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ENERGY TRANSFER BY CHLOROPHYLL *b* IN DETERGENT MICELLES

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SUMMARY

The concentration-dependent depolarization, concentration-dependent quenching, absorption and fluorescence spectra in solutions of chlorophyll *b*-containing detergent micelles with Triton X-100 were studied in a concentration range of $c = 0.4\ \mu M$–$0.6\ mM$ chlorophyll *b* and $c_D = 0.4$–$7.0\ mM$ Triton X-100. The concentration-dependent depolarization obeys Förster's theory of depolarization of fluorescence with a transfer distance parameter $R_0 = 43 \pm 2$ Å. The concentration-dependent quenching is described by an empirical formula for the relative fluorescence yield $\eta/\eta_0 = 1/[1+(c/c_{\frac{1}{2}})^2]$ given by Kelly and Porter (Kelly A. R. and Porter, G. (1970) Proc. R. Soc. Lond. Ser. A. 315, 149–161). With increasing chlorophyll b concentration the red absorption band at 650 nm is shifted toward a longer wavelength and its width increases by 10 nm, the intensity of the long wave fluorescence band increases about 720 nm. The results analysed in terms of these findings lead to the conclusions that chlorophyll *b* molecules are (a) locally concentrated in the micelles up to the concentration range of in vivo conditions, (b) partly in an aggregated state capable for fluorescence, (c) the chlorophyll *b* → chlorophyll *b* homotransfer may be about 3–26 % of the homotransfer chlorophyll *a* → chlorophyll-*a* depending on the ratio of their concentrations.

INTRODUCTION

Pigment-containing detergent solutions have been found to be suitable for studying the photochemical properties of chlorophylls [1–3] and energy transfer between organic dyes [4, 5] as well as between photosynthetic pigments [6, 7]. This advantage of detergent solutions is based on the property of detergent molecules forming micelles at the critical micelle concentration and water-insoluble pigments may be easily incorporated into the micelles. Studies of energy transfer from lutein to chlorophyll *a* [8] as well as between chlorophyll *a* molecules only [9] led to the conclusion that pigment-containing detergent micelles serve as good models of the separate package-type photosynthetic unit.

Energy transfer between chlorophyll *b* molecules [10–13] received relatively less attention than heterogeneous transfer from chlorophyll *b* to chlorophyll *a* or homogeneous transfer between chlorophyll *a* molecules. The aim of the present in-

vestigation was to study the mechanism of energy transfer between chlorophyll *b* molecules incorporated into micelles of the detergent Triton X-100.

METHODS

Chlorophyll *b* was extracted from fresh spinach leaves and purified by chromatography on a sugar column [14]. The pigment was accepted to be pure when the ratio between the blue and red absorption peaks after successive purification reached the value of 2.9–3.0 in diethyl ether [15]. The preparation of micellar solutions of chlorophyll as well as the description of measurements and their evaluation has been given earlier [9]. For the polarization measurements, chlorophyll *b* fluorescence was excited at 640 nm and observed at 662 nm with the excitation and emission monochromators of the spectrofluorimeter MPF-3 set at 8 and 6 nm, respectively. Under these conditions the intensity of scattered light is within the range of instrumental error and does not affect the measurements. For measurements of the relative fluorescence yield, fluorescence was excited at 466 nm. Fluorescence spectra were corrected for reabsorption [16] and the spectral sensitivity of the measuring apparatus. The absorption spectra were recorded on a UNICAM SP 1800 Spectrophotometer. All measurements were carried out at 30 °C.

RESULTS AND DISCUSSION

In our previous paper [9] on chlorophyll *a* fluorescence depolarization, it was shown that this process occurring between pigments incorporated into detergent micelles is caused by energy transfer between pigment molecules within the same micelle. The fluorescence depolarization of chlorophyll *b* is very similar to that obtained with chlorophyll *a*. At detergent concentrations above the critical micelle concentration (0.3 mM) with an increase of the concentration of chlorophyll *b*, the degree of fluorescence polarization drops from the maximal value of 0.28–0.29 and finally complete depolarization occurs. The relative degrees of polarization P/P_0 at different detergent concentrations are shown in Fig. 1. At greater detergent concentrations the

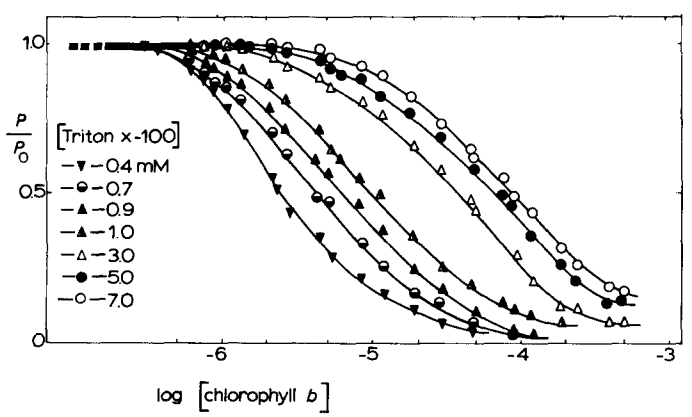

Fig. 1. Relative degrees of polarization of micellar solutions of chlorophyll *b* at different concentrations of the detergent Triton X-100.

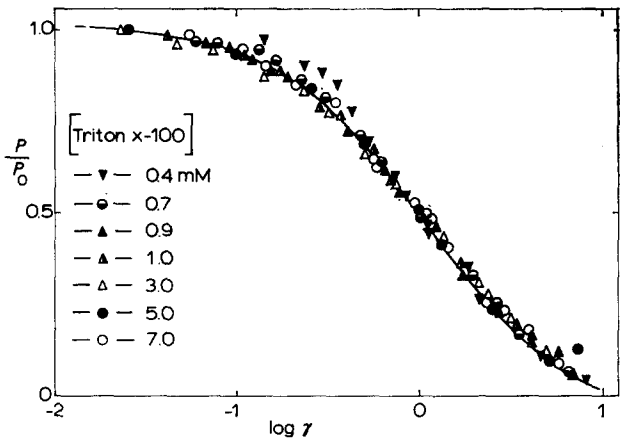

Fig. 2. Experimental data fitted to the theoretical depolarization curve with $\gamma = c/c_0$, where c_0 is the critical concentration at which there is one pigment molecule in a sphere of the radius R_0.

depolarization curves are shifted toward higher chlorophyll *b* concentrations. This result however, considering that the concentration depolarization is the function of pigment concentration, leads to the conclusion that the overall pigment concentrations of the solutions given on the abscissa are not the true concentrations within the micelles. Since all the curves characterize the same process of fluorescence depolarization, they should belong to the same local concentrations of pigments at any given value of P/P_0. This is substantiated by the fact that the experimental points of these curves can be well fitted to the theoretical depolarization curve (Fig. 2).

The critical transfer distance parameter R_0 calculated from the overlap of the absorption and fluorescence spectra was $R_0 = 43 \pm 2$Å, regardless of chlorophyll *b* and detergent concentrations. Using Förster's expression for the critical concentration [17]

$$c_0 = \frac{4000}{4\pi N R_0^3}$$

where c_0 is the critical concentration of pigments at which there is one pigment molecule in a sphere of the radius R_0, (N is Avogadro's number) and substituting $R_0 = 43$ Å, $c_0 = 3$ mM is obtained which, when compared with the c_0 values of the individual depolarization curves (Table I) indicates that the local pigment concentration within the micelles may be higher by several orders of magnitude than the overall concentration of pigments in the sample. Assuming a ratio of 3 : 1 between chlorophyll *a* and chlorophyll *b* concentrations, the usual ratio under in vivo conditions, with $R_0 = 56$ Å and $R_0 = 43$ Å, respectively, the ratio of transfer frequencies chlorophyll *a* → chlorophyll *a* to chlorophyll *b* → chlorophyll *b* calculated according to $n = 1/\tau \cdot (R_0/R)$ [6] is about 100 : 3. However, there are in vivo systems rich in chlorophyll *b* (Photosystem II particles) where this ratio may change by even an order of magnitude. In the case of the chlorophyll *a*/*b* ratio being unity, this value would be 100 : 26.

Fig. 3 shows the relative degrees of polarization of fluorescence P/P_0 as well as

TABLE I

CRITICAL AND HALF-QUENCHING CONCENTRATIONS

Critical concentrations c_0 and half-quenching concentrations $c_{\frac{1}{2}}$ calculated from the curves of depolarization of fluorescence and fluorescence quenching of chlorophyll *b*, respectively.

	Triton X-100 concentration (mM)						
	0.4	0.7	0.9	1.0	3.0	5.0	7.0
c_0 (μM)	2.5	4.8	8.0	11.0	38.0	64.0	85.0
$c_{\frac{1}{2}}$ (μM)	6.2	16.0	25.0	29.0	84.0	130.0	190.0

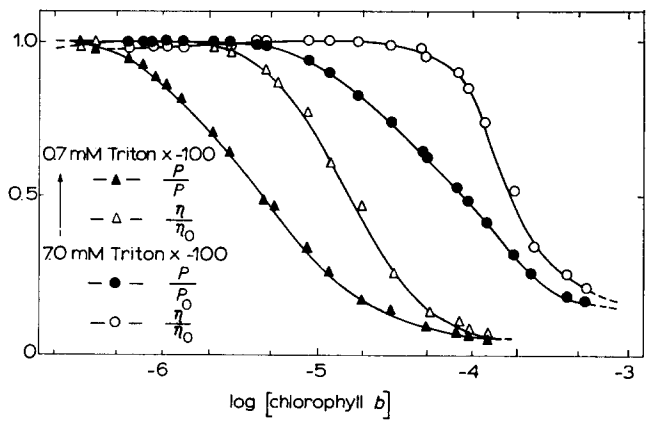

Fig. 3. Relative degrees of polarization of fluorescence P/P_0 and the relative fluorescence yield η/η_0 of chlorophyll *b* at 0.7 mM and 7 mM Triton X-100 concentrations.

the relative fluorescence yield η/η_0 of chlorophyll *b* at 0.7 and 7.0 mM Triton X-100 concentrations. The fluorescence quenching begins to drop at higher concentrations of chlorophyll *b* than the corresponding curves of fluorescence depolarization. This is valid for all detergent concentrations studied and the data comparing the critical concentrations c_0 and the half-quenching concentrations $c_{\frac{1}{2}}$ of the depolarization and quenching curves, respectively, are presented in Table I. Evaluation of the fluorescence quenching data in terms of Förster's theory of concentration quenching of fluorescence [8] leads to the conclusion that the mechanism of fluorescence quenching in this model cannot be described on the basis of this theory. It was found however, that the quenching curves belonging to different detergent concentrations and having different half-quenching concentrations $c_{\frac{1}{2}}$ can be well fitted by the empirical formula [10]

$$\frac{\eta}{\eta_0} = \frac{1}{1+\gamma^2},$$

where $\gamma = c/c_{\frac{1}{2}}$ and $c_{\frac{1}{2}}$ is the concentration of chlorophyll *b* corresponding to the half-quenching concentration of fluorescence (Fig. 4).

The rather close fit of the experimental data to the above empirical expression indicates that fluorescence quenching in this model is a complex process, similar to

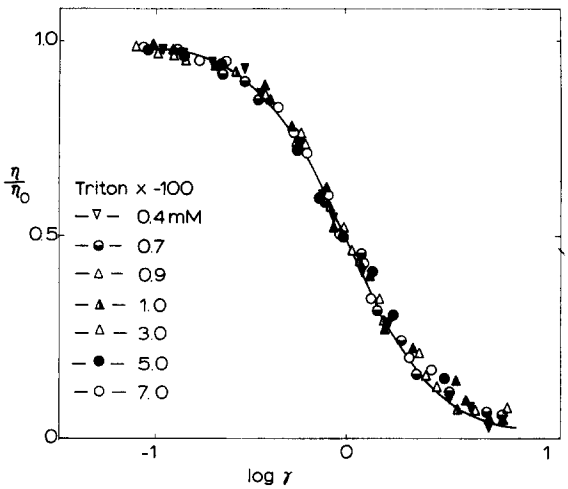

Fig. 4. Experimental data fitted by the empirical expression $\eta/\eta_0 = 1/(1+\gamma)^2$ where $\gamma = c/c_{\frac{1}{2}}$ and $c_{\frac{1}{2}}$ is the half-quenching concentration.

the case of pigments incorporated into a lecithin matrix [10], although the mutual course of the depolarization and quenching curves indicates that quenching is restricted to the process of transfer from the excited monomer to non-fluorescent or poorly fluorescing aggregates (dimer) and/or to their inactive absorption at the exciting wavelength. The presence of such aggregates is postulated by several workers [10–12] but direct proof is scarce.

The increase in local concentration of pigments incorporated into the micelles as compared to the overall concentration in the solutions is about a 1000-fold at a detergent concentration of 0.4 mM (Table I, first column). Therefore, the changes in the absorption characteristics of chlorophyll *b* are very pronounced. Table II shows these changes as the concentration of chlorophyll *b* is increased. The peak of the red absorption band (650 nm) is shifted towards a longer wavelength (654 nm) and the

TABLE II

ABSORPTION CHARACTERISTICS OF CHLOROPHYLL *b* IN 0.4 mM TRITON X-100 SOLUTION

Chlorophyll *b* concentration (μM)	Peak position (nm)		Peak ratios blue/red	Halfwidth (nm)
	blue	red		
2	463	650	2.70	22.5
6	463	650	2.67	23.5
10	463	650	2.61	24.0
50	463	651	2.54	27.0
80	463	652	2.43	28.5
100	463	654	2.39	30.5
200	464	654	2.14	32.5
Chlorophyll *b* in acetone	455	645	2.92	20.5

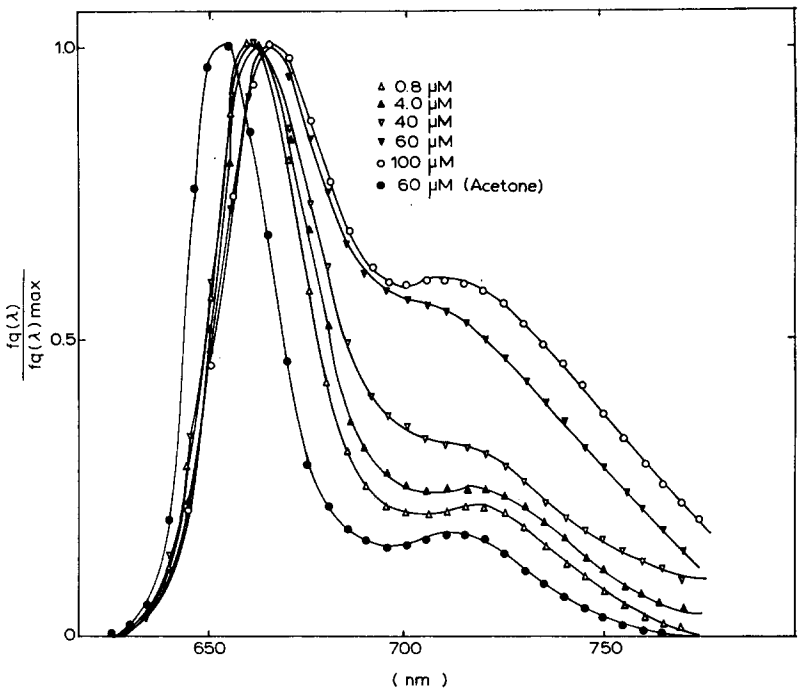

Fig. 5. Chlorophyll *b* fluorescence spectra at a constant Triton X-100 concentration of 0.4 mM. Symbols, concentration of chlorophyll *b*; ●, 60 μM chlorophyll *b* in acetone.

band half-widths (from 22.5 to 32.5 nm) increase by 10 nm as the chlorophyll *b* concentration is increased from 2 to 200 μM. These data, in our opinion, prove that with increasing chlorophyll *b* concentration aggregates absorbing at longer waves are formed in the detergent micelles. This conclusion is corroborated by the fluorescence spectra shown in Fig. 5. As the chlorophyll *b* concentration is increased, the peak of the relative fluorescence spectra ascribed to monomer fluorescence is shifted from 658 nm towards a longer wavelength and the intensity of fluorescence in the long wavelength region is greater compared with the fluorescence peak attributed to the monomer. This means that on increasing the chlorophyll *b* concentration, the formation of poorly fluorescing aggregates (those absorbing at longer wavelengths) serving as quenchers of fluorescence is caused.

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