Journal of Plankton Research Vol.8 no 3 pp.537–548, 1986

Relationship between action spectra for chlorophyll *a* fluorescence and photosynthetic O_2 evolution in algae

A.Neori, M.Vernet[1], O.Holm-Hansen and F.T.Haxo[1]

Food Chain Research Group, Scripps Institution of Oceanography, A-018, and [1]Marine Biology Research Division, Scripps Institution of Oceanography, A-002, University of California, La Jolla, CA 92093, USA

Abstract. Relative excitation spectra of chlorophyll *a* fluorescence are shown to be very close to relative action spectra of photosystem II O_2 evolution in seven micro- and macro-algae of five phyla. The conditions under which this correspondence should hold, based on theoretical considerations, and the applications of this correspondence to interpretation of fluorescence excitation spectra of chlorophyll *a* from natural populations are discussed. The spectra presented are interpreted in relation to the light-harvesting pigments and their association with the two photosystems.

Introduction

Knowledge of the spectral response of algal photosynthesis to light is essential to an understanding of the interactions between light, algae and primary production in the oceans. Photosynthetic O_2 evolution action spectra have commonly been used in laboratory studies to estimate the wavelength dependence of photosynthesis (Haxo, 1960; Fork and Amesz, 1969; Larkum and Barrett, 1983, pp. 69–71). This methodological approach is, however, inconvenient for oceanographic field work due to its complexity, its insensitivity and the susceptibility of some algal species to the manipulations involved. This led to the choice of *in vivo* action (excitation) spectra of chlorophyll *a* (chl *a*) fluorescence as an increasingly useful alternative in biological oceanography (Yentsch and Yentsch, 1979; Mitchell and Kiefer, 1984; Neori *et al.*, 1984). Such applications emphasize the need for a precise definition of the relationship between the two kinds of spectra, and of the conditions which ensure this relationship in a variety of algal pigment types.

Since the pioneer investigations by Dutton *et al.* (1943) and in particular those by Duysens (1952) on the role of accessory pigments and energy transfer in photosynthesis, action spectra for chl *a* fluorescence have become an essential laboratory tool in the study of photosynthesis (e.g. Goedheer, 1972; Butler, 1978; Mathis and Paillotin, 1981). A close relationship between *in vivo* chl *a* fluorescence from photosystem II (PSII) and photochemical activity of PSII was suggested (reviewed by Butler, 1978; Mathis and Paillotin, 1981). Fluorescence from photosystem I (PSI) at room temperature is considered to be negligible. In spite of the large volume of data published on either chl *a* fluorescence action spectra or on photosynthesis action spectra, there are no systematic *in vivo* comparisons between the two, as far as we know.

The conditions under which these two kinds of spectra should be approximately similar can be derived from models of the photochemical apparatus. One, which is often cited, was suggested by Butler (1978, 1980, 1984). According to this model, an excited chl *a* molecule in PSII can lose its energy in one of three ways: (1) a non-radiative thermal

decay, i.e. heat (D); (2) photochemistry – the reduction of an acceptor in a PSII reaction center, leading to CO_2 fixation and O_2 evolution (PII), or (3) chl *a* fluorescence (FII), which includes variable (Fv) and constant (Fo) components.

A fourth pathway, that of energy transfer from the light-harvesting pigments of PSII directly to PSI, or spillover (e.g. Mohanty and Govindjee, 1973), could be added. For our purposes, however, spillover can be considered with pathway 1 (D) above, as such energy dissipation does not result directly in either fluorescence or O_2 evolution (Butler, 1984).

Butler (1980) argued that the yields of both PSII photochemistry (ϕPII) and fluorescence (ϕFII) depend on only one variable – the fraction (A) of open to total PSII reaction centers. ϕPII is highest when A = 1 (that is all reaction centers are open), while ϕFII is highest when A = 0 (when all reaction centers are closed). Two consequences of Butler's theory are: (i) under conditions which keep A at a constant value appropriate for either fluorescence or O_2 evolution, as defined above, the yields ϕPII and ϕFII should stay constant at all wavelengths [see Prézelin and Ley (1980) and Krause and Weis (1984) for possible complications which may be applicable to natural populations]; and (ii) as long as A is constant, photochemistry and chl *a* fluorescence should both be linear functions of the number of light photons absorbed by PSII. As a result, considering that in healthy cells Fo is much smaller than Fv when A = 0 (Mathis and Paillotin, 1981), and of quite similar spectral response (Kitajima and Butler, 1975), the shapes of the action spectra of ϕPII and ϕFII should be rather similar. The value of A can be kept at the desired level in a variety of ways. For action spectra of PSII O_2 evolution, Joliot and Joliot (1968) designed a modulated light beam system in which the value of A could be kept constant by a strong background light beam. For measurements of chl *a* fluorescence action spectra, the value of A can be kept at the desired level (close to zero) by addition of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea). After a short exposure to light, DCMU-treated algae have all PSII reaction centers closed (i.e. A = 0), and ϕFII is at a higher value, which is nearly light independent within the time-scale (minutes) and with the weak excitation light intensities used for fluorescence measurements (Joliot, 1965; Slovacek and Hannan, 1977; Butler, 1978; Mathis and Paillotin, 1981).

In this paper, we compare action spectra for *in vivo* chl *a* fluorescence of DCMU-treated cells, and for PSII photochemistry of normal cells (as measured by O_2 evolution). We test experimentally the predicted relationship between these spectra, under defined conditions, for seven different algae representing the major algal pigment types.

Materials and methods

Microalgae

Unialgal cultures were grown in standard media as indicated below, and were continuously illuminated at low light levels by 'Cool White' fluorescent light, which resulted in light-limited growth rates of 0.1–0.2 doublings day^{-1}. All cultures were actively growing when sampled, as determined by cell counts.

Chroomonas sp. (Provasoli isolate; Cryptophyta), was grown in 'D' medium (Provasoli and MacLaughlin, 1963), in batch culture, shaken daily, illuminated as 10 μE m^{-2} s^{-1} and at a temperature of 18–20°C.

Rhodomonas D3 (Provasoli isolate, SIO code no. CR13; Cryptophyta), was grown under the same conditions as *Chroomonas*.

Glenodinium sp. (Bernard isolate, SIO code no. PY33; Pyrrophyta), was grown in 'GPM' medium (Loeblich, 1975), in a semi-continuous turbidostat, illuminated at 30 μE m^{-2} s^{-1}, at a temperature of 18°C. The culture was stirred by a magnetic stirrer, and bubbled with air.

Chaetoceros gracilis (SIO code no. BA13; Bacillariophyta), a small cell size species, was grown under the same conditions as *Glenodinium* above.

Macroalgae

Healthy, endophyte-free specimens of *Porphyra perforata* (Rhodophyta), and of *Ulva* sp. (probably *californica*; Chlorophyta), were collected from rocky intertidal beaches in La Jolla. Blades were kept at low light levels (20 μE m^{-2} s^{-1}) in unenriched, flowing or frequently changed filtered sea water, and remained in healthy condition for weeks. Spectra of these vegetative blades were measured one or two days after collection.

Phycodrys sp. (strain JS-SC No. 119; Rhodophyta), a thin-bladed species, was grown in an aquarium with flowing filtered sea water, at an irradiance of 3 μE m^{-2} s^{-1}.

In-vivo fluorescence action spectra of chl a

Spectra were measured with a Perkin Elmer MPF 44A spectrofluorometer, and were corrected using the rhodamine B method (Yguerabide, 1968). The excitation band width was 3 nm and emission was set at 680 nm with a 10 nm band width. A Corning CS 2-61 blocking cutoff filter was placed in front of the emission monochromator. Microalgae, suspended in growth medium, were placed in a standard 1-cm fluorescence glass cuvette. At the concentrations used (specified in figure legends), cell densities did not significantly alter spectral shape, as tested on dilution series. For macroalgae, small, visibly homogeneous pieces, with a size of approximately 2 × 5 mm (slightly larger than the focused excitation beam) were cut from very thin vegetative blades. They were placed vertically in a drop of sea water, and held by water adhesion to a black brass block at a 45° angle to the exciting beam and to the emission monochromator line of view. All measurements were made at room temperature (20−22°C), within several minutes after the samples had been withdrawn. Prior to measurement, the sampled algae were incubated for 1 min in white light (~20 μE m^{-2} s^{-1}) in the presence of 50−100 μM DCMU (Joliot, 1965; Papageorgiou and Govindjee, 1968), added as either a methanolic or an ethanolic solution. No deleterious effects were observed for either of the solvents, which were added at 1% v/v or less (see also data of Holt and French in Macdowall, 1949, Table V). The fluorescence yields of the DCMU-treated cells were stable for the duration of the measurements.

Photosynthetic action spectra

Spectra were measured polarographically using a modulated beam, bare platinum oxygen electrode system (Joliot and Joliot, 1968; Joliot, 1972), essentially as described previously (Prézelin *et al.*, 1976). The action spectrum apparatus was modified by the incorporation of a microcomputer which continuously monitored wavelength, light in-

tensity and the modulated oxygen electrode signal. The computer controlled the light level at each wavelength, rotating a calibrated circular gradient UV-VIS-NIR neutral density disk (Oriel Corp., model 28650) in order to keep the monochromatic modulated beam quantum flux incident to the electrode mount at a constant, operator-determined level during a scan. The light detector was a calibrated silicon photodiode (EG and G model UV-444BQ). Final quantum corrections were made with a thermopile (Kettering Scientific Research, Model 68). The monochromatic light was isolated by a Bausch and Lomb 500 mm grating (1200 grooves mm^{-1}) monochromator from a 150 W xenon arc lamp. Entrance and exit slits were 3 and 2 mm respectively, producing a spectral band width of 5 nm. The measurements were taken point by point at 3- to 4-nm intervals, starting at the short wavelength. The monochromator was automatically driven, and stopped for about 30 s at each point. After a few seconds for light level adjustment and signal stabilization, seven readings were taken by the computer at 2-s intervals. The median value of these was selected and stored by the computer as the datum for modulated oxygen evolution at that wavelength. In this operational mode, a full spectrum (375−740 nm) was completed in about 50 min. Slower sweeps, and the collection of more points at each wavelength, did not affect the spectra. Due to the 8-bit resolution of our data acquisition system, wavelength readings were accurate to ± 1.5 nm. A continous bright background beam was provided by a 50 W tungsten lamp, focused through a Calflex-C (IR reflecting) filter and a red or far-red cutoff filter, as defined in the figure legends.

Preparations of microalgae on a recessed electrode were handled as described by Prézelin *et al.* (1976). With macroalgae, a raised electrode was used, so that the retaining dialysis membrane held the algal blade in close contact with the platinum surface. A piece approximately 3 × 3 mm was cut from a vegetative and visibly homogeneous blade, for mounting on the electrode. For each preparation, the intensity of the background beam was adjusted to maximize the modulated signal. The modulated beam intensity was kept in the linear region of a P versus I curve which was run immediately prior to each wavelength sweep. Specific conditions can be found in the figure legends.

Fluorescence and O_2 evolution action spectra were determined on the same day. Aliquots of the same culture or like pieces from the same blade were used. The fluorescence spectra were digitized and stored by the computer. Values between experimental data points were interpolated at 1 nm intervals. To reduce random noise in the data, the action spectra for O_2 evolution had to be smoothed by a moving average of 9 nm, and in some cases (described in figure legends) the average of several replicate spectra had to be calculated. The spectra are given in relative units, and they are scaled to coincide at 480−500 nm.

Results

The action spectra for chl *a* fluorescence and for O_2 evolution are presented as plots of relative response versus wavelength. Our PSII O_2 evolution spectra look similar to previously published PSII spectra from related algae (Vidaver, 1966; Ley and Butler, 1976). In all algae there were only minor differences in the shapes of the fluorescence spectra between normal and DCMU-treated cells, although the absolute fluorescence

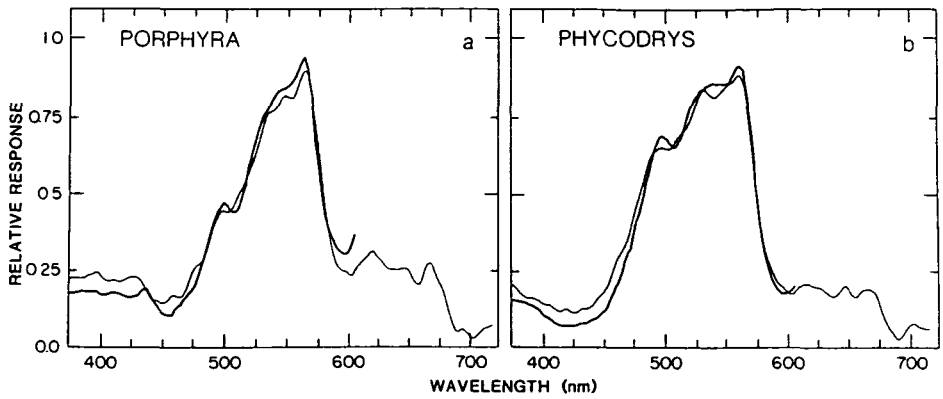

Fig. 1. Action spectra of PSII O_2 evolution (light line), and of *in vivo* chl *a* fluorescence after treatment with DCMU (heavy line): (a) *Porphyra perforata* Background beam intensity was 3 mW cm^{-2} (~ 177 μE m^{-2} s^{-1}), defined by a Corning CS 2-64 red cutoff filter and a Calflex-C filter. Monochromatic beam intensity was 10 μW cm^{-2} at 550 nm (~ 0.46 μE m^{-2} s^{-1}); (b) *Phycodrys* sp. Background beam was defined as in *Porphyra*, with intensity of 1 mW cm^{-2} (~ 57 μE m^{-2} s^{-1}). Monochromatic beam intensity was 2.5 μW cm^{-2} at 550 nm (~ 0.12 μE m^{-2} s^{-1})

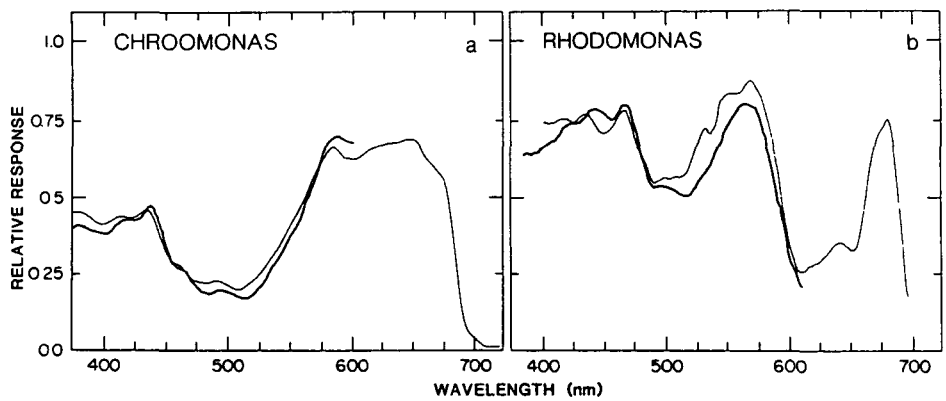

Fig. 2. Action spectra of PSII O_2 evolution (light line), and of *in vivo* chl *a* fluorescence after treatment with DCMU (heavy line): (a) *Chroomonas* sp. Cell density and chl *a* concentration during fluorescence measurement were 8.1 × 10^5 cells ml^{-1} and 0.516 μg ml^{-1}, respectively. Background beam intensity was 1 mW cm^{-2} (~ 60 μE m^{-2} s^{-1}), defined by a Calflex-C filter and a Hoya R-70 far-red cutoff filter Monochromatic beam intensity was 6 μW cm^{-2} at 550 nm (~ 0.28 μE m^{-2} s^{-1}); (b) *Rhodomonas D3*. PSII action spectrum is an average of two spectra. Cell density and chl *a* concentration during fluorescence measurement were 1.9 × 10^5 cells ml^{-1} and 0.48 μg ml^{-1}, respectively. Continuous background beam was defined as in *Chroomonas*, with the intensity of 5 mW cm^{-2} (~ 298 μE m^{-2} s^{-1}). Monochromatic beam intensity was 2.5 μW cm^{-2} at 550 nm (~ 0.17 μE m^{-2} s^{-1}).

values increased about 2.7-fold as a result of the DCMU treatment. In spectra of the former (normal) cells (not shown), the peaks were somewhat higher (<5%), relative to the less-prominent regions of the spectra, in comparison with the latter, DCMU-treated cells.

In the red algae *Porphyra* and *Phycodrys* (Figure 1), the green region (centered at 550 nm) shows high activity while the blue-violet and red regions (centered at 435 nm

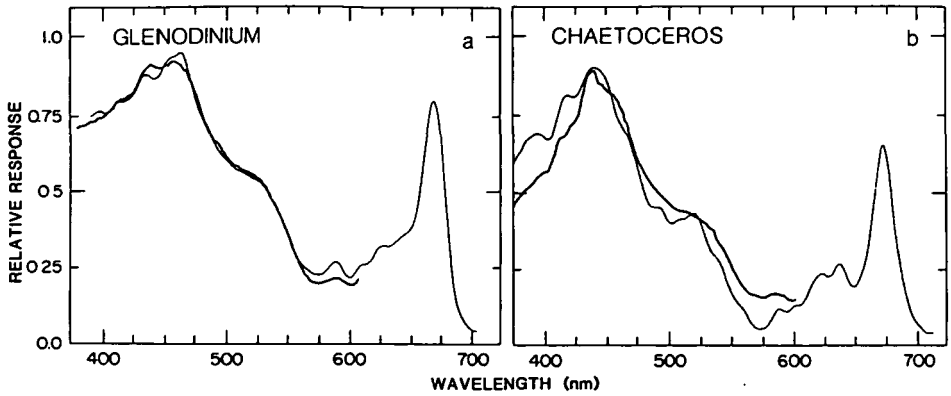

Fig. 3. Action spectra of PSII O_2 evolution (light line), and of *in vivo* chl *a* fluorescence after treatment with DCMU (heavy line): (a) *Glenodinium* sp. PSII spectrum is an average of three spectra. Cell density and chl *a* concentration during fluorescence measurements were 1.7×10^5 cells ml^{-1} and 0.214 $\mu g\ ml^{-1}$, respectively. Continuous background beam was defined as in *Chroomonas* with intensity of 1.5 mW cm^{-2} ($\sim 127\ \mu E\ m^{-2}\ s^{-1}$). Monochromatic beam intensity was 6 $\mu W\ cm^{-2}$ at 550 nm ($\sim 0.4\ \mu E\ m^{-2}\ s^{-1}$); (b) *Chaetoceros gracilis*. Cell density and chl *a* concentration during fluorescence measurement were 10^6 cells ml^{-1} and 0.291 $\mu g\ ml^{-1}$, respectively. Continuous background beam was defined as in *Chroomonas*, with intensity of 3 mW cm^{-2} ($\sim 255\ \mu E\ m^{-2}\ s^{-1}$). Monochromatic beam intensity was 7.5 $\mu W\ cm^{-2}$ at 550 nm ($\sim 0.5\ \mu E\ m^{-2}\ s^{-1}$).

and 675 nm, respectively) show relatively little response. The action spectra for chl *a* fluorescence closely match those for PSII O_2 evolution activity.

In the cryptomonads *Chroomonas* and *Rhodomonas* (Figure 2), the blue and red regions, as well as the green region in *Rhodomonas* (phycoerythrin-rich) and the orange region (centered at 620 nm) in *Chroomonas* (phycocyanin-rich) display prominent activity. The match between the spectra of chl *a* fluorescence and PSSI O_2 evolution is very close in *Chroomonas*. In *Rhodomonas*, the fluorescence spectrum deviates from the PSII O_2 evolution spectrum by up to 20%.

The dinoflagellate *Glenodinium* and the diatom *Chaetoceros* (Figure 3), belong to a collective group of algae containing chl *a* and *c* and a major polyoxy xanthophyll (peridinin and fucoxanthin, respectively). In both, the blue-violet, blue (centered at 470 nm) and red regions show higher activity than in the green region, with a distinct shoulder in the blue-green (centered at 520 nm). The match between the chl *a* fluorescence spectrum and the PSII O_2 evolution spectrum is very close in both algae. Interestingly, the action spectra of the two algae are also very similar to each other and to the spectra of several other diatoms and dinoflagellates we have examined (Neori and Haxo, unpublished). Such a similarity between these two algal orders was noted by Yentsch and Yentsch (1979).

In the chl *a*- and *b*-containing green alga *Ulva* (Figure 4), the most active regions are the red and the blue, with distinct shoulders in the blue-violet and in the orange regions. The match between spectra of chl *a* fluorescence and PSII O_2 evolution is good.

In some of the figures, at wavelengths shorter than 420 nm, the spectra of chl *a* fluorescence increasingly deviate from those of PSSI O_2 evolution. We attribute this discrepancy to selective light scattering by the algae. This scattering increases exponentially with decreasing wavelength at that region when using a standard spectrofluorometer

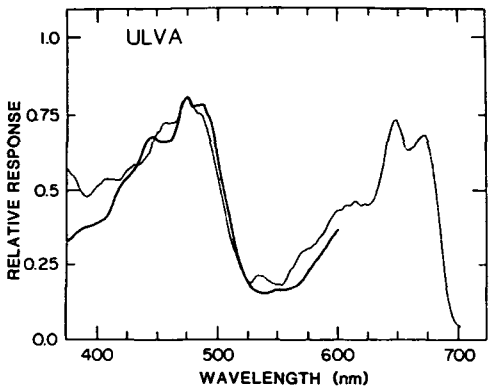

Fig. 4. Action spectra of PSII O_2 evolution (light line), and of *in vivo* chl *a* fluorescence after treatment with DCMU (heavy line) in *Ulva* sp. (probably *U. californica*). Continuous background beam was defined as in *Chroomonas*, with intensity of 2 mW cm^{-2} (~ 170 $\mu E\ m^{-2}\ s^{-1}$). Monochromatic beam intensity was 15 $\mu W\ cm^{-2}$ at 500 nm (~ 1.0 $\mu E\ m^{-2}\ s^{-1}$).

with perpendicular geometry as employed in our study (Latimer and Rabinowitch, 1956). We did not apply a correction as has been done elsewhere (e.g. Thielen and Van Gorkom, 1981), since our measurements showed no appreciable error in the region of primary interest, 435 nm and above.

Discussion

The seven algal species investigated represent some of the major photosynthetic pigment types in the oceans. While all photoautrophic organisms have chl *a* (the bulk of which has *in vivo* absorption peaks close to 436 nm and at 677 nm, see Table I), taxonomic groups characteristically differ in their photosynthetic accessory pigment composition, and in the manner in which these pigments are associated with either PSI, PSII or both. Many aspects of this subject have been recently reviewed by Larkum and Barrett (1983). Our spectral data reflect the great variabilty in pigment composition and function association in the major groups of algae.

In red algae (Figure 1), most of the accessory pigments (mainly biliproteins, see Table I) transfer the excitons of photons absorbed, primarily or exclusively, to chl *a* molecules of PSII. Bulk chl *a*, however, is largely in PSI (Ley and Butler, 1975; Hiller and Goodchild, 1981). As a result, the major spectral regions of chl *a* absorption (blue-violet and red wavelengths) typically show very little PSII activity as compared to their absorption spectra (Haxo, 1969; Larkum and Barrett, 1983). It should be emphasized, however, that light absorbed in these chl *a* regions can be active in photosynthesis through the Emerson enhancement effect, provided there is sufficient light absorbed by PSII to balance the excitation for both photosystems (Haxo, 1961; Larkum and Barrett, 1983).

In cryptomonads (Figure 2) the increased PSII activity in the blue-violet and red regions reflects a more even distribution of chl *a* between the two photosystems (Ingram and Huller, 1983). However, blue-violet and red PSII activity is much lower in cryptomonads than might be expected for their absorption spectra (Haxo and Fork, 1959; Haxo, 1960), indicating that a significant fraction of chl *a* is in PSI (Lichtlé *et al.*, 1980). The high PSII activity in the region of biliprotein absorption (centered at 540–650 nm, see Table

Table I. Pigments expected to make major contributions to whole-cell absorption in the species studied at the observed maxima listed below (peak maxima ±2 nm). Pigments and spectral assignments are based in part on unpublished data and representative citations listed below.

Species	*In vivo* absorption maxima (nm)	Pigment
Porphyra perforata[a,b,c,d]	436, 677	Chlorophyll *a*
(Rhodophyta)	493, 546, 565	R-phycoerythrin
	617	R-phycocyanin
Phycodrys sp.[d,e]	438, 625, 680	Chlorophyll *a*
(Rhodophyta)	498, 540, 566	R-phycoerythrin
	625	T-phycocyanin
Rhodomonas D3[b,d,f,g,h,i,j]	435, 638, 676	Chlorophyll *a*
(Cryptophyta)	460, 632	Chlorophyll c_2
	545, 565	Phycoerythrin type I
	460, 495	Alloxanthin
Chroomonas sp.[d,g,h,j,k,l]	435, 676	Chlorophyll *a*
(Cryptophyta)	460	Chlorophyll c_2
	585, 625, 645	Phycocyanin type I
	460, 495	Alloxanthin
Chaetoceros sp.[m,n]	440, 590, 622, 675	Chlorophyll *a*
(Bacillariophyta)	462, 590, 637	Chlorophylls c_1 and c_2
	525 (broad)	Fucoxanthin
	495	β-Carotene and minor xanthophylls
Glenodinium sp.[o,p]	437, 590, 622, 676	Chlorophyll *a*
(Pyrrophyta)	462, 590, 632	Chlorophyll c_2
	525 (broad)	Peridinin
	497	β-Carotene and minor xanthophylls
Ulva sp.[a,b,q,r]	437, 678	Chlorophyll *a*
(Chlorophyta)	~475, 652	Chlorophyll *b*
	~475	Lutein and other carotenoids

[a]Haxo and Blinks (1950); [b]Fork (1961); [c]Haxo *et al.* (1985); [d]O'Carra and O'hEocha (1976); [e]resembles *Selesseria decipiens*, Haxo and Blinks (1950, Figure 20); [f]Haxo and Fork (1969), like *Rhodomonas lens*, Figure 2; [g]Chapman (1965); [h]Cheng *et al.* (1974); [i]Pennington *et al.* (1985); [j]MacColl *et al.* (1976); [k]like *Hemiselmis virescens*, Haxo (1960); [l]Ingram and Hiller (1983); [m]Mann and Myers (1968); [n]Goedheer (1970); [o]Prézelin and Haxo (1976); [p]Prézelin *et al.* (1976); [q]Fork (1963); [r]Vidaver (1966).

I) indicates that these pigments are affiliated with PSII, perhaps excusively, as in the red algae. In *Rhodomonas*, the peak of activity at 465 nm may be due to chl *a* absorption and suggests the association of this pigment primarily with PSII (see also Ingram and Hiller, 1983). The discrepancy between the spectra of chl *a* fluorescence and of O_2 evolution in the spectral range 530−600 nm (Figure 2b) could result from energy losses by phycoerythrin fluorescence. Biliproteins are generally considered to transfer energy *in vivo* at high efficiency (Glazer, 1981). However, significant fluorescence may be emitted directly from the light-harvesting biliproteins, as can be implied from Yentsch and Yentsch (1979), Harnischfeger and Herold (1981) and Neori (unpublished).

The action spectra for both chl *a* fluorescence and PSII O_2 evolution in a diatom and a dinoflagellate (Figure 3), reflect the involvement of chl *a* as well as the accessory

pigments in PSII light harvesting of these algae. The latter pigments include a polyoxy xanthophyll (fucoxanthine in diatoms, peridinin in dinoflagellates and chl *c*. The action spectra in these groups are quite close to their absorption spectra, due to the association of most of the pigments, except for some of the chl *a*, with PSII (Hiller and Goodchild, 1981; Larkum and Barrett, 1983).

In the green alga *Ulva* the pigment system and photosynthetic responses have been shown to be similar to those of the much-studied *Chlorella* (Vidaver, 1966; Ried 1972). The spectra of chl *a* fluorescence and of O_2 evolution reflect the primary association of chlorophylls *a* and *b*, and perhaps also of some carotenoids, with PSII (Vidaver, 1966; Ried, 1972; Hiller and Goodchild, 1981).

In all seven algae, action spectra of chl *a* fluorescence and of O_2 evolution show close correspondence in the spectral region of 420–600 nm. This suggests that standard spectrofluorometers can be used to acquire information on the photosynthetic activity of PSII in algae. Spectrofluorometers, with their superior sensitivity and stablity, can provide spectra with detail not currently obtainable by oxygen electrodes. These advantages have already facilitated new studies on the role of carotenoids in light-harvesting in cryptomonads (to be presented elsewhere). If the region below 420 nm is of interest, it may be advisable to use a special spectrofluorometer with front surface emission, as in Kiefer and SooHoo (1982), instead of the standard 90° geometry employed in our work. The use of total fluorescence (FII) with DCMU-treated algae, rather than variable fluorescence (Fv), increased the sensitivity of the measurements. DCMU treatment had only minor effects on the spectral shapes, implying that under the conditions used the state of PSII reaction centers (A) of normal cells did not change appreciably during our fluorescence measurements.

The present observations on the close relationship between spectra of chl *a* fluorescence and of PSII photosynthetic activity in cultures can help to interpret similar spectra data for natural populations. Caution should be advised, however, since various factors may distort fluorescence action spectra of chl *a* for natural populations, relative to their PSII action spectra. Such factors include the presence of fluorescent organic and non-organic substances which can occur in natural samples. We would like to point out that the DCMU-induced increase in ϕFII is largest for healthy, photosynthesizing cells (Samuelson and Öquist, 1977; Slovacek and Hannan, 1977). Thus, DCMU treatment can increase the contribution of healthy cells to the fluorescence spectra for samples of natural populations, make these spectra more similar to the PSII photosynthesis spectra.

Large differences between the values of ϕFII for difference components within the algal population can distort fluorescence spectra, if they did not correspond with differences between the relative value of ϕPII for the same components. Rhythms of the kind reported by Prézelin and Ley (1980) can create such a distortion, if they do not apply equally to the entire algal population, especially when the PSII spectra of the different components not similar to each other. The necessary handling procedures of natural samples, such as filtration (Yentsch and Yentsch, 1979; Neori *et al.*, 1984), could create artifacts which should also be considered. Some species of phytoplankton, notably cryptomonads, can break on the filter pad during filtration, and the leaked pigments can significantly alter the fluorescence spectra.

The relationship between PSII action spectra and total photosynthesis action spectra warrants further discussion. In populations comprised of diatoms and dinoflagellates, fluorescence spectra as shown here may well give a good approximation of the spectral response of absorption and of overall photosynthetic potential, since most of the pigments are associated with PSII. On the other hand, in populations dominated by biliprotein-containing red algae and cyanobacteria, there would be a large difference between the spectrum of chl *a* fluorescence and the spectrum for potential overall photosynthesis, due to the preferential association of chl *a* and of the accessory pigments with different photosystems. This is also true of the biliprotein-containing cryptomonads, but to a lesser extent. In such cases, the use of chl *a* fluorescence spectra, which are related to PSII activity alone, can lead to major underestimates of the photosynthetic potentials of populations.

Bearing in mind the possible distortions, whose magnitude and importance cannot be evaluated until measurements of the photosynthetic action spectra for a range of field samples are possible, the present study (as well as additional studies in progress) of laboratory cultures suggest that fluorescence excitation spectra of chl *a in vivo* when applied to such field samples provide a fair measure of PSII activity.

Acknowledgements

We acknowledge with gratitude the inspiration provided by the late W.L.Butler and his help with instrumentation. Supported in part by NSF-DPP grant 82-12362 to O.H.H., DOE contract DE-AT03-82ER60031, and a grant to A.N. O.H.H. and F.T.H. from the Fund for Ocean Research. Travel to the IInd GAP workshop was facilitated by a DOE grant to the convenors, by a UCSD Chancellor's Associates Grant and by the SIO graduate department. We thank Joan G.Stewart for providing specimens of *Phycodrys*, R.A.Lewin for providing specimens of *Porphyra*, D.S.Berns for providing the culture of *Chroomononas*, J.Lance for help with cultures, J. and E.Yguerabide for the use of their spectrofluorometer, R.C.Booth, Y.Blatt and L.Petrosian for technical advice, and T.A.Nichols for the design and construction of our data acquisition system. This study was the partial fulfilment for a Ph.D. degree by A.N.

References

Butler,W.L.. (1978) Energy distribution in the photochemical apparatus of photosynthesis. *Ann. Rev. Plant Physiol.*, **29**, 345–378.

Butler,W.L. (1980) Energy transfer between PSII units in a connected package model of the photochemical apparatus of photosynthesis. *Proc. Natl. Acad. Sci. USA*, **77**, 4697–4701.

Butler,W.L. (1984) Exciton transfer out of open photosystem II reaction centers. *Photochem. Photobiol.*, **40**, 513–518.

Chapman,D.J. (1965) Studies on the carotenoids of the flagellate cryptophyceae, and the chloroplast pigments of the endosymbiotic algae in *Cyanophora paradoxa* and *Glaucocystis nostochinearum*. Ph.D. Thesis, University of California, San Diego, 169 pp.

Cheng,J.Y., Don-Paul,M. and Antia,N.J. (1974) Isolation of an unusually stable *cis*-isomer of alloxanthin from a bleached autolysed culture of *Chroomonas salina* grown photoheterotrophically on glycerol. Observations on *cis*-trans isomerization of alloxanthin. *J. Protozool.*, **21**, 761–768.

Dutton,H.J., Manning,W.M. and Duggar,D.M. (1943) Chlorophyll fluorescence and energy transfer in the diatom *Nitzchia closterium*. *J. Phys. Chem.*, **47**, 308–313.

Duysens,L.N.M. (1952) Transfer of excitation energy in photosynthesis. Ph.D. Thesis, State University of Utrecht, The Netherlands.

Fork,D.C. (1961) Studies on photosynthetic enhancement in relation to chlorophyll *a* activity and accessory pigment function. Ph.D. Thesis, University of California, 128 p.

Fork,D.C. (1963) Observations of the function of chlorophyll *a* and accessory pigments in photosynthesis. In Kok,B. and Jagendorf,A.T. (eds), *Photosynthetic Mechanisms of Green Plants*. National Academy of Sciences—National Research Council, Washington, DC, pp. 352–361.

Fork,D.C. and Amesz,J. (1969) Action spectra and energy transfer in photosynthesis. *Ann. Rev. Plant Physiol.*, **20**, 305–328.

Glazer,A.N. (1981) Photosynthetic accessory proteins with bilin prosthetic groups. In Hatch,M.D. and Boardman,N.K. (eds), *The Biochemistry of Plants*. Academic Press, New York, Vol. 8, pp. 51-96.

Goedheer,J.C. (1970) On the pigment system of brown algae. *Photosynthetica*, **4**, 97–106.

Goedheer,J.C. (1972) Fluorescence in relation to photosynthesis. *Ann. Rev. Plant Physiol.*, **23**, 87–112

Harnischfeger,H. and Herold,B. (1981) Aspects of energy-transfer between phycobilins and chlorophyll in *Chroomonas* spec. (Cryptophycea). *Ber. Deutsch. Bot. Ges. Bd.*, **94**, 65–73.

Haxo,F.T. (1960) The wavelength dependence of photosynthesis and the role of accessory pigments. In Allen,M.B. (ed.), *Comparative Biochemistry of Photoreactive Systems*. Academic Press, New York, pp. 339–360.

Haxo,F.T. (1961) Some implications of recent studies on the role of accessory pigments to photosynthesis in submarine daylight. In *Pacific Science Congr., 10th (Honolulu), Abstracts*, p. 159.

Haxo,F.T. and Blinks,L.R. (1950) Photosynthetic action spectra of marine algae. *J. Gen. Physiol.*, **33**, 389–422.

Haxo,F.T. and Fork,D.C. (1959) Photosynthetically active accessory pigments of cryptomonads. *Nature*, **184**, 1051–1052.

Haxo,F.T., O'hEocha,C. and Norris,P. (1955) Comparative studies of chromatographically separated phycoerythrins and phycocyanins. *Arch. Biochem. Biophys.*, **54**, 162–173.

Hiller,R.G. and Goodchild,D.J. (1981) Thylakoid membrane and pigment organization. In Hatch,M D. and Boardman,N.K. (eds), *The Biochemistry of Plants*, Academic Press, New York, Vol. 8, pp. 2–49.

Ingram,K. and Hiller,R.G. (1983) Isolation and characterization of a major chlorophyll *a/c*2 light-harvesting protein from a *Chroomonas* species (Cryptophyceae). *Biochim. Biophys. Acta*, **722**, 310–319.

Joliot,P. (1965) Etudes simultanées des cinétique de fluorescence et d'émission d'oxygene photosynthétique. *Biochim. Biophys. Acta*, **102**, 135–148.

Joliot,P. (1972) Modulated light source use with the oxygen electrode. *Methods Enzymol.*, **24B**, 123–134.

Joliot,P. and Joliot,A. (1968) A polarographic method for detection of oxygen production and reduction of Hill reagents by isolated chloroplasts. *Biochim. Biophys. Acta*, **153**, 625–634.

Kiefer,D.A. and SooHoo,J.B. (1982) Spectral absorption by marine particles of coastal waters off Baja California. *Limnol. Oceanogr.*, **27**, 492–499.

Kitajima,M. and Butler,W.L. (1975) Excitation spectra for photosystem I and photosystem II in chloroplasts and the spectral characteristics of the distribution of quanta between the two photosystems. *Biochim. Biophys. Acta*, **408**, 297–305.

Krause,G.H. and Weis,E. (1984) Chlorophyll *a* fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. *Photosyn. Res.*, **5**, 139–157.

Larkum,A.W.D. and Barrett,J. (1983) Light harvesting processes in algae. In Woolhouse,H.W. (ed.), *Advances in Botanical Research*. Academic Press, London, Vol. 10, pp. 1–219.

Latimer,P. and Rabinowitch,E. (1956) Selective scattering of light by pigment containing plant cells. *J. Chem. Phys.*, **24**, 480.

Ley,A.C. and Butler,W.L. (1976) Efficiency of energy transfer from photosystem II to photosystem I in *Porphyridium cruentum*. *Proc. Natl. Acad. Sci. USA*, **77**, 3957–3960.

Lichtlé,C., Jupin,H. and Duval,J.C. (1980) Energy transfers from photosystem II to photosystem I in *Cryptomonas rufescens* (Cryptophyceae). *Biochim. Biophys. Acta*, **591**, 104–112.

Loeblich,A.R.,III (1975) A sea water medium for dinoflagellates and the nutrition of *Cachonina niei*. *J. Phycol.*, **11**, 80–86.

MacColl,R., Berns,D.S. and Gibbons,O. (1976) Characterization of cryptomonad phycoerythrin and phycocyanin. *Arch. Biochem. Biophys.*, **177**, 265–275.

Macdowall,F.D.H. (1949) The effects of some inhibitors of photosynthesis upon the photochemical reduction of a dye by isolated chloroplasts. *Plant Physiol.*, **24**, 462–480.

Mann,J.E. and Myers,J. (1968) On pigments, growth and photosynthesis of *Phaeodactylum tricornutum*. *J. Phycol.*, **4**, 349–355.

Mathis,P. and Paillotin,G. (1981) Primary processes of photosynthesis. In Hatch,M.D. and Boardman,N K.

(ed), *The Biochemistry of Plants*. Academic Press, New York, Vol. 8, pp. 98–161.

Mitchell,B.G. and Kiefer,D.A. (1984) Determination of absorption and fluorescence excitation spectra for phytoplankton. In Holm-Hansen,O., Bolis,L. and Gilles,R. (eds), *Marine Phytoplankton and Productivity*. Springer-Verlag, Berlin, pp. 157–169.

Mohanty,P. and Govindjee (1973) Light-induced changes in the fluorescence yield of chlorophyll *a* in *Anacystis nidulans* II. The fast changes and the effect of photosynthetic inhibitors on both the fast and slow fluorescence induction. *Plant Cell Physiol.*, **14**, 611–629.

Neori,A., Holm-Hansen,O., Mitchell,B.G. and Kiefer,D.A. (1984) Photoadaptation in marine phytoplankton: changes in spectral absorption and excitation of chlorophyl *a* fluorescence. *Plant Physiol.*, **76**, 518–524.

O'Carra,P. and O'hEocha,C. (1976) Algal biliproteins and phycobilins. In Goodwin,T.W. (ed.), *Chemistry and Biochemisty of Plant Pigments*. Academic Press, London, Vol. 1, 2nd edn, pp. 328–376.

Papageorgiou,P. and Govindjee (1968) Light-induced changes in the fluorescence yield of chlorophyll *a in vivo*. I. *Anacystis nidulans*. *Biophys. J.*, **8**, 1299–1315.

Pennington,F.C., Haxo,F.T., Borch,G. and Liaaen-Jensen,S. (1985) Carotenoids of Cryptophyceae. *Biochem. Syst. Ecol.*, **13**, 215–219.

Prézelin,B.B. and Haxo,F.T. (1976) Purification and characterization of peridinin-chlorophyll *a*-proteins from the marine dinoflagellates *Glenodinium* sp and *Gonyaulax polyedra*. *Planta (Berl.)*, **128**, 133–141.

Prézelin,B.B., Ley,A.C. and Haxo,F.T. (1976) Effects of growth irradiance on the photosynthetic action spectra of the marine dinoflagellate, *Glenodinium* sp. *Planta (Berl.)*, **130**, 251–256.

Prézelin,B.B. and Ley,A.C. (1980) Photosynthesis and chlorophyll *a* fluorescence rhythms of marine phytoplankton. *Mar. Biol.*, **55**, 295–307.

Provasoli,L. and MacLaughlin,J.J.A. (1963) Limited heterotrophy of some photosynthetic dinoflagellates. In Oppenheimer,C.H. (ed.), *Symposium on Marine Microbiology*. Charles C.Thomas Publishers, Springfield, IL, pp. 105–113.

Ried,A. (1972) Improved action spectra of light reactions I and II. In Forti,G., Avron,M. and Melandri,A. (eds), *IInd International Congress on Photosynthesis Research*. Dr W.Junk N.V. Publishers, The Hague, pp. 763–772.

Samuelson,G. and Öquist,G. (1977) A method for studying photosynthetic capacity of unicellular algae based on *in vivo* chlorophyll fluorescence. *Physiol. Plant.*, **40**, 315–319.

Slovacek,R.E. and Hannan,P.J. (1977) *In vivo* fluorescence determination of phytoplankton chlorophyll *a*. *Limnol. Oceanogr.*, **22**, 919–924.

Thielen,A.P.G.M. and Van Gorkom,H.J. (1981) Quantum efficiency and antenna size of PS IIα, IIβ and I in tobacco chloroplasts. *Biochim. Biophys. Acta*, **635**, 111–120.

Vidaver,W. (1966) Separate action spectra for the two photochemical systems of photosynthesis. *Plant Physiol.*, **41**, 87–89.

Yentsch,C.S. and Yentsch,C.M. (1979) Fluorescence spectral signatures: the characterization of phytoplankton populations by the use of excitation and emission spectra. *J. Mar. Res.*, **37**, 471–483.

Yguerabide,J. (1968) Fast and accurate method for measuring photon flux in the range 2500–6000 Å. *Rev. Sci. Instr.*, **39**, 1048–1052.

Received May 1985; accepted January 1986