Vol. A58 (1980) *ACTA PHYSICA POLONICA* No 3

THE LIGHT ABSORPTION AND FLUORESCENCE YIELD OF THIONINE IN SODIUM LAURYL SULPHATE MICELLAR SOLUTIONS

BY ELIZABETH BÁLINT, I. VASS, J. HEVESI

Institute of Biophysics, József Attila University, Szeged*

AND N. MAREK

Institute of Medical Chemistry, Medical University, Szeged**

(Received November 22, 1979)

The dependence of the light absorption and relative fluorescence yield of $1 \cdot 10^{-6}$–$1 \cdot 10^{-4}$M thionine and 0–$5 \cdot 10^{-3}$ M sodium lauryl sulphate on the local dye concentration on the micelles was investigated. Formula is given for the determination of the local dye concentration from the absorption spectra. Bojarski's method shows that the electronic excitation energy transfer between dye molecules in solutions containing monomeric and dimeric dye molecules obeys Förster's theory above the critical micelle concentration, to a given local dye concentration, and also that the dye aggregates play an important role in the transfer.

1. *Introduction*

Dye–detergent systems are suitable for modelling energy transfer processes occurring in photosynthetic pigment systems during photosynthesis [1–7]. In aqueous solutions of detergents, oriented molecular aggregates of colloidal dimensions, known as micelles, exist above the critical micelle concentration (cmc) [8–9]. If appropriate dyes are added to the micellar solution, the dye molecules will be incorporated into or attached to the micelles, in a way very similar to that of chloroplasts.

In this paper the light absorption spectra and the relative fluorescence yield of thionine (Th) in micellar solutions of sodium lauryl sulphate (SLS) were investigated to obtain information about the "incorporation" of the dye molecules, and about the energy transfer between different dye forms existing in the micelles. The concentrations of Th and SLS were varied from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-4}$ M and from 0 to $5 \cdot 10^{-3}$ M, respectively. The technique for the preparation of the micellar solutions and the methods of measurement are described elsewhere [3, 10, 11].

---

* Address: Institute of Biophysics, József Attila University, H-6722 Szeged, Egyetem u. 2., Hungary.

** Address: Institute of Medical Chemistry, Medical University, H-6720 Szeged, Dóm tér 9., Hungary.

2. *Results and discussion*

The absorption spectrum of Th in aqueous solution has two main bands, attributed to monomers (600 nm, α-band) and dimers (565 nm, β-band). In detergent solutions the absorption spectra of Th show significant changes (Fig. 1). The addition of detergent shifts the maxima to longer waves by 3–5 nm, which is in good agreement with the results of [1]. The absorption spectra at higher detergent concentrations are very similar to these in aqueous solution, but at low detergent concentrations two new bands appear, at 465 and 640 nm (γ- and δ-bands). These are attributed to dye-detergent complexes and to higher dye aggregates. Figure 1 also shows that with decreasing detergent concentration the heights of the α- and β-bands decrease, while those of the γ- and δ-bands increase.

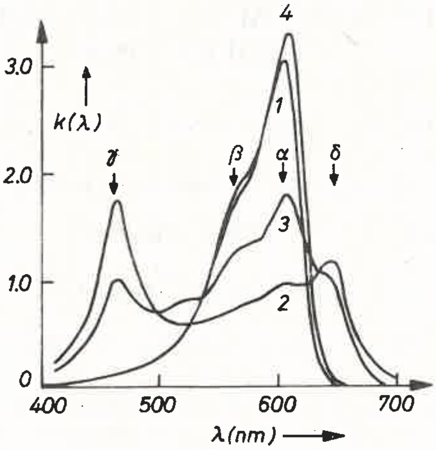

Fig. 1. Absorption spectra of $5 \cdot 10^{-5}$ M thionine at different detergent concentrations (*1* — 0 M: *2* — $2.5 \cdot 10^{-3}$ M; *3* — $3 \cdot 10^{-3}$ M; and *4* — $4 \cdot 10^{-3}$ M SLS)

Lehoczki and Hevesi [12] reported that the dye molecules are primarily situated in micelles, with local dye concentrations exceeding the bulk concentration by three orders of magnitude. The appearance of dimers and aggregated dye forms is, therefore, attributed to high local dye concentrations.

Based on the following considerations the local concentrations of the different dye forms can be determined. According to the Beer-Lambert law:

$$k(\lambda)_{\max} = 2.3 \cdot \varepsilon(\lambda)_{\max} \cdot c, \tag{1}$$

where $k(\lambda)_{\max}$ and $\varepsilon(\lambda)_{\max}$ are the maximum absorption and decadic molar extinction coefficients. The bulk concentration, c and the partial local concentration, c_{loc}, of the different dye forms can be described as follows:

$$c = \frac{n}{V_0} \quad \text{and} \quad c_{\text{loc}} = \frac{n'}{V_{\text{mic}}}. \tag{2}$$

Here n is the number of moles of dye in volume, V_0, of the solution and n' is that in the total volume of micelles (V_{mic}) in which the dye molecules are situated.

Mukarjee's method [13] for conductivity measurements allows one to determine the quantity of detergent in the micelles. Earlier investigations [11, 14] showed that V_{mic} is independent of the dye concentration, and that n is nearly equal to n'. Thus, from Eqs. (1) and (2)

$$c_{\mathrm{loc}} = \frac{V_0 k(\lambda)_{\max}}{V_{\mathrm{mic}} \cdot 2.3 \cdot \varepsilon(\lambda)_{\max}} \equiv A k(\lambda)_{\max}. \tag{3}$$

For a given form of the dye, A is constant.

Since the individual bands overlap appreciably (Fig. 1), $k(\lambda)_{\max}$ for the different bands cannot be found from the spectra measured. Alyentsev's method [15] was employed to resolve the bands. This method is based on the possibility of presenting a composite spectrum as a linear combination of separate bands. The analysis was carried out with a WANG-602 minicomputer. Figure 2 shows the distribution and heights of the components. At higher dye concentrations more significant differences can be found between the measured and calculated values of $k(\lambda)_{\max}$. The true $k(\lambda)_{\max}$ values of the monomer, aggregate and the complex bands, proportional to the appropriate local dye concentration, are plotted in Fig. 3, as functions of the initial dye concentration, at $3.5 \cdot 10^{-3}$ M detergent concentration. Each function can be approximated by two straight lines of different slopes. The break points (B) occur at the same concentration (about $2 \cdot 10^{-5}$ M) of the dye. At higher concentrations the quantity of monomers hardly increases, but the quantity of aggregated forms increases rapidly. The behaviour of the dimer band (not shown in the figure) is similar to that of the monomer band. As regarding the local dye concentration, it can be stated that after a certain bulk the concentration of approximately

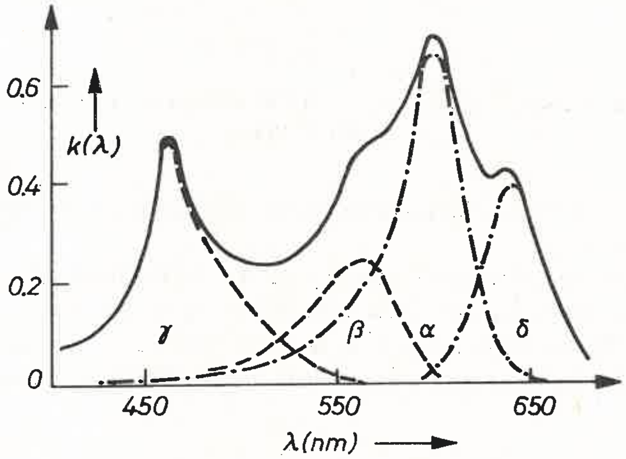

Fig. 2. The resolved absorption spectra of $2 \cdot 10^{-5}$ M thionine with $3 \cdot 10^{-3}$ M SLS in water

$2 \cdot 10^{-5}$ M is a reached, the micelles become "saturated". On further increase of the bulk concentration, the proportion of the monomers decreases. Simultaneously, more and more aggregates and dye detergent complexes are formed. One can say that the properties of the systems investigated are determined mainly by the local concentration.

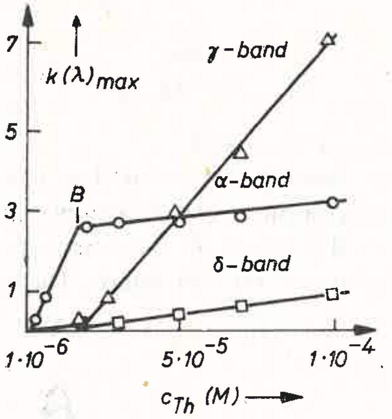

Fig. 3. $k(\lambda)_{max}$ values of absorption bands at $3.5 \cdot 10^{-3}$ M SLS concentration, as functions of the thionine concentration

Equation (3) can explain the concentration quenching curves measured at constant detergent concentrations.

Figure 4 shows the measured quenching curves. As the local concentration of monomers is unknown, $\log k(\lambda)_{max}$, which is proportional to it, see. Eq. (3), is plotted on the horizontal axis. The theory developed by Bojarski [16], which assumes Förster's type of energy transfer [17] and takes into consideration the influence of dimers present in the

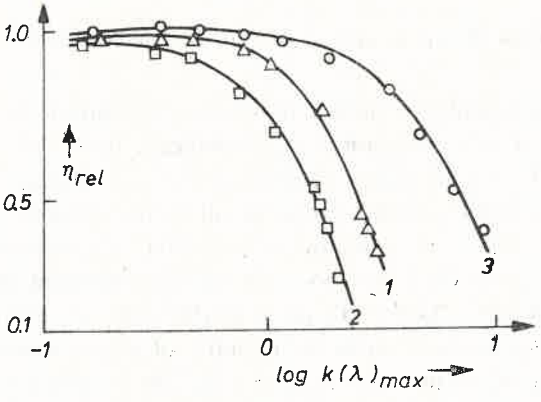

Fig. 4. The measured quenching curves at three different detergent concentrations (*1* — $3.0 \cdot 10^{-3}$ M, *2* — $3.5 \cdot 10^{-3}$ M and *3* — $5 \cdot 10^{-3}$ M SLS)

solution was used to explain the quenching curves measured. Bojarski gives the relative fluorescence yield as:

$$\eta_{\mathrm{rel}} = \frac{1-f(\gamma)}{1-\alpha\alpha_0 f(\gamma)}, \tag{4}$$

where

$$f(\gamma) = \sqrt{\pi}\cdot\gamma\cdot e^{\gamma^2}\cdot[1-\mathrm{erf}\,(\gamma)],$$

$$\alpha = \frac{\gamma_{\mathrm{mon}}}{\gamma},$$

$$\gamma = \frac{\sqrt{\pi\eta_0}}{2}\cdot\left[\frac{c_{l_{\mathrm{mon}}}}{c_{0_{\mathrm{mon}}}} + \frac{Kc^2_{l_{\mathrm{mon}}}}{C_{0_{\mathrm{dim}}}}\right],$$

$$\gamma_{\mathrm{mon}} = \frac{\sqrt{\pi\eta_0}}{2}\,\frac{c_{l_{\mathrm{mon}}}}{c_{0_{\mathrm{mon}}}}.$$

In Eq. (4) K, is the rate constant of the monomer–dimer equilibrium, α_0, is a constant characteristic of the dye, η_0 is the quantum yield of the solutions and, $c_{l_{\mathrm{mon}}}$ is the local concentration of the monomers. The critical concentrations ($c_{0_{\mathrm{mon}}}$ and $c_{0\mathrm{dim}}$) relating to the monomer–monomer and monomer–dimer energy transfers can be calculated from:

$$c_{0_{\mathrm{mon}}} = \frac{3}{4\,\pi N' R^3_{0_{\mathrm{mon}\to\mathrm{mon}}}}; \qquad c_{0_{\mathrm{dim}}} = \frac{3}{4\pi N' R^3_{0_{\mathrm{mon}\to\mathrm{dim}}}},$$

where $N' = 6.023\cdot 10^{20}$, and the R_0-s are the critical distances for the respective energy transfer [11].

If Eq. (3) is taken into consideration, γ can be rewritten as:

$$\gamma = \frac{\sqrt{\pi\eta_0}}{2}\left(\frac{Ak(\lambda)_{\mathrm{max}\cdot\mathrm{mon}}}{c_{0_{\mathrm{mon}}}} + \frac{KA^2k^2(\lambda)_{\mathrm{max}\cdot\mathrm{mon}}}{c_{0_{\mathrm{dim}}}}\right). \tag{5}$$

As in Eqs. (4) and (5), α_0 and A are unknown. These were determined in the following way.

a. From the concentration quenching curves of thionine in aqueous solutions with a known dye concentration α_0 ($= 0.865$) was determined by computer curve-fitting to Eq. (4), taking α_0 as the parameter.

b. Equations (4) and (5) were fitted to the measured quenching curves in the presence of constant detergent concentrations, taking A as the parameter. Fitting was made in the region under the break point (B) concentration, where dye molecules were present in the solution only in monomeric and dimeric forms. For fitting, we chose the value, $\eta_0 = 0.3$, derived from the literature. The energy transfer constants, $R_{0\mathrm{mon}} = 46$ Å, and $R_{0\mathrm{dim}} = 27$ Å, were calculated from Förster's formula; and the critical concentrations were $c_{0\mathrm{mon}} = 4.1\cdot 10^{-3}$ M and $c_{0_{\mathrm{dim}}} = 2.1\cdot 10^{-3}$ M. The dimerization constant ($K = 500\ \mathrm{M}^{-1}$) was calculated from the absorption spectra.

If A is already known, then local concentration of monomers can be calculated from the absorption coefficients, $k(\lambda)_{\max}$, determined by spectrum analyses.

Figure 5 shows the measured and theoretical quenching curve for $3.5\cdot10^{-3}$ M SLS solutions as functions of the logarithm of the calculated local concentration. A significant difference exists between the measured and calculated curve only above a concentration of $2\cdot10^{-5}$ M, where there are more aggregates in the system. In this region the energy transfer is more effective than was calculated when only considering the presence of monomers and dimers.

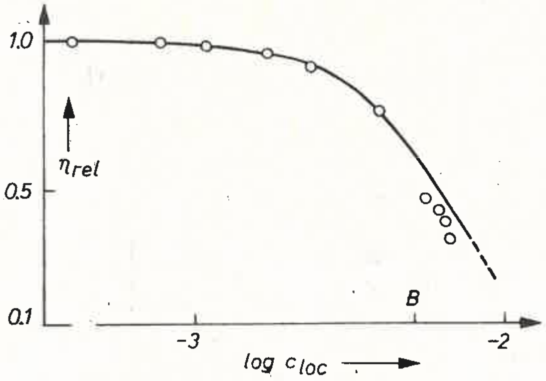

Fig. 5. The measured (○○○) and calculated (———) relative fluorescence yields of thionine solutions at $3.5\cdot10^{-3}$ M SLS in water

3. *Conclusions*

The electronic excitation energy transfer between monomeric and dimeric thionine molecules present in the micelles can be described by Förster's theory up to $5\cdot10^{-3}$ M local dye concentration. From this value upwards, the dye aggregates also play an important role in the energy transfer. Since the emission band of monomers and the absorption band of aggregates overlap considerably, the energy transfer process of monomers to aggregates is responsible for a decrease in the relative fluorescence yield in this region, as shown by a discrepancy from the Förster theory, which was extended by Bojarski, and shown by experiment.

REFERENCES

[1] G. S. Singhal, E. Rabinowitch, J. Hevesi, V. Srinivasan, *Photochem. Photobiology* **11**, 531 (1970).
[2] J. Hevesi, E. Lehoczki, E. Bálint, *Zh. Prikl. Spektrosk.* **13**, 758 (1970).
[3] E. Bálint, E. Lehoczki, J. Hevesi, *Acta Phys. et Chem. Szwged* **17**, 15 (1971).
[4] E. Bor, J. Hevesi, *Acta Phys. et Chem. Szeged* **20**, 357 (1974).
[5] E. Lehoczki, J. Hevesi, L. Tolvaj, *Acta Phys. Pol.* **A46**, 741 (1974).
[6] K. Csatorday, E. Lehoczki, L. Szalay, *Biochem. Biophys. Acta* **376**, 268 (1975).

[7] E. Lehoczki, L. Szalay, *Acta Phys. et Chem. Szeged* **23**, 161 (1977).
[8] I. W. McBain, *Colloid Science*, Heath, Boston 1950.
[9] F. M. Menger, *Acc. Chem. Res.* **12**, 111 (1979).
[10] P. A. Winsor, *Chem. Rev.* **68**, 1 (1968).
[11] J. Hevesi, E. Bálint, E. Lehoczki, *Acta Phys. Pol.* **A38**, 829 (1970).
[12] E. Bálint, J. Hevesi, I. Vass, *Acta Phys. et Chem. Szeged* **23**, 123 (1977).
[13] E. Lehoczki, J. Hevesi, *Acta Phys. et Chem. Szeged* **18**, 11 (1972).
[14] E. Lehoczki, J. Hevesi, *Acta Phys. et Chem. Szeged* **18**, 123 (1972).
[15] P. Mukarjee, K. I. Mysels, *J. Am. Chem. Soc.* **68**, 220 (1946).
[16] M. B. Fok, *Trudy Inst. Fiz. Akad. Nauk SSSR*, **59**, 3 (1972).
[17] C. Bojarski, J. Domsta, *Acta Phys. Hung.* **30**, 145 (1971).
[18] Th. Förster, *Z. Naturforsch.* **4**a, 321 (1949).