Effects of light quality on chlorophyll-forms Ca 684, Ca 690 and Ca 699 of the diatom *Phaeodactylum tricornutum*

LISE CARON [1], HENRI JUPIN [2], and CLAIRE BERKALOFF [1]

[1] Laboratoire de Botanique de l'Ecole Normale Supérieure, 24 rue Lhomond, F-75231 Paris Cedex 05, France
[2] Laboratoire de Biologie Végétale, Université F-66025 Perpignan Cedex, France

Key words: Chlorophyll-complexes, diatom, emission and excitation fluorescence, photosystems.

Abstract. Colored light modifies the relative concentration of chlorophyll-forms of the diatom *Phaeodactylum tricornutum* compared to white-light control. No change in the ratio carotenoids/chlorophylls was observed after 4 days exposure to green light (max: 530 nm), blue light (max: 470 nm) or red light ($\lambda > 650$ nm) of same intensity.

However, the absorption spectra were modified, the content in Ca 684, Ca 690, Ca 699 forms increased in red and green light cultures and photosynthetic unit size of PS II decreased by 30% in green and blue light cultures.

Fluorescence emission and fluorescence excitation spectra according to the Butler and Kitajima method (1975) were carried out for each culture. Ca 669 form was predominant in the two photosystems. The newly appeared far red forms fluoresce at 715 nm like PS I forms.

We conclude that these new forms originated in a rearrangement of PS II forms. They do not transmit excitation energy to reaction center of PS I and are disconnected from the other chlorophyll-forms of the photosynthetic antennae.

Introduction

Changes in the spectral quality of the incident light induce important modifications in the photosynthetic pigments, chloroplast structure and metabolism of diverse cultivated algae [12, 15, 16, 17, 18]. The effects have been correlated in some cases with chromatic adaptation to medium conditions [4]. These modifications have been observed in algae submitted during several weeks to practically monochromatic light. Diatoms, in these culture conditions, showed far red absorbing chlorophyll-forms, in particular Ca 705 [8, 9, 12].

In this study, we show that even a short time of illumination (4 days) in monochromatic conditions induces rapid modifications of the spectro-

Abbreviations: ABS = absorption; Ca = chlorophyll-complex; chla = chlorophyll a; chl c = chlorophyll c; chl t = total chlorophylls; D.C.M.U. = 3- (3, 4 dichlorophényl) 1-diméthyl-urea; d^v = division; F = fluorescence; PS I and PS II = photosystem I and photosystem II

Photosynthesis Research 4, 21–33 (1983)

scopic properties of the diatom *Phaeodactylum tricornutum*. The analysis of these changes by means of fluorescence excitation spectra [5] allow us to define the composition of photosystem antennae.

Material and methods

Axenic cultures of *Phaedactylum tricornutum* (Bhölin) were performed on a complete medium [13] at 19°C and were exposed to alternate 12 H periods of light and darkness. The cells were continuously mixed by bubbling. Light was provided by white fluorescent tubes (Claude U 20 RS) with maxima at 420, 525, 575 nm. The transmission spectra of filters allowing the different colored lights to be obtained are presented in Figure 2 (b^1, c^1, d^1). The light energy was the same in all the cases.

In the euphotic zone of the ocean, light predominantly consists of low intensity blue and green radiations according to water turbidity. To simulate marine irradiances, we have studied the effects of green and blue lights on the growth and the photosynthetic pigments. It is also known that red light induces the formation of far red absorbing chlorophyll-forms in some Diatoms [12].

Cells were counted with a Thoma cell every day at the same hour in order to obtain growth curves. The samples for pigment analysis and fluorescence measurements were taken during the third hour of the light period after 4 days exposure to different lights: at the time, algae grown in white, blue or red light were effectively in the exponential phase and the pigment concentration per cell was stabilized.

The algae were disrupted with a French Press and then were extracted with acetone 90%. Concentrations of chl a and chl c were calculated according to Jeffrey and Humphrey [10].

Low temperature absorption and fluorescence emission spectra have been previously described [1]. Whole cells were filtered on Millipore filters and rapidly cooled in liquid nitrogen (the optical density at 675 nm was adjusted to 0.05). The Gaussian analysis of the absorption spectra was obtained according to French et al. [7]; the maximum wavelength of the gaussian curves are calculated from the fourth derivatives.

The fluorescence excitation spectra were obtained according to the Butler and Kitajima method (1975). The apparatus is as described earlier [14]; the algae have been layered on Millipore filters (1, 7 μg chl a cm^{-2}) after 15 minutes in the dark. The frozen filter has been flashed for 5 to 10 minutes to close the PS II reaction centers.

The fluorescence induction curves were obtained in whole cells at 20°C. The exciting light was passed through a blue filter (438 nm). The fluorescence emission was detected by a photomultiplier with a red filter (Matra 692 nm). Finally, the signal was analysed with an oscilloscope (Tektronix 5103 N). [3].

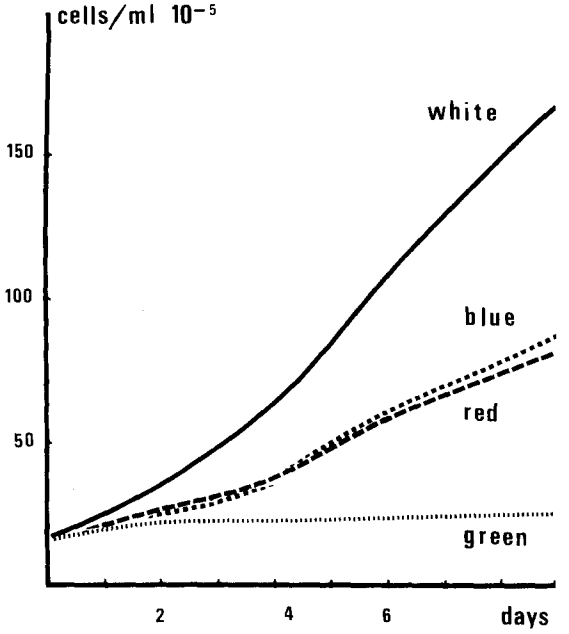

Figure 1. Growth curves of *Phaeodactylum tricornutum* in 4 W/m² white, blue, red and green light (12:12 light-dark cycle) at 19 °C.

Table 1. Chlorophyll-pigments content in cells grown under different light conditions for 4 days (absolute error = ± 0.03 μg × 10^{-7})

Cultures illuminations	white	blue	red	green
chl_t/cell × 10^{-7} μg	2.46	2.92	2.60	3.00
chl_a/cell × 10^{-7} μg	2.10	2.48	2.18	2.45
chl_c/cell × 10^{-7} μg	0.36	0.44	0.42	0.55
chl_a/chl_c	5.8	5.6	5.2	4.5

Results

(1) Growth characteristics (Figure 1)

The algae grown in white light divided more frequently than the other ones (1 d^v/3 days). Cultures in red or blue light grew more slowly (1 d^v/4 days). In green light, algae hardly divided at all (1 d^v/month). The growth curves are presented in Figure 1.

(2) Chlorophyll content (Table 1)

The chlorophyll content per ml was the greatest in cultures grown in white light. In the meantime, culture growth in green, red or blue light increased the amount of chlorophyll per cell. Chlorophyll content per cell was the greatest in algae grown in green light. The ratio chl a/chl c was also modified, being lower in algae grown in green or red light.

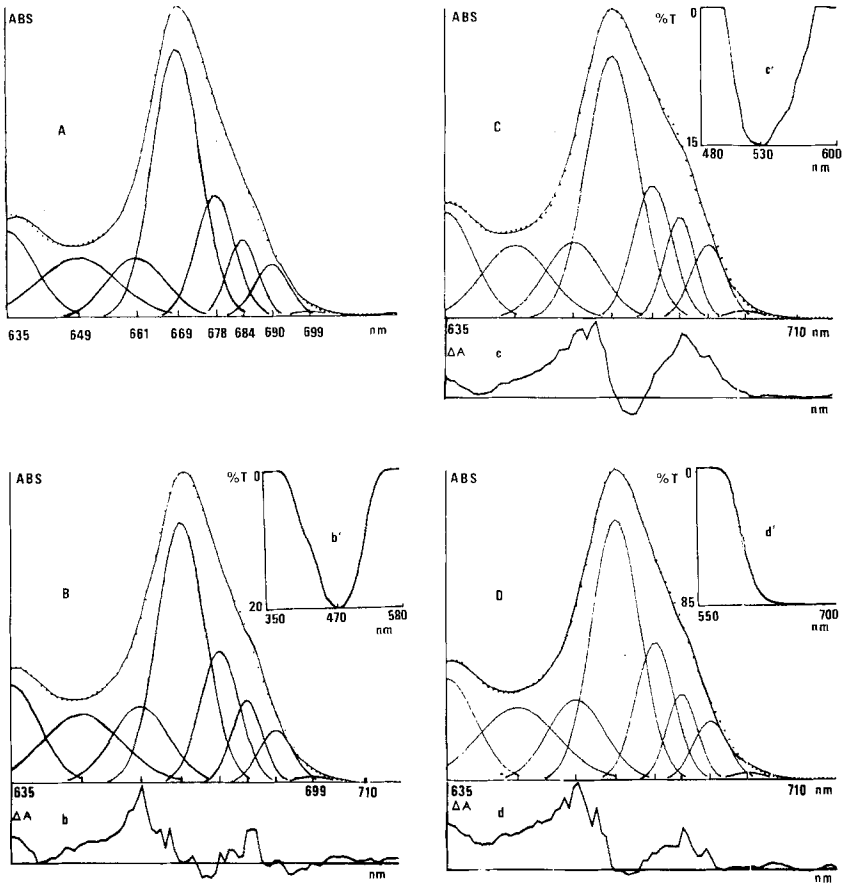

Figure 2. Absorption spectra between 635 and 715 nm at – 196 °C of algae (4 days old) grown in A: white light, B: blue light, C: green light, D: red light and their Gaussian decomposition. – Difference between the absorption spectra of algae cultivated in colored light and the absorption spectrum of algae grown in white light, b: 'blue' minus 'white', c: 'green' minus 'white', d: 'red' minus 'white'. – The passbands of the transmission spectra of utilised filters for the different types of incident light on the cultures, b^1: blue filter, c^1: green filter, d^1: red filter.

(3) Absorption spectra at – 196 °C (Figure 2)

The absorption spectra showed no chromatic adaptation of the carotenoids, for the ratio carotenoids/chlorophylls did not vary. Only the ratio chl a/chl c changed; the cultures in green or red light were enriched in chl c. This result confirms those obtained by chlorophyll titration.

The spectra also showed differences in the range 630–720 nm (Figure 2 (A, B, C, D)). The red absorption band appeared wider in the cultures grown in colored light and especially in those grown under green or red light. The differences between the absorption spectra of algae grown in green or red

Table 2. Percentages of the eight Gaussian components calculated from absorption spectra (635–715 nm) at — 196 °C of the algae cultivated under the four different types of incident light (4 days-old cells) chl a + c = 100%

	chl c	chl a						
λ nm of Gaussian components	635	649	661	669	678	684	690.5	699
white light	7.6	13.5	10.3	39.4	15	7.6	6	0.6
blue light	8	14.6	12.3	36	15.2	7.6	5.6	0.6
green light	8.1	12.4	11.5	36.7	14.3	8.7	7.4	0.8
red light	8	14.9	12.5	34.9	15.2	7.6	6.1	0.8

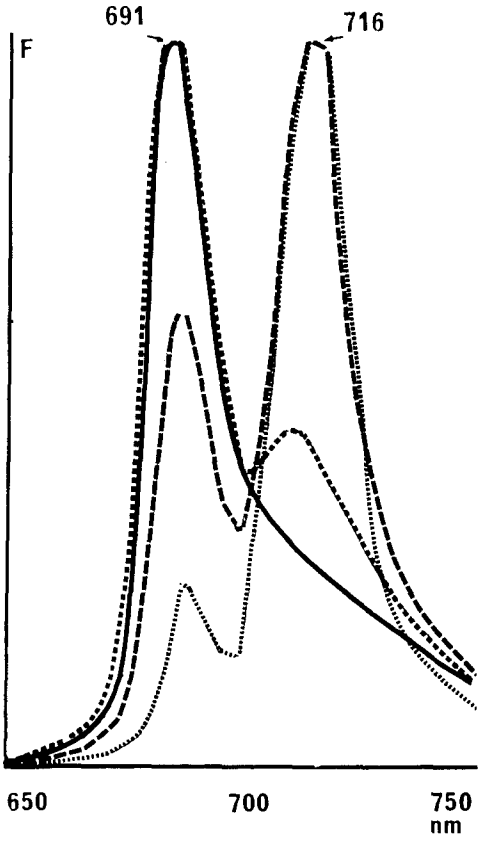

Figure 3. Fluorescence emission spectra at —196 °C of 4 days-old algae grown in: — white light, ••• blue light, – – red light, ... green light.

light and those of algae grown in white light indicated a higher absorption of wavelengths above 668 nm in cultures under monochromatic light (Figure 2b, c, d)). For these long wavelengths diffusion phenomena can be neglected. Eight Gaussian absorption bands allowed a good resolution of the absorption spectra between 635 and 730 nm (multiple correlation coefficient larger than 0.9994) (Figure 2 (A, B, C, D)). The predominant chlorophyll-form was Ca 669 in all the cultures. The algae grown in green or red light presented 30% increase of the Ca 699 complex in comparison with the other cultures. The Ca 684 and Ca 690 also increased in algae grown in green light (15% and 20% respectively (Table 2)).

(4) Fluorescence emission spectra at − 196°C

The spectra showed 2 emission peaks or shoulders: one at 690–692 nm, the other at 715–717 nm (Figure 3). The green light culture showed an emission peak at 716 nm higher than that of the other cultures. The red light culture presented an intermediate form. The 691 nm peak was predominant for the cultures in white or blue light.

The differences can easily be correlated with the absorption spectra characteristics: the far-red absorption increase was accompanied by a high peak of fluorescence emission at 716 nm in algae grown in red or green light. Even during the first few days, the differences between the cultures were observable and persisted with time.

(5) Fluorescence induction at 20°C

The half-increase time of the fluorescence induction curve in presence of D.C.M.U. was different in each culture; in green or blue light cultures the half-increase times were greater than in red or white light cultures (Table 3). It can be concluded that PS II antennae of algae grown in green or blue light were smaller than those of the other cultures. The area included between the induction curve and its asymptote, proportional to the amount of the primary acceptor of PS II, did not vary significantly. Thus, the culture conditions have not appreciable influence on the number of active PS II reaction centers.

(6) Fluorescence excitation spectra at − 196°C

The fluorescence excitation spectra at 715 nm allowed us to obtain action spectra of PS I and PS II (5). The variable part (F_β or F_v) of fluorescence corresponding to action spectrum of PS II did not show any appreciable change related to variations in the quality of incident light (Figures 4 and 5).

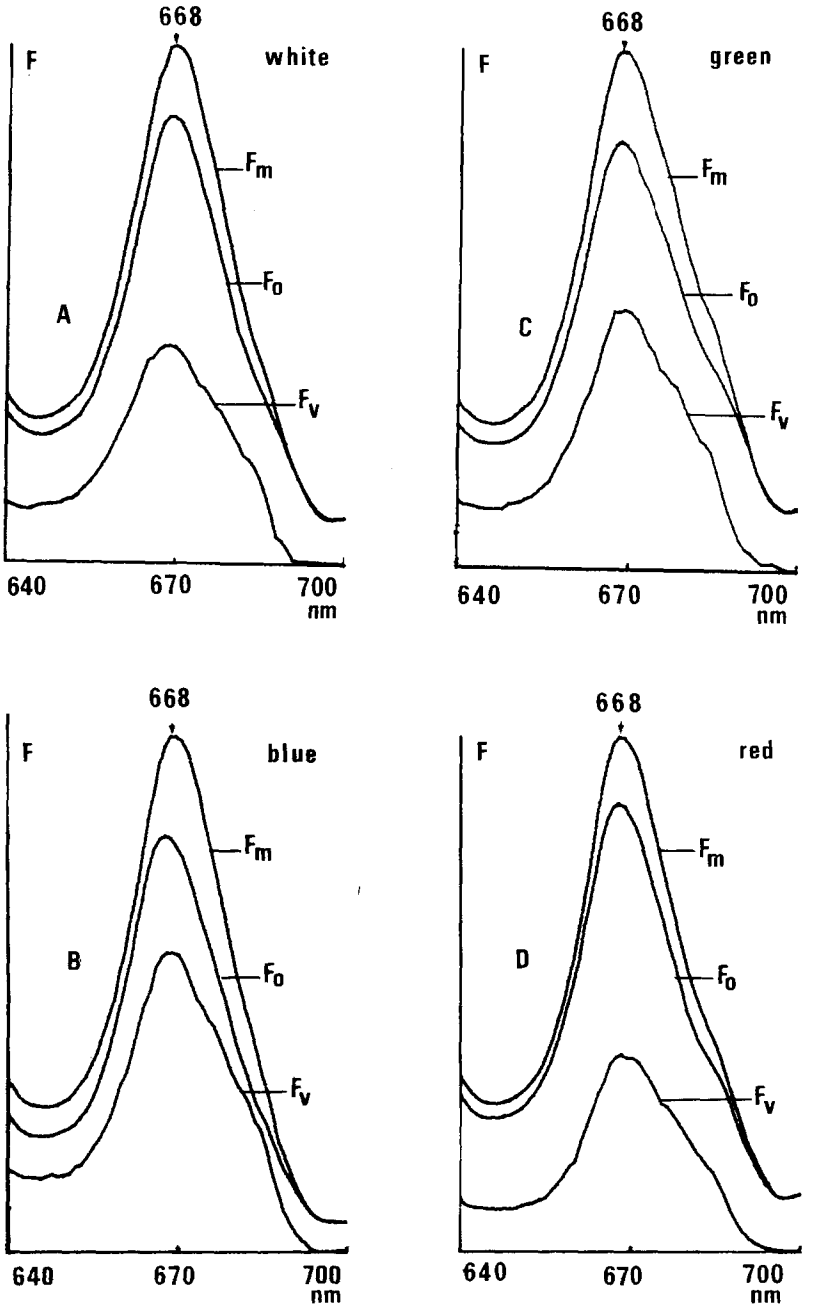

Figure 4. Fluorescence excitation spectra at −196 °C and at 715 nm. F_M: maximum fluorescence after 3 minutes of illumination, F_O: minimum fluorescence (the reaction-centers are open, F_V: variable fluorescence $F_M - F_O$). Whole algae grown for 4 days in A: white, B: blue, C: green, and D: red lights.

Table 3. Characteristics of fluorescence induction curves at 20 °C in the presence of D.C.M.U (4 days-old cells). Area: area included between the curve and its asymptote. t 1/2: half-increase time.
F_o: initial fluorescence level
F_M: maximum fluorescence level
F_V: variable fluorescence $F_M - F_O$

	F_O	F_V	F_O/F_V	t 1/2 ± 5 ms	Area/F_V
White	6.9	4.1	1.7	32	3
blue	5.1	4.2	1.2	40	3.8
green	7.7	4.2	1.8	39	3.4
red	6.7	3.7	1.8	33	3.5

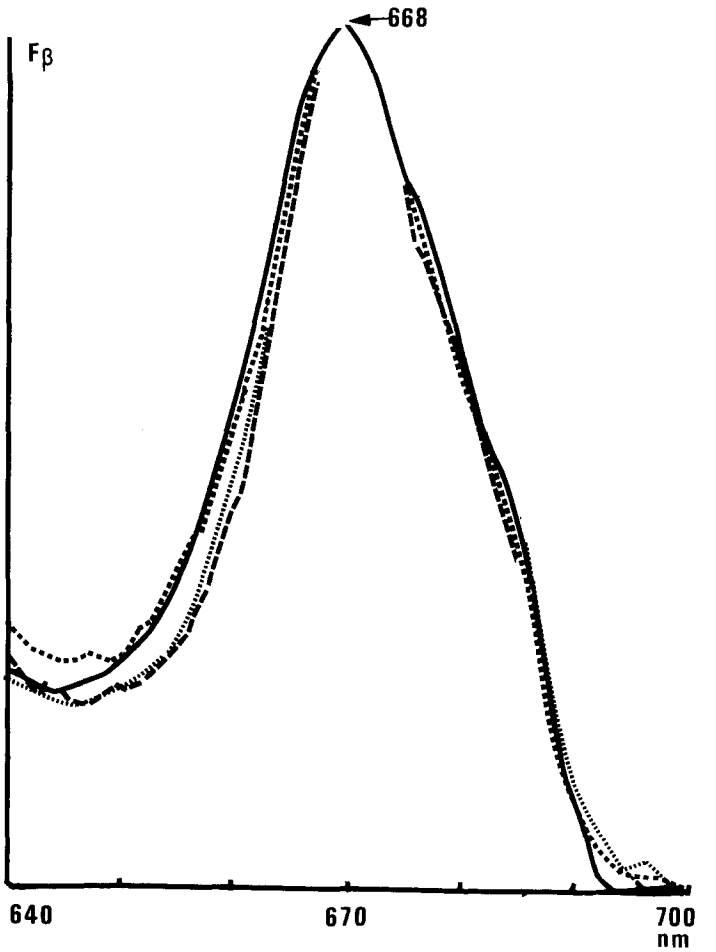

Figure 5. PS II action spectra of 4 days-old cultures corresponding to F_V of the fluorescence excitation spectra normalized at the peaks (668 nm). — white light, ··· blue light, ····· green light, – – red light.

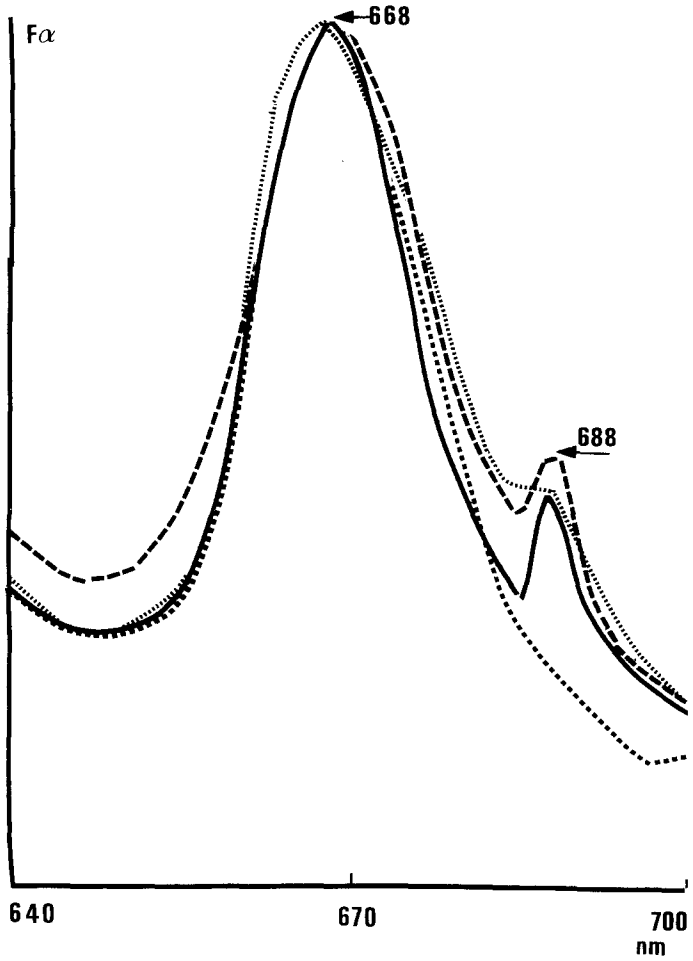

Figure 6. Action spectra of fluorescence at 715 nm at −196 °C obtained by the Butler and Kitajima method (1975) (4 days-old cells). Cultures in —— white light, ----- blue light, ... green light, – – – red light. (Normalized at 668 nm). F_α: evaluated variable fluorescence of PS I (see text).

Action spectra of PS I (F_α) were calculated according to Butler and Kitajima [5]

$$F_\alpha = F_M(715) - \frac{F_M(690)}{F_v(690)} \times F_v(715)$$

F_v (690) and F_M (690) are variable and maximum fluorescence emission of PS II at 690 nm; F_v (715) and F_M (715) are variable and maximum fluorescence emission at 715 nm.

The action spectra of PS I showed maxima at 668 nm for all the cultures.

For the cultures in white, green, red light, the spectra presented another peak at 688 nm which did not appear in the blue light culture. The fluorescence arising from Ca 699 was greater in algae grown in green or red light (Figure 6).

Discussion

The amount of light energy, with which algae are provided, strongly influences their growth. Under weak illumination, (a few watts/m^2) the rhythm of cellular division in Diatoms was much reduced. Our results show that the spectral quality of incident light also modifies the rate of cellular division. In red or blue light, the growth of *Phaeodactylum* was slower than in white light; in green light those algae hardly divided at all.

The light quality also acts on pigment synthesis: the chlorophyll content of the cells was increased in blue, red or green light in comparison with white light. These quantitative modifications did not affect the same chlorophylls in each case:

- red illumination induced a chl c increase,
- green and blue illuminations increased the content of both chl a and chl c

(the cellular level of chl c was higher in algae grown green light). Though the ratio chl a/chl c changed with the light spectra, the ratio carotenoids/chlorophylls did not vary significantly. There was therefore no chromatic adaptation similar to that observed in *Rhodophyceae* and *Cyanophyceae* [4].

These results are in agreement with those of Vesk and Jeffrey (1977 b) who observed no chromatic adaptation in different Diatoms grown in 'blue-green' light.

Absorption spectra exhibited significant differences only for long wavelengths, between 630–717 nm, which are absorbed by chl c and chl a. The absorption bands were analysed by French and al. method [7]. The eight Gaussian components exhibited maxima at wavelengths similar to those observed in spectra of extracted *Fucus* chloroplasts [2]. In *Phaeodactylum* as in *Fucus,* Ca 669 form predominated whereas Ca 678 content was clearly weaker in *Phaeodactylum* (15% of chl a + c) than in *Fucus* (32% of chl a + c). The absorption band in the red spectrum was narrower for the Diatom than for the extracted plastids of the macrophyte. Elsewhere, variations in the spectral light quality of culture conditions induced rapid changes in Chl a-complex contents. Red light, from the fourth day, induced a large increase of Ca 699(+ 30% in comparison with white light) and a much smaller increase of Ca 684 and Ca 690 observable in the absorption spectrum, but no quantifiable with the French's resolution of absorption curves, (Figure 2 and Table 2). Green light induced considerably stimulated formation of the three forms: Ca 684, Ca 690 and Ca 699 (+ 15%, + 20%, + 30% compared to white light).

Parallel to this development, we noted a great increase in fluorescence emission with a peak at 716 nm which was present at 25 °C as well as at − 196 °C for the algae grown in red or green light. The fluorescence induction curves for algae in presence of D.C.M.U. demonstrated an important decrease of the PS II antenna-size when the algae were cultivated under blue or green light.

However, the variable part of the fluorescence excitation spectra was similar whatever the culture, which therefore implies that the action spectrum of PS II was not modified qualitatively; chlorophyll a-forms belonging to PS II were therefore affected in their entirety by blue or green light. Since cellular chlorophyll content did not decrease, we must explain the observed shrinking of the PS II antennae of the cultures in blue or green light by an inactivation of a part of the chlorophylls or by their connection to another antenna.

Action spectra of fluorescence at 715 nm, estimated by the Butler and Kitajima method [5] from fluorescence excitation spectra showed maxima similar to those obtained for the PS II (668 nm). So, unlike in spinach chloroplasts, the action maximum of PS I exhibited no shift towards the longer wavelengths. Only Ca 699 is specific to the PS I. The PS I action spectra of the different cultures differed in respect to absorption by chlorophyll-forms at the far-red end of the spectrum.

Cultures in green or red light presented a strong fluorescence emission at 715 nm. The presence in excitation spectra of a peak not visible in absorption spectra and the great increase in 715 nm fluorescence suggest that new appeared forms have a higher fluorescence yield than the normal fluorescent forms.

Probably these new chlorophyll forms Ca 684, Ca 690 and Ca 699 do not transfer the excitation energy to PS I reaction centers and are deconnected from the antenna. Cultures in white light, which fluoresced weakly at 715 nm, showed an important peak at 688 nm in the excitation spectrum of 715 nm fluorescence, whereas cultures in blue light, which fluoresced heavier at 715 nm, did not present a peak at 688 nm. These differences can be explained by the fact that fluorescence emission and excitation spectra obtained by our methods are relative spectra so that the size of antennae modulate the spectra. The PS II antennae decrease in size in blue light, as shown by fluorescence induction kinetics in presence of DCMU. This fact emphasizes the difference of fluorescence emission between the cultures, decreasing the emission peak at 691 nm. Furthermore, to explain the weak emission of white light cultures at 715 nm, we can admit that the PS I antenna is smaller than the PS I antenna of blue light cultures, the major part of the emission spectrum coming from the inactive form present in the absorption spectrum.

The algae cultivated in blue light showed weak emission of fluorescence at 715 nm, which is carried by small amounts of Ca 690 forms. On the other hand, it should be observed that Diatoms grown in 'Blue-green' light (Maxi-

mum at 480 nm) showed an increase of photosynthetic carbon fixation [11]. Blue light approximates more closely to the natural light conditions existing in sea media. From this we may conclude that cultures in blue light offer the best correspondence to the natural conditions in which *Phaeodactylum tricornutum* live, and therefore allows the photosystems to reach a greatest efficiency.

This research was supported by C.N.R.S. grants (R.C.P. 598 and L.A. 311).

References

1. Bennoun P and Jupin H (1976) Spectral properties of system I deficient mutants of *Chlamydomonas reinhardii.* Possible occurence of uphill energy transfer. Biochim Biophys Acta 440:122–130
2. Berkaloff C, Duval J, Jupin H, Chrissovergis F and Caron L (1981) Spectroscopic studies of isolated-protein complexes of some brown algae thylakoids. G. Akoyunoglou Ed. Proceedings of the fifth international photosynthesis Congress. Vol III: 485–494. Philadelphia. Balaban International Science Services
3. Berkaloff C and Duval JC (1980) Correlated influence of cation concentration and excitation intensity on PS II activity – I. Influence of high salt concentration on spinach chloroplast activity. Photosynthesis Research 1:115–125
4. Bogorad L (1975) Phycobiliproteins and complementary chromatic adaptation. Ann Rev Plant Physiol 26:369–401
5. Butler WL and Kitajima M (1975) Energy transfer between photosystem II and photosystem I in chloroplasts. Biochem Biophys Acta 396:72–85
6. Falkowski PG and Owens TG (1980) Light-shade adaptations. Two strategies in marine phytoplankton. Plant Physiol 66:592–595
7. French CS, Brown JS and Lawrence MC (1972) Four universal forms of chlorophyll a. Carnegie Institution Year Book 70:487–495
8. Goedheer JC (1973) Chlorophyll a forms in *Phaeodactylum tricornutum:* Comparison with other diatoms and brown algae. Biochim Biophys Acta 314:191–201
9. Goedheer JC (1981) Comparison of the long wave chlorophyll fluorescence in various green and blue-green algae and Diatoms. Photosyn Research 2:49–60
10. Jeffrey SW and Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplancton. Biochem Physiol Pflanzen 167:191–194
11. Jeffrey SW and Vesk M (1981) Blue-green light in marine micro-algae: enhanced thylakoid and chlorophyll synthesis. G. Akoyunoglou Ed. Proceedings of the fifth international photosynthesis Congress. Vol VI: 435–442. Philadelphia. Balaban International Science Services
12. Jupin H (1973) Modification de l'importance relative des deux systémes photochimiques chez la diatomée *Detonula* sp. cultivée en lumiére rouge. Physiol Vég II: 507–517
13. Jupin H (1974) Modifications de l'appareil photosynthétique liées à l'apparition de complexes pigmentaires absorbant dans le rouge lointain chez une diatomée *Detonula* sp. Thése Doctorat d'Etat, Sciences. Université Paris VI
14. Lichtlé C, Jupin H and Duval JC (1980) Energy transfers from photosystem II to photosystem I in *Cryptomonas rufescens* (Cryptophycae). Biochim Biophys Acta 591:104–112
15. Vesk M and Jeffrey SW (1977)a Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanophyxis turris.* J Phycol 13:271–279
16. Vesk M and Jeffrey SW (1977)b Effect of blue-green light on photosynthetic pigments and chloroplast structure in unicellular marine algae from six classes.

J Phycol 13:280–288
17. Wallen DG and Geen GH (1971)a Light quality in relation to growth, photosynthethic rates and carbon metabolism in two species of marine plancton algae. Marine Biology 10:34–43
18. Wallen DG and Geen GH (1971)b Light quality and concentration of proteins, RNA, DNA and photosynthetic pigments in two species of marine plankton algae. Marine Biology 13:44–51