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ISOLATION AND CHARACTERIZATION OF DINOFLAGELLATE AND CHRYSOPHYTE CYTOCHROME-*f* (553-4)

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Key Word Index—*Amphidinium carterae*; *Cachonina niei*; *Glenodinium* sp; *Peridinium foliaceum*; *Gonyaulax polyedra*; *dinoflagellate*; *Cricospherae carterae*; chrysophyte; cytochrome-*f* (553-4); isolation and characterization.

Abstract—Cytochrome-*f* (553-4) was isolated from mass cultures of the six dinoflagellates, *Amphidinium carterae* (2 strains), *Cachonina niei*, *Glenodinium* sp., *Peridinium foliaceum*, *Gonyaulax polyedra*, and one chrysophyte, *Cricospherae carterae*. Sonication of whole cell suspensions released the water-soluble protein, which was then purified by vacuum dialysis, salt fractionation and column chromatography. Reduced forms of isolated cytochromes had absorption maxima at 270–6, 316–8, 415–6, 522–3 and 553–4 nm. The α-absorption peak was asymmetrical. MW's, as determined by SDS polyacrylamide gel electrophoresis, ranged from 10700 to 13500. Amino acid analysis of *C. niei* cytochrome-*f* revealed 102 residues, with a composite MW of 10836. Purified cytochromes had isoelectric points ranging from 3·45 to 4·25 and oxidation-reduction potentials ranging from +0·374 to +0·351 V.

INTRODUCTION*

The *f*-type cytochrome associated with photosynthetic electron transport has been isolated and characterized, chemically and physically, in higher plants [1,2], algae [3–14], and bacteria [15]. However, there is only one published report on cytochrome-*f* in chrysophytes and none on dinoflagellate algae. Significant quantities of soluble cytochrome-*f* can now be obtained from these unicellular algae, using large scale culturing techniques [16], and methods developed for the isolation of water-soluble carotenochlorophyll proteins from dinoflagellates [17,18]. Considering the increasing use of cytochromes in evaluating phylogeny [19,20], as well as in characterizing photosynthetic systems, cytochrome-*f* was isolated and characterized from the chrysophyte, *Cricospherae carterae* and six marine dinoflagellates: *Cachonina niei*, *Glenodinium* sp., *Gonyaulax polyedra*, *Peridinium foliaceum*, and *Amphidinium carterae* (2 strains). The two strains of *A. carterae* were examined because they differed in relative amounts of extractable carotenoproteins.

* Abbreviations: SDS, sodium dodecyl sulfate; SIO, Scripps Institution of Oceanography, F. T. Haxo Collection; pI, isoelectric point.

RESULTS AND DISCUSSION

Purification. Vacuum dialysis of the water-soluble extract from the phytoplankton cells resulted in a partial purification of cytochrome-*f*. Low MW proteins, including cytochrome, passed through the dialysis membrane and were collected in the surrounding vessel. When this recovered fraction was subjected to ammonium sulfate precipitation at 50% saturation, the majority of contaminating protein was removed in the pellet. Cytochrome could then be recovered and concentrated by making the supernatant 70% in ammonium sulfate and dissolving the orange pellet in a small volume of distilled water. Final purification of cytochrome was achieved by Sephadex and DEAE ion exchange chromatography. In addition, a purified *C. niei* cytochrome-*f* sample was obtained from Dr. H. W. Siegelman. The sample had been prepared by fractionation of cell-free,

Table 1. Characteristics of dinoflagellate and chrysophyte cytochrome 553–4

	Yield % of wet wt.	λ_{max} (nm) Dithionite reduced α	β	γ	δ	Protein	Aγ/Aα	Em, 7 (V)	pI	MW
DINOFLAGELLATES										
Amphidinium carterae (PY-2)	0·37	553	523	416	318	270	7·0	0·356	3·68	13000
Amphidinium carterae (Plymouth 450)	0·33	554	523	416	318	270	7·0	0·363	3·98	10700
Cachonina niei	0·11	554	523	416	318	274	7·0	0·374	3·55	13000 (11000)*
Glenodinium sp.	0·38	554	522	415	318	274	7·7	0·360	3·67	12200
Gonyaulax polyedra	—	553	522	416	—	—	6·1	—	3·45	13500
Peridinium foliaceum	—	553	522	416	—	—	6·7	0·351	3·45	12500
CHRYSOPHYTE										
Cricospherae carterae	0·13	553	522	416	316	276	7·0	0·359	4·25	12000

* Determined by amino acid analysis.

water-soluble extracts by repeated Sephadex column chromatography. Purity of isolated cytochromes was confirmed by a single band on SDS polyacrylamide gel electrophoresis and isoelectrofocusing on polyacrylamide. Approximately 80% of the solubilized cytochrome was isolated in this manner. Using α-band absorption, an extinction coefficient of 29·4 mM^{-1} cm^{-1}, and MW's determined by SDS polyacrylamide electrophoresis, estimates of cytochrome yields could be expressed in terms of percent wet wt of starting material. The values are listed in Table 1 and are similar to yields reported for the chrysophyte, *Monochrysis lutheri* [8].

Spectral properties. The visible and UV absorption maxima of the purified reduced forms of cytochrome-*f* of the dinoflagellates *A. carterae*, *C. niei*, *Glenodinium* sp., *P. foliaceum*, *G. polyedra* and of the chrysophyte, *C. carterae* are listed in Table 1. Consistent with maxima reported for other algal *f*-type cytochromes [8,9,12], the α-band was observed at 553–554, the β-band at 522–523, and the γ-band at 416 nm. UV maxima occurred at 316–318 and 270–276 nm. All isolated cytochromes had an asymmetrical α-band, with a shoulder at 550 nm. This absorption property is typical of *f*-type cytochromes reported for the red and brown algae [9,12], but a symmetrical α-band has been reported for the chrysophyte, *M. lutheri* [8]. The cytochrome-*f* (Aγ/Aα) 416/553 nm absorbance ratio of 7·0 (Table 1) differs from values close to 5·0 for *c*-type cytochromes but is in agreement with ratios determined for other algal *f*-type cytochromes [11,12].

Oxidized and reduced absorption spectra typical of the isolated cytochrome 553–4 are given in Fig. 1. Peaks in the visible region were depressed in the ferricyanide oxidized spectrum and the γ-band was shifted to 408 nm. The extinction coefficient for the reduced α-band of *C. niei* was calculated to be 29·4 mM^{-1} cm^{-1}, using the MW value of 11000 as determined from amino acid analysis.

Oxidation–reduction potential. The oxidation–reduction potentials ranged from 0·351–0·374 V (Table 1) and are in agreement with values of

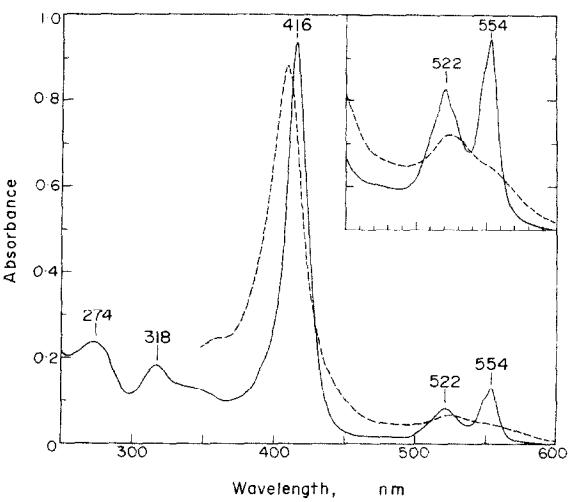

Fig. 1. Absorption spectra of purified *Cachonina niei* cytochrome 554. The oxidized state (– – –) was measured in the presence of 0·1 mM potassium ferricyanide. The reduced state (——) was measured in the presence of sodium dithionite. The 250–380 nm region of the spectrum was measured prior to dithionite reduction. Detailed spectra of the α and β bands are given in the inset.

Table 2. *Cachonina niei* cytochrome-554 amino acid composition

Amino acid	Integral residues
Lysine	7
AE-cysteine	2
Histidine	1
Ammonia	0
Arginine	2
Aspartic acid	13
Threonine	3
Serine	4
Glutamic acid	12
Proline	5
Glycine	12
Alanine	13
Valine	9
Methionine	2
Isoleucine	5
Leucine	4
Tyrosine	5
Phenylalanine	3
Total residues	102
Estimated mw	11000

0·34–0·39 V reported for other algal cytochromes-*f* [5,9,10,12]. This positive potential is indicative of the photosynthetic cytochrome-*f* reported by Davenport and Hill [2].

Isoelectric point. The low isoelectric points (pI) ranging from 3·45–3·98 for the dinoflagellate cytochromes and 4·25 for the chrysophyte cytochrome are all typical of photosynthetic cytochrome-*f* [7–9,12], and distinguish them from the mitochondrial cytochrome-*c* which has a pI value close to 10.

Molecular weight. MW's determined by SDS polyacrylamide gel electrophoresis of cytochromes 553-4 from these algae ranged from 10700 to 13500. A value of 11000 g/mol was obtained from amino acid analysis (Table 2). MW values of cytochrome-*f* reported for other algal groups range from 10500 to 13500 [9].

Amino acid composition. The results of a preliminary amino acid analysis of purified cytochrome 553–4 from *C. niei*, a representative dinoflagellate, are given in Table 2. Comparison of the amino acid composition with that of various algal *f*-type cytochromes demonstrated a close similarity between the dinoflagellate cytochrome-*f* and the cytochrome-*f* isolated from the chrysophyte, *M. lutheri* [8]. The determination of 2 cysteine residues is consistent with values reported for cytochrome-*f* from other algal groups [8,20,21]. The number of amino acid residues of aspartate, glutamate, alanine, half-cystine, valine, phenylalanine, methionine and histidine are equal to the number reported for *M. lutheri*. However, the number of residues of threonine, serine, proline, glycine, isoleucine, leucine, tyrosine, lysine and arginine differ.

The chemical and physical properties of the isolated cytochromes from 6 dinoflagellates and one chrysophyte can be considered typical of lower algae and generally consistent with their phylogenetic relationship [23].

EXPERIMENTAL

Culture conditions. Algae studied and their origins were: *Amphidinium carterae* Hulbert (Plymouth 450), *Amphidinium carterae* Hulbert (R. Holmes from L. Provasoli; SIO code no. PY-2), *Cachonina niei* Loeblich III (IUCC 1564), *Glenodinium* sp. (L. Provasoli, M. Bernard isolate), *Gonyaulax polyedra* Stein (A. Dodson; SIO code no. PY-11), *Peridinium foliaceum* (Stein) Biecheler (IUCC 1688), and *Cricospherae carterae* (Braarud et Fagerland) Braarud (A. Dodson; SIO code no. CHR-1). The algae were grown in modified *Gonyaulax polyedra* media (GPM) [24] through a series of increasing volume discontinuous cultures [16]. Except for *G. polyedra*, the cultures were harvested by continuous flow centrifugation from 176 l. growth vats. Vats were illuminated with cool white fluorescent lamps at approximately 2700 lx, aerated mildly, and maintained at 20°. Vat yields varied between 50 and 150 g wet wt. *G. polyedra* was harvested from 60 fernbach flasks, each containing 1 liter medium, with a cellular wet wt of about 32 g.

Preparation of material. Harvested cells were resuspended in equal amount (W/V) of 0·1 M Tris, pH 8·0, sonicated in an ice bath to greater than 90% breakage with a Branson Sonic Power Model S75. The sonicate, after an initial centrifugation at 81000 *g* for 180 min yielded a pink to brick-red supernatant fraction containing the crude preparation of pigment–protein complexes, soluble cytochrome, and protein. Subjecting the colored supernatant fraction to vacuum dialysis permitted the low MW soluble proteins, including cytochrome, to pass through the dialysis membrane and into the surrounding collecting vessel. The partially purified cytochrome solns were subjected to $(NH_4)_2SO_4$ ppn for further purification. $(NH_4)_2SO_4$ was added to 50% satn which was centrifuged 10000 *g* for 10 min, and the tan pellet was discarded. Supernatant soln was brought to 70% satn, recentrifuged, and the orange ppt containing cytochrome, was recovered and dissolved in a small vol. H_2O. The sample was then chromatographed on a 2 × 15 cm G100 coarse Sephadex column, equilibrated in the cold (5°) with H_2O. The eluted orange–pink band was then applied and concentrated on a 2·5 × 10 cm DEAE 52 ion exchange column, buffered to pH 8·2, and the cytochrome band migrated down the column when 3 ml 0·1 M NaCl was applied. This allowed the separation of cytochrome from a dark protein band at the top of the column. The topmost band was removed by pipette, and the cytochrome was eluted off the remaining column with 0·3 M NaCl. The eluate was refractionated by $(NH_4)_2SO_4$ pptn and the ppt collected by vacuum filtration on 0·45 μm Millipore filters. Redissolved

cytochrome ppt were rechromatographed on a smaller 0·7 × 2 cm DEAE 52 column. The final eluate was dialyzed against H_2O.

Cytochrome-*f ex C. niei* was obtained as a freeze-dried sample from H. W. Siegelman and had been prepared as follows. Supernatant fractions (81 000 *g*) from broken cells containing cytochrome-*f* were fractionated in the cold by Sephadex G100 column chromatography. Elution buffer was 0·005 M Tris pH 8·4 with the addition of 0·2 M NaCl. Cytochrome fractions from several Sephadex columns were recovered, pooled, concentrated, and dissolved in a small volume of buffer. The sample was then rechromatographed on Sephadex, a freeze-dried portion of the isolated cytochrome-*f* was analyzed for amino acid composition at the Brookhaven National Laboratory, and the remainder was used in studies at La Jolla.

Analytical methods. MW's were determined by SDS polyacrylamide gel electrophoresis [24] and isoelectric points were determined by following modified standard procedures [25]. Stable pH gradients were achieved by passing a current of 1 ma/tube through 6% acrylamide gels containing 3% (V/V) ampholyte pH 3–10 with solns of 0·03 M KH_2PO_4 and 0·02 M NaOH as anolyte and catholyte respectively. Oxidation–reduction potentials were measured in a ferri–ferrocyanide system similar to that of Davenport and Hill [2]. For the amino acid composition, *C. niei* cytochrome-*f* was hydrolyzed in evacuated sealed tubes with redistilled HCl (6 N) at 115° for 24 hr and analyzed on an automatic amino acid analyzer.

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