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Title: THE ACTION SPECTRUM OF PHOTOSYNTHESIS OF THE MARINE DIATOM SKELETONEMA COSTATUM (GREVILLE)

Abstract approved: Herbert C. Curf, Jr.

An action spectrum of photosynthesis for Skeletonema costatum (Greville) Cleve, a pelagic, marine diatom was obtained from values of photosynthetic $^{14}$C uptake at various wavelengths of light.

Comparison of the thallus absorption spectrum with the action spectrum of photosynthesis for S. costatum was in accord with theoretical expectations based on Tanada's determination of the quantum yield of photosynthesis for Navicula minima, a freshwater, benthic diatom.
The Action Spectrum of Photosynthesis of the Marine Diatom *Skeletonema costatum* (Greville) Cleve

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THE ACTION SPECTRUM OF PHOTOSYNTHESIS OF THE MARINE DIATOM SKELETONEMA COSTATUM (GREVILLE) CLEVE

INTRODUCTION

In accordance with the criteria of Rabinowitch (1961), the action spectrum of photosynthesis may be defined as the response of photosynthetic organisms, in the light-limited region of photosynthesis, to identical numbers of quanta at different wavelengths within the electromagnetic spectrum.

The photosynthetic equation for plants

\[ 6 \text{CO}_2 + 12 \text{H}_2\text{O} \xrightarrow{\text{hv}} \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 + 6 \text{H}_2\text{O} \]

involves several parameters which might be used to monitor photosynthetic response to light. Of these parameters, the change in oxygen concentration in the environment of the experimental organisms as a result of photosynthesis has been most widely used in determining photosynthetic action spectra.

According to Blinks (1965), Engelmann (1884) obtained the first action spectrum of photosynthesis. Congregation of motile bacteria along a filament of *Spirogyra* spp. as a response to photosynthetic oxygen production was used to measure photosynthesis as a function of various wavelengths of a microspectrum of light cast on the filament.

Ehrke (1932) chemically determined oxygen produced
photosynthetically in response to light isolated with three different colored filters. Values for photosynthesis in the different spectral regions were reported for *Enteromorpha compressa* and *Delesseria sanguinea*.

Baatz (1940), Samgrowsky (1942) and Wassink and Kersten (1946) used manometric techniques for measuring photosynthetic oxygen production in three spectral regions isolated with filters. Values for photosynthesis in the different spectral regions were reported for *Biddulphia sinensis*, *Coscinodiscus excentricus* and *Chaetoceros didymus*, for *Chaetoceros ceratospermum*, *Coscinodiscus excentricus* and *Chlorella spp.*, and for *Nitzschia dissipata* and *Chlorella spp.*, respectively. Fichoff (1939) used a differential manometer to measure oxygen photosynthetically produced by *Chlorella pyrenoidosa* at different wavelengths of light isolated with a "dispersion" filter. Emerson and Lewis (1942, 1943) manometrically measured photosynthetic oxygen production as a function of different wavelengths of light isolated with a monochromator. *Chroococcocus spp.* and *Chlorella spp.* were the experimental organisms.

Several investigators have used polarographic electrodes for measuring photosynthetic oxygen production in response to different wavelengths of light isolated with monochromators. Investigators and their experimental organisms are: Haxo and Blinks (1950) for *Coilodesme californica*, *Delesseria decipiens*, *Myriogramme*

Kok and Hoch (1961) used a mass spectrometer to measure photosynthetic oxygen production in response to wavelengths of light isolated with a monochromator for Anacystis nidulans.

Diatoms form the largest component, both in numbers of individuals and in numbers of species, of marine planktonic plants responsible for the photosynthetic production of organic matter in the ocean. It is desirable to know the characteristics of the photosynthetic system of such an important group of organisms. One of the more ecologically important characteristics of a photosynthetic system is its ability to utilize light over a broad range of wavelengths. Although comparisons of photosynthesis have been obtained in several regions of the spectrum and although the quantum yield of photosynthesis has been determined for a diatom (Tanada, 1951), no quantized action spectrum of photosynthesis has been determined for a diatom.

Previous investigators have almost exclusively used the photosynthetic production of oxygen in determining action spectra of
photosynthesis. The action spectrum determined in this investigation was based on photosynthetic $^{14}$C uptake.

The purpose of this investigation was to obtain an action spectrum of photosynthesis for *Skeletonema costatum* (Greville) Cleve, a ubiquitous, marine, pelagic diatom. The action spectrum of photosynthesis and the *in vivo* thallus absorption spectrum for *S. costatum* were to be compared on the basis of theoretical expectations derived from Tanada's (1951) values for the quantum yield of photosynthesis for *Navicula minima*, a freshwater, benthic diatom. The pigment composition of *S. costatum* was to be determined.
EXPERIMENTAL METHODS

Determination of the Action Spectrum of Photosynthesis

The output of a Bausch and Lomb no. 33-86-20-12 xenon arc lamp was increased with a 33 mm diameter, 16 mm focal length, first surface, aluminum, spherical mirror (Edmund Scientific no. 30.43D) attached with epoxy resin to a machine screw. The mirror was focused to image the arc on itself by adjusting the length of screw projecting from a tapped hole in the source housing. Three 14.7 x 2.2 x 1 cm ceramic magnets arranged outside of the lamp housing maintained the arc at the focal point of the source condenser system (Fig. 1). Line voltage to the lamp power supply was stabilized with a Slotted Line Test Equipment Model SL1 voltage regulator. A Corning glass filter no. 3060, color specification no. 3-75, with 50 percent transmittance at 410 mμ was placed in front of the entrance slit of a Bausch and Lomb no. 33-86-02 visible grating monochromator, coupled to the source housing, to exclude lower order spectra. Output was controlled by adjusting the diaphragm of a condenser (B. and L. no. 33-86-53) at the exit slit of the monochromator. The beam of light from the monochromator was directed along an 18 cm optical path through a 5 mm-thick, plexiglass window mounted in the side of a constant temperature water bath, to a 10 cm quartz spectrophotometer cell suspended in the bath. The stainless steel interior
of the bath was painted flat black. Black plastic tape on the front surface of the plexiglass window limited the area of illumination to 1.68 cm$^2$.

To monitor monochromator output a flat, first surface, rhodium mirror mounted on a vertical, hinged support was swung into the beam. The beam was reflected along an 18 cm optical path to an Eppley circular, 16 junction, bismuthsilver thermopile equipped with a 1 mm-thick quartz window. A cardboard tube with a window of 5 mm-thick plexiglass was placed around the thermopile to reduce transients caused by air currents in the darkroom. A 1 microfarad capacitor was placed in parallel with the thermopile to further reduce transients. The output of the thermopile was monitored with a Keithley Model 150 microvolt-ammeter, the line voltage to which was stabilized with a Sola CVN voltage regulator.

Membrane-filtered sea water was enriched (Guillard and Ryther, 1962), autoclaved and cooled prior to inoculation with S. costatum. Cells were grown in a flask placed in the center of two FC16T10/WWX fluorescent lamps backed by an aluminum reflector. The cells were kept in suspension by a stream of air bubbles and were maintained in continuous light at a temperature of 20°C.

Cells in logarithmic growth were placed in a 2.8 liter Erlenmyer reservoir flask covered with black Visqueen. The flask was placed in a 19°C constant temperature water bath and left for 12 hours.
Cell numbers approximately doubled during this period. Three hundred μCi of $^{14}\text{C} \text{1}^{-1}$ were added as NaH$^{14}\text{C}_3$ to the culture after the 12 hour "conditioning" period.

A preliminary dark uptake curve was constructed from dark $^{14}\text{C}$ uptake values determined at intervals over an initial seven hour period and at 21 hours after addition of $^{14}\text{C}$. Each sample consisted of 31 ml of culture pipetted from the reservoir and filtered onto a Millipore HA 0.8 membrane filter under 176 mm Hg. Each damp filter was glued to an aluminum planchette with rubber cement, dried for five days in a desicator and "counted" five times to 10,000 counts under a Nuclear Chicago Model D-47, gas flow, window detector coupled to a Model C-110B automatic sample changer. Counting efficiency was 25 percent. The rate of dark $^{14}\text{C}$ uptake appeared to become constant after approximately four hours and remained essentially constant to termination of the experiment (Fig. 2).

On consideration of the form of the preliminary dark $^{14}\text{C}$ uptake curve, measurements of photosynthesis were performed after a 12-hour "conditioning" period followed by a six-hour "$^{14}\text{C}$ incubation" period to insure linear dark $^{14}\text{C}$ uptake with time during photosynthetic $^{14}\text{C}$ uptake determinations. Room illumination was provided by a 40 watt incandescent bulb inside a Kodak, Wratten OA safelight with peak output at 560 m.$\mu$. Photosynthetic $^{14}\text{C}$ uptake was determined in a series of experiments by irradiating 31 ml of culture in
a 10 cm spectrophotometer cell for 10 minutes at each experimental wavelength. Metal clamps held the cell in the same alignment in the beam path for each measurement. Irradiated samples were discarded unless the monochromator output was within four percent of the predetermined value at the end of the 10 minute irradiation period. After irradiation, the contents of the reaction vessel were filtered, dried and "counted" in the same fashion as the dark uptake samples. Replicate 31 ml dark $^{14}$C uptake samples were periodically pipetted from the reservoir during each experimental period and treated as above. The mean relative error of twenty pairs of dark $^{14}$C uptake samples was $\pm 1.8$ percent (range $\pm 0.05$ to $\pm 5.0$ percent) where relative error was 50 times the difference between the replicates divided by the mean of the replicates. The graduated pipette used in sampling was considered precise to 0.6 percent. Although the contents of the reservoir were stirred before each sample was drawn, it is possible that as a result of the chain-like mode of growth of S. costatum the distribution of cells in the medium was non random after mixing.

A linear regression of dark $^{14}$C uptake on time was performed for each experiment. It was assumed, for convenience in calculation, that the dark $^{14}$C mechanism operated during photosynthesis. A dark $^{14}$C uptake value for each irradiated sample, based on total elapsed time from the beginning of the experiment was calculated
with the regression formula and subtracted from total $^{14}$C uptake for the irradiated sample to give photosynthetic $^{14}$C uptake as cpm for the 10 minute irradiation period.

A graph (light-curve) of photosynthesis as a function of numbers of quanta supplied at 680 m\(\mu\) was constructed (Fig. 3).

A bandpass of 10 m\(\mu\) and a constant flux of $3.71 \times 10^{14}$ quanta cm$^{-2}$ sec$^{-1}$ were chosen for this investigation in consideration of the light curve of photosynthesis and of the limitations of the equipment. The constant quanta flux at each experimental wavelength was maintained by adjusting the diaphragm on the monochromator condenser until the thermopile output reached a predetermined value, in \(\mu\)volts, for $3.71 \times 10^{14}$ quanta cm$^{-2}$ sec$^{-1}$. For example, calculation of thermopile output, in \(\mu\)volts, for $3.71 \times 10^{14}$ quanta cm$^{-2}$ sec$^{-1}$ at 680 m\(\mu\) is as follows:

$$E = n \hbar \nu = n \frac{c}{\lambda} = \frac{1 \times 6.6256 \times 10^{-27} \text{ erg sec} \times 3 \times 10^{-10} \text{ cm sec}^{-1}}{680 \times 10^{-7} \text{ cm}}$$

Since watt sec = $10^7$ erg,

$$E = \frac{1.987 \times 10^{-19} \text{ erg cm} \times 10^{-7} \text{ watt sec erg}^{-1}}{680 \times 10^{-7} \text{ cm}}$$

Therefore,

$$E = 2.92 \times 10^{-13} \text{ \(\mu\)watt sec quantum}^{-1}$$

And finally,

$$2.92 \times 10^{-13} \text{ \(\mu\)watt sec quantum}^{-1} \times 3.71 \times 10^{14} \text{ quantum cm}^{-2} \text{ sec}^{-1}$$
\[ x \times 0.234 \, \mu\text{volt} \cdot \mu\text{watt}^{-1} \cdot \text{cm}^{-2} = 25.4 \, \mu\text{volts}. \]

Where: \( E \) = energy, \( n \) = number of quanta, \( h \) = Planck's constant, \( v \) = frequency, \( \lambda \) = wavelength in cm, \( c \) = velocity of light and \( 0.234 \, \mu\text{volt} \cdot \mu\text{watt}^{-1} \cdot \text{cm}^{-2} \), the thermopile calibration factor, was supplied by Eppley Laboratories, Inc.

Photosynthetic \( ^{14} \text{C} \) uptake at various wavelengths from 430 m\( \mu \) to 690 m\( \mu \) was determined in a series of four experiments. The wavelength range was limited by monochromator output, a function of the spectral output characteristics of the xenon arc lamp. As a result of differences in growth among clones of \textit{S. costatum}, cell concentrations of from 40,000 to 200,000 cells ml\(^{-1} \) were used in the four experiments. This resulted in different absolute values of dark and photosynthetic \( ^{14} \text{C} \) uptake among the four experiments. The data from these experiments were weighted to place the four action spectrum envelopes in congruence. The mean value of photosynthetic \( ^{14} \text{C} \) uptake at 430 m\( \mu \) for each of three experiments was adjusted to equal the mean value of photosynthetic \( ^{14} \text{C} \) uptake at 430 m\( \mu \) for the fourth or "standard" experiment. The "standard" experiment consisted of three photosynthetic \( ^{14} \text{C} \) uptake measurements at 430 m\( \mu \) with two measurements at each of the other experimental wavelengths (Fig. 4A). "Adjusted" experiments consisted of from one to three photosynthetic uptake measurements at each of several, but not all, experimental wavelengths. Photosynthetic
$^{14}$C uptake data for all wavelengths other than 430 m$\mu$ within each of the three experiments were multiplied by the ratio of mean photosynthetic $^{14}$C uptake at 430 m$\mu$ for the "standard" experiment to mean photosynthetic $^{14}$C uptake at 430 m$\mu$ for each of the respective experiments.

**Determination of Thallus Absorption Spectrum**

A 100 ml volume of a culture of *S. costatum* (about 200,000 cells ml$^{-1}$) was centrifuged at 1000 g. The cells were resuspended in 4 ml of membrane-filtered sea water and placed in a 1 cm path-length spectrophotometer cuvette. The *in vivo* thallus absorption spectrum from 400 m$\mu$ to 700 m$\mu$ was determined with a Bausch and Lomb Model 505 spectrophotometer with 50 Å slits, calibrated with mercury lines (Fig. 4B). Wavelength accuracy was ±0.5 m$\mu$. Opal glass diffusing plates were placed in the path of the exit beams from reference and sample cuvettes to decrease effects of light scattering from suspended cells (Shibata, Benson and Calvin, 1954).

**Determination of Pigment Composition**

A culture of *S. costatum* was harvested by continuous flow centrifugation at 10,000 g in a Sorvall RC-2 refrigerated centrifuge. The algal pellet was extracted by shaking with absolute methanol for several minutes (Strain, Manning and Hardin, 1944) then
centrifuged at 1000 g. The liquid supernatant was poured into a separatory funnel. The pellet was extracted with 50 percent (v/v) absolute, redistilled acetone in methanol. After centrifugation at 1000 g, the liquid supernatant was poured into the separatory funnel. The contents of the funnel were then diluted with an equal volume of 10 percent (w/v) sodium chloride solution. About 20 ml of diethyl ether were added to the funnel. The pigment-containing epiphase was decanted, washed three times with 10 ml aliquotes of 10 percent (w/v) sodium chloride solution to remove acetone and methanol, and centrifuged at 1000 g to remove water. The solvent was then evaporated to dryness in subdued light with a stream of nitrogen. The dried pigments were dissolved in about 0.5 ml of redistilled, absolute acetone.

Riley and Wilson's (1965) thin layer chromatography technique was used to separate the various pigments. A 20x20 cm glass plate was coated with a 0.1 mm layer of silica gel G, then dried and heated at 120° C for 30 minutes. The plate was cooled and placed inside a plexiglass-covered box. The box was flushed with nitrogen after which acetone-pigment solution was spotted on a margin of the plate with a syringe inserted through holes drilled in the cover. The plate was placed in a light-tight tank containing a solution composed of 12 percent (v/v) diethyl amine, 30 percent (v/v) ethyl acetate and 58 percent (v/v) redistilled petroleum ether (b.p. 36.0° C). After
the solvent front traveled 15 cm, the plate was removed from the developing tank and dried. The pigment bands were taken up individually with a Kensington Scientific Corp. fritted glass sample collection tube no. K-40-20. Pigments were eluted from the bands with either 95 percent ethanol or redistilled absolute acetone.

Absorption spectra of the various pigments were determined with a Bausch and Lomb Model 505 spectrophotometer with 5 Å slit widths. Absorption spectra were determined in the eluant.
RESULTS AND DISCUSSION

Action Spectrum of Photosynthesis and Thallus Absorption Spectrum

If the quantum yield of photosynthesis is the same for all wavelengths, the quantized action spectrum of photosynthesis may be expected to exactly parallel the thallus absorption spectrum (Rabinowitch, 1951). Tanada (1951) showed that for the freshwater, benthic diatom *Navicula minima*, the quantum yield of photosynthesis was a constant 0.110 from 680 mμ to 520 mμ, declined to 0.092 at 475 mμ and then increased to 0.104 at 430 mμ. The quantum yield of photosynthesis dropped to 0.070 at 690 mμ. *Skeletonema costatum* possesses the same pigment system (Jeffrey, 1961) found in each of six other species of diatoms, one of which was of the genus *Navicula* (Strain, Manning and Hardin, 1944). It was therefore assumed that the quantum yield of photosynthesis for *S. costatum* was identical with Tanada's quantum yield of photosynthesis results for *N. minima*. It was not possible to obtain an experimental quantum yield of photosynthesis for *S. costatum* in this investigation because the low cell numbers used in the experiments precluded determination of the numbers of quanta actually used in photosynthetic $^{14}$C uptake.

If light scattering effects are minimized, the in vivo thallus absorption spectrum of a plant is directly comparable to the action
spectrum of photosynthesis for the plant (French, 1959). The opal glass diffusing plates used in this investigation have been shown to minimize light scattering effects on the determination of in vivo thallus absorption spectra for algae (Latimer, 1958). We might therefore expect that the quantized action spectrum of photosynthesis for *S. costatum* (Fig. 4A) would follow the thallus absorption spectrum (Fig. 4B) between 680 mµ and 420 mµ, would be lower between 520 mµ and 430 mµ, and would be significantly lower at 690 mµ, relative to the thallus absorption spectrum. We might also expect the value of photosynthetic $^{14}$C uptake to be slightly higher at 675 mµ than at 440 mµ, relative to the absorption of energy at 675 mµ compared with the absorption of energy at 440 mµ. On inspection of the action spectrum of photosynthesis and thallus absorption spectrum for *S. costatum*, these expectations are realized within the limits of experimental error.

Measurements of the in vivo absorption spectra of marine phytoplankton show that their pigment composition is well suited for the absorption of wavelengths of maximum transmission of light in clear oceanic water (Yentsch, 1962). For the oceanic environment, the red absorption peaks of the chlorophylls cannot be active below 10 m while blue light absorbed by chlorophyll penetrates to about 50 m. As a result of the selective spectral absorption of light by sea water, carotenoids must be the principle absorbers of light
below 50 m in the oceanic environment. If an organism is to survive at depths greater than 50 m in the open ocean, light absorbed by carotenoids must be active in photosynthesis. This investigation for the marine, pelagic diatom _S. costatum_ is in accord with Tanada's (1951) findings for the freshwater, benthic diatom _N. minima_ that light absorbed by the carotenoid fucoxanthin in the pigment system of a diatom is active in photosynthesis.

Some criteria for a device for measuring photosynthetically usable light may be deduced from Tanada's (1951) quantum yield of photosynthesis results and from the results of this investigation. For plants, photosynthetic production of organic matter has been shown to be a function of the numbers of quanta, at photosynthetically usable wavelengths, impinging on the plant below the level of light saturation of the photosynthetic system of the plant. Therefore, a device for measuring photosynthetically usable light must give the flux of quanta, at wavelengths usable in photosynthesis, on the surface of the plant. For measuring light usable by phytoplankton, the device must give the flux of wuanta from 700 m\(\mu\) to 400 m\(\mu\) on a 4\(\pi\) surface.

**The Pigment Composition of S. Costatum**

Jeffrey (1961) found chlorophylls a and c, chlorophyllide a, fucoxanthin and two _cis_ isomers, diadinoxanthin, diatoxanthin and
β carotene in *Skeletonema costatum*. Using Riley and Wilson's (1965) technique, I found chlorophyll a, chlorophyllide a, fucoxanthin, distoxanthin and β carotene in *S. costatum* (Table 1). Riley and Wilson isolated these pigments, with the exception of chlorophyllide a and with the addition of chlorophyll c, from *Phaeodactylum tricornutum*. Although a band thought to be chlorophyll c was isolated from *S. costatum* by column chromatography in this investigation, the pigment could not be resolved on TLC plates. Strain, Hardin and Manning (1944) found fucoxanthin and two cis isomers diadinoxanthin, diatoxanthin and β carotene in each of six different species of diatoms, one of which was *Nitzchia closterium* (probably *Phaeodactylum tricornutum*). That two cis isomers of fucoxanthin as well as diadinoxanthin were resolved on TLC plates by neither Riley and Wilson nor in this investigation raises some question as to the analytical potential of Riley and Wilson's (1965) technique. The technique provides, however, a relatively rapid and easy means for separating the major classes of pigments.

The participation of different pigments in photosynthesis is shown by the correlation of wavelengths of maxima in the action spectrum of photosynthesis with the absorption maxima of known pigments isolated from the experimental organism (Smith and French, 1963). Tanada (1951) concluded, after adjusting *in vitro* pigment absorption curves for solvent-induced shifts in absorption maxima
so that the in vitro maxima coincides with maxima in the thallus absorption spectrum, that the continued high quantum yield of photosynthesis in the region of declining absorption by the chlorophyll system (600 m\(\mu\) to 520 m\(\mu\)) is the result of an efficiency in photosynthetic utilization of light absorbed by fucoxanthin about equal to the efficiency of utilization of light absorbed by the chlorophyll system. The results of this investigation appear to be in accord with Tanada's conclusion.
CONCLUSION

The relation between the action spectrum of photosynthesis and the thallus absorption spectrum for *Skeletonema costatum* (Greville) Cleve corresponds to expectations based on Tanada's values for the quantum yield of photosynthesis for the diatom *Navicula minima*. 
Figure 1. Experimental Apparatus.

1. Xenon arc source
2. Spherical mirror
3. Ceramic magnet
4. Carjack, circa 1938
5. Grating monochromator
6. Condenser
7. Thermopile
8. Cardboard tube
9. First surface mirror
10. Plexiglass window
11. Microvolt-ammeter
12. Water bath
13. Controlled temperature water supply
14. Spectrophotometer cell
Figure 2. Dark $^{14}$C Uptake.
Figure 3. Light Curve of Photosynthesis.
Figure 4. A. Action Spectrum of Photosynthesis.
B. In vivo Thallus Absorption Spectrum.
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<th>Reference</th>
<th>Maxima found, m(\mu)</th>
<th>Solvent</th>
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<td>663, 616, 431, 411</td>
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<td>663, 616, 537, 418</td>
<td>Acetone</td>
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<tr>
<td>Fucoxanthin</td>
<td>453</td>
<td>3</td>
<td>453</td>
<td>Ethanol</td>
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<td>Diadinoxanthin</td>
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<td>3</td>
<td>448, 478</td>
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<td>(\beta) carotene</td>
<td>454, 480</td>
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<td>453, 480</td>
<td>Acetone</td>
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3. Strain, Manning and Hardin (1944)
BIBLIOGRAPHY


