FLUORESCENCE CHANGES IN PORPHYRIDIUM EXPOSED TO GREEN LIGHT OF DIFFERENT INTENSITY: A NEW EMISSION BAND AT 693 mμ AND ITS SIGNIFICANCE TO PHOTOSYNTHESIS

By Anne Krey and Govindjee

DEPARTMENT OF BOTANY, UNIVERSITY OF ILLINOIS, URBANA

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Photosynthesis is now widely supposed to require two light reactions for the transfer of one hydrogen atom from water to carbon dioxide.[1] These two reactions may take place in one and the same reaction center,[2] or in two reaction centers.[3, 4] There is evidence, derived from difference spectroscopy,[5] for the existence in the pigment system of certain algae of one "trap," known as P700. It seems to be associated with "reaction I" (reduction of ferredoxin and oxidation of cytochrome). No clear evidence exists for another trap, associated with "reaction II" (reduction of cytochrome and oxidation of water). The results presented below support the existence of this second trap.

With increase in intensity of green light, I_g, the differential fluorescence yield of Porphyridium (dF/dI_g) increases from a lower constant value to a higher constant value, reached when photosynthesis becomes saturated. Figure 1 shows the intensity of fluorescence (measured at 693 mμ by a newly constructed fluorometer[6]) plotted versus intensity of exciting light (544 mμ) for the red alga, *Porphyridium cruentum* (O.D. at 680 mμ = 0.03) suspended in Warburg's carbonate-bicarbonate buffer #11 (with added NaCl). In order to reach the high-intensity range, two light sources of the same spectral composition had to be used simultaneously. One light beam was from an incandescent lamp, filtered through a 544-mμ Bausch and Lomb interference filter (half-band width, 10 mμ) and a green glass filter Schott VG5. The second, also from an incandescent lamp, was obtained by a Bausch and Lomb monochromator (grating size 100 mm × 100 mm); the monochromator, too, was adjusted to provide 544-mμ (half-band width, 10 mμ) green light. The measuring monochromator (with a band pass of 2 mμ) was provided with a sharp cut-off red glass filter (Corning 2–63) in front of the entrance slit. Fluorescence was detected by an EMI 9558-B phototube; the signal was amplified by a Keithley 150A Microvolt-Ammeter and recorded on a Brown recorder.

The results, shown in Figure 1, are in agreement with earlier observations of Franck,[7] Brugger,[8] and those of Duysens and Sweers,[9] Butler,[10] Teale,[11] and Rosenberg and Bigat.[12]

The ratio of the (differential) fluorescence yields at high and low intensities varies with the condition of the culture. Several experiments were made. In cultures (grown in low light intensities) which show a high quantum yield of photosynthesis (*ca.* 0.10), this ratio is 1.8 to 2.0 (at about 690 mμ), whereas in cultures (grown in high light intensities) with a lower quantum yield of photosynthesis (*ca.* 0.05), the ratio is about 1.2; in extreme cases the dF/dI curve becomes linear.

The question is whether the emission spectrum changes with the change in fluorescence yield, as we pass from "low" to "saturating" light. Figure 2 shows the spectral emission curves of Porphyridium (corrected for photomultiplier sensitivity and spectral efficiency of the monochromator), measured by excitation with the two

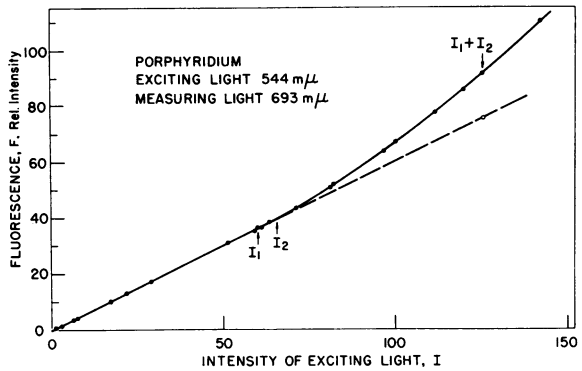

FIG. 1.—Fluorescence intensity (measured at 693 mμ) as a function of intensity of exciting green light (544 mμ) [$F = f(I)$]. Organism: red alga, *Porphyridium cruentum*. Temperature: 22°C. The light intensities used in obtaining the emission spectra shown in Fig. 2 are marked by arrows. I_1 and I_2 refer to the two low-intensity beams; $I_1 + I_2$, to the high-intensity beam. The dashed curve is an extrapolation of the lower linear portion of the $F = f(I)$ curve. The open circle is the expected point for $I_1 + I_2$ if $F = f(I)$ were linear; the difference between the expected (open circle) and the observed value is the additional fluorescence at high intensity.

separate green light beams (with intensities in the lower linear range of the curves in Figure 1; $I_1 = 60$ and $I_2 = 62.5$), and by excitation with the combined beams with total intensity in the upper range of the curve in Figure 1; $I_1 + I_2 = 122.5$). The difference spectrum, also shown in Figure 2 (on a 10 times expanded scale), is obtained by subtracting the sum of the emission spectra, obtained by exciting with the two low-intensity beams, from the emission spectrum obtained by exciting with the two beams together. If the shape of the emission spectrum remained unchanged at high intensity, one would see in the difference spectrum only a peak at 685 mμ. Instead, a new peak at 693 mμ, and two minor peaks, one at 669 mμ (positive) and another one at 660 mμ (negative), appeared. The last two minor bands suggest a shift of the fluorescence band of phycocyanin. Nine experiments were made on very pink-looking Porphyridium cells (having high quantum yield of photosynthesis) and they all showed the same results. Experiments made with DCMU-poisoned cells and with cells having low quantum yield of photosynthesis, do not show any difference emission band.

The fluorescence spectra, shown in Figure 2, have unusually high intensities in the long-wave region. Since the optical density of the algal sample used in these experiments was only 0.03 at 680 mμ, no complications, due to the selective reabsorption of fluorescence in the main red band of chlorophyll *a*, could be expected. The high intensity of fluorescence in the long-wave region has been observed also[6, 13] in the blue-green alga, *Anacystis nidulans*. In our experience, the fluorescence intensity in the long wavelength region in the red alga *Porphyridium cruentum* is consistently higher than in the green alga *Chlorella pyrenoidosa*. However, a combination of the relatively rapid decline in the sensitivity of the photomultiplier (EMI 9558-B) and the smaller magnitude of the signal beyond 730 mμ may have made the measurements in the long-wave region (>730 mμ) less reliable.

The 693-mμ Difference Emission Band in Porphyridium.—The fluorescence difference spectrum is quite different from the familiar fluorescence spectrum in two respects.

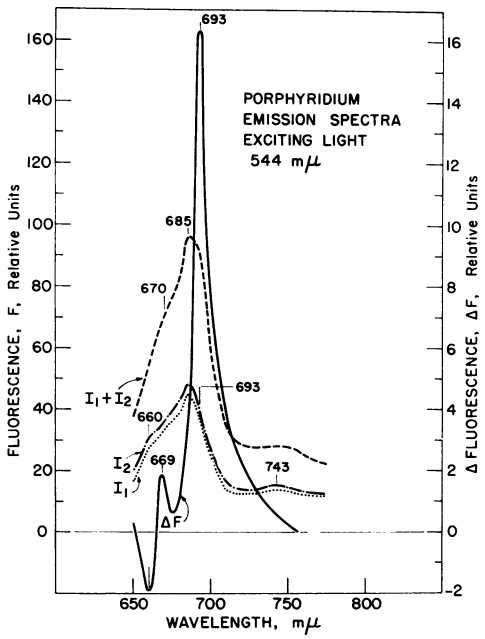

FIG. 2.—Emission spectra obtained in low intensities [I_1 (dotted), and I_2 (dashes and dots)] and in high intensity [$I_1 + I_2$ (dashes)] of green (544 mμ) light in *Porphyridium cruentum*. The solid line (ΔF) is the "difference emission spectrum" (see text).

First, the half-band width of the 693-mμ difference emission band is 10–15 mμ, in contrast to 30 mμ which is the half-band width of the 685-mμ emission band. Experiments with spinach chloroplasts[14] suggest that the 685-mμ band may be a complex band, composed of at least two bands. Because of this, a comparison of the sharpness of the 685-mμ and 693-mμ bands is not very meaningful. Still, the 693-mμ band appears remarkably sharp, and we do not quite understand why this is so. (The half-band width of chlorophyll *a* in solution is around 18 mμ.)

The second distinctive characteristic of the fluorescence difference spectrum is the location of the new band on the long-wave side of the 685-mμ band. This suggests that a pigment with an absorption maximum on the long-wave side of that producing the 685-mμ emission band is responsible for the 693-mμ emission band (*vide infra*).

The apparent absence of a vibrational satellite band needs to be confirmed.

When photosynthesis is light-saturated, light energy reaching the reaction center (energy trap) cannot be utilized efficiently for chemical purposes. It is logical to assume that this may lead to a loss of excitation energy, by fluorescence. If this were so, and if the absorption spectra of the trap and the bulk pigments were somewhat different, one would expect the emission spectrum, measured at high light intensity, to be different from the emission spectrum measured at low light intensity.

The 693-mμ difference band in the fluorescence spectrum can be tentatively attributed to fluorescence from trap II, and not from trap I, because the action spectrum of the stimulation of fluorescence, measured by Butler,[15] was that of system II. Green light causes excitation mainly of system II. If we accept the "separate-package model" of photosynthesis (cf. ref. 16), we expect to see also fluorescence

emanating mainly from system II. When the two pigment systems are "separated" by the methods of Boardman and Anderson,[17] the particles containing system II show a fluorescence band at 698 mμ at 77°K; this band is absent from particles containing system I only.[18] The 698-mμ emission band observed in whole cells[13] and chloroplast fragments[14] at liquid nitrogen temperature also may be due to system II (possibly to trap II); it might be caused by the same molecules that cause fluorescence at 693 mμ at room temperature, as observed in this report. Furthermore, trap I is believed[13] to have a fluorescence band at 718 mμ (at 77°K) and the distance between the 718-mμ and 698-mμ band is too wide to attribute both of them to the same species. In addition, the intensity of the 718 mμ and 698 mμ bands decreases at different rates when the cooled (77°K) sample is allowed to warm up gradually.[14] The 698-mμ band disappears by the time temperature has risen to about 140°K, whereas the 718-mμ band persists at still higher temperatures. This also suggests that these two bands at 77°K belong to different pigment complexes.

We suggest that the traps "I" and "II" behave differently in light-saturation. The trap "I" (P700) is bleached (probably photooxidized[3]); it remains bleached at high light intensity because its reduction is limited by a slow dark enzymatic reaction. This "trap" cannot produce excess fluorescence under these conditions. Trap "II," on the other hand, is assumed to be complexed with an oxidant (such as ferricytochrome; cf. Franck and Rosenberg[2]). It sensitizes the transfer of electrons from H_2O to the cytochrome, if the latter is in the oxidized state. When the cytochrome is accumulated in the reduced state in saturating light, no electron transfer takes place through the sensitizing chlorophyll to cytochrome, and the chlorophyll stays colored. We believe that the 693-mμ fluorescence band emanates from "photochemically frustrated" trap II under these conditions; the 685-mμ fluorescence band is due to the bulk of chlorophyll *a*. The above discussion implies that the 693-mμ difference emission band may be due to chlorophyll *a*. The question remains: How do we know that this band is not due to phycobilins?

If the 693-mμ fluorescence is "trap II fluorescence," its appearance must be universal, since the basic photosynthetic mechanism seems to be the same in all plants. We measured the fluorescence spectra of the blue-green alga *Anacystis nidulans*, excited with different intensities of 605-mμ light. Results similar to those described above for Porphyridium were obtained. A major difference peak at 693 mμ and minor peaks at 670 mμ (positive) and 658 mμ (negative) were clearly observed. Experiments with other organisms, including Chlorella and spinach chloroplasts, are in progress.

Summary.—A major band at 693 mμ appears when the fluorescence of Porphyridium excited by green light of high intensity is compared to that of the same cells excited by green light of low intensity. It is suggested that this new band may be due to "trap II" becoming fluorescent when photosynthesis is light-saturated.

The minor positive (669 mμ) and negative (660 mμ) bands, appearing at the same time, may be due to a shift of the fluorescence peaks of phycocyanin.

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*RNA METABOLISM IN PUPAE OF THE OAK SILKWORM, ANTHERAEA PERNYI: THE EFFECTS OF DIAPAUSE, DEVELOPMENT, AND INJURY**

BY ROBERT H. BARTH, JR., PETER P. BUNYARD, AND TERRELL H. HAMILTON†

THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

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In this communication we report a partial characterization of the RNA of the oak silkworm, *Antheraea pernyi*—specifically, its separation by means of sucrose gradient centrifugation into the heavier ribosomal fractions, and the lighter "messenger" and "transfer" fractions. We have further determined the relative rates of RNA synthesis by studying the incorporation of labeled uridine into the several fractions during pupal diapause, during the early stages of adult development, and following injury to diapausing pupae. Finally, we record the effects of actinomycin D on RNA metabolism under each of these conditions. Two different tissues are compared, viz., the wing hypodermis and the fat-body.

Materials and Methods.—(1) *Management of experimental animals, injuries, and in vivo labeling:* Pupae of *Antheraea pernyi* employed in these experiments were in one of two different physiological states: (*a*) diapausing pupae maintained by a 12-hr daily photophase at 25°C; and (*b*) lengthily chilled pupae with development blocked by storage at 2–3°C. Upon exposure to 25°C the latter pupae showed visible initiation of development within 48 hr. The early stages of development