RED DROP IN THE QUANTUM YIELD OF FLUORESCENCE OF SONICATED ALGAE

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ABSTRACT The change of the quantum yield of fluorescence, Φ, with the frequency of exciting light, was investigated in *Chlorella*, *Anacystis*, and *Porphyridium* suspensions, and in sonicates from these cells prepared under aerobic and anaerobic conditions. In case of *Chlorella*, sonicates were made in acid and in alkaline media (pH 4.65 and 7.80). In the alkaline medium, a drop of Φ towards the longer waves was found to begin at 1.466×10^4 cm^{-1} (682 nm) in sonicates, and in suspension. In the acid medium, the drop began at 1.471×10^4 cm^{-1} (680 nm), 1.418×10^4 cm^{-1} (705 nm), and 1.389×10^4 cm^{-1} (720 nm) in suspension, anaerobic sonicate, and aerobic sonicate, respectively. The results indicate that the cause of the change in the red drop is preferential destruction of a long-wave component of chlorophyll *a* (such as Chl *a* 693). The amount of this component remaining after sonication is larger in alkaline than in acid sonicates. With *Anacystis* and *Porphyridium*, only alkaline suspensions (pH 7.80) could be used for sonication, because in acid medium, the phycobilin-chlorophyll complex is rapidly broken and phycobilin extracted from the cell. In *Anacystis*, the red drop begins at 1.562×10^4 cm^{-1} (640 nm) and 1.538×10^4 cm^{-1} (650 nm) in suspension and sonicate, respectively; in *Porphyridium*, it starts at 1.550×10^4 cm^{-1} (645 nm) in both cases. These results suggest that sonication in alkaline medium (pH 7.80) destroys some Chl *a* 693 in *Anacystis*, but not in *Porphyridium*.

INTRODUCTION

The "red drop" of the quantum yield of fluorescence in *Chlorella* suspensions was first noted by Duysens (1); he observed it at 675 nm. Weber (2) found no such decline up to 680 nm; but Szalay et al. (3) again observed it, beginning at about 680 nm. According to Das and Govindjee (4), the drop is shifted from 680 to 720 nm upon sonication under aerobic condition at pH 4.60. They suggested that sonication causes preferential loss of a long-wave, nonfluorescent form of chlorophyll, Chl *a* 693 (probably identical with French's (5) Chl *a* 695). Sonication of *Chlorella* suspensions in an anaerobic, alkaline medium (pH 7.80) produced no similar shift of the red drop. Systematic investigations of the effect of sonication on the red drop

of *Chlorella pyrenoidosa*, *Anacystis nidulans*, and *Porphyridium cruentum* are presented in this paper.

EXPERIMENTAL

Culturing

Chlorella pyrenoidosa, *Anacystis nidulans*, and *Porphyridium cruentum* cultures were grown in an inorganic medium. *Chlorella* was grown over a combination of fluorescent and incandescent lamps, at 22°C; *Anacystis* over fluorescent and incandescent lamps at 25°C, and *Porphyridium* over fluorescent light at 18°C (see reference 6). Air containing 5% CO_2 was bubbled through the cultures. The cells were harvested after a 20–80-fold increase in population; 4–6 day old cultures were used.

Sonication

Before sonication, the algae were centrifuged for 5–15 min at 3950 rpm to precipitate all cells. The supernatant was rejected, and the residue resuspended, either in culture medium at pH 4.65 or in phosphate buffer (0.06 M Na_2HPO_4, 0.007 M KH_2PO_4, and 0.1 M KCl) at pH 7.80. The suspension (50 ml) was placed in the chamber of the sonicator (Raytheon ultrasonic generator, Model D. F. 101, Raytheon Co., Waltham, Mass.) operating at 10 kc and 250 w and sonicated for 1 hr. For anaerobic sonication, argon, purified by passage through alkaline pyrogallol, concentrated H_2SO_4, and water, and dried with calcium chloride, was bubbled through the suspension for 1 hr; the inlet and the outlet of the chamber were then closed and the suspension sonicated. With cooling by ice water, the "macroscopic" internal temperature in the sonication vessel stayed at about 14–15°C. The pH was not changed by sonication.

Spectral Measurements

Adsorption spectra of the suspensions and of the sonicates were measured in the integrating sphere attachment of a Bausch & Lomb 505 spectrophotometer (Bausch & Lomb Inc., Rochester, N. Y.). Fluorescence (which is measured together with transmission in the integrating sphere) was neglected, because the yield of fluorescence is only of the order of 3% in sonicates as well as in the suspension. The cell used for absorption measurements was 1 cm deep.

The *emission and the excitation spectra of fluorescence* were measured by means of a spectrofluorometer constructed in our laboratory (7–9) with a bandwidth of 6.6 nm. The absorbances for the path length of the exciting light in the fluorescence vessel (d = 0.1 cm) derived from absorbances measured for 1 cm path length, were converted to per cent absorptions. The emission spectra (excited by blue light, λ_{ex} = 440 nm) were corrected for the spectral response of the system: (photomultiplier EMI 9558B plus analyzing monochromator). The excitation spectra (determined for total emission) were corrected for spectral variations of the incident quantum flux. Variations in the emission spectrum associated with changes in the frequency of exciting light (see Williams et al.[1]), were neglected.

The relative quantum yields of total chlorophyll *a* fluorescence as function of wavelength of exciting light were obtained by dividing the ratio of fluorescence intensity to incident intensity, observed at each λ_{ex}, by the corresponding per cent absorption value. These relative quantum yields, Φ_λ/Φ_{680}, were plotted (Figs. 1, 2, 5, and 6) as functions of the wave number of the exciting light.

[1] Williams, W. P., N. R. Murty, and E. Rabinowitch. Paper in preparation.

The red drop of fluorescence was determined also by application of the Stepanov-Ketskeméty (10) theory, as described elsewhere (3). In this method, the function:

$$F(\bar{\nu}) = 3 \log \bar{\nu} - \log [f_e(\bar{\nu})/K(\bar{\nu})] = \frac{h\bar{\nu}c}{kT} \log e + \text{constant} \qquad (1)$$

(where $f_e(\bar{\nu})$ and $K(\bar{\nu})$ denote the relative emission intensities and absorption coefficients, and h, k, c, T are Planck constant, Boltzmann constant, velocity of light, and the temperature of the medium, respectively) is plotted against the wave number, $\bar{\nu}$. The plot appears linear in the shortwave region, but the linearity breaks down at the longer waves. Such deviations are due, according to Ketskeméty, to spectral variations in the quantum yield of fluorescence, $\Phi(\bar{\nu})$ (assumed to be constant in equation 1). One can determine the $\Phi(\bar{\nu})$-values by requiring function 2 to be linear.

$$F'(\bar{\nu}) = 3 \times \log \bar{\nu} + \log \Phi(\bar{\nu}) - \log [f_e(\bar{\nu})/K(\bar{\nu})] = \frac{h\bar{\nu}c}{kT} \log e + \text{constant}. \qquad (2)$$

Measurements were made on cell suspensions, aerobic sonicates and anaerobic sonicates' at pH 4.65 and at pH 7.80 in the case of *Chlorella*, and at pH 7.80 only in the case of *Anacystis* and *Porphyridium* (because at the lower pH the loss of phycobilins is too rapid).

RESULTS AND DISCUSSION

Chlorella pyrenoidosa

Figs. 1 and 2 show the directly measured red drop curves of fluorescence yield for pH = 4.65 and pH 7.80. In Fig. 2 (pH 7.80), the drop begins at about 1.466×10^4 cm^{-1} (682 nm); its course is practically the same in all three systems studied. In other words, in alkaline medium, 1 hr of sonication does not affect the red drop. In Fig. 1 (pH 4.65), however, important differences appear between the three curves. The red drop begins at about 1.471×10^4 cm^{-1} (680 nm) in the suspension and only at about 1.418×10^4 cm^{-1} (705 nm) in the acid anaerobic sonicate. In the acid aerobic sonicate, there is almost no red drop (at least, up to 1.392×10^4 cm^{-1}, i.e.

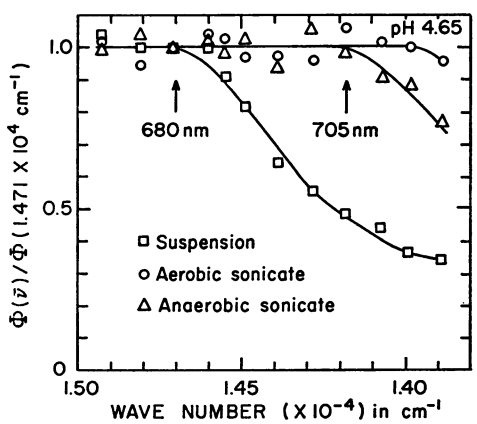

FIGURE 1 Relative quantum yield of fluorescence, $\Phi(\bar{\nu})/\Phi(1.471 \times 10^4$ cm$^{-1})$ calculated from the excitation and absorption spectra, for a suspension of *Chlorella pyrenoidosa* and its aerobic and anaerobic sonicates at pH 4.65.

718 nm). This increase in the quantum yield of fluorescence in the far-red part of the spectrum upon sonication at pH 4.65 suggests the loss (perhaps, by pheophytinization) of a nonfluorescent (or less fluorescent, see Williams et al.[1]) form of chlorophyll *a*, absorbing in the long-wave region (such as Chl *a* 693 or Chl *a* 695). These findings are in accordance with previously reported results (4).

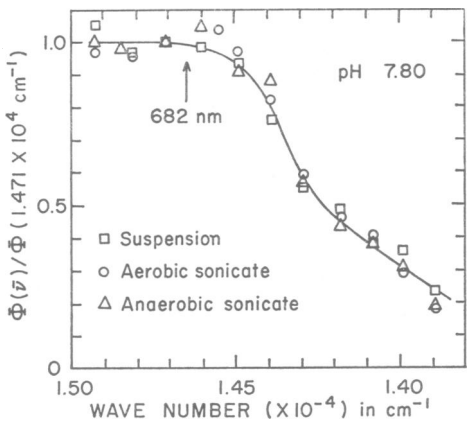

FIGURE 2 Relative quantum yields of fluorescence, $\Phi(\bar{\nu})/\Phi(1.471 \times 10^4\ cm^{-1})$ calculated from the excitation and absorption spectra for a suspension of *Chlorella pyrenoidosa* and its aerobic and anaerobic sonicates at pH 7.80.

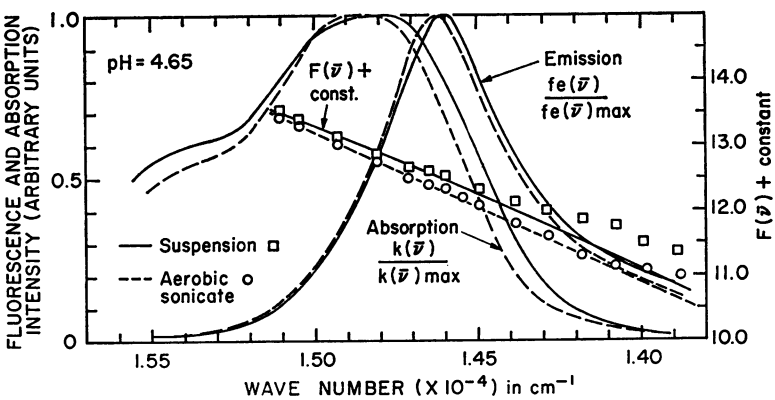

FIGURE 3 Spectra of absorption $[k(\bar{\nu})/k(\bar{\nu})_{max}]$ and of emission, $[f_e(\bar{\nu})/f_e(\bar{\nu})_{max}]$ of a suspension of *Chlorella pyrenoidosa* and of its aerobic sonicate (prepared at pH 4.65), in relative units; and the corresponding plots of $[F(\bar{\nu}) + \text{constant}]$ vs. $\bar{\nu}$ in equation 1.

At pH 4.65, the red drop of the quantum yield begins later in the aerobic than in the anaerobic sonicate. This suggests that oxidation of the "nonfluorescent" pigment component plays a certain role in this shift. However, at pH 4.65, the red drop is strongly shifted even in the anaerobic sonicate. This indicates that hydrogen ions in the medium play an even more important role in this effect. The smallness of the difference between the red drops in aerobic and anaerobic sonicate at pH 7.80 confirms that pH has more influence on the shift than O_2.

Figs. 3 and 4 show the absorption and emission curves of *Chlorella* suspension and aerobic sonicates, at pH 4.65 and 7.80; the red drop, calculated from these spectra according to equation 2, is shown by squares and circles in these figures. The spectra of anaerobic sonicates fall between the two curves shown in the figures.

The peak position in the absorption spectrum of alkaline sonicates (pH 7.80) remains unchanged by sonication, both under aerobic and anaerobic conditions; but in acid solution (pH 4.65) the peak is shifted in the aerobic sonicate from $1.479 \times 10^4\ cm^{-1}$ (676 nm) to $1.488 \times 10^4\ cm^{-1}$ (672 nm). In the anaerobic sonicate (pH 4.65), the peak lies between those of the suspension and the aerobic sonicate. These results again confirm that during sonication, both O_2 and hydrogen ions contribute to preferential destruction of the non- (or weakly-)fluorescent, long-wave form of chlorophyll *a*. The sharpening of the absorption band may be due, especially in

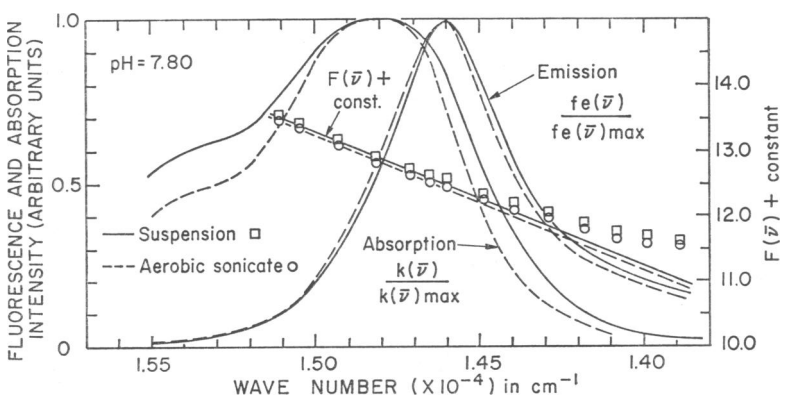

FIGURE 4 Spectra of absorption, $[k(\bar{\nu})/k(\bar{\nu})_{max}]$ and of emission, $[f_e(\bar{\nu})/f_e(\bar{\nu})_{max}]$ of a suspension of *Chlorella pyrenoidosa*, and of its aerobic sonicate (prepared at pH 7.80), in relative units; and the corresponding plots of $[F(\bar{\nu}) + \text{constant}]$ vs. $\bar{\nu}$ in equation 1.

alkaline sonicate, to the disappearance of the "sieve effect" upon sonication (as shown by Das et al. (11).

The peaks of all fluorescence spectra lie between $1.462 \times 10^4\ cm^{-1}$ and $1.458 \times 10^4\ cm^{-1}$ (684–686 nm), in good agreement with the value given previously (4) for the suspension and the aerobic sonicate. Thus, the emission spectrum is essentially unchanged by sonication under all conditions used. Even though sonication in acid medium destroys preferentially a minor pigment component, its disappearance does not affect substantially the fluorescence spectrum, because this component contributed only little to the emission prior to sonication. If the observed minor shift of the emission peak (by about 1.5 nm, indicated in Fig. 3) is real, it can be attributed to the disappearance of a weak fluorescence band (F700), belonging to Chl 693 (see Williams et al.[1]). After sonication in acid medium, the shift of the emission spectrum towards shorter wavelengths is more pronounced than in

alkaline medium—another indication that, in acid medium, Chl *a* 693 is destroyed more effectively than in alkaline medium (pH 7.80). Actually, 35–37% of the total pigment is destroyed by sonication in the acid medium (pH 4.65) as compared to only 8–10% in alkaline medium (pH 7.80).

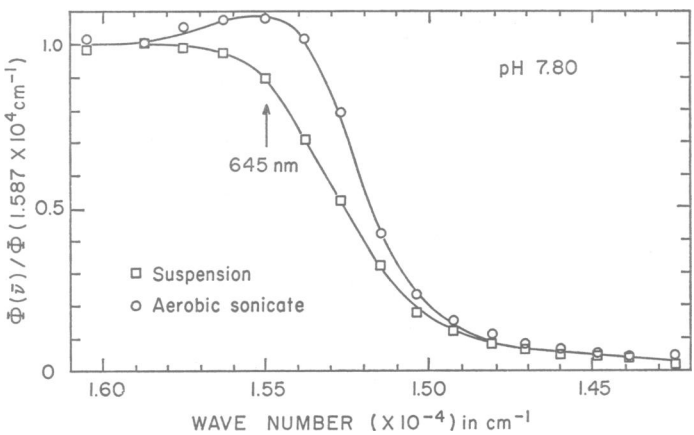

FIGURE 5 Relative quantum yields of fluorescence, $\Phi(\bar{\nu})/\Phi(1.587 \times 10^4\ cm^{-1})$, calculated from excitation and absorption spectra, for a suspension of *Anacystis nidulans* and for its aerobic sonicate prepared at pH 7.80.

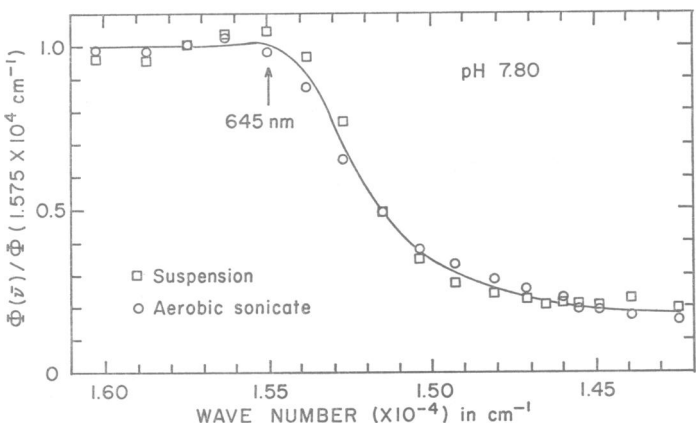

FIGURE 6 Relative quantum yields of fluorescence $\Phi(\bar{\nu})/\Phi(1.575 \times 10^4\ cm^{-1})$, calculated from the excitation and absorption spectra, for a suspension of *Porphyridium cruentum* and its aerobic sonicate prepared at pH 7.80.

The circles and squares in Figs. 3 and 4 denote the values of $F(\bar{\nu})$ + constant for the aerobic sonicates and the suspension, respectively; the straight lines are fitted to their positions in the shortwave region by the method of least squares. The location of the red drop derived from equation 2 appears to be in good agreement with the results of direct measurements (Figs. 1 and 2).

Closer analysis of the *shape* of the absorption band under different conditions may give additional information, e.g. about possible changes in the relative amounts and location of the absorption peaks of the two main components of chlorophyll *a* in vivo, Chl *a* 670 and Chl *a* 680 (see Cederstrand et al. (12).

Anacystis nidulans and Porphyridium cruentum

Figs. 5 and 6 show the directly measured red drop curves of fluorescence yield of *Anacystis* and *Porphyridium* cell suspensions and their aerobic sonicates at pH 7.80. Fig. 5 shows that in *Anacystis*, the red drop begins at about 1.562×10^4 cm^{-1} (640 nm) in the suspension, i.e. somewhat earlier than in the aerobic sonicate, where it begins at 1.538×10^4 cm^{-1} (650 nm). The red drop curve of anaerobic sonicate almost coincides with that of aerobic sonicate. In *Porphyridium* (Fig. 6), the red drop begins at about the same place, 1.550×10^4 cm^{-1} (645 nm), both in cell suspension and in the sonicate. As in *Anacystis*, the red drop curves of the aerobic and the anaerobic sonicate coincide.

These results suggest that even in alkaline medium (pH 7.80), on sonication, a small amount of a pigment (with an absorption band at or beyond 650 nm) is destroyed in *Anacystis*, while in *Porphyridium*, sonication has no such effect. The latter finding is analogous to that obtained with *Chlorella*. The function Φ for *Anacystis* and *Porphyridium* was calculated also from equation 2. The results again were in good agreement with those obtained from direct measurements.

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