Biochimica et Biophysica Acta, 376 (1975) 268–273

BBA 46875

ENERGY TRANSFER BY CHLOROPHYLL *a* IN DETERGENT MICELLES

K. CSATORDAY, E. LEHOCZKI and L. SZALAY

Biological Research Center, Szeged, Institute of Biophysics, József Attila University, Szeged (Hungary)

(Received August 13th, 1974)

SUMMARY

The process of energy transfer was studied in the chlorophyll *a*-containing detergent micelle, serving as a possible model of the photosynthetic unit. Chlorophyll *a* was added to aqueous solutions of the detergent Triton X-100 and incorporated into the micelles. The energy transfer process was studied by investigating the concentration depolarization of fluorescence of chlorophyll *a*. On the basis of the experimental depolarization curves as well as the value of the Förster parameter $R_0 = 56$ Å calculated from the overlap of absorption and fluorescence spectra it was concluded that energy transfer between chlorophyll *a* molecules in this model follows the Förster-type mechanism of inductive resonance. Furthermore it was found that the local concentration of chlorophyll *a* in the micelles is higher by 1–3 orders of magnitude than its overall concentration in the solution and by choosing the appropriate ratio between the concentration of chlorophyll *a* and the detergent it is possible to reach the in vivo chlorophyll concentration of 0.1 M within the micelles. Thus the chlorophyll-detergent micelle model may be applied as a model of the separate package-type photosynthetic unit.

INTRODUCTION

For studying energy transfer processes of photosynthesis various models e.g. monolayers of photosynthetic pigments [1–3] and pigmented lipid vesicles [4] and bilayers [5] are employed. It has been found that energy transfer between pigments in such models can be described on the basis of Förster's theory [6–8] of dipole-dipole interaction. Another model i.e. pigment-containing detergent solutions have been extensively used to study the photochemical properties of chlorophylls [9–11]. The advantage of such a system is that the detergent molecules form micelles at a certain concentration, called the critical micelle concentration and water-insoluble pigments are easily incorporated into the micelles, reminiscent of pigments in the chloroplast. Detergent micelles containing organic dyes [12, 13] and photosynthetic pigments [14, 15] have been used to study excitation energy transfer.

The aim of the present investigation was to study the mechanism of energy transfer between chlorophyll *a* molecules incorporated into micelles of the detergent

Triton X-100, as well as the conditions that enable this system to serve as a model of photosynthesis.

METHODS

Chlorophyll *a* was extracted from fresh spinach leaves and purified by chromatography on a sugar column [16]. The detergent Triton X-100 was the product of Rohm and Haas. Micellar solutions of chlorophyll *a* were prepared by diluting a stock solution (0.01 M) of Triton X-100 to the necessary concentration after a known amount of dried chlorophyll *a* dissolved in acetone had been added. Each of the final solutions contained 2 % acetone. The detergent concentration of the solutions was varied from 0.1 mM to 7 mM including the critical micelle concentration which is 0.3 mM. The chlorophyll concentration of the solutions ranged from 0.8 μM to 0.4 mM. The prepared solutions were stirred for one minute and then incubated for 1 h at 40 °C. The solutions were kept in the dark at 4 °C. The method of preparation described allows the solutions to remain stable for days without appreciable changes in their optical characteristics.

Absorbance spectra were recorded on the Unicam SP 1800 spectrophotometer, samples having been placed in the secondary sample position. Fluorescence spectra as well as the degree of polarization of fluorescence were measured on the Perkin Elmer MPF-3 spectrofluorimeter. The degree of polarization was calculated using the equation

$$P = \frac{h(\lambda)I_{\parallel} - I_{\perp}}{h(\lambda)I_{\parallel} + I_{\perp}} \tag{1}$$

where $h(\lambda)$ is a factor that accounts for the polarizing effect of the instrument itself. For the polarization measurements fluorescence was excited in the region of the main red band of absorbance at 660 nm and measured at 680 nm. The excitation and emission monochromators were set at 8 and 6 nm bandwidths, respectively. Under these conditions the intensity of scattered light is within the range of instrumental error and does not effect the polarization measurements.

The absorption and fluorescence spectra were used to calculate the Förster parameter R_0/the average distance between molecules when the probability of transfer is equal to that of emission [7] which is given by the equation

$$R_0^6 = \frac{9000 \ln 10\, x^2 c^4}{128\, \pi^5 n^4 N}\, \Phi \int_0^{\infty} f(v)\, \varepsilon(v) \frac{\mathrm{d}v}{v^4} \tag{2}$$

where x^2 is a factor accounting for the mutual orientation of the molecules, c the velocity of light, Φ the fluorescence yield, n the refractive index of the medium, N Avogadro's number, $f(v)$ the normalized fluorescence spectrum and $\varepsilon(v)$ the molar decadic extinction coefficient and v is the frequency.

All measurements were carried out at 30 °C.

RESULTS AND DISCUSSION

Detergent solutions of chlorophyll *a* have a negligible degree of polarization (0.01–0.02) at detergent concentrations below the critical micelle concentration regardless of chlorophyll *a* concentration. At detergent concentrations above the critical micelle concentration however, the degree of polarization changes depending on the chlorophyll concentration and can reach a maximal value of 0.25–0.26. At a given detergent concentration (above the critical micelle concentration) the degree of polarization decreases with increasing chlorophyll *a* concentration from the initial value of 0.25–0.26 and complete depolarization occurs at high chlorophyll *a* concentrations.

In order to prove that rotational depolarization plays a negligible role in causing depolarization of fluorescence the viscosity of the samples was varied by addition of glycerol. Table I shows the changes in the degree of polarization as the glycerol content of the samples is increased to 50 %. In this case the detergent concentration of the sample was 3 mM. Without glycerol the degree of polarization is 0.22 which means, that the chlorophyll molecules are rigidly fixed in the micelles during the lifetime of the excited state. As the glycerol content of the solution is increased, the substantial increase in the viscosity is not followed by a correspondingly substantial increase in the degree of polarization as would be expected if rotational depolarization played a significant role. The observed slight increase in the degree of polarization is probably due to the fact that the rotation of the micelles which can be neglected owing to their great volume as compared with that of a chlorophyll *a* molecule, is further decreased as a result of the increased viscosity of the surrounding medium.

TABLE I

DEGREE OF POLARIZATION OF FLUORESCENCE OF CHLOROPHYLL *a* IN 3 mM SOLUTION OF TRITON X-100 CONTAINING VARIOUS AMOUNTS OF GLYCEROL

Glycerol content (%):	0	10	20	30	40	50
P:	0.22	0.23	0.24	0.25	0.25	0.26

Consequently depolarization of fluorescence of chlorophyll *a* incorporated into micelles is caused by energy transfer between pigment molecules belonging to the same micelle, while the great distance between micelles renders energy transfer between pigment molecules attached to different micelles highly improbable.

Fig. 1 shows the relative degrees of polarization P/P_0 at six different detergent concentrations. It is interesting to note that at greater detergent concentrations the depolarization curves are shifted toward higher chlorophyll *a* concentrations. Insofar as the concentration depolarization curve is the function of chlorophyll concentration i.e. the average distance between chlorophyll *a* molecules all curves should characterize the same chlorophyll *a* concentration at a given value of P/P_0.

Concentration values indicated on the abscissa give the overall concentrations of chlorophyll *a* in the samples. As far as local concentrations are concerned within

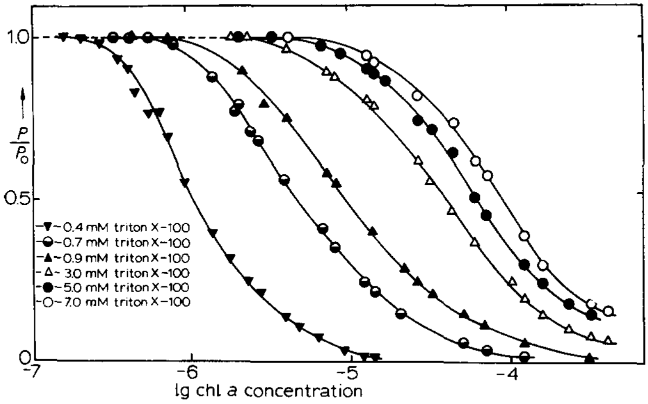

Fig. 1. Relative degrees of polarization of micellar solutions of chlorophyll *a* at different concentrations of the detergent Triton X-100.

the detergent micelle they must be the same at any given value of P/P_0 regardless of the corresponding chlorophyll *a* and detergent concentrations.

Fig. 2 shows the experimental points of all depolarization curves fitted to the theoretical depolarization curve with $c_0 = 1$ mM, c_0 being the critical concentration of pigments at which there is one pigment molecule in a sphere of the radius R_0. The value of P/P_0 for the theorecital depolarization curve at pigment concentrations equalling c_0 is 0.505 [17].

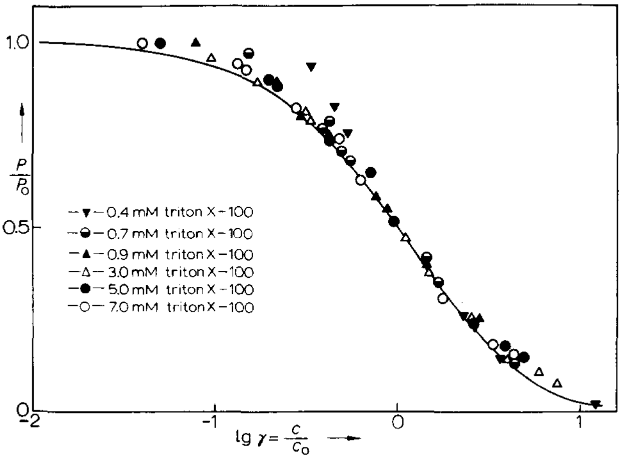

Fig. 2. Experimental data fitted to the theoretical depolarization curve with $\gamma = c/c_0$; where $c_0 =$ 1 mM.

It can be seen from Table II that the depolarization curves corresponding to the various detergent concentrations have different critical concentration values c_0. At the same time the fit to the theoretical depolarization curve seems to be sufficiently good to justify the conclusion that Förster's theory may be applied to energy transfer

TABLE II

CRITICAL CONCENTRATIONS c_0 CALCULATED FROM THE CURVES OF DEPOLARIZATION OF FLUORESCENCE OF CHLOROPHYLL *a* IN SOLUTIONS OF TRITON X-100

Triton concn (mM)	0.4	0.7	0.9	3.0	5.0	7.0
c_0 (μM)	1.0	4.6	9.0	39.0	60.0	90.0

processes in this model. It also indicates that internal conditions may be alike in solutions with different external parameters such as detergent and pigment concentrations.

The critical transfer distance parameter R_0 calculated from the overlap of absorption and fluorescence spectra by using Eqn 2 was $R_0 = 56 \pm 2$ Å, regardless of chlorophyll *a* and detergent concentrations. For these calculations the value of the orientation factor was chosen to be $x^2 = 2/3$, corresponding to a random distribution of pigment molecules. The R_0 value for an orientation factor $x^2 = 4$, that is for a maximally ordered arrangement of pigment molecules would be $R_0 = 75 \pm 3$ Å. Using Förster's expression for the critical concentration [7]

$$c_0 = \frac{3000}{4\pi N R_0^3} \tag{3}$$

and substituting $R_0 = 56$ Å and $R_0 = 75$ Å we get $c_0 = 2.2$ mM and $c_0 = 0.9$ mM respectively. Comparing these results with the values of c_0 presented in Table II, one may conclude without making any assumptions about the mode of incorporation of chlorophyll *a* into the micelle that true pigment concentration within the micelles should be higher by 1–3 orders of magnitude than the overall concentration of pigments in the sample. The calculated values of c_0 explain why the fit to the theoretical depolarization curve with $c_0 = 1$ mM was satisfactory. However, it is most probable that the pigments are randomly distributed within the micelles as substantiated by the fact that complete depolarization occurs, whereas an ordered arrangement would result in residual polarization.

From these results it may be stated that energy transfer between chlorophyll *a* molecules incorporated into Triton micelles may be interpreted on the basis of dipole-dipole interaction. Such energy transfer can be observed throughout a wide range of chlorophyll *a* concentrations owing to the fact that the detergent micelles are capable of concentrating the pigment molecules within themselves.

Thus a substantial increase in local pigment concentration occurs, and by choosing the appropriate pigment and detergent concentrations even in vivo pigment concentrations of the order of 0.1 M can be reached. This is important because energy transfer occurs between pigment molecules belonging to the same micelle, therefore, the process of transfer may be similar to that which could be functioning in the separate package type photosynthetic unit in vivo.

REFERENCES

1 Tweet, A. G., Bellamy, W. D. and Gaines, G. L. (1964) J. Chem. Phys. 41, 2068–2077
2 Trosper, T., Park, R. B. and Sauer, K. (1968) Photochem. Photobiol. 7, 451–469

3 Costa, S. M. de B., Froines, J. R., Harris, J. M., Leblanc, R. M., Orger, B. H. and Porter, G. (1972) Proc. R. Soc. London, A 326, 503–519

4 Colbow, K. (1973) Biochim. Biophys. Acta 314, 320–327

5 Strauss, G. and Tien, H. T. (1973) Photochem. Photobiol. 17, 425–431

6 Förster, T. (1947) Z. Naturforsch. 2b, 174–182

7 Förster, T. (1951) Fluoreszenz Organischer Verbindungen, Vandenhoeck und Ruprecht, Göttingen

8 Förster, T. (1948) Ann. Physik. 2, 55–75

9 Massini, P. and Voorn, G. (1968) Biochim. Biophys. Acta 153, 589–601

10 Shaposnikova, M. G., Drozdova, N. H. and Krasnovsky, A. A. (1971) Biokhimiya 36, 704–711

11 Hertogs, M. and Wessels, J. S. C. (1965) Biochim. Biophys. Acta 109, 610–613

12 Singhal, G. S., Rabinowitch, E., Hevesi, J. and Srinivasan, V. (1970) Photochem. Photobiol. 11, 531–545

13 Hevesi, J., Lehoczki, E. and Bálint, E. (1970) Zh. Prikl. Spektr. U.S.S.R. 13, 458–463

14 Teale, F. W. J. (1958) Nature 181, 415–416

15 Zenkevitch, E. I., Losev, A. P. and Gurinovitch, G. P. (1972) Molekulyarnaya Biologiya 6, 824–833

16 Jacobs, E. E., Vatter, A. E., and Holt, A. S. (1954) Arch. Biochem. Biophys. 53, 228–238

17 Szalay, L. (1964) Ann. Physik, 7, 221–228