Biochimica et Biophysica Acta, 314 (1973) 191–201

BBA 46586

CHLOROPHYLL *a* FORMS IN *PHAEODACTYLUM TRICORNUTUM*: COMPARISON WITH OTHER DIATOMS AND BROWN ALGAE

J. C. GOEDHEER

Biophysical Research Group, Institute of Physics, State University, Utrecht (The Netherlands)

(Received March 9th, 1973)

SUMMARY

The long-wave chlorophyll *a* forms in *Phaeodactylum tricornutum* (688 and 703 nm) change into a short-wave form, 670 nm, as a result of incubation with 55% glycerol, freeze–thawing, short ultraviolet irradiation and, probably, chloroplast preparation. This short-wave form is non-fluorescent. Fluorescence polarisation measurements indicate that the long-wave chlorophyll *a* molecules are oriented parallel to each other. Although "labile" long-wave chlorophyll *a* receives energy from Photosystem II pigments at room temperatures and follows the induction phenomena of fluorescence, it is indicated by afterglow experiments that it probably does not participate in Photosystem II.

Long-wave chlorophyll forms in *Fucus* are stable and probably are related to Photosystem I.

INTRODUCTION

In the fluorescence spectrum of *Phaeodactylum tricornutum* (a diatom) grown at relatively low light intensities, the main room temperature maximum is located at 708 nm. This fluorescence band is most likely emitted by a long-wave chlorophyll *a* form which shows an absorption shoulder around 703 nm[1]. Fluorescence polarisation measurements indicated that absorption in the long-wave part of the spectrum gave rise to a pronounced polarisation, suggesting an orientation of this long-wave form[2].

Heating the cells at 40 °C for 10 min, exposure to ethanol for several hours or ultraviolet irradiation results in a loss of the 708-nm fluorescence as well as in a loss of long-wave absorption. Also during preparation of chloroplasts, either by French press treatment, grinding or ultrasonic vibration the long-wave fluorescence and absorption disappears[1,3–5].

With intact cells at room temperature the time course of fluorescence induction at about 710 nm is similar to that at 680 nm, while addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) affected both emissions in a same way. Brown[1] concluded from these phenomena that the long-wave chlorophyll *a* form in *Phaeodactylum* participates in System II of photosynthesis.

At liquid-nitrogen temperature the long-wave emission is enhanced and shifted

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

towards 713 nm. At this temperature the fluorescence spectrum resembles that measured with various green, blue-green and red algae, like *Chlorella*, *Synechococcus* and *Porphyridium*[6]. Spectra of algae measured at temperatures between 77 and 300 °K show that the fluorescence around 720 nm increases gradually when the temperature is lowered[7]. With these algal species usually about 20% of total emission around 710–720 nm at room temperature should be ascribed to a chlorophyll *a* form responsible for the major band at 77 °K[8]. Here the long-wave emission is not abolished by moderate heating, ultraviolet irradiation or various methods of chloroplast preparation. At room temperature the 720-nm fluorescence does not follow kinetics of the 685-nm emission[9]. After fractionation of chloroplasts by detergents or French press treatment and subsequent differential centrifugation into fractions assumed to contain mainly Photosystem I and II particles, respectively, the long-wave fluorescence band is present in the former ones, while in some cases also a long-wave absorption shoulder can be measured at 77 °K[4,10].

Thus, in contrast to *Phaeodactylum*, in these organisms the long-wave absorption shoulder and the low-temperature fluorescence band around 720 nm seem to be closely connected to System I of photosynthesis.

The absorption and fluorescence spectra of various other diatoms and brown algae, however, often differ considerably from those in other groups of chlorophyll *a*-containing photosynthetic organisms[5,11]. In the low-temperature fluorescence spectrum, no separate 710–720-nm maximum is found, while at 77 °K the absorption maximum is located around 670 nm. This suggests that in these species the percentage of long-wave chlorophyll *a* belonging to System I is low. Therefore, to obtain information about the long-wave chlorophyll *a* forms in *Phaeodactylum*, its spectral properties were compared with diatoms and brown algae lacking these special forms. From the results of absorption, fluorescence and afterglow measurements, presented in this paper, conclusions are drawn about structural and functional aspects of these chlorophyll *a* forms.

METHODS

Phaeodactylum tricornutum was grown in artifical sea water at an average temperature of 18 °C and an average light intensity of 20 lux. *Nitschia palea*, another diatom, was grown at 27 °C in a light cabinet (14 h light, 10 h dark) at an intensity of 150 lux. Brown algae were kindly provided by the "NIOZ" (Dutch Marine Research Institution) at Den Helder.

Absorption spectra were determined in a Cary 14 R spectrofotometer. Spectra at 77 °K were recorded with a Cary low-temperature attachment or a Dewar vessel with windows on each side. The Cary attachment was provided with flat windows, also under 90° with the transmitted beam. This enabled the use of this attachment in the fluorescence polarisation measurements at low temperature. With these measurements the samples had to be a "clear glass" to avoid depolarisation due to scattering. To this purpose 55% glycerol was added to 0.02 M phosphate buffer solution (pH 7.3) containing the diatoms and frozen immediately after mixing. As glycerol may affect the spectra (see Results) absorption and fluorescence spectra were compared with samples frozen on filter paper without glycerol in the Dewar vessel. Little difference was found, provided freezing occurred immediately after mixing.

Fluorescence spectra and fluorescence excitation spectra were determined with an apparatus described earlier[6]. For the excitation spectra the incandescent lamp was replaced by a 100-W tungsten halogen lamp. Chloroplast suspensions were prepared by grinding the cells 5 min with carborundum at 4 °C in phosphate buffer. Chloroplasts and chloroplast fractions were separated from intact cells and carborundum by 2 times centrifugation at 3500 × *g* for 20 min.

In some experiments the cells were ground in buffer containing 1 M sucrose. Separation of chloroplasts from cells then occurred by 2 times centrifugation at 12000 × *g*.

Chloroplast preparation was also performed by ultrasonic vibration and by pressume gradient application ("French press").

Action spectra for chlorophyll afterglow were determined for the long-living component. They were measured with a circulating flow method, allowing the measurement of afterglow at 0.2 s after illumination of low intensity[12]. As a light source a 150-W tungsten halogen lamp was used. The volume of the illuminated cuvette was about 6 times that of the sampling circuit, allowing nearly continuous illumination of the cells. Indication of spectral composition of the afterglow was obtained by addition of interference filters between detection vessel and photomultiplier. Here saturating white light was used for excitation, and afterglow was measured after 10 min, allowing induction phenomena to be completed. Freeze–thawing experiments occurred by freezing cells in liquid nitrogen for 5 min and thawing them in running tap water of 13 °C.

Photobleaching in red light occurred with cells kept at 4 °C and illuminated with a 150-W projector with a 2-mm Schott RG 5 filter, transmitting red light ($\lambda > 650$ nm). Effects of ultraviolet irradiation were measured after the cells had been irradiated with a 100-W Bausch and Lomb mercury lamp.

RESULTS

In Fig. 1 the absorption, fluorescence emission and fluorescence excitation spectra of the diatoms *P. tricornutum* and *N. palea* are given for 14-day-old cultures, at 300 (a, b and c) and at 77 (d, e and f) °K. The largest spectral differences are seen in the fluorescence emission spectra. The overall fluorescence yield of *Phaeodactylum* is appreciably higher than that of *Nitschia*. With an equal absorption of about 40% at 547 nm (mainly due to fucoxanthol) the ratio of fluorescence intensity of *Phaeodactylum* to that of *Nitschia* was about 1.3 at 685 nm, and about 6 at 708 nm at 300 °K. At 77 °K these ratios were about 0.4 and 30.

The additional long wave absorption band of *Phaeodactylum*, shown in a difference spectrum between both species with absorption equalized at 672 nm, is high from 688 to 703 nm (Fig. 2a). In low-temperature spectra this difference is composed of bands at 690 and 707 nm. This additional absorption disappears when chloroplasts are prepared with the usual methods (Fig. 2b). Also when grinding the cells in buffer containing 1 M sugar or mannitol these bands are not retained during preparation. With increase of age of cells both the 703-nm absorption band and the 688-nm one increase (Fig. 2c).

The absorption difference spectrum of *Nitschia* cells measured at 300 °K appears to be higher between 640 and 660 nm than that of *Phaeodactylum*. This may be due

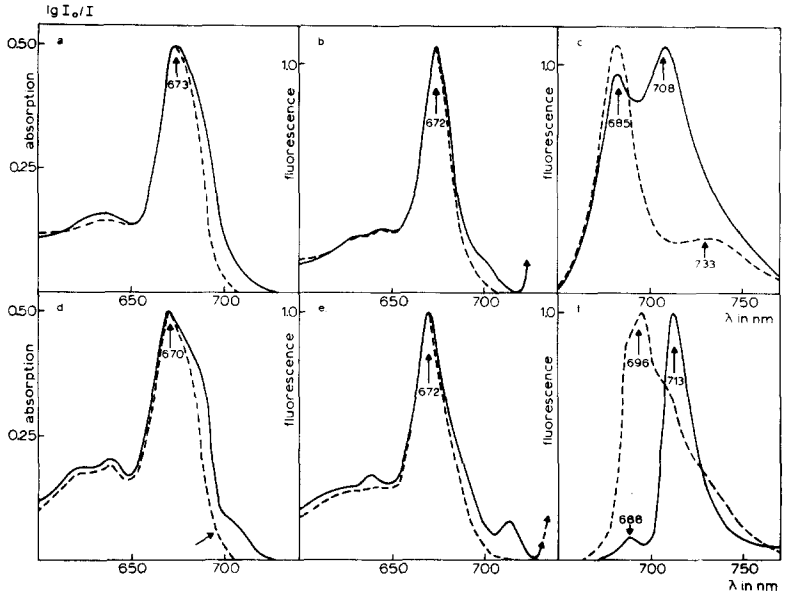

Fig. 1. Spectra of cell suspensions of the diatoms *Phaeodactylum* (———) and *Nitschia palea* (– – –). (a) Absorption at 300 °K. (b) Fluorescence excitation at 300 °K. (c) Fluorescence emission at 300 °K. (d) Absorption at 77 °K. (e) Fluorescence excitation at 77 °K. (f) Fluorescence emission at 77 °K.

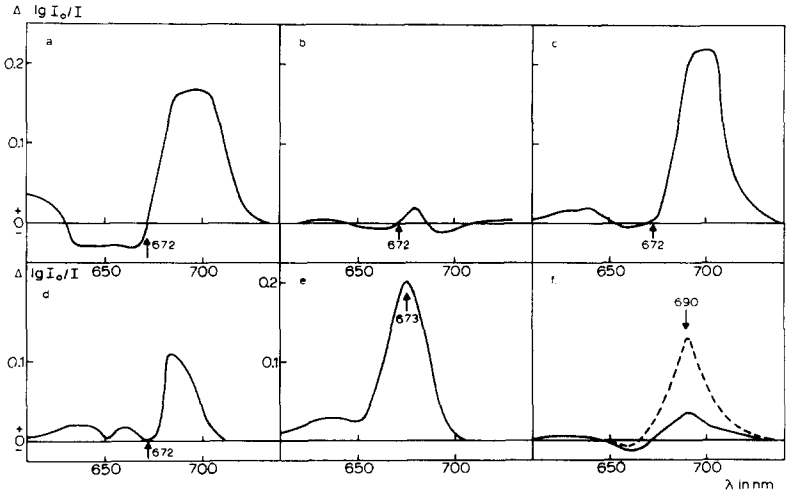

Fig. 2. (a) Absorption difference spectrum between a sample of *Phaeodactylum* and *Nitschia* cells equalised at 672 nm and measured at 300 °K. (b) Same as (a) of chloroplast preparations prepared by grinding. (c) Absorption difference spectrum between a 1.5-month- and a 14-day-old culture of *Phaeodactylum*. (d) Absorption difference spectrum between a sample of *Nitschia* cells and chloroplasts. (e) Absorption difference spectrum between a sample of *Phaeodactylum* chloroplasts kept in the dark and an identical sample photobleached in red light ($\lambda > 650$ nm) at 2 °C. (f) Absorption difference spectrum between a sample of *Phaeodactylum* cells kept in the dark and an identical sample irradiated with ultraviolet light for 1.5 (———) or 3 (– – –) min.

either to the presence of extra short-wave absorption in *Nitschia* or to artifacts, caused by higher scattering values and a different "sieve effect"[13] with intact cells of different dimensions.

The fluorescence excitation spectra, measured for the long-wave emission, show that the 688-nm absorption shoulder is rather low at 77 °K. This suggests that the efficiency of energy transfer from this band to the long-wave absorption band is appreciably below 100%. It seems unlikely that there is a shorter-wave emission to be ascribed to the 688-nm absorption band, which does not pass the cut-off filter in the fluorescence beam. This may be seen as follows. The far-red band in the fluorescence excitation spectrum is much more pronounced than in the absorption spectrum. In old cultures of *Phaeodactylum* the 707-nm band in the fluorescence excitation spectrum at 77 °K may amount to nearly 50% of the 670-nm band (*cf.* Fig. 5). The half width value and shape of the former band is similar to that of the 713-nm band in the fluorescence emission spectrum—resulting either from 437- or from 547-nm incident light—whereas if part of the fluorescence should be due to the 688-nm absorption, the fluorescence band should be much broader.

Absorption and fluorescence spectra of chloroplasts obtained from the brown algae *Laminaria digitata* and *Laminaria saccharosa* were similar to those of *Nitschia*, given in Fig. 1. Absorption and fluorescence spectra of chloroplasts obtained from *Fucus serratus* show appreciably more long-wave chlorophyll absorption and fluorescence than the former species, apparently this absorption and fluorescence is not annihilated by chloroplast preparation (Fig. 3).

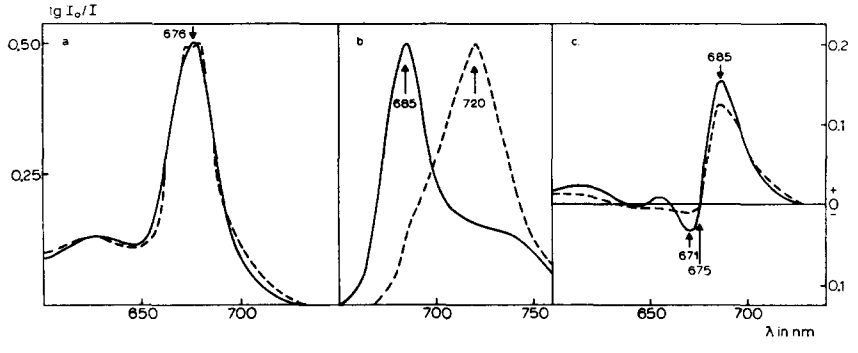

Fig. 3. Absorption and fluorescence spectra of a chloroplast suspension of the brown algae *F. serratus*. (a) Absorption spectra measured at 300 (———) and 77 (– – –) °K. (b) Fluorescence spectra measured at 300 and 77 °K. (c) Absorption difference between a sample of *Fucus* and *Laminaria* chloroplasts (equalized at 675 nm) at room (———) and at liquid-nitrogen (– – –) temperature.

In contrast to fluorescence and absorption properties of most other species of algal cells measured earlier (green, blue-green and red algae), the long-wave absorption and fluorescence bands in *Phaeodactylum* disappear when the diatoms are stored in the glycerol–buffer mixture used for low-temperature measurements. Fig. 4 shows absorption, fluorescence and absorption difference spectra of a sample immediately after mixing, after 30 min and after 1 h storage in the dark at 22 °C.

The figures indicate that the absorption between 680 and 710 nm decreases, while around 670 mn an increase occurs. The fluorescence drops considerably at all

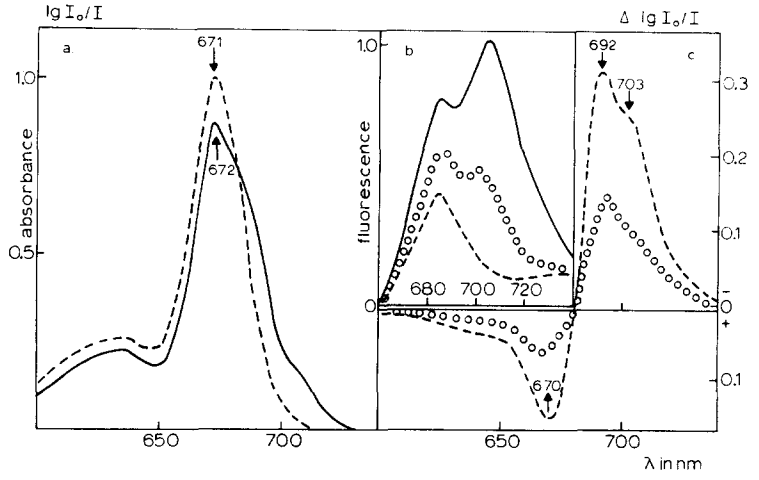

Fig. 4. Absorption (a) and fluorescence emission (b) spectra at 300 °K of samples of *Phaeodactylum* cells suspended in 55% glycerol and 45% 0.02 M phosphate buffer (pH 7.3), measured immediately after mixing (——), after 30 min (○○○), and after 1 h (– – –) storage in the dark. (c) Absorption difference spectra between these samples.

wavelengths. After prolonged storage the fluorescence spectrum is similar to that of chloroplast suspensions and lacks the 708-nm band. Most probably the strongly fluorescing long-wave chlorophyll form absorbing around 703 nm and 688 nm is changed into a non-fluorescing form with maximum around 670 nm. Similar changes in absorption and fluorescence spectra are found when the cells are frozen and subsequently thawed. After one time freeze–thawing the 710-nm fluorescence band decreases gradually by a factor of 14 upon 30 min storage in the dark at 20 °C, while fluorescence at 685 nm decreases by a factor of 3. Storage at 4 °C greatly reduced the decrease of the 710-nm band.

Also irradiation with ultraviolet light results in comparable changes (Fig. 2f). With short irradiation times a positive branch is found in the absorption difference spectrum, indicating increase in absorption around 665 nm. With increase in time of irradiation absorption around 670 nm also decreases.

The newly found non-fluorescing 670-nm band resulting from storage in 50% glycerol, freeze–thawing and short ultraviolet irradiation and possibly also chloroplast preparation has a maximum at slightly shorter wavelength than the main chlorophyll *a*. The area in the difference spectrum of the 670-nm absorption is lower than that of the disappearing 688- and 703-nm bands. Extraction of normal and glycerol-treated "old" cells with 80% acetone yielded, when measured spectroscopically, the same chlorophyll *a* content to within 5%. Chromatograms of pigments extracted from untreated cells, cells treated with glycerol and chloroplasts showed only a single chlorophyll *a* band and no measurable percentage of pheophytin. The fraction of chlorophyllide *a*, which did not move much in the chromatogram with the eluent used, was higher in the treated cells and chloroplasts than in the untreated cells. Spectroscopically chlorophyllide is nearly identical to chlorophyll *a*. Irradiation of chloroplasts with strong red light or prolonged ultraviolet irradiation resulted in a decrease at all

wavelengths. The shape of the absorption difference spectrum was similar to that of the absorption spectrum (Fig. 2e).

As shown in Fig. 5, low-temperature fluorescence action spectra in polarised light show, with *Phaeodactylum* cells, a fairly good polarisation in the long-wave bands. In the 670-nm region and in that of chlorophyll *c* around 635 nm the absorption and polarisation values are lower. Spectra taken at room temperature also show relative high fluorescence polarisation in the long-wave part of the spectrum. With *Nitschia* cells exact polarisation spectra could not be obtained due to the low intensity of far-red emission. Values obtained with interference filters showed that very low polarisation occurred. As light scattering at cell walls results in a depolarisation, the intrinsic polarisation values will be higher. However, chloroplast preparations with a high long-wave absorption could not be obtained.

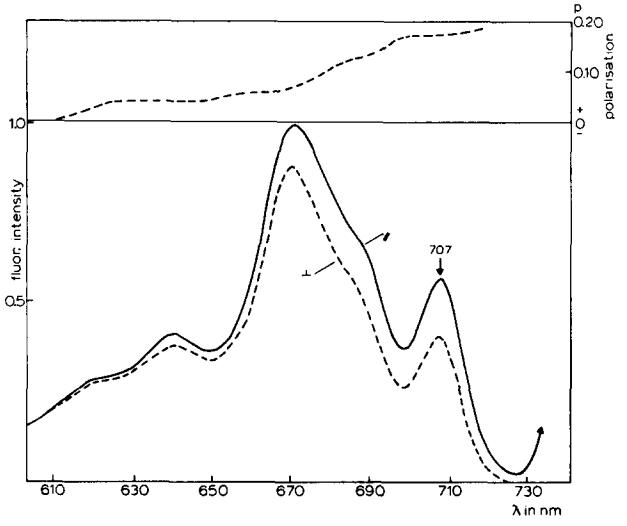

Fig. 5. Fluorescence action spectra of *Phaeodactylum* cells (1.5-month-old culture) in light polarised parallel (———) and perpendicular (– – –) to the direction of polarisation of incident light and measured at 77 °K. In the upper part the value of fluorescence polarisation $[p=(I_{\parallel}-I_{\perp})/(I_{\parallel}+I_{\perp})]$ is given.

The measurements of Brown[1], which showed that with intact cells the fluorescence induction curve at 710 nm about equals that at 685 nm and is equally affected by DCMU, were confirmed. These phenomena, however, do not necessarily indicate that the long-wave chlorophyll *a* forms in *Phaeodactylum* participate in Photosystem II. As these forms are easily destroyed by chloroplast preparation, Hill reaction could not be used as indicator of System II activity. With intact cells afterglow is assumed to be emitted by System II pigments[12,14]. Thus, action spectra of afterglow may be a measure of System II activity, provided the intensity of excitation is sufficiently low to prevent luminescence quenching by System I absorption[12]. In Fig. 6 the action spectra for chlorophyll afterglow, measured with the circulating flow method at 0.2 s after illumination, is given for *Phaeodactylum* and *Nitschia* cells. Very little difference is seen for these samples, although absorption and fluorescence spectra differ considerably (Fig. 1). With old cultures (1.5 months) of *Phaeodactylum*, which contain

appreciably more long-wave chlorophyll *a*, the action spectrum also is similar to that of *Nitschia*, though the afterglow intensity is much less. Similar action spectra were also obtained with chloroplast suspensions of the brown algae *L. digitata* and *F. serratus*. This suggest that the long-wave chlorophyll *a* forms do not participate in System II activity, but according to the fluorescence emission spectrum in *Phaeodactylum*, drain an appreciable fraction of energy by System II pigments.

The afterglow emission values for different wavelengths, measured with interference filters, is given in Table I. It is shown that at 710 nm afterglow is higher in *Phaeodactylum* than in *Nitschia*, but this difference is much lower than in the fluorescence spectrum.

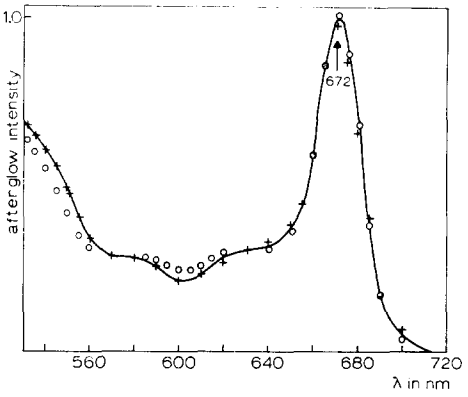

Fig. 6. Action spectrum for chlorophyll afterglow of *Phaeodactylum* (+) and *Nitschia* (○) cells measured 0.2 s after illumination with the circulating flow method.

TABLE I

SPECTRAL DISTRIBUTION OF AFTERGLOW OF *PHAEODACTYLUM* AND *NITSCHIA* MEASURED WITH INTERFERENCE FILTERS ($\lambda_{\frac{1}{2}}$ ABOUT 9 nm)

Afterglow values are arbitrarily put to 1.0 at 685 nm.

	Afterglow value			
	685 nm	*695 nm*	*710 nm*	*740 nm*
Phaeodactylum	1.0	8.8	7.0	5.5
Nitschia	1.0	6.0	3.2	2.5

DISCUSSION

The most marked phenomenon resulting from cell breakage, incubation in 50% glycerol, freeze–thawing or a short period ultraviolet irradiation is the complete disappearence of the long-wave fluorescence band measured at room temperature, while the 685-nm band, taking into account the overlap with the 710-nm one, is little affected. This is coupled with a decrease in absorption on the long-wave side of the red chlorophyll band and in increase around 670 nm. The extraction experiments showed that the disappearence of these "labile" long-wave chlorophyll *a* forms in

Phaeodactylum is probably not caused by a destruction of chlorophyll. Also a molecular change in the porphin head of the chlorophyll molecule seems, in view of the results from chromatography, less likely. This suggests that only the special conditions prevailing in intact cells and leading to the "labile" long-wave forms, are changed by the treatments described.

The nature of such special conditions needs some consideration. Pigment aggregation *in vitro* in monolayers and crystals results in a long-wave shift of the red absorption band and disappearence of fluorescence (*cf.* ref. 15). In some cases shifts up to 740 nm can be observed[16,17]. Chlorophyll–water–pheophytin aggregates in *n*-octane show an absorption band at 712 nm[18]. The absence of a measurable amount of pheophytin in the chromatograms from cells and chloroplasts indicates, however, that it is unlikely that the "labile" long-wave chlorophyll, which may amount to 10–15% of total chlorophyll according to absorption difference spectra, is represented by such a complex. This does not exclude that the energy acceptor of System I, P700 is represented by such a complex (*cf.* ref. 19), as this pigment from is present in a very low concentration.

Apart from aggregation in fixed units a red shift of the absorption band may also result from pigment orientation. This shift is also caused by dipole interaction. With *Phaeodactylum* the excitation spectra in polarised light show relatively high polarisation values for both long-wave bands. Provided the particle does not rotate during the mean lifetime of fluorescence, high polarisation values are brought about either by a very low "local" pigment concentration, which does not allow energy transfer between adjacent pigment molecules, or by an oriented system of closely packed pigment molecules in which energy transfer does not alter the direction of emission. In view of the high long-wave band in the excitation spectrum, indicating a large number of molecules, it seems likely that polarisation of fluorescence here is caused by a parallel orientation of the long-wave molecules. A change in environmental conditions could disturb the orientation and, with it, the special properties caused by this orientation: long-wave shift of absorption band, fluorescence yield, and polarisation. The spectral properties of the disturbed system may resemble those of colloidal chlorophyll *a* in buffer solution: absorption around 670 nm and absence of fluorescence. The occurrence of the long-wave forms then, is closely connected to the structural integrity of the chloroplast. The relatively high proportion of chlorophyllide *a* in the chromatograms of treated cells and chloroplasts is probably caused by the presence of a highly active chorophyllase in this organism[20,21]. It is possible that the pigment orientation is destroyed by a separation of phytol and the porphin head of the molecule. The gradual decrease in long-wave fluorescence band upon storage in the dark after freeze–thawing at 20 °C but less at 4 °C seems to point into this direction.

From the fluorescence excitation spectra it follows that light absorbed in the "labile" 688-nm band (C_a 688*) is not effectively transferred to the band of longest wavelength which is responsible for fluorescence emission. This is in contrast to what was found to happen in *Euglena*, where also a labile 688-nm band is found. It may be explained by assuming that in *Phaeodactylum* the fluorescence yield of C 688* is low. The yield of the labile 703-nm form (C_a 703*–F 708*) is appreciably higher than that of the usual System II chlorophyll *a*, at room as well as at liquid-nitrogen temperature.

In view of the difference in shape between absorption and flourescence excitation spectra, it may be concluded that energy transfer from fluorescing System II chlorophyll *a* (C_a 672–F 685) to C_a 703*–F 708* does not exceed 25% in relatively young cultures at room temperatures, in spite of the high 708-nm band in the fluorescence emission spectrum. The fluorescence yield (and probably lifetime) of the C_a 703* –F 708* form, however, appreciably exceeds that of C_a 672–F 685 and consequently a high 708-nm emission band results at 300 °K. This indicates that with this organism fluorescence yield is not related to chlorophyll content.

If the lifetime of System II fluorescence (F 685) is shortened during induction processes, the efficiency of energy transfer from C_a 672–F 685 to C_a 703*–F 708* also decreases and as a result the induction of the long-wave emission band follows that of the short-wave one. An energy transfer of about 25% may be accomplished by a spatial separation between the long-wave forms and System II chlorophyll *a*. Aging of cells results in an increase in the percentage of chlorophyll *a* present in the labile long-wave forms, in particular the C_a 703*–F 708* one.

The action spectra of chlorophyll afterglow suggests that the oriented long-wave chlorophyll *a* forms do not participate in System II of photosynthesis. They drain, however, part of the energy absorbed by Photosystem II pigments. In this respect it may be remarked that both yield of afterglow and yield of oxygen production was found to decrease when the cells accumulate more "labile" long-wave chlorophyll *a*. These forms could function as protecting agents in case of saturating intensity of irradiation of whole cells.

High-intensity photobleaching in red light of *Phaeodactylum* chloroplasts, where the long-wave forms are absent, affected, according to Fig. 2f, the whole chlorophyll band. This is in contrast to most other algal chloroplast suspensions, where primarily chlorophyll *a* of System I was found to be bleached[11].

Long-living afterglow is assumed to arise from excitation of System II chlorophyll *a*, probably by chemical back reactions. Once chlorophyll is in its excited state again, light emission can occur and energy transfer to other fluorescing molecules is possible. Hence, although the long-wave forms do not show afterglow due to their own absorption, indirect excitation after back reactions from System II products may result in their presence in the luminescence emission spectrum.

F. serratus, as shown in Fig. 3, is an example of a brown alga which contains chloroplasts with an appreciable fraction of "stable" long-wave chlorophyll *a*. Chloroplast preparation by the usual methods (short ultraviolet irradiation or incubation in 50% glycerol) did not affect the shape of the spectra markedly. Absorption and fluorescence spectra of this organism resemble those of various blue-green and red algae. An absorption difference spectrum between *Fucus* chloroplasts and *Laminaria*, *Nitschia* or *Phaeodactylum* chloroplasts shows a band at 684 nm with a shoulder around 703 nm. These bands disappear preferentially during photobleaching in red light[11]. The action spectrum of chlorophyll afterglow in *Fucus* is similar to that given in Fig. 6. In the fluorescence action spectrum of the high 720-nm emission band, measured at 77 °K, a band around 684 nm with shoulder at about 704 nm is observed. From these spectral properties it may be concluded that the "stable" 684-nm band and 703-nm shoulder in *Fucus* belong to the System I pigment system, and are similar to those in other groups of algae.

Thus, though the spectral properties of these "stable" long-wave forms closely

resemble those of the "labile" ones, the functional and structural relation to the chloroplast is quite different.

REFERENCES

1 Brown, J. S. (1967) *Biochim. Biophys. Acta* 143, 391–398
2 Goedheer, J. C. and Gulyaev, B. A. (1971) *Proc. 1st Eur. Biophys. Congr.*, Part IV, pp. 43–47, Verlag Wiener Med. Acad., Baden
3 Brown, J. S. (1968) *Carnegie Inst. Wash. Yearb.* 67, 528–534
4 Brown, J. S. (1969) *Biophys. J.* 9, 1542–1552
5 Sugahara, K., Murata, N. and Takamyia, A. (1971) *Plant Cell Physiol.* 12, 377–385
6 Goedheer, J. C. (1964) *Biochim. Biophys. Acta* 88, 304–317
7 Cho, F. and Govindjee (1970) *Biochim. Biophys. Acta* 205, 371–378
8 Goedheer, J. C. (1972) *Annu. Rev. Plant. Physiol.* 23, 87–112
9 Lavorel, J. (1962) *Biochim. Biophys. Acta* 60, 510–523
10 Ogawa, T., Vernon, L. P. and Mollenhauer, H. H. (1969) *Biochim. Biophys. Acta* 172, 216–229
11 Goedheer, J. C. (1970) *Photosynthetica* 4, 97–106
12 Goedheer, J. C. (1962) *Biochim. Biophys. Acta* 64, 294–308
13 Duysens, L. N. M. (1956) *Biochim. Biophys. Acta* 19, 1–12
14 Haug, A., Jaquet, D. D. and Beall, H. C. (1972) *Biochim. Biophys. Acta* 283, 92–99
15 Ke, B. (1966) in *The Chlorophylls* (Vernon L. P. and Seely G. R., eds), pp. 253–279, Academic Press, New York
16 Ballschmiter, K. and Katz, J. J. (1969) *J. Am. Chem. Soc.* 91, 2261–2265
17 Jacobs, E. E., Vatter, A. E. and Holt, A. S. (1954) *Arch. Biochem. Biophys.* 53, 228–238
18 Norris, J. R., Uphaus, R. A., Cotton, T. M. and Katz, J. J. (1970) *Biochim. Biophys. Acta* 223, 446–449
19 Katz, J. J. (1973) *Naturwissenschaften* 60, 32–39
20 Barrett, J. and Jeffrey, S. W. (1964) *Plant Physiol.* 39, 44–47
21 Jeffrey, S. W. (1972) *Biochim. Biophys. Acta* 279, 15–33