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Three photosynthetic antenna porphyrins in a primitive green alga

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Fluorescence excitation spectra (between 400–500 and 610–700 nm) for chlorophyll emission from particles and detergent extracts of the primitive green microalga, *Mantoniella*, were measured. The results showed that the prophyrin, magnesium 2,4-divinylpheoporphyrin a_5, which this alga accumulates in addition to Chl *b*, also can transfer excitation energy to Chl *a*, and therefore act as antenna for photosynthesis. Evidence was found that magnesium 2,4-divinylpheoporphyrin a_5 has a Soret band near 450 nm in vivo which further increases the light-harvesting capacity of these algae growing deep in the open ocean.

Introduction

Mantoniella squamata is a member of a large group of primitive, green, motile microalgae (Prasinophytes) which have been well studied morphologically [1] but not in terms of biochemical function. Ricketts [2] found that certain members of this group including *Mantoniella* and *Micromonas* contain the protochlorophyll- or Chl *c*-like pigment, magnesium 2,4-divinylpheoporphyrin a_5 in addition to Chl *a* and Chl *b*. Magnesium 2,4-divinylpheoporphyrin a_5 is an intermediate in chlorophyll biosynthesis in higher plants [3,4] as well as in some photosynthetic bacteria [5]. To detect magnesium 2,4-divinylpheoporphyrin a_5 in both higher plants and bacteria, it is necessary to use etiolated tissue or inhibitors of chlorophyll synthesis, but in some of the Prasinophytes it appears as a stable component in amounts ranging from 2–9% of the Chl *a* [2].

Magnesium 2,4-divinylpheoporphyrin a_5 is similar in structure and absorption properties to both protochlorophyllide and Chl c_2 [2,3]. Chl c_2 is a major antenna porphyrin in several large groups of algae including the diatoms, brown algae and dinoflagellates [6]. We observed a distinct absorption band near 633 nm (in addition to the broad 625 nm Chl *a* band) in both intact and broken cells of *Mantoniella* [7,8]. A similar band can be seen in absorption spectra (77 K) of the diatom, *Phaeodactylum*, but not in spectra of higher plants or green algae [9–11]. Logically, therefore, the 633 nm band in *Mantoniella* is from magnesium 2,4-divinylpheoporphyrin a_5. Support for the Chl *c*-like antenna function of magnesium 2,4-divinylpheoporphyrin a_5 came from experiments in which detergent treated *Mantoniella* membranes were separated into Photosystem I and light-harvesting pigment-protein fractions [7,8]; magnesium 2,4-divinylpheoporphyrin a_5 partitioned with the other antenna pigments.

Fluorescence excitation spectroscopy is a useful technique for determining energy transfer between chromophores bound to intrinsic membrane proteins [9]. In this paper we measured fluorescence excitation spectra of both intact and broken *Mantoniella* cells for the emission of Chl *a* near 720 nm at 77 K. This result indicates that magnesium 2,4-divinylpheoporphyrin a_5 is able to

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Abbreviation Chl, chlorophyll

transfer absorbed light energy to Chl *a*, and thus act as antenna pigment for photosynthesis.

The absorption maximum of magnesium 2,4-divinylpheoporphyrin a_5 in the blue spectral region in vivo is difficult to discern because of overlap with the chlorophylls and carotenoids. Therefore, we treated *Mantoniella* membranes with enough detergent to prevent energy transfer between the three porphyrins, and measured a fluorescence excitation spectrum for each one at its red emission maximum.

Materials and Methods

Mantoniella squamata (University of Texas Culture Col. No. 990) was grown in 3 l batches of enriched natural sea water medium over fluorescent lamps and bubbled with air for 7–9 days at about 20°C. The cells were harvested by centrifugation and broken in a French pressure cell. The broken cell membranes were washed with 5 mM EDTA and again with 2 mM Tris-maleate buffer (pH 7). Washed sediments were stored at −80°C. Chlorophyll concentration was estimated according to the method of Jeffrey and Humphrey [12] for phytoplankton in which Chls *a*, *b* and *c* may be present together. Magnesium 2,4-divinylpheoporphyrin a_5 and Chl *c* have similar maxima and extinction coefficients [2,6].

A portion of frozen pellet was suspended to about 400 μg total chlorophyll per ml water. Aliquots of this suspension were diluted with either water or 1% Nonidet P-40 (a detergent similar to Triton X-100). The final Nonidet concentration was less than 0.1%. A chlorophyll concentration of about 3 μg/ml was dilute enough to avoid reabsorption artifacts in the fluorescence spectra.

Fluorescence excitation and emission spectra were measured either at 20°C or 77 K with a Perkin-Elmer (MPF-3L) fluorometer. Absorption spectra were measured at the same two temperatures in a Cary 17 spectrophotometer fitted with a scattering transmission attachment. Both instruments are interfaced with a Hewlett-Packard minicomputer for storing and correcting the spectra for instrument sensitivity.

Results

Fig. 1 shows absorption (a) and fluorescence excitation (b) spectra of *Mantoniella* particles, and

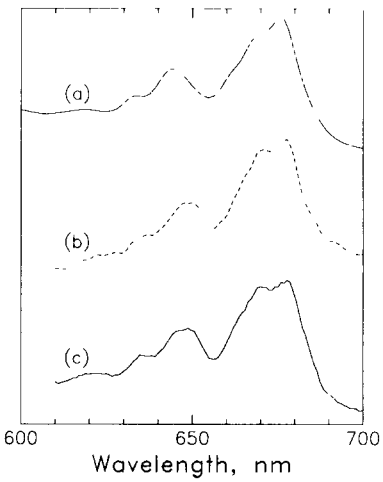

Fig 1 *Mantoniella* chloroplast particles (a) absorption, (b) fluorescence excitation (slitwidth, 3 nm) for emission at 720 nm (slitwidth, 9 nm), (c) same as (b) with intact cells, measured at 77 K.

an excitation spectrum (c) of intact cells between 610 and 700 nm for emission at 720 nm, all measured at 77 K. An unusual characteristic of *Mantoniella* is its lack of any fluorescence emisison maximum beyond 700 nm at 77 K either from intact cells, membranes or semi-purified Photosystem I complexes [7,8]. We chose to measure a broad emission band near 720 nm in order to extend the excitation spectra to 700 nm. However, the excitation spectrum was the same in the 600–660 nm region when emission at the Chl *a* maximum (683 nm) was measured.

The shapes of the two excitation spectra from intact (Fig. 1c) and broken (Fig. 1b) cells are the same with distinct maxima at 678 and 670 nm for Chl *a*, at 648 nm for Chl *b*, and near 633 nm for Magnesium 2,4-divinylpheoporphyrin a_5. The absorption spectrum of intact cells (not shown) was the same as that of the broken cells (Fig. 1a) except for some flattening of the Chl *a* peaks caused by the high pigment concentration in each chloroplast. As noted before [7,8], the absorption maximum of Chl *b* near 643 nm in *Mantoniella* is anomalous because Chl *b* absorbs near 648 nm in other green plants [11]. To check against an instrumental artifact, the same absorption and fluorescence excitation spectra were measured of particles from the green alga, *Chlamydomonas*, both of a wild-type strain and a Chl *b*-less mutant. As

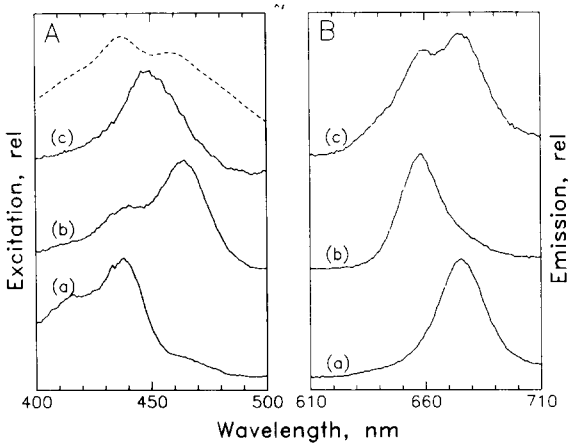

Fig 2 Nomdet extract of *Mantoniella* particles. (A) Absorption (broken line), fluorescence excitation spectra (slitwidth, 4 nm) for emission at (a) 675 nm, (b) 658 nm, (c) 635 nm, (slitwidth, 3 nm) (B) Fluorescence emission spectra (slitwidth, 2 nm) from excitation at (a) 435 nm, (b) 470 nm, (c) 450 nm (slitwidth, 5 nm) measured at 20°C

expected, we found an excitation band coincident with the absorption band near 649 nm in spectra of the wild type and no similar band in the mutant.

To determine the absorption maxima of the three porphyrins in the blue spectral region where there is considerable overlap bteween them as well as with carotenoids, we solubilized the membrane particles with sufficient detergent to prevent energy transfer between the pigments. Fig. 2B shows fluorescence emission spectra of the detergent extract when excited by light at 435 (a), 470 (b) or 450 (c) nm. Curves a and b clearly show the emission maxima of Chls *a* and *b* at 675 and 658 nm, respectively. Exciting light at 450 nm is not only absorbed by Chls *a* and *b*, but also by magnesium 2,4-divinylpheoporphyrin a_5 which causes the shoulder on the emission spectrum near 635 nm (curve c).

Fig. 2A shows the corresponding fluorescence excitation spectra for emission at 675 (a), 658 (b) or 635 (c) nm together with the absorption spectrum (broken line) of the same detergent extract. The three excitation spectra show maxima at 438 nm for Chl *a* (a), 464 nm for Chl *b* (b), and 448 nm for magnesium 2,4-divinylpheoporphyrin a_5 (c). By this procedure, we are able to deduce the blue absorption maximum of magnesium 2,4-divinylpheoporphyrin a_5 near 450 nm in vivo.

Discussion

The fluorescence excitation spectra in Fig. 1 show that magnesium 2,4-divinylpheoporphyrin a_5 which absorbs at 633 nm in vivo can transfer energy to Chl *a* and participate as an antenna pigment for photosynthesis. One may speculate upon the evolutionary pathway which led these primitive eucaryotic algae to form Chl *b* rather than Chl *c* like many other deep-growing marine plants. Chl c_2 is identical to magnesium 2,4-divinylpheoporphyrin a_5 except for a lack of two hydrogen atoms. Thus the diversion of a part of the magnesium 2,4-divinylpheoporphyrin a_5 on the Chl *a* biosynthetic pathway to Chl c_2 is easy to imagine. Both Chl c_2 and magnesium 2,4-divinylpheoporphyrin a_5 have very high ratios (7–10) of their Soret to red band absorption coefficients, making them very efficient antenna in blue light. Chl *b*, on the other hand, is thought to have arisen by mutation independently in several different algal groups [13], and its absorption greatly adds to light-harvesting capacity in the orange-red spectral region. The early Prasinophytes may have started on a Chl *c*-forming pathway, but abandoned it in favor of Chl *b* and an environment more enriched in red light. Further experiments may show a difference in pigment content depending on light quality during growth of *Mantoniella*.

Both *Micromonas* and *Mantoniella* have a relatively large amount of Chl *b* (Chl *a*/*b* smaller than 2), and observing its absorption maximum in vivo at 643 nm rather than at 648 nm like in other green algae requires some explanation. We have extracted the Chl *b* from *Mantoniella* into 90% acetone and purified it by HPLC. It ran at the same rate and had the same absorption spectrum as standard Chl *b* from spinach. Therefore, to observe the fluorescence excitation maximum of Chl *b* in *Mantoniella* at 648 nm is not surprising. A possible explanation for the difference between the absorption and fluorescence excitation of Chl *b* may be that a protein-bound form of Chl *b* is present but inactive in energy transfer.

The separate bands of Chl *a*, Chl *b* and magnesium 2,4-divinylpheoporphyrin a_5 could be distinguished from each other and the carotenoids by fluorescence excitation spectroscopy of detergent-treated membranes. The detergent treatment was

sufficient to inhibit energy transfer between the pigments, but not to solubilize them completely. The absorption maximum of magnesium 2,4-divinylpheoporphyrin a_5 in 90% acetone is at 441 nm [2] whereas the excitation peak found here is at 448 nm (Fig. 2Ac). A red shift is usual when pigments form a complex with proteins. Mann and Myers [14] estimated that the blue absorption maximum of Chl *c* shifted from 443 nm in 90% acetone to 460 nm in vivo. Although the detergent treatment caused a small blue shift of all the pigments in vivo, the presence of magnesium 2,4-divinylpheoporphyrin a_5 most certainly enhances the light-absorbing capacity of these algae and may help them to survive at considerable depths in the oceans where only blue light can penetrate [15].

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