination was found to be similar to the "15 min" one. Fig. 8B also gives a fluorescence action spectrum of a bean leaf after 4 h of illumination. This spectrum showed a broadening of the red chlorophyll band after cooling, but no shoulder at 695 mμ and no activity of chlorophyll *b*.

(2). Carotenoids. At low temperature no activity of carotenoids was seen in the "15 min" spectrum or in the "1 h" spectrum. After 4 h of illumination two bands were seen, at about 475 mμ and at about 520 mμ. These bands were seen also in the room temperature action spectrum of a 4 h illuminated leaf.

After 7 h of illumination a broad band with a maximum at around 480 mμ was measured. This band was similar in shape to the band at these wavelengths in Chlorella.

DISCUSSION

Chlorophyll

Though in the room temperature fluorescence action spectra of intact blue-green algae no red chlorophyll band was seen (*cf.* also DUYSENS[14]) chlorophyll activity was nearly hidden by the broad phycocyanin band. Removal of phycocyanin by grinding or heating resulted in the appearance of a red chlorophyll band. The violet band could also be measured in intact cells.

With all cells and leaves capable of photosynthesis, cooling resulted in a broadening, or a doubling, of the red chlorophyll band, towards the long wavelength side. Apparently a chlorophyll form absorbing at around 680 mμ, tentatively called C_a680, became active in the low temperature fluorescence.

Except for aged Euglena cells, in dilute suspensions no band or shoulder at around 695 mμ was seen, either in the room temperature or in the liquid nitrogen temperature action spectrum. The measured fluorescence at liquid nitrogen temperature, however, was in the wavelength region beyond 720 mμ, and was assumed to be emitted mainly by some far red chlorophyll *a* form, *e.g.* C_a695 (*cf.* GOEDHEER[2]). It thus should be concluded that a rather efficient energy transfer occurs from the chlorophyll forms C_a670 and C_a680 to the far red form C_a695 at these temperatures.

At room temperature fluorescence is assumed to be emitted mainly by a short wave chlorophyll form (C_a670–F686). This assumption agrees well with the observation that the chlorophyll form C_a680 was absent in the room temperature fluorescence action spectrum.

Some other results of action spectra can be understood in terms of the above conception.

(a). After a 15-min or a 1-h irradiation of etiolated bean leaves, the 670 mμ chlorophyll band in the fluorescence action spectrum was sharpened rather than broadened as a result of cooling. The liquid nitrogen fluorescence emission spectra showed that the fluorescence bands also are sharpened, but no new bands are formed[2]. New maxima only arise after about 4 h of greening. We assume that the C_a680 and C_a695 chlorophyll *a* forms are not yet produced after 1 h.

Bean leaves were found to be capable, with our growing conditions, of performing photosynthesis only after about 4 h of greening. It thus seems likely that the presence of the complex chlorophyll system is a prerequisite for photosynthetic activity.

(b). Aged Euglena cells showed a separate band at 695 mμ in the low temperature fluorescence action spectrum, but also bands at 680 and 670 mμ. At room temperature C_a695 was seen only as a shoulder on a band with maximum at 674 mμ. These action spectra make us suggest, that in Euglena C_a695 receives an appreciable amount of energy from C_a670 and C_a680 even at room temperature, but mainly from the first component.

(c). The low temperature action spectra for heated blue-green algae showed, instead of a broad maximum, a sharp peak at 682 mμ. This can be explained by assuming, that the fluorescence capacity of C_a670 is destroyed at a lower temperature than that of C_a680, this in contrast to the absorption bands. Indeed it was found that, in the low temperature emission spectra, the 686 mμ band disappeared after heating to 70°, while the 696 mμ band was much less decreased.

(d). The high-density fluorescence action spectra, when measured at low temperatures, differed appreciably from the absorption spectra, at least on the long wavelength side. This may be explained as follows.

As can be seen from the presence of bands at 686 and 696 mμ in the fluorescence emission spectrum—which bands are ascribed to C_a670 and C_a680—energy transfer from these forms to C_a695 did not occur without losses. As the low temperature fluorescence beyond 740 mμ is assumed to be emitted mainly by C_a695, this chlorophyll form will be more marked in the high density action spectrum than the other forms. This results in a peak at about 700 mμ, on the long wave side of C_a695. Such an action spectrum was shown by Chlorella and the other green algae measured (*cf.* Fig. 2B). The fluorescence action spectrum of a fully green bean leaf, measured by BUTLER[15] shows a similar shape.

The fact that such a spectrum of blue-green and red algae was more complex, could have been due to the presence of a comparatively high percentage of C_a680 in these algae. In the cultures used for these experiments, a high fluorescence band F696 was measured at low temperatures, indicating a relatively low energy transfer from C_a680 to C_a695. In such a way the "trough" in the high density action spectrum on the long wave side of C_a680, can be explained. The chlorophyll form C_a695 then accounts for the first far red peak (Fig. 2A). The second peak can be interpreted in two ways. First it may be due to another far red chlorophyll form. Second it may result from a difference in slope of C_a670 and C_a680. If C_a680 is much steeper at low temperature than C_a670 (some indication for this may be derived from Fig. 6) there will be some wavelength in the far red where C_a670 exceeds C_a680. The gradual disappearance of this second band with increasing temperature may be an argument in favour of the latter possibility.

It should be remembered that the exact location of the bands depends on absorption ratios, and thus differs with different pigment density.

Phycobilins

In the fluorescence action spectrum of Synechococcus the phycocyanin band, as shown in Fig. 1, showed a marked shoulder at about 645 mμ. This shoulder was absent in the action spectrum of extracted phycocyanin. It sharpened into a maximum after cooling to liquid nitrogen temperature. With Anacystis such a shoulder was much less pronounced at room temperature, but in the low temperature spectrum it could be seen clearly. Also in the absorption spectrum there was some difference between shapes of the phycocyanin bands in Synechococcus and Anacystis, while the absorption bands were broader than those of extracted phycocyanin. Thus, in the fluorescence action spectrum as well as in the absorption spectrum, of both species the phycocyanin band *in vivo* was broadened as compared to the phycocyanin band *in vitro*. This fact accounts, at least partly, for the obscuring of the red chlorophyll band in the action spectra.

To explain this broadening we might postulate the presence of several phycocyanin "forms" *in vivo*. Three peaks were found in the phycocyanin region in low temperature action spectra. Therefore we assume three "forms": Phy 620, Phy 630 and Phy 650, a nomenclature analogous to that for chlorophyll. It may be remarked that a pigment with absorption maximum at about 650 mμ also appears to be measured in low temperature derivative absorption spectra[16].

The absorption spectra of phycocyanin freshly extracted from Synechococcus and Anacystis were found to be similar, though not completely identical, if measured at pH 6. The differences *in vitro*, however, were much smaller than the ones *in vivo*. Thus the phycocyanin "forms" *in vivo* do not seem to differ much when they are extracted from the cell content. Their difference might be due only to difference in interaction; that is, to their location within the photosynthetic structure. O'HEOCHA[17] found a pigment called allophycocyanin with absorption maximum at 650 mμ to be present in various blue-green and red algae. It might be that "Phy 650" equals allophycocyanin, while for some reason or another this pigment does not show up in the absorption difference spectra of extracted phycocyanins.

The fluorescence maximum of phycocyanin *in vivo* was located at 655–660 mμ, while the fluorescence maximum of freshly extracted phycocyanin (buffered at pH 6.0) was located at 653 mμ. In view of the location at about 618 mμ of the absorption band of phycocyanin *in vitro*, the fluorescence maximum *in vivo* was most probably due to a short wave "Phy" form, and not to a possible "Phy 650".

The hypothesis could be made that the short wave "Phy" forms transfer light energy to C_a670, while "Phy 650" transfers energy to C_a680. In such a way phycocyanin could be active as an accessory pigment in both chlorophyll systems of photosynthesis. Although some experiments (changes in fluorescence action spectrum upon heating or upon addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea) could be explained by this assumption, it does not as yet seem to be in accordance with other observations.

Carotenoids

The absence of carotenoid bands in the room temperature fluorescence action spectrum of blue-green and red algae is in sharp contrast to the activity of these pigments in green algae and higher plants. Comparison of absorption spectra of lamellae of blue-green algae (Fig. 3A) with the absorption spectrum of chloroplast fragments of the chlorophyll *b* deficient green alga Tribonema (Fig. 5A) indicates that carotenoid absorption was about equal for both species. The fluorescence action spectrum of chloroplast fragments of Tribonema showed marked activity of carotenoids (bands at 470 and 490 mμ), while in the action spectrum of lamellae of blue-green algae no activity was seen. With chlorophyll *b* containing cells the action spectrum was affected by the presence of the Soret band of chlorophyll *b* at 475 mμ, but here also carotenoid activity seemed considerable.

Extraction of Synechococcus and Tribonema lamellae and grana with methanol and petroleum ether showed that with Synechococcus (and with other blue-green and red algae) the major fraction of carotenoids was hypophasic, while with the green algae an appreciable fraction was epiphasic. This suggests that the major fraction of the Synechococcus carotenoids are xanthophylls while an appreciable fraction of the Tribonema ones are carotenes (*cf.* KARRER AND JUCKER[18]). From this result it is tempting to assume, that carotenes transfer their energy to chlorophyll C_a670—which form is responsible for room temperature fluorescence—while the efficiency of transfer from xanthophylls is negligible. Such an hypothesis fits in with results from etiolated leaves. As is seen in Fig. 8, in the "15-min" (or "1-h") spectrum no bands were seen in the region of carotenoid absorption, though these pigments dominate the absorption spectrum (*cf.* also BUTLER[19] and GOEDHEER[8]). The major fraction of these carotenoids

are xanthophylls. Carotenes appear to be formed later in the developing stage of photosynthesis[11]. After 4 h of greening, a band was visible at about 470 mμ, and a broader one at 520 mμ. The band at about 470 mμ is tentatively ascribed to chlorophyll *b*, which has its violet band at about this wavelength. The nature of the 520 mμ band is unknown. After 6–7 h of greening a band appeared at about 490 mμ, which band together with the 470 mμ one formed a broad maximum. This band in the action spectrum is ascribed to carotene. The 520 mμ band then disappeared in the slope of the high 470–490 mμ band.

With blue-green and red algae two bands were visible in the liquid nitrogen action spectrum, which were not visible in the room temperature one.

These bands, located at about 475 and 520 mμ, were in the neighbourhood of corresponding bands in the absorption spectrum of myxoxanthophyll. This pigment, described by KARRER AND JUCKER[18] is present in some blue-green algae. In Anacystis and Porphyridium, however, no measureable amounts of myxoxanthophyll could be detected, in contrast to Synechococcus and Oscillatoria, but the mentioned bands were present in all four species in an about equal amount. Therefore nothing can be said as yet about the nature of the pigment(s) responsible for these bands. As the 475 and 520 mμ bands became visible at about the same temperature as the fluorescence bands F696 and F720 (—100° to —120°), they most probably transfer their absorbed light to either C_a680 or C_a695. The absence of the main carotenoid bands in the liquid nitrogen action spectrum shows that the major fraction of carotenoids also does not transfer energy to these chlorophyll forms.

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