Photoadaptation in Marine Phytoplankton

CHANGES IN SPECTRAL ABSORPTION AND EXCITATION OF CHLOROPHYLL a FLUORESCENCE

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ABSTRACT

The optical properties of marine phytoplankton were examined by measuring the absorption spectra and fluorescence excitation spectra of chlorophyll a for natural marine particles collected on glass fiber filters. Samples were collected at different depths from stations in temperate waters of the Southern California Bight and in polar waters of the Scotia and Ross Seas. At all stations, phytoplankton fluorescence excitation and absorption spectra changed systematically with depth and vertical stability of the water columns. In samples from deeper waters, both absorption and chlorophyll a fluorescence excitation spectra showed enhancement in the blue-to-green portion of the spectrum (470–560 nm) relative to that at 440 nm. Since similar changes in absorption and excitation were induced by incubating sea water samples at different light intensities, the changes in optical properties can be attributed to photoadaptation of the phytoplankton. The data indicate that in the natural populations studied, shade adaptation caused increases in the concentration of photosynthetic accessory pigments relative to chlorophyll a. These changes in cellular pigment composition were detectable within less than 1 day. Comparisons of absorption spectra with fluorescence excitation spectra indicate an apparent increase in the efficiency of sensitization of chlorophyll a fluorescence in the blue and green spectral regions for low light populations.

Light is often a limiting factor for the growth of marine phytoplankton. Hence it is expected that photoadaptation is an important feature of phytoplankton physiology. Photosynthetic quantum yields of phytoplankton in natural waters have frequently been determined to indicate cellular responses to ambient light conditions (see [5] for a discussion). It is important in such studies to know the absorption and photosynthesis action spectra of the algal populations to understand how efficiently they are able to use the total ambient light. This is of particular concern at depths where light intensity limits phytoplankton growth.

The light environment in the water to which the algae have to adapt is relatively simple: intensity decreases with depth and is associated with modifications to the light spectrum. The spectral distribution of irradiance shows a broad blue-green maximum below the surface. With increasing depth, the peak narrows and shifts slowly toward blue in clear ocean water (12). As the concentrations of phytoplankton pigments in the water increase, their high violet and blue absorption diminishes the light in that region of the spectrum and shifts the maximal irradiance toward the green region (1). This was a basic environmental justification for the Engelmann theory of complementary chromatic adaptation (4, 15, 24).

Field studies have shown that phytoplankton at depth have decreased maximal rates of photosynthesis (25) and increased cellular concentrations of Chl a (2, 13). These two features yield dramatic decreases in assimilation number with depth (8, 25). Recent studies of algal cultures indicate that adaptation to low light involves increases in the cellular concentration of PSI reaction centers (20, 21) and PSII reaction centers (9), as well as the size of the photosynthetic unit (8, 20). Low light-adapted cells show an increased ratio of accessory pigments to Chl a as well as relative enhancement of the green and blue regions in the spectra of absorption, Chl a fluorescence excitation, and oxygen evolution (15, 17, 22, 26).

Studies of photoadaptation processes in natural aquatic environments have been limited mainly to measurements of the photosynthetic response of the phytoplankton to differing light intensities (e.g. 16, 25) and algal pigment content (2). Some information exists on increases in the ratio of accessory pigments to Chl a in shade-adapted natural populations (15), but the effect of these changes on the action spectra of photosynthesis usually was not measured. Photosynthesis action spectra have not been measured routinely at sea due to problems associated with (a) physiological artifacts resulting from cell concentration, (b) the length of time required to complete a measurement (1 h or more), and (c) difficulty of using action spectra instrumentation on board ship. These problems may be circumvented to some extent by measuring the action spectra of Chl a fluorescence as representative of the photosynthesis action spectra (15, 30).

We present here the use of corrected excitation spectra of Chl a in the study of photoadaptation in natural marine phytoplankton. Fluorescence and absorption of cultures have been used for some time to study energy transfer from accessory pigments to Chl a (6) and corrected fluorescence spectra have been used for quantitative studies on energy transfer processes (7, 15). Our studies combine these proven methods with recent improvements in the techniques of optical measurements on filtered samples, in order to discern spectral differences between phytoplankton populations from different depths.

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MATERIALS AND METHODS

Data were collected during two cruises in antarctic waters—in the Scotia (Vulcan VII) and Ross (Glacier 1982) Seas, and a cruise in the Southern California Bight (SCBS3 [18]). Water samples were obtained with Niskin bottles mounted on a rosette which was equipped with a CTD sensor. Total downwelling irradiance was measured with a quantum scalar irradiance meter (Biospherical Instruments, Inc., San Diego, California; models QSR 240 or 250), and compared continuously with a reference probe on deck. After being withdrawn from the Niskin bottle, the water samples were kept covered with a black cloth in a cool place until filtered, usually within 1 h of sampling. One-half to 2-L samples were filtered through 24-mm glass fiber filters (Whatman GF/F). The filters were immediately frozen at -20°C until fluorescence could be measured. Fluorescence on the filters was measured within hours (Scotia Sea), 8 to 10 weeks (SCBS samples), or after 5 to 6 months (Ross Sea).

Incubation experiments were done in incubators on the decks of the ships. Surface sea water was pumped through the incubators to maintain the temperature. Sunlight irradiance was attenuated, using either plastic or metal neutral density screens. Light intensity in the bottles as a fraction of the incident sunlight irradiance was measured with a quantum scalar irradiance meter (model QSL 100, Biospherical Instruments). In each incubation experiment, a large water sample from a 30-L Niskin or from two 5-L Niskins from one depth was subdivided between a few glass bottles. All transfers were done in dim light to avoid exposure of the algae to high light intensities. Most experiments on the SCBS cruise started at night. On the Ross Sea cruise the experiments started in the evening, when ambient light was close to its minimum for the day. The bottles were shaken well before each sampling, and the samples were immediately filtered and stored frozen as described above.

Fluorescence excitation spectra for the samples from the Scotia Sea and California waters were measured in a fluorometer which was a modification of the vertical light path spectrofluorometer as described by Kieber and SooHoo (14). The components included a Bausch and Lomb high intensity monochromator (33-86-76) and a Bausch and Lomb 45-w tungsten-halide light source. Fluorescent light intensity for the sample and blank were measured with a gamma 20-20-10 photomultiplier and 2900 Autophotometer. The photomultiplier was mounted above a dichroic filter which reflected the excitation beam down onto the sample and transmitted the Chl a red fluorescence. A 680-nm bandpass filter (Melles-Griot 03-FIV-065) was placed in front of the phototube. The photometer was interfaced, after amplification, to a Digital Equipment Corporation PDP 1103 computer which digitized, averaged, and smoothed the data. The optical configuration for the fluorescence measurements was chosen to facilitate system calibration and thereby allow calculation of system-corrected, fluorescence excitation spectra. Calibration was accomplished using the fluorescence dye, rhodamine-B, as a quantum counter (29). The system correction spectrum was a smoothed average of three-B spectra which was suppressed slightly (below 10% maximal deviation) for true photon flux as determined with a calibrated EG&G UV 444 BQ silicon photodiode. The raw fluorescence spectra were divided by this system correction spectrum in order to give the corrected relative fluorescence excitation spectra. Because of the spectral limitations of the rhodamine-B correction system and the dichroic filter, our spectra were restricted to excitation wavelengths less than 560 nm. During measurement, the sample filters were kept frozen by metal discs which had been precooled on dry ice. Absorption for these samples was measured in a Cary 219 spectrophotometer with modified Shibata method using a wet glass fiber filter in the reference beam.

The fluorescence spectra of the Ross Sea samples were measured in a Perkin-Elmer MFP 44A fluorescence spectrophotometer, using the correction attachment. The GF/F filters were mounted on a hollow brass block. The block was precooled on dry ice and was kept frozen by a piece of dry ice inside it.

The fluorescence spectra are presented in arbitrary units except where indicated otherwise. When spectra were compared or averaged, they were first normalized to a constant integrated area between 400 to 560 nm (4).

Continuous vertical profiles of Chl a fluorescence were made as follows: the water, pumped up by a submersible pump, was passed in parallel through two Turner Designs model 10-005 flow-through fluorometers. One was equipped with a standard Chl a measurement system in which excitation was by blue light with the peak at 435 nm, 55 nm width at half-maximum, and excitation falling below 1% of maximum at 370 and 475 nm (blue lamp, excitation filters—Corning 5-60, reference filter—Corning 3-66, emission filter—Corning 2-64). The other fluorometer, with the same emission and reference filters, had a green excitation with the peak at 535 nm, 50 nm width at half-maximum, and excitation falling below 1% of maximum at 475 and 575 nm (Turner Designs G-13 green lamp, Corning 4-96 and Corning 3-71 filter combination). The analog signals from the two fluorometers as well as from the depth transducer were digitized and recorded on a HP-85 desk-top computer. Correlation was tested with the Kendall Tau test (28).

RESULTS

In Figure 1 we compare average spectra of absorption, fluorescence excitation of Chl a, and fluorescence yield for high and low light samples from four vertically stratified stations. The four high light spectra were averaged at 5 nm intervals and standard deviation of the mean was calculated for each point. The same was repeated for the low light spectra. The fluorescence yield spectra was calculated by dividing the values of the average fluorescence excitation of Chl a by the corresponding average of absorption. It is apparent that fluorescence excitation in the blue to green regions is more pronounced in the low light samples than in the high light samples.

An examination of the spectral data from the three areas of our study exhibited considerable variability with depth, but always with the same trend of relative increases in the green and blue-green excitation with depth. In order to quantify these variations, we calculated a ratio from the fluorescence spectra. It is generally accepted that absorption at 440 nm is due mainly to Chl a absorption, that peaks or shoulders at 470 to 490 nm are often due mainly to Chl b and c absorption (23), and that absorption at 550 nm is due to xanthophylls and biliprotein pigments (17, 23). A majority of the algae in the three cruises belonged to taxa with a Chl a-c-xanthophyll pigment system although some of the SCBS cruise stations had a large fraction of Chlorophyta cells with a Chl a-b system and unidentified nanoplankton. The Ross Sea samples also had a large undefined nanoplankton fraction by taxometry are not available to us for further examination at this time. Based on this information and upon our observations of high variance in the 470 to 490 nm and the 550 nm regions of the spectrum, we defined a fluorescence ratio (F ratio) as the ratio of Chl a fluorescence excited at 480 and 550 nm to fluorescence excited at 440 nm. We feel that this ratio reflects changes in the sensitization of Chl a fluorescence by accessory pigments relative to the sensitization by Chl a itself.

In Figure 2 we present examples of stations from the Scotia Sea (a) and the SCBS cruise (b), where the F ratio and water density are plotted as a function of depth. In these as well as most other stations the F ratio increased with depth, the increase

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3 Abbreviations: SCBS, Southern California Bight Study; CTD, conductivity temperature depth; LHCP, light-harvesting Chl-protein.
being larger at stations that are highly stratified. The significance of the correlation between the $F$ ratio increases and depth was tested statistically for the nine stations of the SCBS cruise and the seven stations in the Scotia Sea. We tested the significance of the difference between a constant $F$ ratio with depth and a continuously increasing ratio. Some of the deepest samples showed decreasing $F$ ratios, which were related to the high phaeopigment contents in the same bottles, but still they were included in the analysis. When we omitted these bottles or compared average $F$ ratios for the mixed layer with the values below the mixed layer, our significance levels were higher since virtually all stations showed an increasing trend in the $F$ ratios with depth. The test for deviation from binomial expectations for the Kendall Tau test was found to be significant with $p < 0.05$ and $p < 0.055$, for the SCBS and Scotia Sea cruises, respectively. For the SCBS cruise data the extent of the increase in the $F$ ratios with depth was larger in the stratified off-shore stations than in the less stratified on-shore ones, which indicated to us that the changes might be related to adaptation to various depths when vertical mixing rates are low. During the Ross Sea cruise we also measured continuous vertical profiles of in vivo Chl a fluorescence excited by violet and green light. In this case we used the ratio of fluorescence excited by green (535 nm) to fluorescence excited by violet (435 nm) light (referred to as $F_c$).

Changes in the $F_c$ and $F$ ratios with depth were generally similar (Fig. 3) and showed a clear qualitative correlation with the water density profiles (Figs. 2 and 3). This latter correlation, which has been observed repeatedly in all cruises to date, suggests some causality, i.e. when the water is unstable, the residence time of the cells at any depth is insufficient for the induction of significant photoadaptation.

We regard the agreement between the vertical profiles of the two analyses (Fig. 3) to be encouraging, considering the different wavelengths and the experimental procedures used to obtain values for $F$ and $F_c$. The decrease of the ratios in the deepest samples (Fig. 3b) may be explained by high concentrations of phaeopigments which were measured in those samples. Detrital particulates containing phaeopigments and accessory pigments would lower the $F$ ratio because energy absorbed by accessory pigments in these particulates would not be transferred to Chl $a$, whereas Chl degradation products still absorb light at 440 nm and fluoresce above 660 nm. The results presented so far show that the $F$ ratios increase with depth in the water column. We hypothesize that it is the inverse relationship for light with depth which is the causative factor. This is supported by plotting the $F$ ratio and the corresponding $F_c$ ratio (an analogous ratio for absorption spectra) from the SCBS cruise samples as a function of light intensity at their
corresponding depths (Fig. 4). To minimize the variability between stations, we normalized the ratios at each depth to the value measured at the sea surface for that station. Data in this figure also show that the rate of increase of the A ratios with decreasing light intensity is less than the rate of increase of the F ratios.

To examine the contribution of photoadaptation to the measured spectral differences, we incubated natural populations on deck at various light intensities. We present one representative of 8 incubation experiments which were carried out during the three cruises (Fig. 5). Changes in F ratio are shown as a function of time. The initial water for the incubation was taken from the 4% light level. As seen in spectra of the 1% and 100% light treatments after 22 h incubation (Fig. 6), the samples incubated at the higher intensities have reduced absorption and excitation at blue-to-green wavelengths relative to the violet wavelengths. As in Figure 1, these changes in fluorescence are also significantly larger than the changes in absorption, indicating differences in efficiency of Chl a fluorescence sensitization. From our incubations we estimate the time needed for the appearance of a detectable F ratio change to be approximately 6 light h, and for full adaptation to be 2 d or longer.

**DISCUSSION**

Filtering and freezing of marine algae for fluorescence measurements is almost unavoidable in oceanographic research. Marine particles are in dilute suspension and must be concentrated in order to measure their optical properties. Spectrofluorometers are not usually available on board ship and the demands of field schedule often require storage of the samples before analysis. The effects of these procedures on the quality of the data must be considered. Studies with laboratory cultures indicate little significant differences between fluorescence excitation spectra of algae in suspension and on filters. There are optical problems which flatten the excitation spectra when the concentration of Chl a on the filter exceeds 2.5 μg per cm² (18). Our samples contained less than 1.0 μg Chl a per cm².

The effects of freezing are more complex since they introduce small changes in the shape of the excitation spectra—mostly by varying the green region relative to the violet by 10 to 20%. Such variability is not enough to change significantly the relationship between samples which were taken at the same time and handled identically. Using fresh samples, fluorescence excitation spectra as well as fluorescence yield spectra typically show the enhancement in the blue-to-green region of the spectrum with depth (18). The basic information in the fluorescence spectra is preserved despite freezing. Our use of averages of several samples (Fig. 1) reduces the effect that random variability might have on interpretation. The significant correlation of the increase in the F ratio with increasing depth and with decreasing light intensity (Figs. 2–4, 6) and the fact that we seldom get the opposite trend both in fresh and in frozen samples, indicate that the broadband information which we derive from the spectra is real. The similarities shown in Figure 3 between in vivo and filtered-frozen...
samples, measured on two completely different instrumentation systems and calculated by independent methods, together with the correlation of F ratio changes with water vertical stability, corroborate our analysis and interpretation.

The changes in spectral absorption and spectral excitation of Chl a fluorescence for natural marine particles (Fig. 1) could be caused by a number of factors, including changes in the species of phytoplankton with depth, adaptation of cells to ambient light levels, temperature and nutrient concentrations, or by contributions from detrital pigments. For example, Yentsch and Yentsch (30) have recorded uncorrected spectra for the excitation of Chl a fluorescence and have ascribed changes to variations in the taxonomic composition of the phytoplankton crop. The large differences in absorption and excitation spectra between the planktonic Chrysophyta, Chlorophyta, Dinophyta, Bacillariophyta, and Cyanobacteria suggest a possible influence of taxonomic composition upon fluorescence properties of natural crops. Shi-

mura and Fujita (26), in laboratory work following earlier field findings (27), suggested nitrate uptake limitation under high light conditions as the reason for spectral shifts in absorption, Chl a fluorescence excitation, and fluorescence yield spectra in cultures of Phaeodactylum tricornutum.

We argue for several reasons that the changes reported here are predominantly caused by photoadaptation. First and most importantly, the changes in absorption and excitation observed within the water column (Fig. 1) are very similar to changes induced by the incubation of the sampled crop at differing light intensities (Fig. 6). Since the response is relatively rapid, it is unlikely that these changes were caused by changes in the taxonomic composition of the sampled crop. Furthermore, since differing light levels were achieved by neutral density filters, our results confirm earlier reports (4, 27) that changes in the spectral composition of the light field were not a necessary stimulus for photoadaptation.

Second, floristic analyses of waters sampled in the Southern California Bight revealed no pattern in the distribution of species which could account for the spectral changes with depth. Species composition did change within the water column but without consistency (D. G. Redalje and F. M. H. Reid, unpublished results). Third, nutrient supply does not appear to be a significant factor. Increases in the accessory pigment absorption and excitation of Chl a fluorescence with depth were seen in Antarctic Seas where all nutrients, including ammonium, were in high concentrations throughout the water column. Although increases with depth in accessory pigment sensitization were less in the polar waters than in temperate waters, regional differences in light fields and depth of vertical mixing rather than nutrient fields appear to best explain this difference.

Our data show that the physiological response of phytoplankton to low light levels involves (a) an increase in the fluorescence excitation of Chl a by accessory pigments relative to the excitation by Chl a itself, and (b) an increase in the absorption by accessory pigments relative to absorption by Chl a. The spectral changes in fluorescence excitation are larger than the changes in absorption, so that the spectral variation in fluorescence yield is larger for shade-adapted cells than for sun-adapted cells. What could account for these observations? The accessory pigments absorbing in the 470 to 560 nm region may have different energy transfer efficiency depending upon degree of photoadaptation. For example, sun-adapted cells may have relatively higher concentrations of photoprotective carotenoids than shade-adapted cells (3). Since the absorption of these carotenoids overlaps that of other accessory carotenoids, such changes in concentration will cause decreased efficiencies in the blue-to-green region for sun-adapted
cells. The increases in fucoxanthin content, reported for shade-adapted Skeletonema costatum (10; R. S. Alberte, personal communication), if compensated by a decrease in the content of less efficient accessory or protective pigments, would increase the apparent fluorescence yield in the blue-to-green region. Halldal (11) and Öquist (19), having made similar observations to ours in Chlorella, have proposed that the spectral variability was caused by changes in the efficiency of energy transfer from accessory pigments to Chl a. Shade-adapted cells of the dinoflagellate Glenodinium sp. exhibited a significant relative increase of their O$_2$ evolution action spectrum in the blue-to-green region which was not accompanied by an increase of similar magnitude in their absorption spectrum (22). The relative increase was attributed to an increase in the concentration of the peridinin-Chl-protein.

There are other possible explanations, not addressed by our data, of photoadaptive responses involving the distribution of pigments between PSI, PSII, and the light-harvesting pigment proteins (LHCP, peridinin-Chl-protein, fucoxanthin-Chl-protein, and phycobiliproteins [15]).

Better understanding of the photoadaptation response of phytoplankton living under low light conditions will require simultaneous action spectra of fluorescence excitation of Chl a and of photosynthesis, and detailed chromatographic analyses of pigments or pigment-protein complexes.

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LITERATURE CITED

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SCBS 18, EXPERIMENT 4, 22 hrs

FIG. 6. The spectral characteristics of two filtered samples from the same incubation experiment as in Figure 5, after 22 h at (a) full sun light and (b) at 1% of full sun light.


