ENERGY TRANSFER IN A LIGHT-HARVESTING CAROTENOID–CHLOROPHYLL c–CHLOROPHYLL a–PROTEIN OF PHAEODACTYLM TRICORNUTUM

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Abstract—The marine diatom Phaeodactylum tricornutum was readily disrupted in 0.1 N Tris–HCl buffer, pH 7.8, in a Braun Model MSK cell homogenizer at 0–5°C. Treatment of the suspension with sodium lauryl sarcosinate (3 molecules per 10000 daltons of protein) at 5°C in the dark and subsequent centrifugations produced a pigmented, protein fraction whose excitation spectrum exhibited energy transfer from carotenoids to chlorophyll a (Chl a). Disruption of the pigment–protein complex by heating in 1% sodium dodecylsulfate resulted in loss of energy transfer. For each Chl a molecule this fraction had 1 Chl a, 4 fucoxanthin, and 6.7 accessory pigment molecules. Presence of the accessory complex of Photosystem II in this preparation is suggested by the high xanthophyll content. Further, based on Chl a concentrations, this fraction had about 18 times more apparent fluorescence emission at 680 nm when excited at 470 nm than the intact cells.

INTRODUCTION

The light-harvesting role of carotenoids in photosynthesis by absorption of light and energy transfer to chlorophyll a (Chl a) became apparent from the initial observations of Dutton et al. (1943) in diatoms and of Goedheer (1972) in higher plants. Fluorescence yields of diatoms are the same whether the incident light is absorbed by Chl a or fucoxanthin, and excitation spectra of chloroplasts of higher plants show transfer of energy. Undoubtedly, separation and characterization of light-harvesting carotenoid complexes from chloroplasts are important for elucidation of the structure–function relationship of these pigments in vivo.

Previously, reaction center and accessory complexes of photosynthetic units have been isolated from chloroplasts with the aid of detergents such as Triton X-100 (Vernon et al., 1971), digitonin (Wessels et al., 1971), and sodium dodecylsulfate (SDS) (Thornber, 1975). More recently, solubilization of light-harvesting complexes of dinoflagellates consisting of peridinin–Chl a–protein (PCP), which exhibited a high degree of energy transfer, was achieved in the absence of detergent either through grinding cells frozen at 77 K and then thawing (Haxo et al., 1976; Siegelman et al., 1977) or by disruption of the organisms through sonication (Prezelin and Haxo, 1976) at 0–5°C.

The freeze-grinding technique did not liberate the pigment–protein complexes of Phaeodactylum tricornutum. Also, excitation spectra of dispersions obtained by treatment of cells with 1% Triton X-100 failed to show as much energy transfer from carotenoids to Chl a (at Chl a concentrations of 1 mg/l) as the intact cells. However, we did find that cells which were first fragmented at 0–5°C in a Braun Model MSK cell homogenizer, then reacted with a sparing amount of sodium lauryl sarcosinate (SLS) and finally centrifuged, yielded a pigment–protein complex whose excitation spectrum showed efficient energy transfer from carotenoids to Chl a pigments. We report here details of the isolation and properties of the solubilized pigment protein complex.

MATERIALS AND METHODS

Growth conditions. The diatom P. tricornutum (642, Bohlin) was acquired from the culture collection of algae at the University of Texas at Austin. It was grown at 18°C in the ASP-2 medium of Provasoli et al. (1957) with added soil extract according to the procedure of Mann and Myers (1968). To provide cells of uniform properties and analyses for study, growth conditions were held constant. Cells were grown continuously in a 5 l cylindrical glass growth chamber (70 cm in height), aerated with 5% CO₂ in air and harvested periodically during the late log phase growth at cell counts of 5 × 10⁸ cells/ml. Continuous light for photosynthesis was provided by a bank of 13 cool-white fluorescent lights. The light intensity, measured with a United Detector Technology light meter, was 24 W/m².

Harvesting. One to two liters of P. tricornutum cells were harvested and then filtered through 12 layers of gauze to remove possible clumps of cells. Cell counts were determined with a Levy chamber haemocytometer. Organisms...
grown under the above conditions had calculated, weight-
averages (moisture-free basis) of 40 pg per cell. All manipu-
lations of pigments and pigment-proteins were conducted in
semi-darkness at 5°C with solvents or buffers that had been
sonicated to remove dissolved oxygen.

Cell disruption. Cells were disrupted by abrasive action
in a Braun model MSK (Bromwll Scientific) cell homogen-
izer. Cells, 5-6 g fresh weight, were dispersed in 0.1 N
Tris-HCI buffer, pH 7.8, and added to a thick-walled glass
homogenizer bottle (45 ml vol); after adjusting the final vol
to 20 ml, an equal vol of glass beads (1 mm diam) was
added.

The precooled bottle and homogenizer were kept cool
with CO₂, that was taken from a cylinder fitted with a riser
tube and concentrations of among acids were determined.

The bottom part of the resulting pellet was essen-
tially colorless, but the upper part had a thin layer of pig-
ment material. The homogenized-treated material in the
supernatant [HC(1000 g, sup.)] was yellow to yellow-
brown and contained about 67% of the cell pigment. Two
milliliters of 1 N SLS were added to 20 ml of HC(1000 g, sup.)
by means of a pipette. Results of analyses of this suspension and sub-
sequent fractions derived from it were expressed on an
initial cell basis: these values may be viewed in terms of isolation yields.

The HC suspension was centrifuged at 1000 g for 30 min
at 5°C. The bottom part of the resulting pellet was essen-
tially colorless, but the upper part had a thin layer of pigment-
material. The homogenized-treated material in the
supernatant [HC(1000 g, sup.)] was yellow to yellow-
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Protein analyses. Protein contents were determined by
6 N HCl hydrolysis of samples for 24 h and subsequent
amino acid analyses. Appropriate volumes of hydrolysate
were injected into a single column, physiological fluid high
pressure liquid chromatography (HPLC) Glencos MM-100
Micro Column Amino Acid Analyzer with a 1-10 mmol
range. Concentrations of amino acids were determined by
integration with a digital computer, and protein concen-
trations were calculated from these values.

Pigment analyses. Chlorophyll and carotenoid pigments of
P. tricornutum were extracted, prepared for chromatog-
ographic analysis and separated on powdered sugar
columns by elution with appropriate solvents by the
methods of Strain et al. (1944). Individual pigments were
identified by their chromatographic behavior on thin-layer
plates of cellulose according to methods of Jeffrey (1961).
Finally, standard solutions of pigments were prepared and
their concentrations were determined from their absorp-
tion curves and coefficients (Jeffrey, 1972; Johansen et al.,
extinction coefficient of 42,4,1 g cm in acetone at 630 nm
was used to calculate the total Chl c of our standard sol-
ution. This value is an average of extinction coefficients of
Chl c₁ and c₂ reported by Jeffrey (1972).

Acetone extracts of pigments were analyzed by HPLC
(Eskins et al., 1977), which is both rapid and sensitive and
permits resolution of Chl c₁ and c₂. A Waters Associates
HPLC equipped with a model 440 absorbance detector,
WISP inject system and a 30 cm X 3.9 mm ID μBonda-
pack C₁₈ column thermostated at 25°C were used. Pig-
ments were eluted from the column with a gradient system
starting with 90% methanol (10% water) and ending with a
1:1 vol/vol ratio of methanol to ethyl acetate. The gradient
was obtained by means of a Waters Associates model 600 solvent programmer set on the linear mode so
that a 1:1 ratio was reached after 20 min at a pumping rate
of 1 ml/min. Elution was monitored at 236 nm. Profiles of
pigment separations were made on a recorder, and areas
under the curves were integrated by an on-line digital com-
puter as described by Butterfield et al. (1978).

Standard solutions of pigments in 80% acetone were
injected onto the HPLC column. Standard curves were
made from areas under profiles of pigments and their total
concentrations. Linear portions of curves were used to
determine pigment concentrations of injected samples.

Pigments were extracted from cells or pigmented pro-
teins with 100% acetone for 30 min at -20°C in the dark
to minimize possible formation of chlorophyllide a by
action of chlorophyllase on Chl a. Residues were separated
from extracts by centrifugation at 2000 g for 15 min. All
pellets were colorless.

Spectra. Absorption spectra of pigments were measured
on a Cary spectrophotometer model 14 PM, and the spec-
trum of the pigment-protein complex was on a Beckman
DB spectrophotometer equipped with an integrating
sphere to minimize scattering effects. Fluorescent spectra
were measured with an Amino Bowman spectropho-
fluorometer with a Corning Red Glass Filter 2030 placed in
the emission beam just before a S-20 spectral response
photomultiplier tube. Spectral slit widths of 1 mm (equival-
to to a 5.5 nm bandpass) were used. Correction factors
for spectral calibration of the Xe lamp source were determined
at every 5 nm (Udenfriend, 1962) and used to quantum
correct excitation spectra. Fluorescence measurements
were made at Chl a concentrations of less than 0.1 mg/l, a
collection range which gave a linear relationship
between fluorescence and pigment concentration under
our conditions.

RESULTS AND DISCUSSION

Phaeodactyllum tricornutum cells are considerably more resistant to disruption by the liquid nitrogen
freeze–thaw method than dinoflagellate cells (Haxo et al., 1976); most cells were still intact even after several
cycles of freezing and thawing. We found, however, that disruption of cells at 0–5°C in a Braun Model
MSK cell homogenizer produced yellow dispersions which emitted 7–8 times more apparent fluorescence
(based on Chl a concentrations) at 680 nm when excited at 470 nm than did intact cells.

Quantitative determination of total pigments per
cell was made on HC since extraction from intact
cells was incomplete. The HPLC pigment-profile for
HC of P. tricornutum showed the presence of two Chl
c pigments, fucoxanthin, neofucoxanthin, diadinoxan-
thin, diatoxanthin, Chl a and carotene (Table 1). A
minor peak which eluted after fucoxanthin and two
other minor peaks, which eluted before Chl a, were
neither identified nor included in our calculations.
Phoepthrinys, which if present, should have eluted just
after carotene (Eskins et al., 1977), were not observed
in these studies. Chlorophyll c₁ and c₂ were first
detected by Dougherty et al. (1966) and later were
isolated by Budzikiwicz and Taraz (1971) and by
Strain et al. (1971) from P. tricornutum. In our work
Table 1. Amounts of pigments, calculated in picograms and picomoles on a cell basis were determined by HPLC for P. tricornutum cells that had been first homogenized (HC) in a Braun homogenizer and then centrifuged at 1000 g. Amounts were also determined for the supernatant that had been first treated with 0.1% SLS for 1 h and then centrifuged at 44000 g. Weights of pigments may be regarded on a yield per initial cell basis.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>HC (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>HC(1000 g) Pellet (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>HC(1000 g) Supernatant (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>HC(1000 g) Total (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>HC(1000 g, sup.) (SLS, 44000 g) Pellet (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>HC(1000 g, sup.) (SLS, 44000 g) Supernatant (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
<th>HC(1000 g, sup.) (SLS, 44000 g) Total (pmol 10&lt;sup&gt;5&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl c</td>
<td>0.072 (11.75)</td>
<td>0.015 (2.45)</td>
<td>0.0635 (10.35)</td>
<td>0.0785 (12.8)</td>
<td>0.041 (6.7)</td>
<td>0.0225 (3.65)</td>
<td>0.0635 (10.35)</td>
</tr>
<tr>
<td>Chl a</td>
<td>0.277 (31.05)</td>
<td>0.082 (9.19)</td>
<td>0.158 (17.71)</td>
<td>0.240 (26.9)</td>
<td>0.121 (13.56)</td>
<td>0.0093 (1.04)</td>
<td>0.1303 (14.6)</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>0.302 (58.75)</td>
<td>0.113 (21.98)</td>
<td>0.2014 (39.18)</td>
<td>0.314 (61.16)</td>
<td>0.199 (38.71)</td>
<td>0.018 (4.0)</td>
<td>0.2170 (42.71)</td>
</tr>
<tr>
<td>Neofucoxanthin</td>
<td>0.097 (18.9)</td>
<td>0.0264 (5.13)</td>
<td>0.074 (14.39)</td>
<td>0.100 (19.52)</td>
<td>0.0613 (11.92)</td>
<td>0.0081 (1.57)</td>
<td>0.0694 (13.49)</td>
</tr>
<tr>
<td>Diadinoxanthin</td>
<td>0.0018 (0.40)</td>
<td>0.0007 (0.15)</td>
<td>0.0012 (0.27)</td>
<td>0.0019 (0.42)</td>
<td>0.0010 (0.22)</td>
<td>0.00025 (0.05)</td>
<td>0.00125 (0.27)</td>
</tr>
<tr>
<td>Diatoxanthin</td>
<td>0.0037 (0.74)</td>
<td>0.0010 (0.20)</td>
<td>0.0026 (0.52)</td>
<td>0.0036 (0.72)</td>
<td>0.0025 (0.50)</td>
<td>0.00003 (0.06)</td>
<td>0.0028 (0.56)</td>
</tr>
<tr>
<td>Carotene</td>
<td>0.0309 (5.76)</td>
<td>0.0108 (2.01)</td>
<td>0.0210 (3.91)</td>
<td>0.0318 (5.92)</td>
<td>0.0169 (3.15)</td>
<td>0.0024 (0.44)</td>
<td>0.0193 (3.59)</td>
</tr>
<tr>
<td>Total</td>
<td>0.7844 (127.35)</td>
<td>0.2489 (41.11)</td>
<td>0.5217 (86.33)</td>
<td>0.7706 (127.44)</td>
<td>0.4427 (74.76)</td>
<td>0.06085 (10.81)</td>
<td>0.5036 (85.57)</td>
</tr>
</tbody>
</table>

*Value also includes diadinoxanthin and diatoxanthin.

Energy transfer

areas under the Chl c<sub>1</sub> and c<sub>2</sub> profiles were added and calculated as Chl c.

About 67% of the pigment and 91% of the protein were found in the supernatant after centrifuging the fragmented cells at 1000 g for 30 min at 5°C. Fragmented cells contained 13.25 pg of protein per cell; after centrifugation, the supernatant contained 12.1 pg per cell-equivalent. The supernatant, which undoubtedly contained much protein not related with pigment complexes, was found to emit 9–10 times more apparent fluorescence (based on Chl a concentrations) at 680 nm when excited at 470 nm than did intact cells.

Since HC(1000 g, sup.) could be sedimented at 20000 g, it was apparent that the pigment protein complex must have a very high mol wt. If the mol wt were in the order of 20–40000 daltons, then it can be assumed to be either in some highly aggregated state or associated with membranes. Use of Triton X-100 to dissociate HC(1000 g, sup.) into possible smaller particles resulted in dispersions exhibiting considerable loss of fluorescence when excited at 470 nm. The detergent apparently disturbed the orientation of pigments responsible for energy transfer. We found, however, that use of low concentrations (0.1%) of SLS (3 molecules per 10000 daltons of protein) for 1 h at 5°C in the dark produced suspensions that did not have diminished fluorescence. Further, centrifugation of the suspension at 44000 g for 1 h at 5°C produced a pellet (Table 1) which when resuspended in 0.1 N Tris–HCl buffer, pH 7.8, and recentrifuged at 2000 g gave a supernatant that had 18.4 times more apparent fluorescence (based on Chl a concentrations) at 680 nm when excited at 470 nm than intact cells. Increased fluorescence of successive fractions in isolation of the complexes is indicative of increased solubilization of the complex. Further, the ratio of one pigment molecule to 10000 daltons of protein suggests that this fraction is relatively free of membrane material.

Total isolation time beginning with harvested cells was 6–8 h. Pigments in the isolated fraction constituted about 34% of the pigments present in the initial cells. Pigment composition of this fraction is given in Table 2. The concentration of Chl c<sub>1</sub> is essentially the same as that of Chl c<sub>2</sub>. The total concentration of Chl c, c<sub>1</sub> and c<sub>2</sub> is the same as that of Chl a. Thin-layer chromatography of these pigments in diethyl ether by the procedure of Jeffrey (1961) shows the absence of chlorophyllide a.

Table 2. Mole concentrations and ratios of pigments contained in 5 ml of light-harvesting pigment protein complex obtained from the pellet reported in column 2, Table 1 by resuspension in 0.1 N Tris–HCl buffer, pH 7.8, and subsequent recentrifugation at 2000 g. The complex was contained in the supernatant.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Amount (nmol)</th>
<th>Based on carotene</th>
<th>Nearest integer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl c</td>
<td>16.35</td>
<td>2.98</td>
<td>3</td>
</tr>
<tr>
<td>Chl a</td>
<td>16.80</td>
<td>3.06</td>
<td>3</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>65.66</td>
<td>11.96</td>
<td>12</td>
</tr>
<tr>
<td>Neofucoxanthin</td>
<td>22.70</td>
<td>4.14*</td>
<td>4*</td>
</tr>
<tr>
<td>Diadinoxanthin</td>
<td>0.03</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diatoxanthin</td>
<td>0.015</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Carotene</td>
<td>5.49</td>
<td>1.00</td>
<td>1</td>
</tr>
</tbody>
</table>

*Value also includes diadinoxanthin and diatoxanthin.
Surprisingly, the molar ratio of the major carotenoid, fucoxanthin, to Chl a in the complex is 4, the same as the ratio of peridinin to Chl a in the pigment–protein complex of Amphidinium carterae (Haxo et al., 1976). Holdsworth and Arshad (1977) isolated a pigment–protein complex from P. tricornutum that contained Chl a, Chl c and fucoxanthin in 2:1:1 molar proportions; in comparison, the molar ratio of Chl c to Chl a in our complex (Table 2) is 1 and the ratio of total accessory pigment to Chl a is 6.7. Isolation of the pigment–protein complex of Holdsworth and Arshad involved use of a French press and 4% sodium dodecylbenzene sulfonate. Apparently complex compositions are influenced by species or by detergents and methods of cell disruptions.

Recently Anderson and Baret! (1979) isolated fucoxanthin–Chl a–Chl c–protein complex from Acrocystis paniculata by disruption of cells with 1% Triton X-100, and reported that it had a Chl a to Chl c molar ratio of 2.

Relative fluorescence emission of our complex at various excitation wavelengths vary with Chl a concentrations similarly to the fragmented cells. Fig. 1. The absorption spectrum as well as the quantum corrected excitation spectrum (Chl a concentration: 0.034 mg/l) of the fraction reported in Table 2, normalized at 440 nm, are given in Fig. 1. Although some differences occur in the region of 470 nm, similarity of the spectra are indicative of energy transfer from carotenoids to Chl a. A similar relationship was also noted between absorption and excitation spectra of the PCP complex of Haxo et al. (1976).

Denaturation of the PCP complex by boiling in 1% SDS for 2 min completely abolished intra-complex energy transfer even though some peridinin and Chl a molecules were still bound to the apoprotein (Song et al., 1976). Similarly, loss of energy transfer also was demonstrated by boiling the P. tricornutum pigment–protein complex in 1% SDS for 2 min. Quantum corrected excitation spectra, normalized at maxima, of the pigment–protein complex, Table 2, in 0.1 N Tris (pH 7.8) buffer at 25°C before and after denaturation by boiling in 1% SDS for 2 min.

Figure 2. Quantum corrected excitation spectra, normalized at maxima, of the pigment–protein complex. Table 2, in 0.1 N Tris (pH 7.8) buffer at 25°C before and after denaturation by boiling in 1% SDS for 2 min.

Phaeodactyllum tricornutum cells yield fluorescence emission at 720 and 695 nm when excited at 470 nm at liquid nitrogen temperatures (Fig. 3). These
emission peaks are consistent with those observed in a variety of photosynthetic organisms that are attributed to PS I and II, respectively (Rijersberg et al., 1979). The low temperature emission spectrum (Fig. 3) of the pigment protein complex (Table 2) is virtually void of emission in the region of 720 nm. The emission peak, however, shifted from 695 to 690 nm. Similar shifts have been noted by Burke et al. (1978) in the case of Chlamydomonas reinhardtii.

Conceivably, the P. tricornutum pigment–protein complex, which was separated under relatively mild conditions and contains all the pigments present in the initial cells, is the total light harvesting pigment–protein complex of the organism. The high Chl c to a ratio in the separated complex relative to the cells may suggest absence of the photosystem centers.

In summary, a method has been described for extraction of a high molecular weight, Chl c-rich, light harvesting pigment–protein from P. tricornutum that demonstrates energy transfer from carotenoid to Chl a. Differences between excitation and absorption spectra in the region of 470 nm suggest possible inefficiency of energy transfer due to natural pigment arrangements and/or loss of activity due to the method of isolation.

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REFERENCES


