Flash Spectroscopy of Porphyridium 1, 2

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A current question concerning the role of the 515 μ meter absorption change in photosynthesis is its occurrence in various classes of organisms. Duyseens (2) first reported a reversible increase in absorption at 515 μ meter in Chlorella irradiated with red light. The predominant change in Porphyridium cruentum (3) was a decrease in absorption at 420 μ meter, attributed to the photooxidation of cytochrome f or a similar cytochrome; only a minor change was observed in this organism at 515 μ meter.

Witt (7), using the flash technique, observed a fast, reversible increase in absorption at 515 μ meter in Chlorella and other green plants. Müller et al. (6) summarized evidence indicating that absorption changes at 515 and 475 μ meter are closely related to the photoreduction of plastoquinone, stating that these changes could be observed only in green plants. They also tabulated various other absorption changes observed in the flash spectroscopy of chloroplasts and algae.

Kok (5) used a rotating-disk technique to investigate light-induced absorption changes in various classes of photosynthetic organisms. He reported on an incidental observation with a very dilute suspension of Porphyridium (5).

The objective of this study was to investigate flash-induced absorption changes in Porphyridium cruentum, especially the occurrence of the 515 μ meter change.

Materials and Methods

Porphyridium cruentum was grown in SWM medium (Allen, M. B. List of cultures maintained by the Laboratory of Comparative Biology, Kaiser Foundation Research Institute, 1960), with the omission of agar. Chlorella pyrenoidosa, Emerson strain was grown in a medium prepared essentially as described by Allen (1). Air containing 4% CO2 was bubbled through the cultures, which were illuminated with fluorescent light in a 12 hour light-12 hour dark cycle and maintained at 24°. The algae were concentrated by centrifugation and resuspended in 0.1 m NaHCO3 prior to measurements.

The apparatus has been described elsewhere (4, p 717). In these experiments the detector was an RCA 1P28 multiplier phototube. The algal suspension was excited with 1 millisecond (to 1% of peak height) flashes of red light (>625 μ meter—Corning filter 2–58). In Porphyridium, phycocyanin was excited as well as chlorophyll, though to a lesser extent. A Corning 5–56 filter was located between the algal suspension and the phototube, to exclude most of the actinic light and luminescence. The absorption changes were measured with a monochromatic detecting beam having a half-width of 5 to 11 μ meter at the wavelengths corresponding to the principal (statistically significant) peaks. The output of the phototube as a function of time was displayed on an oscilloscope screen. Measurements were made on magnified photographs of the oscilloscope trace.

Results and Discussion

The spectrum of flash-induced absorption changes in Porphyridium is shown in figure 1, where the initial magnitude of the absorption changes following a flash is plotted (mean of 3 spectra). Owing to the variability encountered in such measurements (see table I), we performed t tests to determine the statistical significance that could be attached to the maxima and minima in figure 1 (where |ΔA| ≥ 1 × 10^-4). The probability of obtaining a value equal to that found for a given peak, if the true mean is 0, is given in table I, where mean values of the various absorption changes are compared with values esti-

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1 Received Jan. 27, 1964.
2 This research was supported by the Air Force Office of Scientific Research under Grant AF-AFOSR-349-63.
mated from Duysens’ steady-state difference spectrum (3).

The absorption changes found were similar to those reported by Duysens. In both spectra, the major feature was a negative peak at 420 mμ, while the second largest peak, near 400 mμ, was positive. Statistical evidence (table I) indicated that a real positive absorption change occurred at 520 mμ. The changes observed were generally smaller than those found by Duysens, especially when the absorbance of the cell suspension is considered. His pretreatment, keeping the cells in the dark for about half a day in order to enhance the changes, may partially account for these differences.

The half-lives of the back reactions through which the various pigment systems are restored in the dark are given in table I for changes statistically significant at the 5% or higher level; the decay was exponential (single component), as indicated by 3 plots of the logarithm of the change vs. time for each wavelength.

The difference between half-lives at 420 and 450 mμ was not statistically significant. This indicates that both changes may involve the same pigment, apparently cytochrome f or a similar cytochrome oxidized in the light (3, 6). A positive peak at about 400 mμ and a smaller negative peak at 555 mμ are also expected when cytochrome is oxidized (3, 6). A small change consistently observed at 550 mμ approached but did not reach the 5% level of significance (table I). The change at 395 mμ decayed with a much shorter half-life than that found at 420 mμ (the probability of obtaining such a large difference in half-lives if the true difference were 0, determined from a t test, was less than 0.005). Since the decay curves did not suggest more than one component of the same sign, it would appear that the 395 mμ peak is not simply related to the change at 420 mμ. The discrepancy in half-lives could, however, be explained, if a greater positive change at 395 mμ were partially compensated by a longer-lived negative change. It is readily shown that the decay of such a net change would be nearly exponential for several pairs of values of the magnitude and half-life of the negative change. However, since no direct evidence has been brought forth for such a negative change, this explanation is merely tentative.

The half-life of the back reaction at 520 mμ, 11 milliseconds, is in good agreement with the values of other investigators for Chlorella (5, 6). The relative absorbance change at 520 mμ, ΔA/ΔA_580 was 2.4 × 10⁻⁴ (A_580 = 1.29). In similar measurements with Chlorella, a mean absorbance change of 13.3 × 10⁻⁴ was obtained at 515 mμ, which corresponds to ΔI/I = 0.003 in Witt’s terminology (8, p 201); ΔA/ΔA_580 was 3.0 × 10⁻⁴, nearly the same as in Porphyridium.

The ratio of the 520 mμ peak height to the major peak at 420 mμ in Porphyridium, 0.32, was 4 times that estimated from Duysens’ spectrum. The 520 mμ change was not accompanied by a smaller negative change at about 475 mμ as in Chlorella (2, 6). The anticipated change at 475 mμ is apparently compensated by the broad positive change between 435 and 500 mμ accompanying the photooxidation of cytochrome (3). The magnitude of the minimum between the 450 and 480 mμ maxima, present in both our spectrum and that of Duysens, is in agreement with this hypothesis. In accord with expectation the 520 mμ peak was broad in Porphyridium. The half band width, 26 mμ, closely compares with values estimated for Chlorella (2, 5, 6). These results suggest that the 520 mμ pigment plays a similar role in the photosynthesis of green and red algae.

Several factors may account for the less conclusive findings of other investigators (3, 5) bearing on the 520 mμ change in Porphyridium. Thus, it appears that our set of conditions was more favorable for the operation of the 520 mμ pigment system than that selected by Duysens. A better understanding of the effects of genotype, culturing conditions, pretreatment (3), suspension density (5), and of the experimental arrangement may eventually reconcile such seemingly contradictory findings.

**Summary**

The spectrum of flash-induced absorption changes in Porphyridium agreed qualitatively with Duysens’ steady-state difference spectrum.

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Table I. Flash-Induced Absorption Changes in Porphyridium Compared with the Difference Spectrum of Duysens

<table>
<thead>
<tr>
<th>Wavelength mμ</th>
<th>ΔA × 10⁻⁴</th>
<th>Probability p of obtaining value of ΔA found if true mean is 0</th>
<th>Half-life of back reaction millisecond</th>
<th>From Duysens (3) ΔA × 10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>395</td>
<td>+ 5.7 ± 0.8</td>
<td>0.025 &gt; p &gt; 0.010</td>
<td>5.0 ± 0.4</td>
<td>+ 13 at 400 mμ</td>
</tr>
<tr>
<td>420</td>
<td>- 9.6 ± 0.3</td>
<td>0.005 &lt; p &lt; 0.001</td>
<td>10.2 ± 0.5</td>
<td>- 40</td>
</tr>
<tr>
<td>450</td>
<td>+ 3.4 ± 0.4</td>
<td>0.025 &gt; p &gt; 0.010</td>
<td>8.9 ± 2.3</td>
<td>+ 4</td>
</tr>
<tr>
<td>480</td>
<td>+ 2.5 ± 0.7</td>
<td>0.100 &gt; p &gt; 0.050</td>
<td>...</td>
<td>+ 3</td>
</tr>
<tr>
<td>500</td>
<td>- 1.8 ± 0.6</td>
<td>0.100 &gt; p &gt; 0.050</td>
<td>...</td>
<td>0</td>
</tr>
<tr>
<td>520</td>
<td>+ 3.1 ± 0.6</td>
<td>0.050 &gt; p &gt; 0.025</td>
<td>11.0 ± 0.8</td>
<td>+ 3</td>
</tr>
<tr>
<td>550</td>
<td>- 1.5 ± 0.7</td>
<td>0.200 &gt; p &gt; 0.100</td>
<td>...</td>
<td>- 7 at 555 mμ</td>
</tr>
<tr>
<td>560</td>
<td>+ 1.0 ± 0.2</td>
<td>0.100 &gt; p &gt; 0.050</td>
<td>...</td>
<td>+ 2 at 565 mμ</td>
</tr>
</tbody>
</table>

* Mean of 3 determinations ± standard error.
The major absorption change attributed to the photooxidation of cytochrome, a negative peak at 420 m\(\mu\)m, was the predominant change in Porphyridium. A smaller positive peak at 450 m\(\mu\)m, associated with cytochrome, was also observed. A positive change at 395 m\(\mu\)m, attributed to cytochrome, decayed with a significantly shorter half-life than that at 420 m\(\mu\)m. A tentative explanation was advanced.

Several lines of evidence were presented for the similarity of the 520 m\(\mu\)m absorption change observed in Porphyridium to the 515 m\(\mu\)m change in Chlorella.

**Literature Cited**


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**Conditions Determining Effects of Far-Red and Red Irradiations on Flowering of Pharbitis nil**

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Flowering of Pharbitis nil Chois. (Ipomoea nil L. Roth.) seedlings occurs in short photoperiods but is inhibited by far red at the end of the photoperiod (7, 10). Dependence of this response on conditions during the photoperiod was appreciated but had not been examined. The purpose here was to investigate the conditions during the photoperiod that influence flowering and to examine the nature of phytochrome action both immediately after the photoperiod and at various later times in the dark period.

**Materials and Methods**

Seed of *P. nil* for these experiments was from a supply used by Nakayama et al. (7). The seeds were macerated for about 40 minutes in concentrated sulfuric acid, then rinsed overnight in water, sown in wet perlite the next morning, and placed in an incubator at 27.5° in darkness. Forty-eight hours later the seedlings were transplanted to Knop's solution containing microelements and ferric citrate. For the 1962 experiments they were precultivated under continuous light for 2½ days in the greenhouse. The natural light was supplemented at night with incandescent-filament light, following the procedure of Nakayama et al. (7). For the 1963 experiments, to which the tables of this paper refer, the precultivation was done in a growth room with daily cycles of 10 hours of fluorescent light (1,000 ft-c) and 14 hours of incandescent light (80 ft-c). With these modifications seedling growth and chlorophyll formation were improved. The precultivation under continuous light was separated from the first inductive cycle by a short night of 7 hours. After 3 inductive cycles the seedlings were returned to the greenhouse, where they received continuous light until the flowering response was measured.

The average number of flower buds per plant and the number of plants bearing terminal buds, which are formed only under optimal conditions of induction, were recorded. There were 9, 12, or 18 plants per treatment. Control plants grown under continuous light after germination in the dark all remained strictly vegetative. Temperatures during the inductive cycles were 25° during the photoperiods and 22° during the nights.

The far-red radiation was obtained by filtering...