INHIBITION OF PHOTOSYNTHESIS IN CERTAIN ALGAE BY EXTREME RED LIGHT

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ABSTRACT This paper shows that in *Porphyridium cruentum* and in *Chlorella pyrenoidosa* (but apparently not in *Anacystis nidulans*) "extreme red" light (> 720 mμ) can inhibit photosynthesis produced by "far red" light (up to 720 mμ). From the action spectrum of this phenomenon, it appears that an unknown pigment with an absorption band around 745 mμ must be responsible for it.

INTRODUCTION

It was shown in preceding papers (9, 10) that when the unicellular red alga *Porphyridium cruentum* is illuminated with light filtered through a sufficiently dense aqueous solution of mixed phycobilins (filter PB-95), no photosynthesis can be observed.[1] However, the addition of orange supplementary light induces a definite photosynthetic activity attributable to this "far red" light (9, 10): the rate of photosynthesis in orange + far red light is found to be markedly greater than in orange light alone. The occurrence of this effect (5, 6) indicates that phycobilin-filtered light does produce some excitation of chlorophyll *a*. As a matter of fact, PB-95-filtered light contains wavelengths down to about 660 mμ (9, 10), while chlorophyll *a in vivo* absorbs up to 720 mμ. According to Emerson and co-workers (1, 5, 6), these algae, when exposed to monochromatic light (bands isolated by a grating monochromator, band half-width about 10 mμ), do in fact produce measurable photosynthesis up to 700 mμ.

To resolve this apparent discrepancy, it was suggested that the "extreme red" radiation,[2] which is present in the PB-95-filtered light, may be able to *inhibit* photo-

[1] For the transmission spectrum of this filter, see reference 10.

[2] In order to distinguish between the "long-wave" light used in the study of the Emerson effect, and the inhibitory radiation of still longer wavelengths, we define as "far red" the radiations with wavelengths 680 to 720 mμ (the long-wave limit of chlorophyll *a* absorption), and as "extreme red" the radiations extending from 720 up to 800 mμ.

synthesis caused by shorter-wave light. To check this assumption, we investigated the effect of monochromatic "extreme red" light on photosynthesis produced by "far red" light.

METHODS

Algae were cultivated as described in earlier papers from this laboratory (for a summary, see Govindjee *et al.* (7)) and photosynthesis was measured with Emerson's differential manometer (4).

Two light beams were projected onto the bottom of the manometric vessel. The far red beam, isolated from the light of an incandescent lamp by an interference filter (Farrand filter with maximum transmission at 700 mμ), served to produce photosynthesis. The extreme red beam consisted of monochromatic bands (band half-width, about 10 mμ) emerging from the Emerson and Lewis grating monochromator. Light energy was measured with a bolometer, as described by Emerson and Chalmers (4).

The results in Tables I and II were reduced to equal numbers of absorbed quanta by means of absorption measurement with an integrating spectrophotometer built and operated by C. Cederstrand.

RESULTS

A. *Preliminary Experiments.* To make sure that the apparent discrepancy described in the introduction, was not due to differences in the algal material used in the two sets of experiments, we made an experiment in which the same cell suspension was irradiated alternatively with a 10-mμ wide band of 680-mμ light, and with light transmitted through the PB-95 filter. The results, adjusted to equal numbers of quanta absorbed from both beams, are given in Table I.

Table I shows that, in one and the same cell suspension, 680-mμ light produces substantial photosynthesis while absorption of the same amount of light filtered through the PB-95 filter, is unable to do so.

With this point established, we proceeded to check the hypothesis attributing

TABLE I

PHOTOSYNTHESIS OF *Porphyridium cruentum* IN LIGHT OF 680 mμ AND IN LIGHT TRANSMITTED BY PB-95 PHYCOBILIN FILTER

Oxygen evolution, μl/hr.	
In 680-mμ light	In light transmitted by PB-95
+0.26	−0.55
+0.32	−0.26
+0.39	+0.22
+0.74	+0.18
+0.73	+0.21
Mean +0.49 ± 0.10	−0.04 ± 0.15

The actual rate of absorption of light transmitted through filter PB-95 was 0.1 to 0.2 μeinstein/hour, depending on the density of the cell suspension; the rate of absorption of light from the 680-mμ beam was about six times higher. After ascertaining that light saturation was not approached in the latter case, the values obtained in the 680-mμ beam were divided by a factor of 6 for comparison with those obtained in the PB-95-filtered beam.

the inefficiency of phycobilin-filtered light to inhibition of photosynthesis produced by far red light, by extreme red radiation. Radiation at 750 mμ was found in fact to have a marked depressing effect on photosynthesis produced by light of 680 to 720 mμ; this was reported in a preliminary note (8). The extent of inhibition varied for different cultures, as shown in Table II. These variations presumably are due to slight differences in the culturing conditions. Although these conditions were kept as constant as possible, minor variations undoubtedly have occurred, as indicated by perceptibly different growth rates of the various cultures. The physiological age of the cells used was therefore not exactly the same, despite equal periods of cultivation.

B. *Action Spectrum of the Inhibition Effect.* The action spectrum of the inhibitory effect was then studied. First, the rate of photosynthesis in far red light was determined as follows. Two series of measurements, each consisting of eight 1-minute readings in the dark, were alternated with two similar series of readings in the presence of far red light. The first three readings in each set were always discarded. If after 3 minutes, the steady state was not reached, the measuring period was extended until five consistent readings were obtained. The photosynthetic rate in far red light was calculated by subtracting the average of the dark readings from that of the readings in far red light. During each inhibition experiment, this result was checked at regular intervals—as a rule, once an hour.

Two series of readings with extreme red light were then alternated with two series of readings in which this light was combined with far red light. The difference between the averages of the two series was assumed to be the "light action" of the

TABLE II

INHIBITION BY EXTREME RED LIGHT OF PHOTOSYNTHESIS PRODUCED BY FAR RED LIGHT

The pairs of values in each horizontal line are reduced to equal rates of absorption of light quanta.

Rate of photosynthesis, μl O_2/hour		
Far red light 680 to 720 mμ	Far red light + extreme red light 750 mμ	Inhibition per cent
Porphyridium cruentum		
4.01	2.23	44
1.87	0.29	85
1.85	0.29	84
3.30	2.04	38
1.08	0.84	22
Chlorella pyrenoidosa		
2.61	1.98	24
2.52	1.80	29
2.52	2.07	18

far red light in the presence of extreme red light. The per cent difference between this light action and that measured in far red light alone is plotted for the red alga *Porphyridium cruentum* in Fig. 1 as function of wavelength. All the experiments performed with this species are included in this figure.

The efficiency of far red light appears in Fig. 1 to be *enhanced* by supplementary light of 700 to 720 mμ. This is readily explained because our "far red" light, trans-

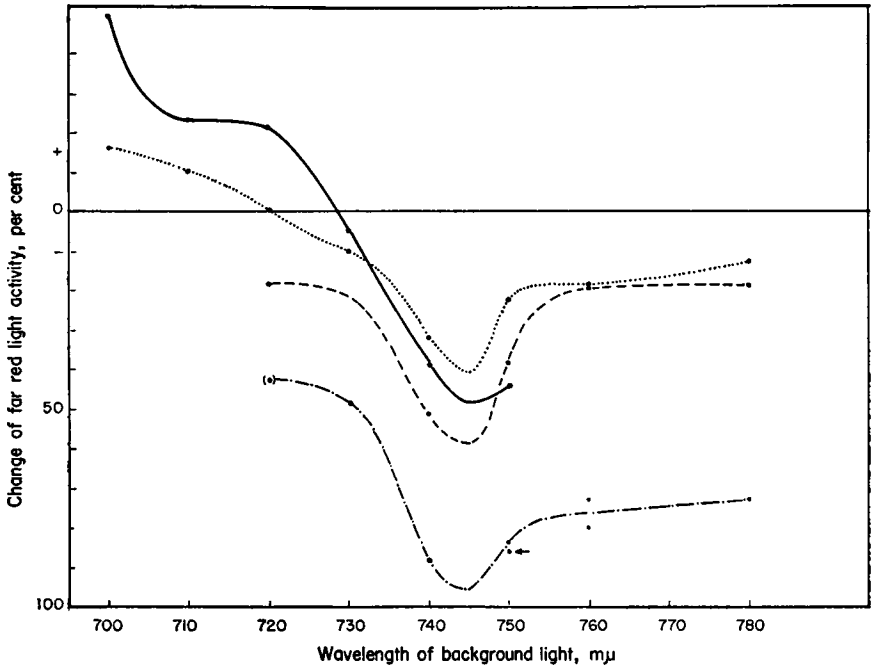

FIGURE 1 Action spectra of stimulation and inhibition of photosynthesis in *Porphyridium cruentum* caused by the addition of monochromatic light bands (half-width, 10 mμ) of different wavelengths, to far red light of about 700 mμ. Arrow indicates a separate experiment. Brackets (.) designate a less reliable measurement. Curves are drawn through the experimental points in as symmetrical way as consistent with the data. The suggested location of the minima is therefore somewhat arbitrary.

mitted by the 700-mμ interference filter, represents a relatively broad band (half-width, about 20 mμ), which includes some light considerably below 700 mμ. In other words, what is measured between 700 and 720 mμ in Fig. 1 is simply the previously known "Emerson effect."

Supplementary light of 730 to 780 mμ, on the other hand, *decreases* the photosynthetic efficiency of far red light; the effect is strongest at 740 to 750 mμ. The reason why the inhibition does not entirely disappear at still longer wavelengths may lie in the distortion of the spectrum in this region, where the resolving power of our grating becomes increasingly poor.

Similar observations with *Chlorella pyrenoidosa* are illustrated by Fig. 2, which

shows three typical experiments. Why the minimum in the curves is sometimes shallow and sometimes deep (between 20 and 100 per cent inhibition) and why it is sometimes found at 740 mμ and sometimes at 745 mμ, we do not know, except that the results suggest differences between individual cultures.

So far, no similar inhibition effect could be observed with suspensions of the blue-green alga *Anacystis nidulans*.

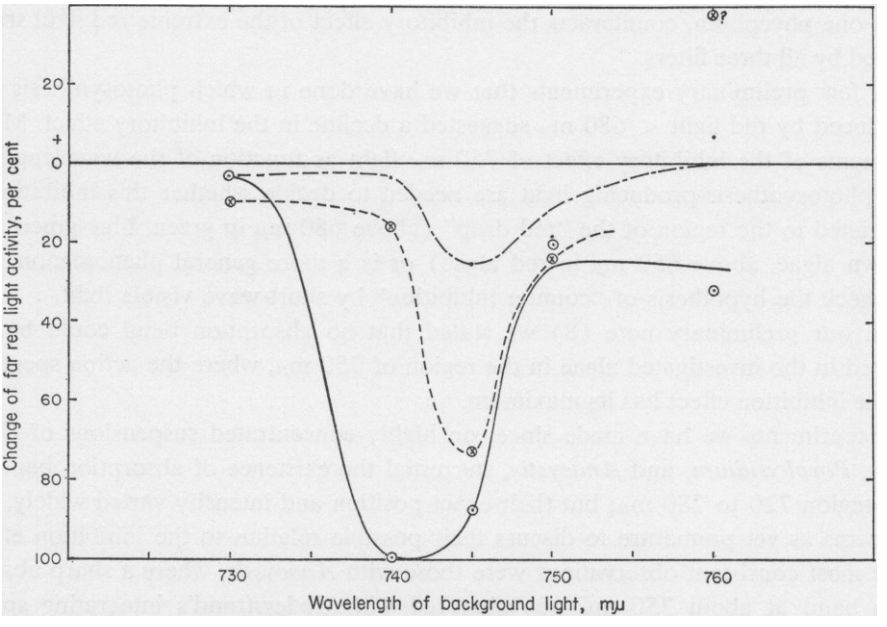

FIGURE 2 Action spectra of the inhibition of photosynthesis in *Chlorella pyrenoidosa* caused by the addition of monochromatic light of different wavelengths to "far red" light of about 700 mμ. ⊗ ? is a questionable point. Curves are drawn in the same way as in Fig. 1.

DISCUSSION

The above described experiments suggest the existence in some algae of a pigment with an absorption maximum at 740 to 750 mμ, whose excitation can inhibit photosynthesis caused by far red light (680 to 720 mμ).

The question arises: how can cells photosynthesize with high yield in white light, which usually contains considerable amounts of the "inhibitory" 740 to 750 mμ radiation? In the first place, it remains to be seen whether the inhibition affects only the photosynthetic action of far red light (> 680 mμ), or extends to shorter wavelengths. A preliminary experiment suggested that no inhibition occurs when photosynthesis is produced by *blue* light. Shorter-wave radiations may conceivably counteract the inhibitory effect of the 740 to 750 mμ radiation. At least, this conclusion is suggested by earlier experiments (*cf.* reference 10), in which light trans-

mitted through either a solution of phycocyanin (without phycoerythrin) or a solution of phycoerythrin (without phycocyanin) was found to produce measurable photosynthesis in *Porphyridium,* even though about the same amount of extreme red radiation was transmitted through these filters as through the filter PB-95 behind which no measurable photosynthesis could be observed. This difference, left unexplained in (10), can be now explained by assuming that the much larger amount of shorter-wave light (< 650 mμ), transmitted by the filters containing only one phycobilin, counteracts the inhibitory effect of the extreme red light transmitted by all three filters.

A few preliminary experiments that we have done in which photosynthesis was produced by red light < 680 mμ suggested a decline in the inhibitory effect. Measurements of the inhibitory effect of 750 mμ light as function of the wavelength of the photosynthesis-producing light are needed to decide whether this inhibition is restricted to the region of the "red drop" (above 680 mμ in green, blue-green, and brown algae, above 650 mμ in red algae) or is a more general phenomenon, and to check the hypothesis of "counter inhibition" by short-wave visible light.

In our preliminary note (8) we stated that no absorption band could be observed in the investigated algae in the region of 750 mμ, where the action spectrum of the inhibition effect has its maximum.

Experiments we have made since, on highly concentrated suspensions of *Chlorella, Porphyridium,* and *Anacystis,* suggested the existence of absorption bands in the region 720 to 780 mμ; but their exact position and intensity varied widely, and it seems as yet premature to discuss their possible relation to the inhibition effect. The most consistent observations were those with *Anacystis,* where a sharp absorption band at about 750 mμ was observed with Cederstrand's integrating sphere spectrophotometer[8] and with the Beckman DU spectrophotometer; but this was the alga in which we have not yet found any evidence of an inhibition effect.

Using the Cary spectrophotometer to compare the absorption spectra before and after illumination with light of different wavelengths, we have observed increases of absorption in the region of 700 to 780 mμ and decreases in absorption in region of 620 to 680 mμ caused by preillumination with red light (660 mμ). This made us think of the findings of Butler and co-workers (2, 3) who reported that a "phototropic" pigment, absorbing at 660 or at 730 mμ, depending on light exposure, is present in (and can be extracted from) plant organs showing photomorphogenic effects. However, the position of the peaks in the difference spectrum and their intensity varied too much to warrant speculations on the existence of a similar "phototropic" pigment in the investigated algae without further systematic study.

The inhibitory effect of extreme red light on photosynthesis suggests that this light creates some kind of an "energy sink." A "physical" energy sink might be

[8] Details of instrument will be published later by Cederstrand.

created by chlorophyll being converted to an inactive form, with a lower excited energy level; or by a pigment other than chlorophyll being converted to a form suitable for trapping the excitation energy. A "chemical" energy sink might be due to deactivation, by extreme red light, of some enzyme or another catalytic component involved in photosynthesis; or to activation of an agent catalyzing some back or side reactions. Perhaps, crystalline chlorophyll, which has an absorption band around 740 mμ, is present in these algae in minute quantities, and provides the "energy sink" responsible for inhibition.

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