DELAYED LIGHT ACTION SPECTRA OF SEVERAL ALGAE IN VISIBLE AND ULTRAVIOLET LIGHT*, ‡

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ABSTRACT

Action spectra for delayed light production by several algae were determined from 250 to 750 mμ incident light.

In the visible portion of the spectrum the action spectra resemble those reported by previous workers for photosynthesis and light emission. Blue-green algae had a maximum at 620 mμ, red algae at 550 mμ, whereas green and brown algae have action spectra corresponding to chlorophyll and carotenoid absorption.

In the ultraviolet portion of the spectrum delayed light is emitted by algae down to 250 mμ incident light.

The action spectra of the different algae are not alike in the ultraviolet portion of the spectrum.

This indicates that pigments other than chlorophyll must be sensitizing or shielding the algae in the ultraviolet region.

INTRODUCTION

Strehler and Arnold (10) showed that after a period of illumination, light was given out by plants for as long as 2 minutes, with a very low intensity. The action spectrum of the production of the delayed light was shown to be the same as the action spectrum of photosynthesis in both algae and higher plants (10). Later experiments demonstrated that the emission spectrum of the delayed light is the same as the fluorescent spectrum of chlorophyll in the living plant, and that it was chlorophyll which emitted the delayed light (1).

In the present experiments, the production of delayed light by several marine and fresh water algae was used to determine the action spectrum from 250 to 750 mμ.

Materials and Methods

The fresh water algae were from the culture collection of Dr. William Arnold of Oak Ridge, Tennessee. The blue-green alga, *Anacystis nidulans*, was grown in the

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Gerloff *et al.* (6) Chu No. 10 medium. The red alga, *Porphyridium cruentum*, was grown in the Punnett and Chambers adaptation of R. H. Swain's medium. Both of these species were grown at 20°C. and illuminated with daylight type fluorescent tubes. A green alga, *Chlorella pyrenoidosa* (Emerson's strain), was grown in Knop's solution at 20°C. under red fluorescent tubes with 5 per cent carbon dioxide in air.

The following marine algae were obtained from the culture collection of Dr. John H. Ryther of The Woods Hole Oceanographic Institution: a green alga, *Dunaliella euchlora*; a chrysophyte, *Monochrysis sp.*; and, two diatoms, *Phaeodactylum tricornutum* and *Coscinodiscus sp.* All marine algae were grown in an enriched sea water medium at 20°C., with 5 per cent carbon dioxide in air, and illuminated with red fluorescent tubes.

TABLE I

Transmission of Standard and Modified Knop's Medium between 225 and 460 mμ

mμ	Standard Knop's medium	Modified Knop's medium (minus Fe salt, 0.0001 per cent)
460	95.3	99.6
400		
375	88.8	99.2
350	75.5	99.2
325	56.0	94.5
300	37.2	82.0
275	22.8	91.8
250	19.0	77.1
225	24.5	

(1 cm. cell, Beckman DU spectrophotometer)

Cells were centrifuged out of the culture medium and transferred, after washing, to Knop's medium minus the iron salt. The modified Knop's medium was made up with 2.5 per cent NaCl for the marine algae. Table I gives the transmission of standard and modified Knop's medium in the ultraviolet portion of the spectrum. The action spectrum was made with a suspension transmitting 85 to 95 per cent of the light from 250 to 750 mμ.

Optical Methods

Since the method of measuring delayed light production has already been adequately described (1, 2, 10), only the modifications in equipment are described here

Light from a 2 kilowatt xenon high pressure arc lamp operated from a Miller D.C. arc welder was focussed on the entrance slit of a Hilger monochromator which has apertures of $f_{1.9}$ and f_6. The intensity of illumination was controlled with an iris diaphragm. The relative light intensity was determined with an Eppley thermopile connected to a low resistance galvanometer.

Cells were pumped from a darkened container through black rubber tubing making two 180° bends, into a square quartz tube (3.5 mm. O.D.) held vertically in a light-tight aluminum case against the exit slit of the monochromator. The thermopile was

mounted directly in back of the quartz tube. From the square quartz tube the cell suspension flowed through black rubber tubing, in the dark, to a flat spiral of glass tubing held in a light-tight aluminum case in front of a photomultiplier which measured the intensity of the delayed light. From the flat spiral of glass tubing the cell suspension flowed to a Maisch metering pump, and back into the darkened flask.

Delayed light was measured using between 0.13 and 0.18 $mm.^3$ of cells per cc. The flow rate of the cell suspension was 36 cc. per second; the velocity, 120 cm. per second. The intensity of the emitted light was measured by the photomultiplier approximately 0.2 second after excitation. The action spectra were made at about one-quarter of the saturating light for each algal species.

The photomultiplier (No. 6217, S 10 response) was mounted inside a metal tube which ran horizontally through a metal tank. The tank was insulated and packed with dry ice to reduce the dark current. The voltage on the photomultiplier was 850 volts. Current from the photomultiplier was measured with a vibrating reed electrometer. The collecting anode was connected to the input of the reed by a 10^8 ohm resistor, and to ground through a 0.0005 microfarad condenser with a high resistance. The reed input was connected to the feed-back lead through a 10^{11} ohm resistor.

Experiments were made with the shutter alternately open for 1 minute and then closed for 1 minute. During the time that the shutter was closed the wave length drum was changed to the next setting, and the entrance and exit slits of the monochromator were adjusted to give a 50 A dispersion at each wave length. The simple experiment of stopping the pump and getting no signal from the cell suspension shows that the light is delayed light and not fluorescence. It also shows that there is no light scattering down the suspension and into the measuring cell.

RESULTS

Figs. 1 through 7 give separate determinations of the delayed light action spectrum of several algae. Collection of this data was started at a wave length of 440 mμ, taking the even multiples of ten down to 250 mμ, up on the odd multiples to 750 mμ, and then back to 440 mμ on the even ones. This was done to minimize any effects of changes in the cell suspension that might take place in the $1\frac{1}{2}$ to 2 hours of the run. Curves showing the data can be compared with one another and with those published for the delayed light action spectrum of *Chlorella* (10) and of the blue-green and red algae (2).

In Figs. 1 through 7 the ordinate is the relative effectiveness per incident quantum in producing the delayed light. The intensity of the emitted light was divided by the galvanometer readings to give an initial slope. The initial slope at each wave length setting divided by the wave length gave the relative values in quanta. These values were plotted as a function of wave length at 10 mμ intervals from 250 to 750 mμ.

DISCUSSION

The action spectrum for the blue-green alga, *Anacystis nidulans* (Fig. 1), shows a broad band at 620 mμ, and a smaller band at 400 mμ. In the visible

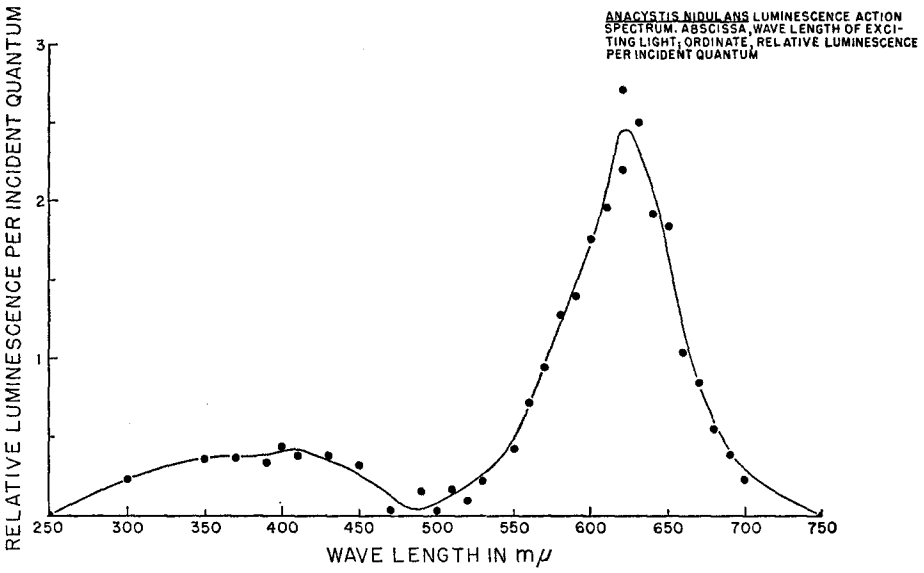
ANACYSTIS NIDULANS LUMINESCENCE ACTION SPECTRUM. ABSCISSA, WAVE LENGTH OF EXCITING LIGHT; ORDINATE, RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
3
2
1
250
300
350
400
450
500
550
600
650
700
750
WAVE LENGTH IN mμ

FIG. 1

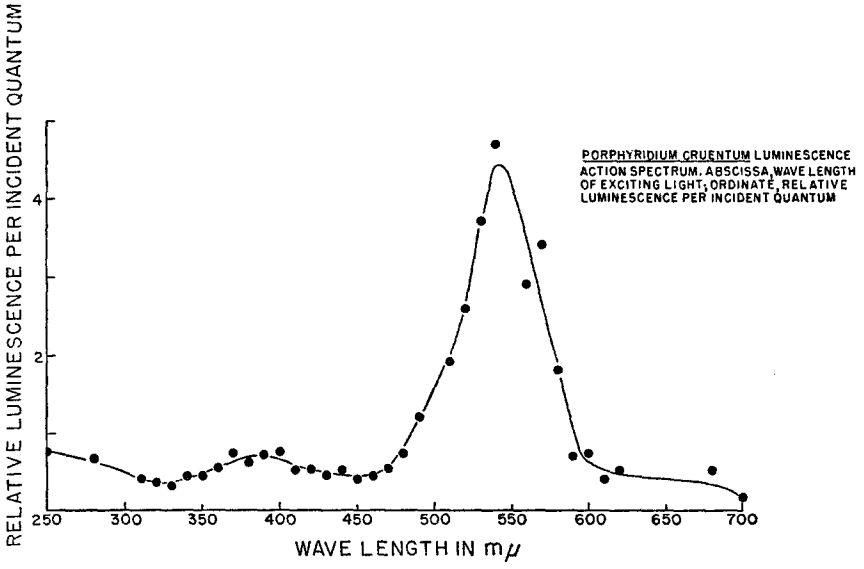
PORPHYRIDIUM CRUENTUM LUMINESCENCE ACTION SPECTRUM. ABSCISSA, WAVE LENGTH OF EXCITING LIGHT; ORDINATE, RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
4
2
250
300
350
400
450
500
550
600
650
700
WAVE LENGTH IN mμ

FIG. 2

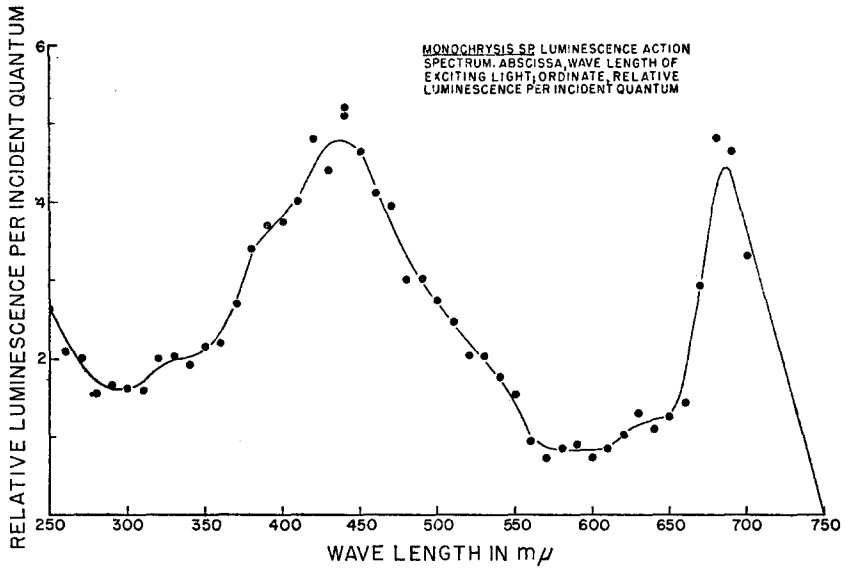
MONOCHRYSIS SP LUMINESCENCE ACTION SPECTRUM. ABSCISSA, WAVE LENGTH OF EXCITING LIGHT; ORDINATE, RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
6
4
2
250
300
350
400
450
500
550
600
650
700
750
WAVE LENGTH IN mμ

FIG. 3

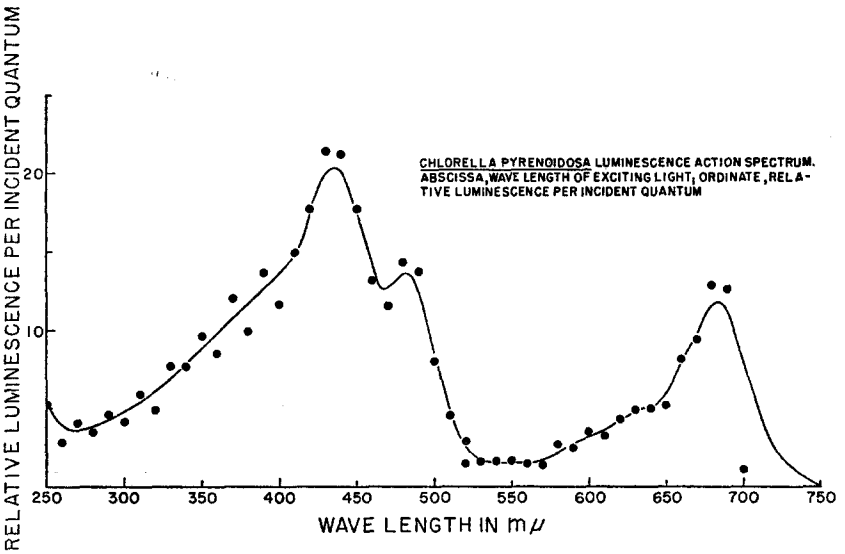
CHLORELLA PYRENOIDOSA LUMINESCENCE ACTION SPECTRUM. ABSCISSA, WAVE LENGTH OF EXCITING LIGHT; ORDINATE, RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
20
10
250
300
350
400
450
500
550
600
650
700
750
WAVE LENGTH IN mμ

FIG. 4

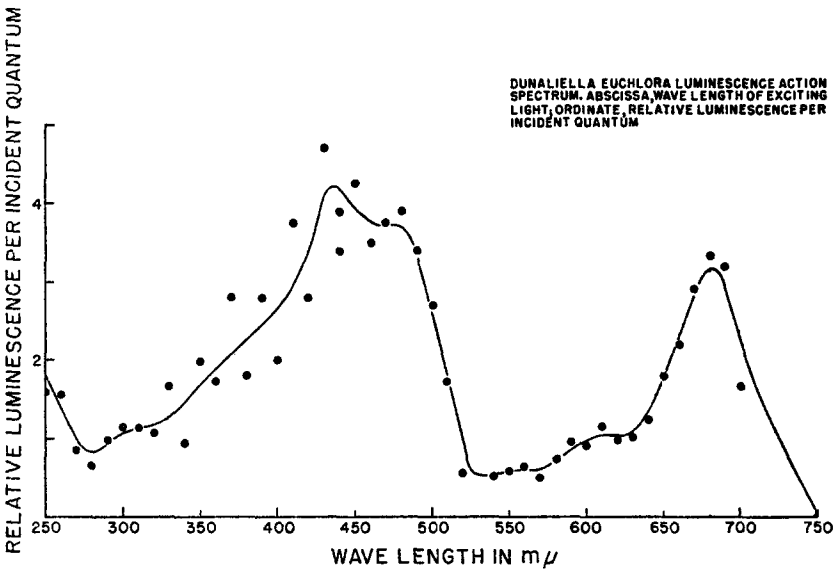
DUNALIELLA EUCHLORA LUMINESCENCE ACTION SPECTRUM. ABSCISSA, WAVE LENGTH OF EXCITING LIGHT; ORDINATE, RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
2
4
250
300
350
400
450
500
550
600
650
700
750
WAVE LENGTH IN mμ

FIG. 5

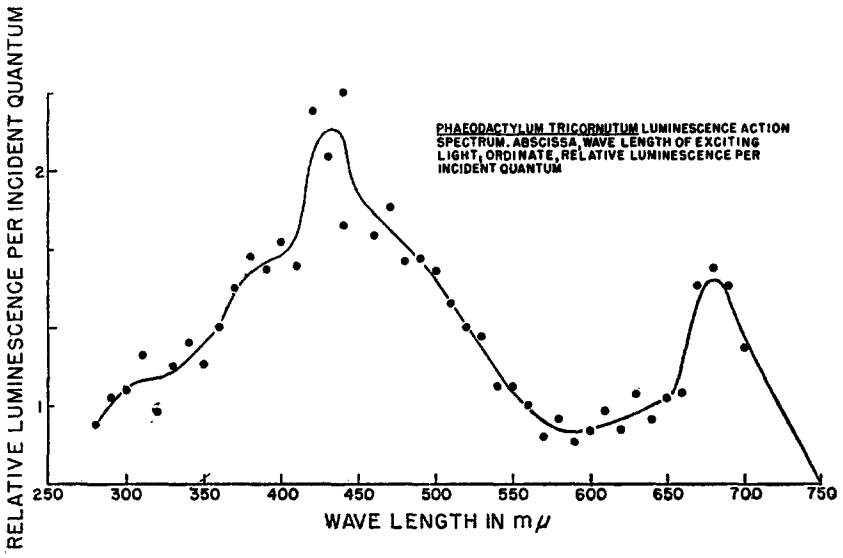
PHAEODACTYLUM TRICORNUTUM LUMINESCENCE ACTION SPECTRUM. ABSCISSA, WAVE LENGTH OF EXCITING LIGHT; ORDINATE, RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
RELATIVE LUMINESCENCE PER INCIDENT QUANTUM
1
2
250
300
350
400
450
500
550
600
650
700
750
WAVE LENGTH IN mμ

FIG. 6

part of the spectrum the curves agree with the action spectrum for photosynthesis and chlorophyll fluorescence in blue-green algae described by Duysens (3) and Haxo and Blinks (8), but not with the action spectrum for photosynthesis in the blue-green algae, *Chroococcus*, given by Emerson and Lewis (5) in which the chlorophyll is fully active.

The action spectrum for the red alga, *Porphyridium cruentum*, is given in Fig. 2. The action spectrum for the delayed light production shows a peak at 550 mμ, a flat shoulder between 600 mμ and 660 mμ, and a fall to zero at 700

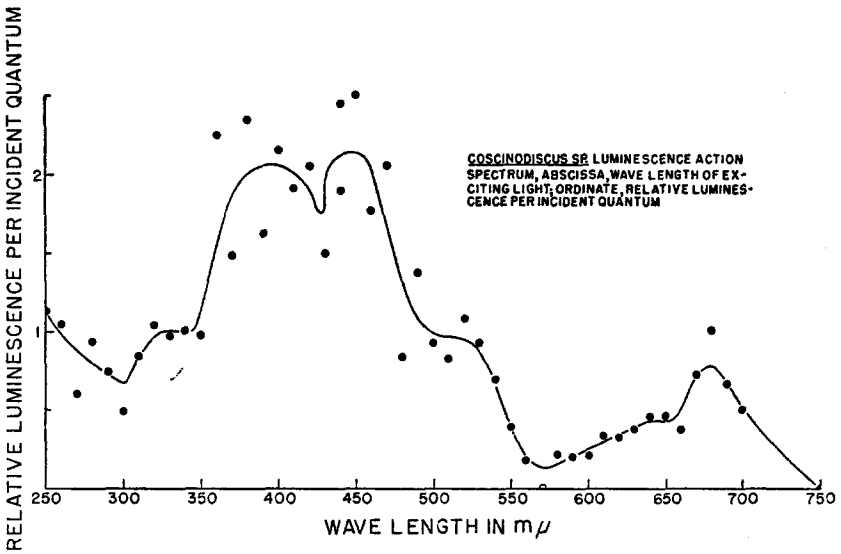

FIG. 7

mμ. In the ultraviolet part of the spectrum there is a flat shoulder between 360 and 400 mμ, and an increase at 250 mμ.

In both the blue-green and red algae, the action spectra are those of the accessory pigments. The low yield in the region in which chlorophyll *a* is mainly absorbing demonstrates that the path of energy transfer, as Duysens (4) suggests, is from phycoerythrin *via* phycocyanin to chlorophyll *a*.

Figs. 3 through 5 show delayed light action spectra for fresh water and marine green and golden green algae with peaks at 680 mμ and 440 mμ. In the ultraviolet part of the spectrum there is a gentle shoulder from 440 mμ down to 280 mμ, and a rise at 250 mμ.

The action spectra for two diatoms (Figs. 6 and 7) demonstrate the broad band of fucoxanthin absorption and the 680 mμ peak of chlorophyll absorption.

The action spectra in the visible part of the spectrum for algae have been discussed by previous workers. In the ultraviolet part of the spectrum the

action spectra indicate that the pigments are still active. That plants can photosynthesize in this region has already been suggested (9), and Harris and Zscheile (7) showed that the chlorophylls have several minor absorption peaks in the spectral region 280 to 380 mμ. The increase at 250 mμ may be an experimental quirk, but it is tempting to think of a protein absorption of the light energy in this part of the spectrum.

I wish to thank Dr. Alexander Hollaender for making possible my visit to the Oak Ridge National Laboratory. I am deeply indebted to Dr. William Arnold of Oak Ridge for permitting the use of all the apparatus in these measurements and for his kind interest, cooperation, and most helpful suggestions in all phases of the work. Thanks are also due to Mrs. Helen Sherwood of Oak Ridge, for her help in making the determinations. Finally, I wish to thank Dr. John H. Ryther of the Woods Hole Oceanographic Institution whose continual enthusiasm helped make this project possible.

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