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Comparison of chlorophyll far-red and red fluorescence excitation spectra with photosynthetic oxygen action spectra for photosystem II in algae

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ABSTRACT: The shapes of excitation spectra for chlorophyll *a* fluorescence in the far-red (730 nm) were compared under physiological conditions to those for chlorophyll *a* fluorescence in the red (685 nm) and to action spectra for photosynthetic oxygen production in diversely pigmented algae. Species examined as representatives of the prominent oceanic light harvesting systems were *Chaetoceros gracilis, Glenodinium* sp., *Ulva* sp., *Porphyridium cruentum* and *Chroomonas* sp. Qualitatively, for any one alga, all 3 action spectra exhibited broadly similar spectral features, suggesting initial light harvesting for photosynthesis by the same major pigments, i.e. those commonly associated with photosystem II. As such, measurement of F_{730} fluorescence (in preference to F_{685}) may provide a useful and facile alternative to oxygen action spectra in assessing the full photosynthetic spectral performance (320 to 700 nm) of individual phytoplankton species or assemblages.

INTRODUCTION

The spectral characteristics of marine algae are important determinants of light attenuation and photosynthesis in the aquatic environment. Spectral absorption properties are largely functions of the kinds, amounts and packaging of pigments present in the algal cell, whereas action spectra reflect fractional absorption and conversion efficiency by photoreactive pigments of the photosynthetic apparatus. Laboratory comparisons of spectral absorption and action spectra for O_2 production in different colored algae (for review, see Larkum & Barrett 1983, Haxo 1985, Lüning & Dring 1985) have been particularly useful in identifying and evaluating the effectiveness of pigments associated with the O_2-evolving system of photosynthesis. Such data are essential for predicting photosynthetic spectral performance in submarine light fields.

Action spectra for chlorophyll *a* (chl *a*) fluorescence have been widely used to elucidate the role of accessory pigments in the photosynthetic energy transfer in cultured algae (Dutton et al. 1943, Duysens 1952, Goedheer 1972). Such studies suggested that fluorescence measurements might provide much of the same information as O_2 action spectra. In order to document this relationship more closely under physiological conditions, Neori et al. (1986) recently made a detailed wavelength comparison of action spectra for excitation of chl *a* fluorescence and action spectra for modulated O_2 production in algae representing the major oceanic pigment systems. Over the common wavelength interval examined, 390 to 610 nm, the shapes of the excitation spectra for chl *a* red fluorescence at 685 nm (F_{685}) and the action spectra for photosystem II (PS II) were in good agreement. The F_{685} measurements could not include the spectral interval 610 to 700 nm because the fluorescence correction standard commonly employed

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(rhodamine B) cannot be used above 610 nm (Yguerabide 1968), and also because of the difficulty in blocking the excitation beam when its wavelength approached that of the measured emission at 685 nm. Such considerations have also limited the spectral range in field studies (Yentsch & Yentsch 1979, Mitchell & Kiefer 1984). While these difficulties can be minimized with specialized laboratory instrumentation (e.g. Govindjee & Yang 1966, Owens et al. 1987), such instrumentation is not generally available to the marine biologist, particularly under field conditions.

In order to extend the range of the standard in vivo chl fluorescence technique (F_{685}) to include the orange and red regions, it is necessary to compare the far-red band of chl *a* emission ($F > 710$) with F_{685} emission characteristics as well as with action spectra for modulated oxygen production. In this paper we provide a direct comparison of these methods in a variety of cultured algae having different antenna pigment systems. The comparison also provides a broader base for evaluating the applicability and limitations of fluorescence measurements of algal populations under field conditions, some aspects of which have recently been considered (Neori et al. 1986).

MATERIALS AND METHODS

Algae. *Chaetoceros gracilis* (S.I.O. code # BA 13; Bacillariophyta) was grown as described previously (Neori et al. 1986) at 60 $\mu E\ m^{-2}\ s^{-1}$ to a cell density of 2×10^5 cells ml^{-1}.

Glenodinium sp. (S.I.O. code # PY 33; Pyrrhophyta) was grown in a 250 ml flask, on a shaker, in 50 ml of GPM medium (Loeblich 1975) at 30 $\mu E\ m^{-2}\ s^{-1}$ to a cell density of 3.6×10^5 cells ml^{-1}.

Porphyridium cruentum (S.I.O. code # RH 11; Rhodophyta) was grown in the same medium and under the same conditions as for *Glenodinium,* to a cell density of 7.2×10^5 cells ml^{-1}.

Chroomonas sp. (Provasoli strain; Cryptophyta) was grown as described previously (Neori et al. 1986), at 270 $\mu E\ m^{-2}\ s^{-1}$, to a cell density of 7.45×10^5 cells ml^{-1}.

Ulva sp. (probably *californica*; Chlorophyta) was collected from sides of submerged rocks in a mid-tidal area near La Mission, Baja California. Blades were held for 2 d at low light in unenriched, flowing filtered seawater prior to the fluorescence and O_2 action spectra determinations, which were carried out on the same blade piece.

Photosynthetic action spectra. Action spectra of PS II were measured with a modulated-beam, bare platinum O_2 electrode system (Joliot & Joliot 1968), as modified by Prézelin et al. (1976) and by Neori et al. (1986). The microalgal coverage on the electrode did not exceed 1 cell layer (checked microscopically), thus avoiding mutual shading. Our general experience with this system has been that major features of action spectra for individual species are quite reproducible between repeated scans of the same mount, between aliquots of the same culture (as employed here for *Porphyridium cruentum* and *Chroomonas* sp.) and between different batch cultures grown in like manner. In the case of *Glenodinium* sp. and *Chaetoceros gracilis,* O_2 production and fluorescence action spectra were measured on different batches. Because *C. gracilis* gave inexplicably noisy O_2 signals, an average of 2 rather than a single spectrum was used (Fig. 3).

Fluorescence. All algal samples were pretreated with DCMU (3-[3,4-dichlorophenyl]-1,1-dimethylurea) and fluorescence measured in a Perkin Elmer MPF 44 A spectrofluorometer as detailed elsewhere (Neori et al. 1984). Except for *Chaetoceros gracilis* which was thinly dispersed on a glass-fiber filter and the thin-bladed macrophyte *Ulva* sp. which was moist-mounted directly on a holding plate, measurements were made in a 1 cm cuvette on dilute cell suspensions whose color was barely discernible by eye.

For measurements at 685 nm (F_{685}) the spectrofluorometer was set in the ratio mode with rhodamine B in the reference compartment. Emission and excitation half band widths of 10 and 3 nm, respectively, were employed and a Corning 2–61 cutoff filter (5, 33, and 72 % transmission at 605, 615, and 625 nm, respectively) blocked the light beam entering the emission monochromator. The F_{685} chl *a* fluorescence spectra were corrected by dividing the measured spectra by the rhodamine B spectrum of the instrument (Yguerabide 1968).

For measurements at 730 nm (F_{730}), emission and excitation half band widths were 10 and 5 nm respectively. A blocking Hoya R-72 cutoff filter (5, 33, and 72 % transmission at 705, 718, and 740 nm, respectively) was placed in front of the emission monochromator. The spectrofluorometer was set in the energy mode. System spectral correction was provided by the thermopile method (Argauer & White 1964). The configuration of the thermopile (Kettering Scientific Res. model 68) was such that the light probe fit snugly within the spectrofluorometer's cuvette holder with its detector positioned at the focus of the exciting beam. Light-energy values were recorded at 5 nm intervals. These values, after conversion to relative quantum flux units (energy times wavelength), were interpolated linearly at 1 nm intervals. The resulting correction spectrum resembled closely that reported earlier for a similar 150 W xenon-arc light source (Yguerabide 1968). The raw F_{730} spectra were digitized at 1 nm intervals into a microcomputer and were divided by the thermophile correction spectrum to obtain the corrected F_{730} chl *a* fluorescence excitation spectra.

RESULTS AND DISCUSSION

Action spectra for excitation of F_{685} and F_{730} nm chl *a* fluorescence and for modulated O_2 production in each of the algal pigment types are given in Figs. 1 to 5, the curves being arbitrarily scaled to each other and, in part, overlapping. Plotted in this manner, it is readily apparent that for a given alga all action spectra are qualitatively similar in general shape. Major variations between species are relatable to the differing spectral prominences of their dominant accessory pigments (except for *Porphyridium cruentum*; see Table 1 in Neori et al. 1986).

In the case of *Chaetoceros gracilis* (Fig. 3) and *Chroomonas* sp. (Fig. 5) agreement in positions of the red peaks is less satisfactory and for most algae (Figs. 2 to 5) spurious peaks near 468 and 490 nm are evident in the F_{730} spectra. Some of these curve deviations could

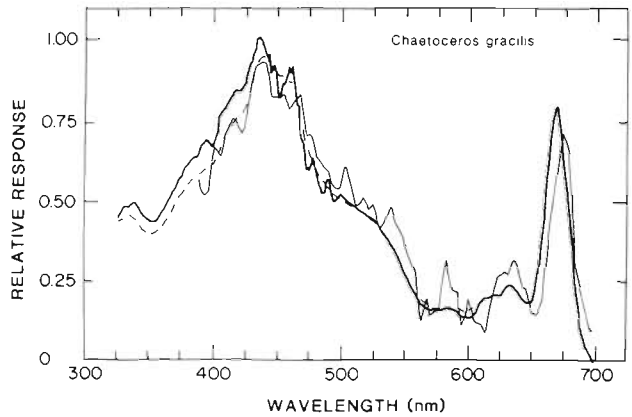

Fig. 3. *Chaetoceros gracilis*. Action spectra for chlorophyll *a* fluorescence at 685 nm (F_{685}) (dashed line); chlorophyll *a* fluorescence at 730 nm (F_{730}) (heavy line); and photosystem II photosynthetic O_2 evolution (light line). The photosynthetic curve is the average of 2 spectra. Note that the 685 nm fluorescence spectrum does not extend beyond 610 nm

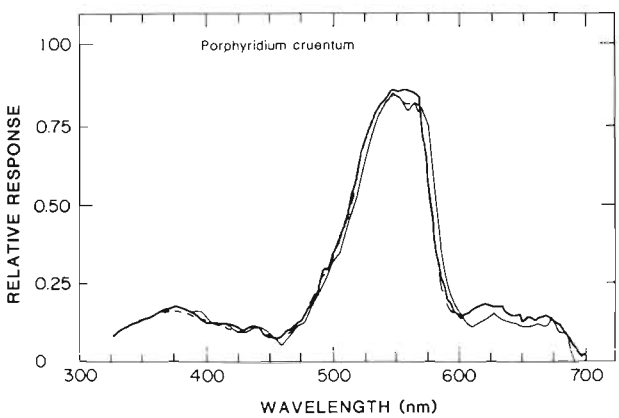

Fig. 1. *Porphyridium cruentum*. Action spectra for chlorophyll *a* fluorescence at 685 nm (F_{685}) (dashed line); chlorophyll *a* fluorescence at 730 nm (F_{730}) (heavy line); and photosystem II photosynthetic O_2 evolution (light line). Note that the 685 nm fluorescence spectrum does not extend beyond 610 nm

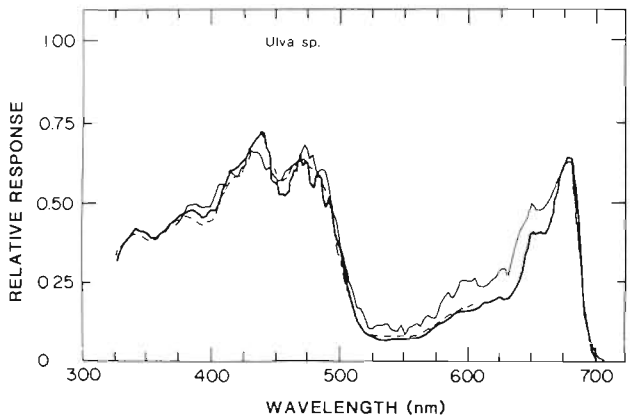

Fig. 4. *Ulva* sp. Action spectra for chlorophyll *a* fluorescence at 685 nm (F_{685}) (dashed line); chlorophyll *a* fluorescence at 730 nm (F_{730}) (heavy line); and photosystem II photosynthetic O_2 evolution (light line). Note that the 685 nm fluorescence spectrum does not extend beyond 610 nm

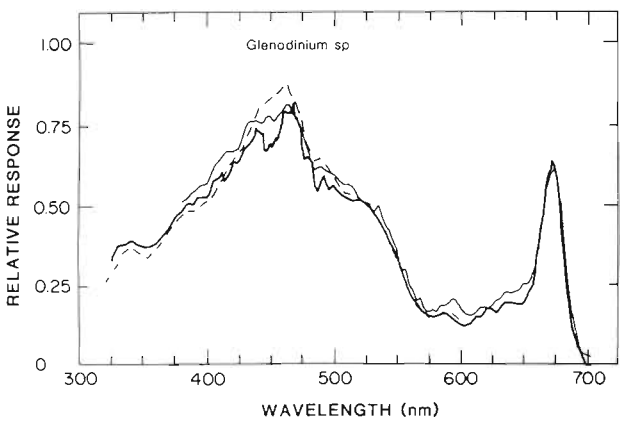

Fig. 2. *Glenodinium* sp. Action spectra for chlorophyll *a* fluorescence at 685 nm (F_{685}) (dashed line); chlorophyll *a* fluorescence at 730 nm (F_{730}) (heavy line); and photosystem II photosynthetic O_2 evolution (light line). Note that the 685 nm fluorescence spectrum does not extend beyond 610 nm

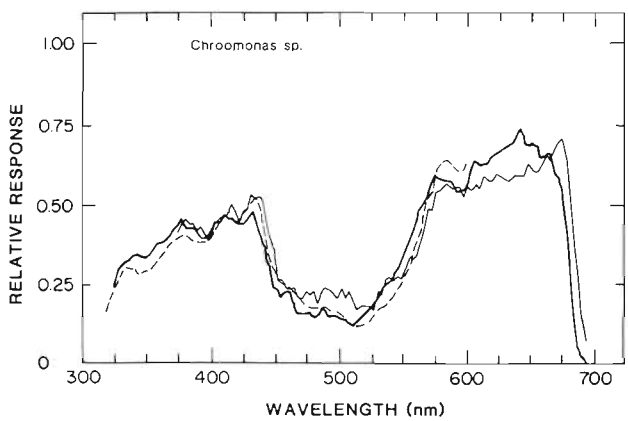

Fig. 5. *Chroomonas* sp. Action spectra for chlorophyll *a* fluorescence at 685 nm (F_{685}) (dashed line); chlorophyll *a* fluorescence at 730 nm (F_{730}) (heavy line); and photosystem II photosynthetic O_2 evolution (light line). Note that the 685 nm fluorescence spectrum does not extend beyond 610 nm

arise from the use of very thin suspensions for the O_2 measurements (low signal to noise ratio) and inadequacies in resolving corrections for actinic light at wavelengths of very sharp emission bands of the xenon arc lamp. However the possibility cannot be disregarded that real, albeit small, differences exist between the O_2 and the fluorescence spectra in some species.

The spectra for *Porphyridium cruentum* (Fig. 1) show excellent agreement with each other at all wavelengths and are in good agreement with the action spectrum for modulated O_2 evolution reported by Ley & Butler (1977) employing comparable instrumentation. All 3 action spectra closely resemble the absorption spectrum of the biliprotein mixture present, which is dominated by B-phycoerythrin (Haxo et al. 1955). The close similarity between the F_{730} spectrum and the F_{685} spectrum further suggests there is very little, if any, spectral contribution to F_{730} by PSI-absorbed light. This appears to be the general situation in rhodophytes (Larkum & Barrett 1983). An exception was reported by Fork et al. (1982) for *Porphyra perforata* (an observation we have confirmed) in that the F_{730} excitation spectrum includes a significant spectral contribution from PSI absorption.

In the dinoflagellate *Glenodinium* sp. (Fig. 2) and the diatom *Chaetoceros gracilis* (Fig. 3), broadened activity across the spectral interval 490 to 560 nm is seen in all action spectra, reflecting major absorption by the characteristic accessory xanthophylls present, peridinin and fucoxanthin, respectively. In *Glenodinium* sp. the correspondence in peak positions and curve shapes in regions of chl *a* and chl *c* absorption are particularly evident in both O_2 and F_{730} spectra. In *Chaetoceros gracilis* the O_2 action spectrum was noisy and could not be improved without using undesirably dense cell mounts on the electrode. The overall shape of this curve, however, resembles closely a smoothed curve for O_2 production we reported earlier for this species (Fig. 3b in Neori et al. 1986). Both O_2 action spectra correspond fairly well with the F_{730} and F_{685} curves, except that the chl *a* peak at 673 nm in the F_{730} spectrum is blue-shifted to 665 nm.

In the chlorophyte *Ulva* sp. (Fig. 4), the agreement between the fluorescence and the O_2 spectra is not as good as in *Glenodinium* sp. Although peak and shoulder features of these spectra correspond to major absorptions by chl *a*, chl *b* and carotenoids, the relative magnitudes of the F_{730} and the O_2 spectra differ by as much as 30 %. In the phycocyanin-rich cryptomonad *Chroomonas* sp. (Fig. 5), excellent agreement was observed in curve shapes and location of activity peaks through the spectral interval 320 to 580 nm but divergences are evident at longer wavelengths. As plotted, light absorbed by phycocyanin-645 (550 to 670 nm) appears to be more effective in generating F_{730} fluorescence than oxygen evolution; further, the O_2 action spectrum showed a distinct peak at 678 nm which was not evident in the fluorescence excitation spectrum. The basis for these effects has not been resolved.

We find it significant that in all 5 species the F_{730} spectra reveal the important features of pigment activity above 610 nm which also appear in the O_2 spectra. This includes intervals of prominent contributions to absorption by phycocyanin in *Porphyridium cruentum* (610 to 660 nm) and *Chroomonas* sp. (610 to 670 nm); chl *c* in *Glenodinium* sp. and *Chaetoceros gracilis* (ca 610 to 640 nm); chl *b* in *Ulva* (ca 610 to 660 nm), and chl *a* in all algae (650 to 700 nm).

The good correlation between the shapes of the O_2 and the F_{730} spectra suggests that the pigments which spectrally dominate F_{730} chl *a* fluorescence at room temperatures in these algae are similar to those associated with PS II. In this connection it is noteworthy that the earlier studies by Goedheer (1965, 1981) on chlorophyll fluorescence at room temperature in e.g. *Anaebaena* and *Chlorella* were interpreted as showing participation of PS II pigments in F_{715} emission. The extent to which this relationship is dependent upon growth conditions is an important consideration, particularly in field applications. We are encouraged, however, that in the several species we have examined, the relationship appears to persist in cells grown under varying irradiance and nutrient regimes (Neori 1986).

Although the molecular origins of $F > 710$ emission remain uncertain (Wong & Govindjee 1981, Peyriere et al. 1984, Wendler et al. 1984), our results indicate that measurement of F_{730} can be used to gain important and otherwise unobtainable information on the extended spectral characteristics of photosynthetic potential in natural and laboratory populations of algae. For instance, it would not be feasible to document the photosynthetic action spectra of natural populations as a function of depth in ocean profiles by using the modulated O_2 electrode system. As presently employed, this method involves specialized and sensitive equipment, highly trained personnel, a requirement for gentle concentration of phytoplankton from large water volumes and problems with intolerance of some algal species to the bare platinum electrode. However, F_{730} spectra can be measured with commercially available instrumentation, on station, at a rate of several samples per hour and with relatively little training.

The results of such a profile can give information on the spectral shapes of photosynthetic activity associated with absorption by major pigments and their change with depth or with time. When compared to the in vivo absorption spectra of the same samples (which can be measured on the same filter as in Neori et al. 1984), one can quantitatively assess the amount of light

that is absorbed by photosynthetically active pigments compared to total light absorption. It should be emphasized, however, that the 'overlap' of the 3 spectra (Figs. 1 to 5) reflects only the *relative* spectral response, and not the magnitude of each signal. Thus, integration of the area under the F_{730} curve cannot be arbitrarily set to equal the rate of oxygen production. Quantification of the signals has not been attempted and might prove difficult in the present instrumental arrangement. However, with the methods developed by Mitchell & Kiefer (1984) and Mitchell (1987), it should be possible to estimate relative photosynthetic quantum yields of the samples.

It is evident from our data that measurements of chlorophyll *a* fluorescence may offer a useful alternative to spectral measurements of PS II activity by modulated O_2 production. The F_{685} excitation spectra offer superior sensitivity, methodological simplicity, and a rather established correlation with PS II photosynthetic activity, but do not assess the orange to red region of the action spectrum. The F_{730} chl fluorescence action spectra, on the other hand, combine the methodological advantages of fluorescence with the broader spectral range of the modulated O_2 electrode technique. In future applications the resolution, accuracy and sensitivity of the F_{730} fluorescence method could be improved by the use of a more sensitive thermopile, a photomultiplier having an enhanced far-red response and a high-resolution data acquisition system.

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