Identification of the Photosynthetic Pigments of the Tropical Benthic Dinoflagellate *Gambierdiscus toxicus*

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ABSTRACT—Photosynthetic pigments of the Florida isolate of Gambierdiscus toxicus *were investigated to aid in characterizing this strain and to assist in comparisons with Pacific Ocean isolates. The pigments were separated using thin-layer chromatography (TLC). Tentative pigment identifications were made from visible absorption maxima (in two solvents) and partition coefficients (hexane: 95 percent methanol). The TLC revealed the presence of 10 pigment bands. The chlorophylls* a *and* c_2 *were the major chlorophylls present. The major carotenoid was peridinin, followed in abundance on a weight basis by diadinoxanthin, dinoxanthin, and B-carotene.* Gambierdiscus toxicus *also contained a water soluble peridinin-chlorophyll* a*-protein complex. A trichromatic method was used to quantify the amount of total carotenoids, chlorophyll* a*, and chlorophyll* c*. The Florida isolate of this species differs from the published data for the Pacific isolate of this species in having only the* c_2 *form of chlorophyll* c *and qualitatively more carotenoids.*

Introduction

Gambierdiscus toxicus, a marine benthic dinoflagellate, is currently of interest to toxicologists since it has been found to produce toxins that have been implicated in ciguatera poisoning (Yasumoto et al., 1977, 1979). While numerous reports have focused on the structure and mechanism of action of the toxins associated with *G. toxicus*, relatively few have addressed fundamental questions regarding the nontoxin biochemistry and physiology of this organism.

In this paper we add additional data that can be used by others in characterizing their *G. toxicus* strains as well as in comparative studies with other dinoflagellate species. Pigment composition of microalgae has been used as a taxonomic criterion for a number of years (Strain et al., 1944; Goodwin, 1952; Riley and Wilson, 1967; Norgard et al., 1974). Studies on chloroplast pigment patterns of photosynthetic dinoflagellates have assisted biologists in grouping these organisms on biochemical data (Jeffrey et al., 1975) in addition to the classical groupings based on morphology.

There has been one published study on the chloroplast ultrastructure coupled with data for some of the pigments of *G. toxicus* (Durand and Berkaloff, 1985). In their study, however, the carotenoids were not completely characterized, either qualitatively or quantitatively. Additionally, they report the unusual occurrence of chlorophyll c_1. In this paper we identify the major photosynthetic pigments of the Florida isolate of *G. toxicus* and compare them with the data for the Pacific strain of this species.

Materials and Methods

Gambierdiscus toxicus was isolated from an intertidal environment on the southern coast of Florida by A. R. Loeblich III in 1983 and designated strain F8. Strain F8 was later grown for pigment analysis in 1.5 liter batches of GPM medium (Loeblich, 1975) adjusted to 31‰ salinity in 2.8 liter Fernbach flasks[1]. The previous paper in this conference (Loeblich and Indelicato, 1986) explains culture procedures and conditions.

Cultures were harvested by continuous-flow centrifugation at the end of the exponential phase of growth. The resulting cell pellet was sonicated in acetone and periodically shaken to facilitate the extraction of the chloroplast pigments. The pigment-containing acetone extract was repeatedly drawn off the cell debris, and fresh acetone was added until the acetone fraction was nearly colorless. The acetone extract was then briefly centrifuged to remove particulate cell debris from the preparation and evaporated to dryness under a stream of nitrogen at less than 40°C.

Dried pigments were dissolved in 180 µl of carbon disulfide and spotted repeatedly onto activated silica gel thin-layer chromatographic (TLC) plates, using 20 µl micropipettes. Development took place in a mixture of hexane/acetone (6:4) in a sealed chamber.

Developed plates were scanned at 470 nm using a Helena Quick-scan R & D scanning densitometer. The readout was used to provide an accurate means of locating the center of each pigment band to aid in the calculation of R_f values. The densitometer integrated the areas under the peaks from which relative percentages for each carotenoid were calculated.

Pigment fractions were then dissolved in ethanol or acetone. An absorption spectrum for each pigment fraction was produced over the visible light range

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

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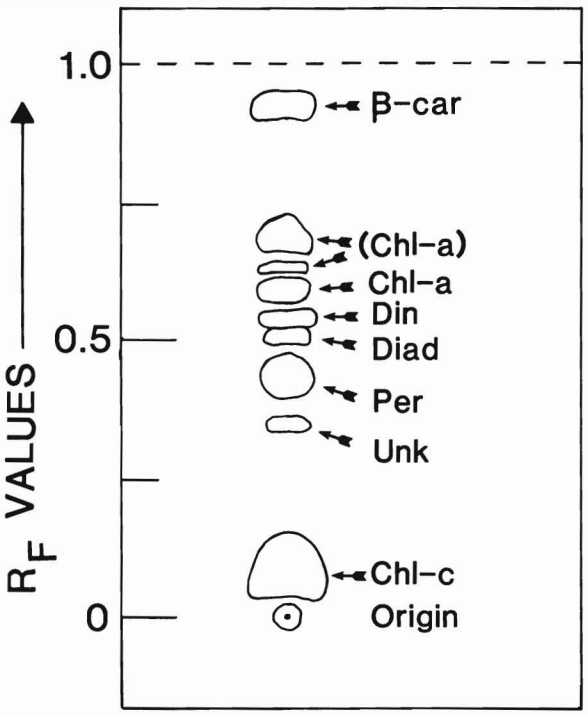

Figure 1.—Representation of a thin-layer chromatographic plate showing separation of *Gambierdiscus toxicus* photosynthetic pigments.

Table 1.—*Gambierdiscus toxicus* chloroplast pigment R_f values and absorbtion maxima.

Pigment[1]	Color	R_f value[2] (acetone: hexane)	Absorption maxima[3] (ethanol) (acetone)
Origin	Brown	0.00	454, 590, 665 No data
Chlorophyll-*c*	Grass-green	0.09	445, 587, 636 450, 583, 633
Unknown	Brown-green	0.35	No data
Peridinin	Red-orange	0.44	473 470
Diadinoxanthin	Yellow-orange	0.51	[4](409), 431, 457 (413), 438, 460
Dinoxanthin	Yellow	0.52	(405), 429, 457 (404), 429, 456
Chlorophyll-*a*	Green	0.56-0.57	413, 504, 535, 615, 666 412, 505, 535, 615, 666
(Chlorophyll-*a*)	Gray-green	0.62	410, 506, 535, 613, 669 No data
(Chlorophyll-*a*)	Gray-green	0.64-0.66	413, 510, 540, 612, 670 412, 507, 536, 610, 668
B-carotene	Yellow	0.91-0.98	429, 451, 478 (404), 429, 453, 475

[1]Pigments are listed in order of increasing mobility.
[2]These values were determined using a developing solvent consisting of 40 parts acetone and 60 parts hexane.
[3]For each pigment, the absorbtion maxima as measured in ethanol are on the first line and the absorbtion maxima as measured in acetone are on the second line.
[4]Absorption maxima given in parentheses are values for shoulders in the spectrum which could not be defined as a clear peak.

(350-750 nm) using a Beckman model 35 spectrophotometer. Partition coefficient values were determined, using the method of Petracek and Zechmeister (1956), to aid in the identification of pigments. The trichromatic method of Jeffrey et al. (1966) was employed to obtain values for the relative weight percentages of carotenoids and chlorophylls present in a whole-cell extract (95 percent acetone) prepared from the cells of *G. toxicus*.

Results

Thin layer chromatography revealed the presence of 10 pigment fractions extracted from the cells of *G. toxicus* (Fig. 1). Of these 10 fractions (numbered in order of elution), four had obvious chlorophyll affinities (fractions 9, 4, 3, 2), four were carotenoids (fractions 7, 6, 5, 1), and two others (fractions 8, 10) were unknown. The chromatographic and spectral properties of these fractions are presented in Table 1.

The major pigment within the cells of *G. toxicus* as measured by percent composition is chlorophyll-*a* (fraction 4) (Table 2). Spectral properties of two other fractions (2, 3), which are gray-green and have slightly higher R_f values than chlorophyll-*a*, suggest that these are degradation products of chlorophyll-*a*. Together, chlorophyll-*a* and its two degradation products make up 47.6 percent by weight of the total pigments in this species (Table 2).

Chlorophyll-*c*2 (fraction 9) is also found in large amounts in *G. toxicus* cells, constituting 16.33 percent of the total pigment weight (Table 2). This pigment's color is grass-green and was far less mobile than chlorophyll-*a* when chromatographically developed in an acetone:hexane solvent (Fig. 1). There was no evidence of chlorophyll-*c*1 in this strain of *G. toxicus*.

Of the four carotenoid pigments found in *G. toxicus*, peridinin (fraction 7) was present in the greatest amount. Peridinin constituted 23.0 percent of the total cellular pigments and 63.6 percent of the total carotenoids by weight (Table 2). Peridinin is easily recognized by its bright red-orange color and its characteristic broad absorption maximum at around 473 nm. It is the last carotenoid to be eluted during chromatographic separation in an acetone:hexane solvent (Fig. 1).

Table 2.—Percent total pigments, percent total carotenoids, and carotenoid partition coefficients for *Gambierdiscus toxicus*.

Pigment	Percent total pigments	Percent total carotenoids	Partition coefficient (hexane: acetone)
Chlorophyll-*a*	47.6		
Chlorophyll-*c*2	16.3		
Peridinin	23.0	63.6	3:97
Diadinoxanthin	4.9	13.6	5:95
Dinoxanthin	4.9	13.6	6:94
B-Carotene	3.3	9.1	

A yellow pigment fraction, which was the most mobile of all pigments contained in *G. toxicus*, and which traveled with the solvent front (R_f value = 0.91-0.98), was identified as B-carotene. Of the major pigments of *G. toxicus*, B-carotene was found to constitute only 9.1 percent of the total carotenoids and 3.3 percent of the total pigment content (Table 2).

The two major yellow xanthophylls produced by this organism had nearly identical spectral properties and R_f values (Table 1, Fig. 1). The first of the two to develop during chromatographic separation was yellow-orange and partitioned between hexane and 95 percent methanol in the ratio of 5:95 (hexane: methanol) (Table 2). This pigment has been identified as diadinoxanthin. The second xanthophyll to elute was bright yellow and had a partition coefficient ratio of 6:94 (hexane:methanol) and has been identified as dinoxanthin. Based on densitometric scan data, both xanthophylls are found in approximately equal amounts in the cell, together composing 9.8 percent of the total pigment content and 27.2 percent of the total carotenoids of *G. toxicus* by weight (Table 2). See Table 3 for the carotenoid and chlorophyll pigment ratios on a weight and molar basis.

Table 3.—Pigment ratios for *Gambierdiscus toxicus*.

Pigments	Wt. ratio	Mol. ratio
Chlorophyll-*a*:Chlorophyll-*c*2	2.91	1.98
Total chlorophyll:Total carotenoid	1.77	2.82
Peridinin:Chlorophyll-*a*	0.48	0.68
Total carotenoid:Chlorophyll-*a*	0.76	

Occasionally, after centrifugation, freeze-thawing, or filtration of *G. toxicus* cells, an orange water-soluble pigment appeared in the supernatant. From spectral data, this orange pigment has been identified as a peridinin-chlorophyll-*a* protein complex.

Discussion

Comparison of our data concerning the chloroplast pigment composition of *G. toxicus* with that of other dinoflagellate species reveals that *G. toxicus* possesses a pigment content which is very similar to that of other dinoflagellates belonging to the gonyaulacoid lineage. Chlorophyll-*a*, chlorophyll-*c*2, peridinin, dinoxanthin, diadinoxanthin, and B-carotene have been found in all photosynthetic dinoflagellates of this lineage. Those species (belonging to the peridinioid lineage) that harbor a photosynthetic endosymbiont (Jeffrey et al., 1975) are atypical as some of the pigments may belong to the symbiont derived from a different algal division: e.g., fucoxanthin in *Peridinium balticum* (Tomas and Cox, 1973). Jeffrey et al. (1975) noted that all of the peridinin containing photosynthetic dinoflagellate species studied contained an unknown "pink" pigment, which remained at the origin during thin layer chromatography. Thin-layer chromatography of *G. toxicus* pigments revealed this same fraction (1), although in *G. toxicus* this pigment was brown. Spectral data and immobility in a nonpolar solvent suggest this to be the peridinin-chlorophyll-*a* protein complex. These protein-pigment complexes act in a light harvesting capacity (Prezelin and Haxo, 1976) and appear to be an integral part of the dinoflagellate photosynthetic apparatus. These photosynthetic complexes have been observed in other dinoflagellates such as *Glenodinium* sp. (Prezelin, 1976), *Gonyaulax polyedra* (Prezelin and Haxo, 1976), *Amphidinium carterae* (Haxo et al., 1976; Siegelman et al., 1976), *Ceratium furca* (Meeson et al., 1982), and *Heterocapsa* spp. (Watson and Loeblich, 1983).

Durand and Berkaloff (1985) reported the presence of both chlorophyll-*c*1 and chlorophyll-*c*2 in *G. toxicus*. Our results disagree as we found only chlorophyll-*c*2. Presence of chlorophyll-*c*1 and *c*2 in dinoflagellates whose major carotenoid is peridinin has been seen in only one species, *Prorocentrum cassubicum* (Jeffrey, 1976); all other photosynthetic dinoflagellates have only chlorophyll *a* and chlorophyll-*c*2. The absence of a second form of chlorophyll *c* in our isolate, and the lack of other reports of this pigment in any dinoflagellate that is morphologically related to *G. toxicus*, suggests strongly that the report by Durand and Berkaloff (1985) should be reconfirmed.

Additionally, *P. cassubicum* belongs to a dinoflagellate lineage that shows affinities to the dinophysioids rather than to the gonyaulacoids to which *G. toxicus* belongs. Durand and Berkaloff (1985) found no evidence for the presence of an internal symbiont as an explanation for the occurrence of the second form of chlorophyll *c*. There remains the possibility that the fraction they identify as "chlorophyll-*c*1" is a chlorophyll degradation product that could result from photooxidation. Such degradation products may occur if pigments are not analyzed under reduced light conditions and in a nonoxidizing (nitrogen) atmosphere. The discrepancies between the pigment pattern for the Florida and Pacific isolates of *G. toxicus* suggest that it may be necessary to analyze more isolates before a clear understanding of the apparent variability can be reconciled.

Durand and Berkaloff (1985) reported only two carotenoids, the xanthophylls diadinoxanthin and peridinin, from the Pacific *G. toxicus*; their study dealt mainly with the ultrastructure and chlorophyll pigmentation. No carotenes were reported for the Pacific isolate. The apparent differences in the carotenoid pigmentation between the Florida and Pacific isolates may disappear when a more detailed analysis of the Pacific form is published.

The properties of fractions 2 and 3 are similar to pheophytin-*a*, a magnesium-deficient chlorophyll molecule, which has been reported in *Peridinium cinctum* (Strain et al., 1944) and in Pacific Gyre phytoplankton samples (Jeffrey, 1975). It is not known whether pheophytin-*a* occurs naturally or if it is a laboratory artifact.

Jeffrey et al. (1975), in a survey of dinoflagellate pigments, showed a range for peridinin, the major dinophycean carotenoid, of 54-68 percent of the total carotenoid fraction. The value of 64 percent which we recorded for *G. toxicus* is within this range. Similar results exist for the ratio of peridinin to chlorophyll-*a* and for the ratio of total carotenoids to chlorophyll-*a* where ranges of

0.32-0.50 and 0.60-0.74 are found, respectively. *Gambierdiscus toxicus* exhibits values of 0.48 for peridinin: chlorophyll-*a* and 0.76 for total carotenoids:chlorophyll-*a* (Table 3).

Although the relative percentages of pigments may vary from species to species, the basic components of the dinoflagellate photosynthetic apparatus are present in all species for which data is available; such is the case with *G. toxicus*. See Jeffrey et al. (1975) for a review of dinoflagellate pigmentation.

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