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A new spectrally distinct component in preparations of chlorophyll *c* from the micro-alga *Emiliania huxleyi* (Prymnesiophyceae)

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A new chlorophyll *c* pigment designated chlorophyll c_3 has been isolated from the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae) using reverse-phase high-performance thin-layer chromatography (HPTLC). Its spectral properties were compared with chlorophylls c_1 and c_2 from standard sources. Visible absorption maxima of the new pigment in diethyl ether were at 451, 585 and 625 nm with band ratios of 30.77, 3.79 and 1.00, respectively. Chlorophyll c_2 was present in approximately equal proportions to chlorophyll c_3, with maxima in diethyl ether at 447, 579 and 628 nm and band ratios of 12.26, 1.17 and 1.00, respectively. No chlorophyll c_1 was detected. The visible absorption spectra of the magnesium-free derivatives of both chlorophylls c_2 and c_3 from *E. huxleyi* in acetone were also recorded. The new chlorophyll c_3 pigment was chromatographically and spectrally distinct from a similar pigment, magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester, present in prasinophyte algae, with which it could have been confused.

Introduction

Two chlorophyll *c* compounds, chlorophylls c_1 and c_2, are widely distributed in the chromophyte algae [1]. The first separations of the naturally occurring compounds by thin-layer chromatography (TLC) using special polyethylene absorbents [2–4] allowed chromatographic and spectral properties of these light-harvesting pigments to be defined. Extinction coefficients were determined after crystallization of the purified derivatives [4], and spectrophotometric equations were derived for quantitative assays [5]. The two chlorophyll *c* pigments, after esterification with diazomethane, were also separated and crystallized [6].

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The chemical structures of chlorophylls c_1 and c_2 were elucidated first in unresolved mixtures of the two components [7,8] and were later confirmed using the separated compounds [9,10]. The two pigments differ only in two hydrogen atoms, chlorophyll c_1 (magnesium tetradehydro-pheoporphyrin a_5 monomethyl ester), which has an ethyl group, and chlorophyll c_2 (magnesium hexadehydro-pheoporphyrin a_5 monomethyl ester), which has a vinyl group at position C_4 in ring II of the chlorophyll macrocycle (Fig. 1). Although the compounds are called 'chlorophylls' [11,12] they differ from chlorophylls *a* and *b* in several important respects; they are porphyrin rather than chlorin derivatives, with ring IV unsaturated; they contain an acrylic rather than propionic side chain at C_7; and they occur as free acids (not esterified to phytol).

The distribution of the two components was

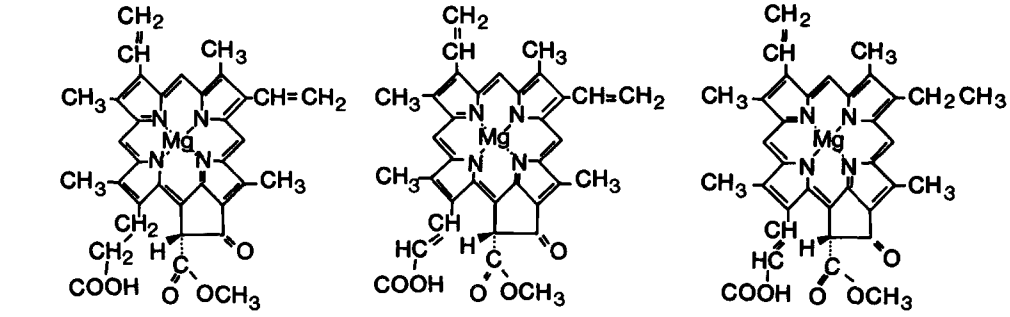

Fig. 1. Structures of chlorophylls c_1 and c_2 and the closely related magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester.

determined in 76 species of algae from seven classes, using a special polyethylene TLC system [1]. Both chlorophylls c_1 and c_2 were present in most of the algae examined, brown seaweeds (17 species examined), diatoms (25 spp.) prymnesiophytes (5 spp.), cryptomonads (1 sp.), chloromonads (1 sp.), xanthophytes (3 spp.) and fucoxanthin-containing dinoflagellates (2 spp.). Chlorophyll c_2, but not chlorophyll c_1, occurred in the peridinin-containing dinoflagellates (20 spp.) and cryptomonads (9 spp.). These results suggested that chlorophyll c_2 was universally distributed in the chlorophyll *c*-containing algae [1]. More recently, however, chlorophyll c_1 alone has been found in nine out of 25 species of freshwater chrysophytes [13], while the other 16 chrysophyte species examined contained both chlorophylls c_1 and c_2.

Another chlorophyll *c*-like pigment, magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester, is found in some members of the green algal class, Prasinophyceae [14], and acts as a light-harvesting pigment in photosynthesis [15].

The present work describes a new chlorophyll *c* pigment (here designated chlorophyll c_3), in chlorophyll *c* preparations from the coccolithophorid *E. huxleyi* (Prymnesiophyceae). This pigment, first isolated in two strains of the chrysophyte *Pelagococcus subviridis* by Vesk and Jeffrey [16], was detected in *E. huxleyi* as an unusual chlorophyll *c* spectrum during spectrographic monitoring of pigment fractions using the high-performance liquid chromatography (HPLC) method of Wright and Shearer [17]. A detailed visible spectral analysis of the new chlorophyll c_3 compound and its companion chlorophyll c_2 from *E. huxleyi*, as well as their magnesium-free derivatives, are presented in this paper. The new chlorophyll c_3 pigment is spectrally and chromatographically different from chlorophylls c_1, c_2 and magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester.

Materials and Methods

Algae

Micro-algal cultures of the coccolithophorid *E. huxleyi* (CSIRO Culture Code CS-57, obtained from Dr R.R.L. Guillard as clone BT6), the prasinophyte *Micromonas pusilla* (CS-86; UTEX, LB 991), the diatom *Amphiprora hyalina* (CS-28, obtained from J. Jordan, FCRG 70) and the dinoflagellate *Amphidinium carterae* (CS-21; Halifax, Canada), were used as sources of the standard chlorophyll *c* compounds. The brown seaweed, *Sargassum flavicans*, was collected in November, 1986 from the same site, at Port Hacking, Sydney, as was used for the first chlorophyll c_1 and c_2 studies [2–4].

Cultures were grown in 250-ml or 5-l Erlenmeyer flasks illuminated from beneath by Philips daylight fluorescent tubes at a light intensity of 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ on 12/12 h light/dark cycles [18]. The culture medium f_2 was used for *Emiliania* and *Amphiprora* [19]. G medium was used for *Micromonas* and *Amphidinium* [20]. Cells were harvested at the end of log phase by centrifugation at 2000 $\times g$ for 5 min for small volumes, or with a continuous-flow plankton centrifuge (8000 $\times g$) for large volumes.

Pigment extraction

The harvested microalgae were extracted in 100% acetone. After centrifugation (2000 × *g* for 3–5 min), an equal volume of diethyl ether (Analytical Reagent grade, peroxide-free) was added to the acetone extract and then mixed gently with 20 vol. of cold (5 °C) 5% NaCl solution. The diethyl ether hyperphase containing the pigments was collected, concentrated under a stream of nitrogen, dried with a little solid NaCl and stored at −15 °C until used for chromatography.

For pigment extraction from the brown seaweed (*S. flavicans*), selected fronds were dipped in boiling seawater (buffered with 0.5 M ammonium acetate) for 20 s, chilled in ice-cold seawater for 10 s, immersed in methanol for 10–15 s until the first traces of carotenoid release were seen, and then transferred to 100% acetone for 5–10 min for extraction to take place. Several changes of acetone were necessary for complete release of the pigments, which left the fully extracted fronds a pale buff colour. The acetone extract was then filtered or centrifuged, and pigments transferred from acetone to diethyl ether for chromatography, as described above.

Chromatography

Three types of chromatographic systems were employed: (1) washed cellulose TLC [21] to obtain a 'whole' chlorophyll *c* fraction, (2) polyethylene TLC (PRX-1025, Polysciences Inc., [4]), for separation of chlorophylls c_1 and c_2 and (3) reverse-phase bonded-silica HPTLC (Merck, RP-8) for separation of the new chlorophyll *c* pigment. The developing solvent systems were *n*-propanol/petroleum ether (60–80 °C) (1.5 : 98.5, v/v) for cellulose TLC; acetone (100%) for polyethylene TLC; and methanol/water (9 : 1, v/v) for the new HPTLC RP-8 plate. All solvents used were Analytical Reagent grade.

Visible absorption spectra were recorded with a Cary Model 17 or a Shimadzu Model PRI spectrophotometer, on pigment fractions eluted from chromatograms with either peroxide-free diethyl ether or acetone.

The magnesium-free derivatives of chlorophyll *c* were prepared without volume change by adding a minute trace of 0.5 N HCl on the tip of a spatula to an acetone solution of chlorophyll *c* in a micro-cuvette [22]. After several minutes the reaction was completed and the spectrum was recorded.

Results

S. flavicans (brown seaweed) and *Amphiprora hyalina* (diatom) were used as the two reference algae containing both chlorophylls c_1 and c_2; *Amphidinium carterae* (dinoflagellate) was the reference 'chlorophyll c_2 only' alga [1]; and *M. pusilla* was the source of the chlorophyll *c*-like pigment, magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester [14].

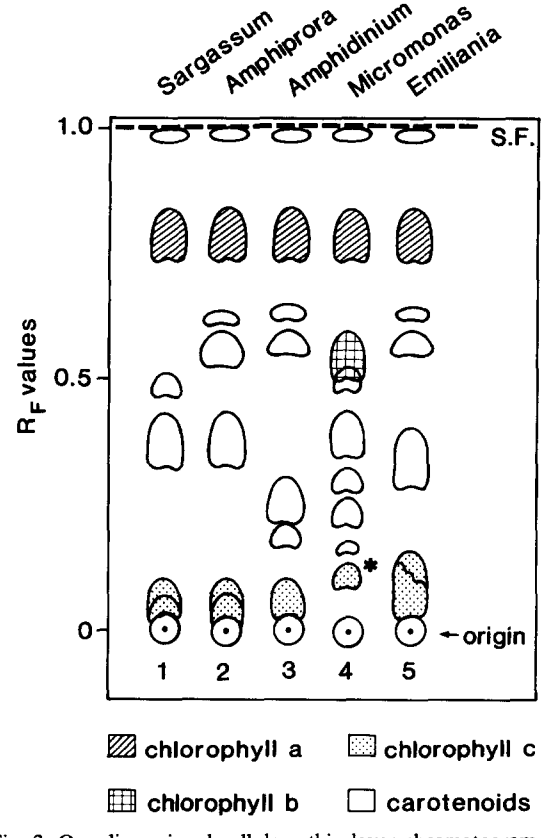

Fig. 2. One-dimensional cellulose thin-layer chromatogram of chlorophylls *a*, *c* and carotenoids of five algae, using *n*-propanol/light petroleum (60–80 °C) (1.5 : 98.5, v/v) as developing solvent. 1, *S. flavicans* (brown seaweed); 2, *Amphiprora hyalina* (diatom); 3, *Amphidinium carterae* (dinoflagellate); 4, *M. pusilla* (prasinophyte); 5, *E. huxleyi* (coccolithophorid); S.F., solvent front. * Magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester.

The separations of the total chlorophyll *c* fraction from *E. huxleyi* and the reference algae on the one-dimensional washed cellulose TLC plate are shown in Fig. 2. The light-green polar chlorophyll *c* fraction (containing either chlorophyll c_1, c_2 and/or c_3) remained just above the origin in all species ($R_F = 0.05$), with the light-green magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester from *M. pusilla* travelling slightly ahead ($R_F = 0.11$). *Emiliania* showed an extended chlorophyll *c* zone. While overlapping fractions were detected within the one chlorophyll *c* zone from *Sargassum, Amphiprora* and *Emiliania,* no further separation of these fractions was possible with this system. Cellulose TLC, however, provides a convenient method of obtaining a 'clean' chlorophyll *c* fraction free from other pigments.

The resolution of the light-green chlorophyll *c* fraction of each species on thin layers of polyethylene is shown in Fig. 3. Both chlorophylls c_1 and c_2 were present in the seaweed *Sargassum* (No. 1) and the diatom *Amphiprora* (No. 2); only chlorophyll c_2 was present in the dinoflagellate *Amphidinium* (No. 3), and magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester from the prasinophyte *Micromonas* (No. 4) ran slightly ahead of chlorophyll c_1 on polyethylene TLC. R_F values of 0.36, 0.24 and 0.48 were obtained for chlorophyll c_1, chlorophyll c_2 and the magnesium 2,4-divinylpheoporphyrin a_5 derivative, respectively. *Emiliania* (No. 5) showed only one very extended chlorophyll *c* zone on polyethylene, with no resolution of separate fractions.

The chromatographic separation of pigments from the five species on the reverse-phase bonded-silica HPTLC system (Merck, RP-8) is shown in Fig. 4. Chlorophyll *a* remained at the origin and chlorophyll *c*, being more polar, ran ahead of all other pigments. Carotenoids formed intermediate zones. Chlorophylls c_1, c_2 and magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester all had the same R_F value (0.45) on the RP-8 HPTLC plate. This was shown by the presence of

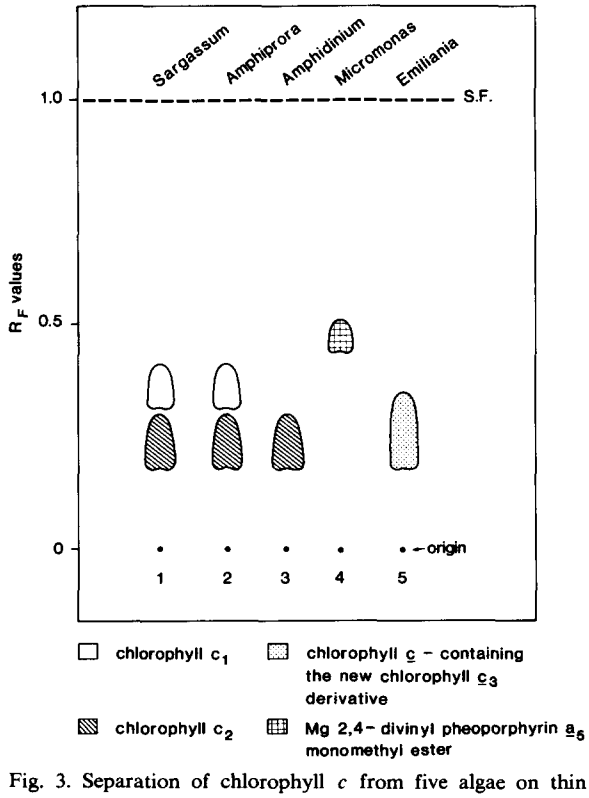

Fig. 3. Separation of chlorophyll *c* from five algae on thin layers of polyethylene (PRX-1025) with 100% acetone as developing solvent. Algal extracts 1 to 5 as for Fig. 1. S.F., solvent front.

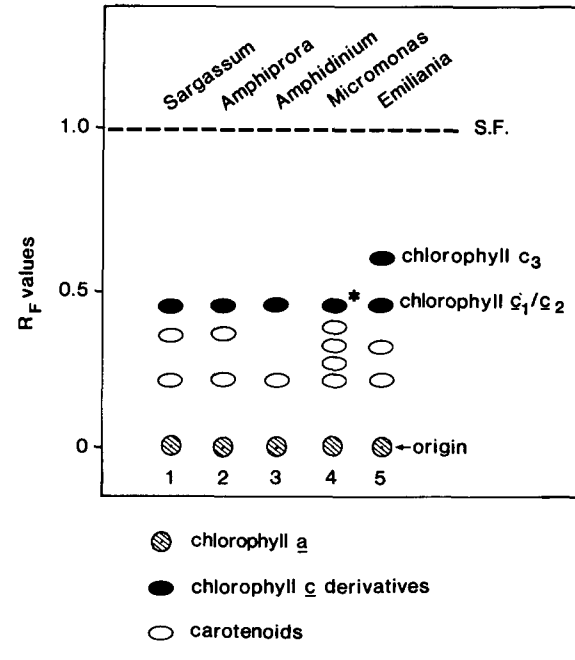

Fig. 4. Separation of chlorophyll *c* fractions on HPTLC plates (Merck, RP-8) from five algae, using methanol/water (9:1, v/v) as developing solvent. Algal extracts 1 to 5 as for Fig. 1. S.F., solvent front. * Magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester.

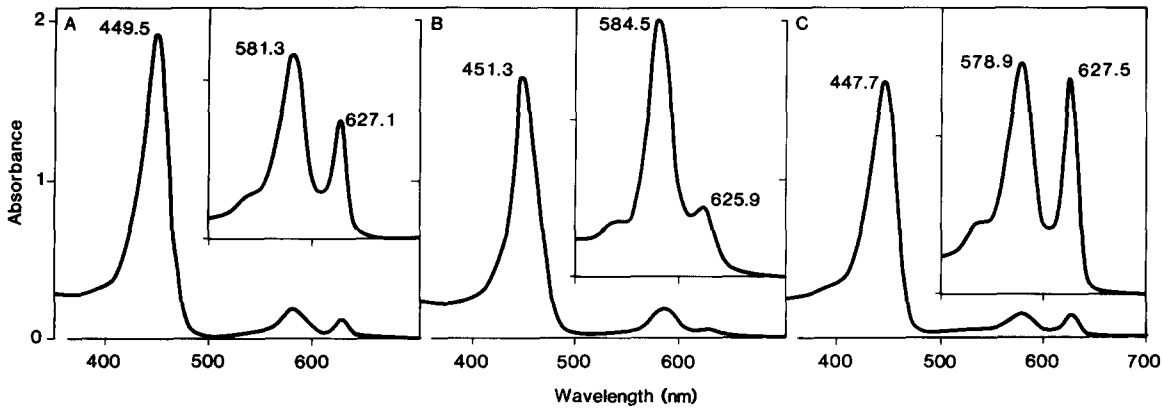

Fig. 5. Visible absorption spectra of chlorophyll *c* fractions from *E. huxleyi*. (A) The 'total' chlorophyll *c* fraction from cellulose TLC, (B) the fast-running ($R_F = 0.59$) chlorophyll *c* fraction, and (C) the slow-running ($R_F = 0.45$) chlorophyll *c* fraction, from the RP-8 HPTLC plate. Insets show details of the red bands. Solvent, diethyl ether.

only one chlorophyll *c* zone ($R_F = 0.45$) from extracts of *Sargassum* (1) and *Amphiprora* (2), which contain both chlorophylls c_1 and c_2 (Fig. 3); one chlorophyll *c* zone ($R_F = 0.45$) from extracts of *Amphidinium* (3), which contains only chlorophyll c_2; and one zone ($R_F = 0.45$) from extracts of *Micromonas* (4), which contains magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester. However, in *Emiliania* (5), two light-green zones separated: a fast-running polar fraction ($R_F = 0.59$) and a slower-running fraction ($R_F = 0.45$) similar in R_F value to that of the chlorophyll *c* of the other species.

The visible absorption spectra in diethyl ether of the chlorophyll *c* fractions from *Emiliania* were then studied (Fig. 5). Fig. 5(A) shows the spectrum of the 'total' chlorophyll *c* fraction from cellulose TLC, Fig. 5(B) shows the spectrum of the

TABLE I

ABSORPTION MAXIMA AND BAND RATIOS OF CHLOROPHYLL *c* FRACTIONS IN DIETHYL ETHER AND ACETONE FROM *E. HUXLEYI*, COMPARED WITH THOSE OF MAGNESIUM 2,4-DIVINYLPHEOPORPHYRIN a_5 MONOMETHYL ESTER FROM *M. PUSILLA*, AND STANDARD CHLOROPHYLLS c_1 AND c_2 FROM *S. FLAVICANS* [3]

Chlorophyll *c* fraction	Solvent	Absorption maxima (nm) band No.			Band ratios		
		III	II	I	III:I	II:I	I:I
'Whole' chlorophyll *c* (cellulose plate)	diethyl ether	449.5	581.3	627.1	15.50	1.55	1.00
	acetone	450.5	582.7	628.5	14.03	1.50	1.00
Chlorophyll c_3 ($R_F = 0.59$; RP-8 plate)	diethyl ether	451.3	584.5	625.9	32.10	3.79	1.00
	acetone	451.7	584.3	626.7	30.77	3.50	1.00
Chlorophyll c_2 ($R_F = 0.45$; RP-8 plate)	diethyl ether	447.7	578.9	627.5	12.26	1.17	1.00
	acetone	449.1	580.7	629.3	10.42	1.17	1.00
Magnesium 2,-4-divinyl-pheoporphyrin a_5 monomethyl ester (RP-8 plate)	diethyl ether	437.1	573.9	623.5	9.72	0.54	1.00
	acetone	437.3	575.3	624.5	8.22	0.44	1.00
Standard chlorophyll c_1 (*S. flavicans*)	diethyl ether	444	578	628	9.93	0.67	1.00
	acetone	443	580	630	6.71	0.58	1.00
Standard chlorophyll c_2 (*S. flavicans*)	diethyl ether	448	582	629	14.1	1.15	1.00
	acetone	444	581	630	9.64	0.83	1.00

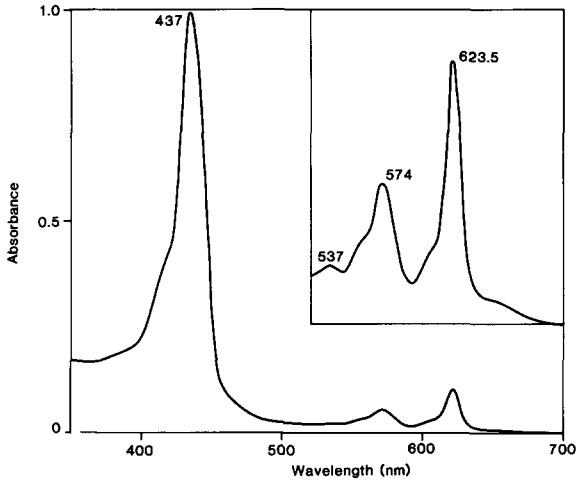

Fig. 6. Visible absorption spectra of magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester from *Micromonas pusilla* in diethyl ether. The inset shows details of red bands.

fast-running ($R_F = 0.59$) chlorophyll *c* fraction from the RP-8 plate, and Fig. 5(C) shows the spectrum of the slow-running chlorophyll *c* fraction ($R_F = 0.45$) from the RP-8 plate. The inset shows the detailed structure of the red bands of each chlorophyll *c* fraction. Absorption maxima and band ratios of the chlorophyll *c* fractions from *Emiliania* are listed in Table I, and compared with those of the closely related pigment, magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester from *M. pusilla*, whose full visible spectrum in diethyl ether is given in Fig. 6. Spectral data from the original description of chlorophylls c_1 and c_2 [3] are included in Table I for comparison.

The visible absorption spectra in acetone of both chlorophyll *c* fractions separated from *E. huxleyi* on the RP-8 plate and their magnesium-free derivatives (obtained without significant volume change) are shown in Fig. 7. The absorption spectrum of the fast-running ($R_F = 0.59$) new pigment, here designated chlorophyll c_3 (distinct from the chlorophyll *c* alteration product c_3 in Ref. 3) and its corresponding pheoporphyrin c_3 are shown in Fig. 7(A), with details of the red absorption bands shown in Fig. 7(B). Similarly, the absorption spectrum of the slow-running ($R_F = 0.45$) derivative and its corresponding pheoporphyrin are shown in Fig. 7(C), with details of the red absorption bands shown in Fig. 7(D). The absorption spectra and band ratios of the slow running zone from *E. huxleyi* in diethyl ether and acetone and that of its magnesium-free derivative in acetone clearly establish this fraction as chlorophyll c_2.

It was important to check that the new chlorophyll c_3 compound was not the chlorophyll *c*-like pigment (magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester, Fig. 1) found in some prasinophytes [14]. Pigment extracts of the prasinophyte, *M. pusilla*, were checked in all the chromatographic systems used in the present work, and the absorption spectra and chromatographic properties of its chlorophyll *c*-like pigment were compared to those of chlorophylls c_1, c_2 and c_3 (Table I and Fig. 6). The *Micromonas* pigment ran with a slightly higher R_F value than chlorophylls c_1 and c_2 on both cellulose and polyethylene TLC (Figs. 2 and 3), and had the same R_F value as both chlorophylls c_1 and c_2 on the RP-8 plate (Fig. 4), with no trace of a fast-running chlorophyll c_3 fraction. Spectrally, magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester was distinct from chlorophylls c_1, c_2 and c_3, with band ratios in diethyl ether of 9.72, 0.54 and 1.00 at maxima of 437.1, 573.9 and 623.5 nm respectively. While its band ratios were closest to those of chlorophyll c_1, they bore little resemblance to those of chlorophyll c_3. Absorption maxima of magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester were all shifted several nanometers to shorter wavelengths from those of chlorophylls c_1, c_2 and c_3, and the structure of the red bands (Fig. 6, insert) was also significantly different.

Discussion

The major characteristics of the new chlorophyll c_3 derivative are its high polarity compared to chlorophylls c_1 and c_2 (Fig. 4) and the relatively low absorption of the 630 nm band compared to those of the 580 nm and Soret bands, giving band ratios of 30.77, 3.79 and 1.00 for the Soret, 580 and 630 nm bands, respectively, in diethyl ether. When compared to band ratios of the original chlorophyll c_1 and chlorophyll c_2 from *S. flavicans*, (Table I and Refs. 2 and 3), it is clear that the present spectral and chromatographic data suggest a new type of chlorophyll *c* compound. The spectral characteristics are closer

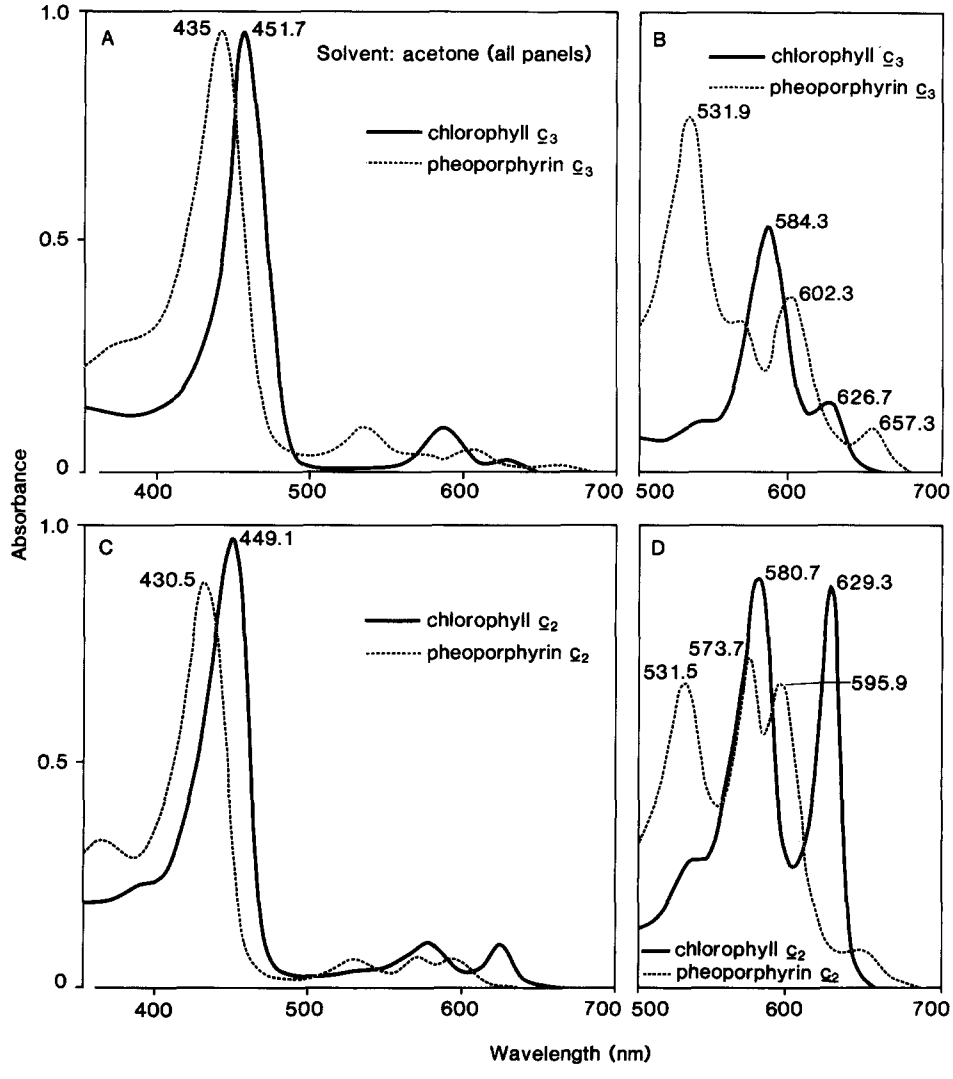

Fig. 7. Visible absorption spectra of chlorophyll *c* fractions from *E. huxleyi* isolated from the RP-8 HPTLC plate with their magnesium-free derivatives, obtained without significant volume change. Solvent, acetone. (A) The fast-running ($R_F = 0.59$) chlorophyll *c* fraction (———) and its magnesium-free derivative (· · · · · ·), and (B) details of the red bands. (C) The slow-running ($R_F = 0.45$) chlorophyll *c* fraction (———) and its magnesium-free derivative (· · · · · ·), and (D) details of their red bands.

to chlorophyll c_2 than c_1, with absorption maxima in diethyl ether at 451.3, 584.5 and 625.9 nm. However, the new compound has both Soret and 580 nm bands shifted several nanometers from those of chlorophyll c_2 to longer wavelengths, while the 630 nm band is shifted several nanometers to shorter wavelengths.

It is also clear that the chlorophyll *c*-like pigment from *M. pusilla* (magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester) is spectrally unlike chlorophylls c_1, c_2 and c_3, both in position of absorption maxima, and intensity of the absorption bands. Wilhelm et al. [23] tentatively identified chlorophyll *c* in *Micromonas* sp. (CCAP LB1965/4), but we believe this pigment to be magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester, since it co-chromatographs on polyethylene and cellulose thin-layer chromatograms with the corresponding pigment from the *M. pusilla* used in the present work (Jeffrey, S.W., unpublished data).

The present data suggests that chlorophyll c_3 is

not an artifact either of extraction or of the new RP-8 chromatographic system. Firstly, freshly prepared extracts of *Sargassum flavicans* (the species originally used for the separation and description of chlorophylls c_1 and c_2, [3]), did not show the new pigment when run on the RP-8 plate (Fig. 4). Neither the diatom *Amphiprora hyalina,* which contains chlorophylls c_1 and c_2 [1], nor the dinoflagellate *Amphidinium carterae*, which contains chlorophyll c_2 only, revealed a chlorophyll c_3 fraction when chromatographed on the RP-8 plate. It is therefore clear that the RP-8 plate does not change or degrade chlorophylls c_1 or c_2 to the c_3 form. Secondly, the contribution of the unusual chlorophyll c_3 spectrum to the spectrum of the 'total' chlorophyll c fraction of *E. huxleyi* (Fig. 5(A) and Table I) can be seen in the chlorophyll c eluted from the cellulose TLC plate before separation into its two component fractions. The Soret band and 580 nm band ratios of this unresolved chlorophyll c were significantly greater (15.50 and 1.55, respectively) than those of either standard chlorophylls c_1 or c_2 or unresolved mixtures of these pigments (see Ref. 1). Thirdly, the pigment was still present when *E. huxleyi* was extracted in methanol, rather than acetone, using the rapid method of Wright and Shearer [17].

Chlorophyll c_3 does not separate from chlorophyll c_2 on the polyethylene (PRX-1025) TLC plate (Fig. 3, chromatogram 5), which has hitherto been the only method available for chlorophyll c_1 and c_2 separations [1–3,13]. Instead, a larger and more extended chlorophyll c_2 zone than usual is seen. Obviously, the chlorophyll c_3 is masked by the chlorophyll c_2 fraction. It is important that anyone using the polyethylene TLC system for chlorophyll c fractionation should check the spectral properties (particularly band ratios) as well as R_F values of their fractions, in order to ascertain the presence or absence of chlorophyll c_3. A new HPLC method of separating the three chlorophylls c_1, c_2 and c_3 has now been developed and details will be available shortly (Jeffrey, S.W. and Collins, A., unpublished data).

A number of chlorophyll c alteration products were noted in the early chlorophyll c studies [3,4]. None of these pigments had the unique spectral properties of the chlorophyll c_3 described here.

To date, chlorophyll c_3 has been found in *E. huxleyi* (present work), in two strains of the minute unicellular alga, *Pelagococcus subviridis* (Chrysophyceae) [16], and in three strains, (one subtropical and two Antarctic) of the prymnesiophyte *Phaeocystis pouchetii* (Jeffrey, S.W., unpublished data). In each of these prymnesiophytes, chlorophyll c_3 is found, together with unusual fucoxanthin pigments [24]. It has also been found in several tropical and subtropical diatoms (Stauber, J.L., and Jeffrey, S.W., unpublished data), in Southern Ocean phytoplankton samples (Wright, S.W., unpublished data) and in a natural bloom of the microflagellate *Corymbellus aureus* (Prymnesiophyceae) (Dr. W.W.C. Gieskes, personal communication).

In the chlorophyll c_3-containing species that have been rigorously examined, chlorophyll c_3 is accompanied by approximately equal amounts of chlorophyll c_2 and apparently replaces chlorophyll c_1. Accurate quantitation of the new chlorophyll c_3 cannot be carried out until the extinction coefficient has been determined. Because the absorbance of chlorophyll c_3 at 630 nm (the wavelength used for chlorophyll c determination) is relatively low, significant errors in determining chlorophyll c_3 may result from using the extinction coefficients for chlorophylls c_1 and c_2 [4,5].

The presence of chlorophylls c_1 and c_2 in different pigment protein complexes of brown algae suggested a light-harvesting function for these pigments [25]. Haxo [26] recently confirmed that the chlorophyll c in *E. huxleyi* (clone BT6 = CS-57 used here) contributed significantly to the alga's light-harvesting ability. The discovery of a new chlorophyll c_3 pigment in this strain, and the absence of chlorophyll c_1, raises the question of whether chlorophyll c_2 or c_3, or both, function as light-harvesting pigments in *E. huxleyi*. The function of the multiple chlorophyll c pigments in the photosynthetic mechanisms of the chromophyte algae now needs to be seriously addressed.

Four pigments are now identified as members of the chlorophyll c family, namely c_1, c_2, c_3 and magnesium 2,4-divinylpheoporphyrin a_5 monomethyl ester. The structure of chlorophyll c_3 is presently under investigation, and when this is known terminology for the four pigments will be proposed.

Note added in proof

Wilhelm, C. (1987) Biochim. Biophys. Acta 892, 23–29) suggests that the chlorophyll *c*-like pigment in the prasinophyte *Mantoniella* (= *Micromonas*) may be chlorophyll c_1, but this does not agree with our evidence presented here.

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