BBA 43197

Chlorophyll absorption and fluorescence in *Ochromonas danica**

The flagellate alga, *Ochromonas danica*, has an unusual three-peaked chlorophyll *a* absorption band in the red region of the spectrum[1]. The only other known alga with a similar absorption spectrum is Euglena grown under light-limiting conditions[2].

The main chlorophyll fluorescence emission maximum in Ochromonas measured at either 20° or —196° is at 691 nm (refs. 3, 4). This peak wavelength position also is unusual except for Euglena[5].

In Euglena, the maxima of absorption and fluorescence emission vary according to different growth conditions, especially light intensity. However, the spectra of Ochromonas were independent of any culture conditions that produced chlorophyll.

O. danica (Cambridge Culture Col., 933/2 Pringsheim) was cultured in the medium of ALLEN, GOODWIN AND PHAGPOLNGARM[6]. Cultures were grown in air with or without 3 % CO_2 and illuminated from incandescent or fluorescent lamps giving light intensities from about 4000 to 7500 lux. Ochromonas grew well on 0.5 % glucose in light or darkness, but dark-grown cells did not form chlorophyll.

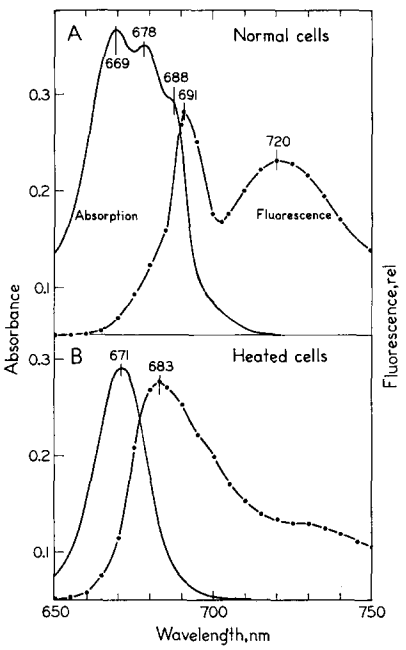

Fig. 1. A. Absorption (———) and fluorescence emission (–·—·–) spectra of *O. danica* measured at —190°. Excitation at 436 nm. B. Same as A after heating the cells at 40° for 20 min.

The equipment for measuring absorption and fluorescence has been described[7,8]. The absorption spectrophotometer was designed to minimize errors due to light scattering. The fluorescence emission spectra were corrected automatically to plot

* Department of Plant Biology, Carnegie Institution of Washington, Publication No. 439.

relative quanta per unit wavelength interval. For emission spectra samples were diluted or used in thinner layers until further dilution caused no change in the shape of the spectrum.

Absorption and fluorescence emission spectra of Ochromonas at —190° are shown in Fig. 1A. The three absorption maxima at 669, 678, and 688 nm cannot be resolved at room temperature with the usual spectrophotometer, but they have been detected by derivative spectrophotometry[1]. The 691-nm emission peak is in the same position when measured at either 20° or —190°, but the 720-nm emission band appears only at low temperature.

After heating the cells to 40° for 20 min, the spectra measured at —190° (Fig. 1B) changed to a single absorption maximum at 671 nm and a main fluorescence band at 683 nm with 700- and 730-nm shoulders.

Absorption spectra of acetone and ether extracts made from cells before and after heating showed that this treatment changed chlorophyll to phaeophytin within the cell[9]. Thus the spectra in Fig. 1B are due to phaeophytin rather than chlorophyll *a*.

Although none of the various conditions for growing Ochromonas affected the chlorophyll spectrum, this organism is unusually fragile. Any mild deleterious treatment such as freezing and thawing, allowing the culture to age for 2 or 3 days beyond its logarithmic growth phase, or heating to 40° for 20 min damages the cell sufficiently to change the chlorophyll to phaeophytin.

In Chlorella and most other algae the chlorophyll *a* absorption consists of two bands having maxima at approx. 670 and 680 nm. These algae show a fluorescence emission maximum at about 685 nm that is thought to come from the 670-nm absorbing form of chlorophyll[10]. A comparison of the absorption and emission spectra in Fig. 1A suggests that although the short-wavelength-absorbing form of chlorophyll, at 669 nm in Ochromonas, may be fluorescing near 685 nm, the observed emission peak at 691 nm results from reabsorption of fluorescence by the unusually high long-wavelength absorption. Actually in many algae the fluorescence emission maximum observed may be at a longer wavelength than the true maximum due to internal reabsorption[11].

Department of Plant Biology,
Carnegie Institution of Washington,
Stanford, Calif. 94305 (U.S.A.)

JEANETTE S. BROWN

1 M. B. ALLEN, C. S. FRENCH AND J. S. BROWN, in M. B. ALLEN, *Comparative Biochemistry of Photoreactive Systems*, Academic Press, New York, 1960, p. 33.
2 J. S. BROWN AND C. S. FRENCH, *Biophys. J.*, 1 (1961) 539.
3 C. S. FRENCH, J. S. BROWN, M. B. ALLEN AND R. F. ELLIOTT, *Carnegie Inst. Washington Yearbook*, 58 (1959) 327.
4 M. BRODY, S. S. BRODY AND J. H. LEVINE, *J. Protozool.*, 12 (1965) 465.
5 C. S. FRENCH AND R. F. ELLIOTT, *Carnegie Inst. Washington Yearbook*, 57 (1958) 278.
6 M. B. ALLEN, T. W. GOODWIN AND S. PHAGPOLNGARM, *J. Gen. Microbiol.*, 23 (1960) 93.
7 C. S. FRENCH, *Carnegie Inst. Washington Yearbook*, 66 (1967) 175.
8 C. S. FRENCH AND M. A. KOERPER, *Carnegie Inst. Washington Yearbook*, 65 (1966) 492.
9 J. H. C. SMITH AND A. BENITEZ, in K. PAECH AND M. V. TRACEY, *Modern Methods of Plant Analysis*, Springer-Verlag, Berlin, 1955, p. 142.
10 J. C. GOEDHEER, *Biochim. Biophys. Acta*, 88 (1964) 304.
11 J. S. BROWN AND M. R. MICHEL-WOLWERTZ, *Biochim. Biophys. Acta*, 153 (1968) 288.

Received February 5th, 1968