

DEGREE OF POLARIZATION IN DYE-DETERGENT MODEL SYSTEMS

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The degree of fluorescence polarization and the absorption spectra of sodium-lauryl-sulphate (SLS) and thionine (Th) micellar solutions were measured. A simple consideration shows that the local concentration of dyes present in the micelles is proportional to the absorption coefficient. According to measurements the degree of polarization and the absorption characteristics of the systems are determined by the local concentration in the micelles. These dye concentrations (calculated from the concentration depolarization in micellar systems on the base of the FÖRSTER-ORE's theory) are higher by 2 to 3 orders of magnitude than the bulk dye concentration and it seems to be similar to the pigment concentration present in the chloroplasts. The energy transfer between dye molecules can be adequately described by FÖRSTER's theory.

Introduction

Dye-detergent systems are suitable for modelling energy transfer processes occurring during the photosynthesis [1]—[2]. In aqueous solution of detergents aggregates of colloidal dimensions — micelles — are formed above the critical-micelle-concentration (c.m.c.) [3]. If appropriate dyes are added to the micellar solution, the dye molecules will be incorporated into micelles [4], with local concentration exceeding the bulk concentration of the solution [5]. The average distance of the dye molecules, commensurable with those of *in vivo* systems (in chloroplast) can be reached.

In the present paper the degree of fluorescence polarization and the absorption spectra of sodium-lauryl-sulphate (SLS) — thionine (Th) micellar solutions have been investigated in order to obtain information about the local dye concentration and the migration of electronic excitation energy within the micelles.

Materials and methods

The solutions examined were prepared as described in an earlier paper [6]. The concentrations of SLS and Th were varied between $0-8 \cdot 10^{-3}$ M and $1 \cdot 10^{-6}-2 \cdot 10^{-4}$ M, respectively. More details about the determination of the absorption spectra are given in [2]. The degree of fluorescence polarization was determined by using a photoelectric apparatus, constructed in the Institute of Medical Chemistry on the base of the description of KETSKEMÉTY et al. [7]. The calculations were performed by a minicomputer, type Wang—600.

Results and discussion

The absorption spectra of the aqueous solutions of the systems ($k(\lambda)$) have two absorption bands with maxima at 600 nm (α -band) and 565 nm (β -band) attributed to the monomer and to the dimer forms of the dye dissolved in the solution, respectively. In detergent solutions — above the c.m.c., found to be $3.5 \cdot 10^{-3}$ M concentration of the detergent — new absorption bands appear at 460 nm (γ -band) and at 640 nm (δ -band) attributed to dye-detergent complexes and to higher dye-aggregates, respectively (see Fig. 1). Dimer and aggregated form of the dye appear in consequence of high local concentration present in the micelles.

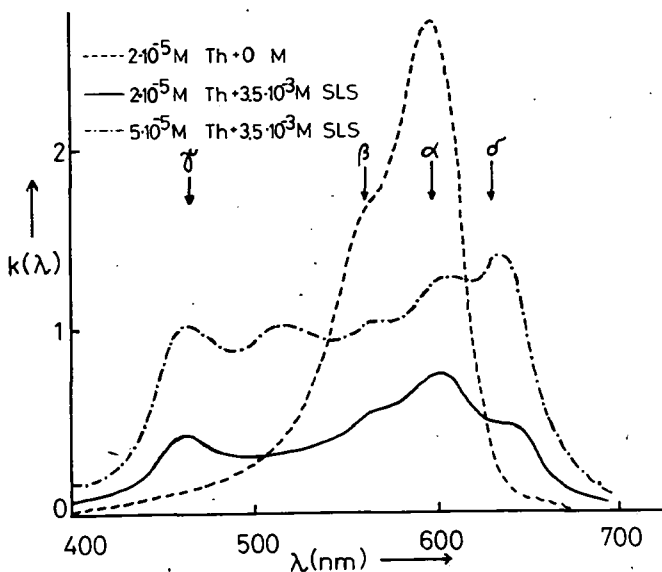


Fig. 1.

The $k(\lambda)_{\max}$ values of the absorption bands are proportional to the local concentration of the corresponding dye form, provided no overlap of the bands exists [8]. In solutions examined only the monomer form of dye are fluorescent [9], therefore the fluorescence polarization depends on the local concentration of the monomers. The bands of the absorption spectra have significant overlap, thus the true values of $k(\lambda)_{\max}$ in the different bands, can not be found directly from the measured spectra. Therefore the measured spectra were analysed by the method of ALJENCEV [10].

The true $k(\lambda)_{\max}$ values of the different band — proportional to the local dye concentration — are plotted in Fig. 2 as a function of the dye concentration weighed in at $4.5 \cdot 10^{-3}$ M detergent concentration. Fig. 2 shows that the curves can be approximated by two straight lines having different slopes. With respect to the local dye concentration it can be stated that after reaching a certain dye concentration (shown in Fig. 2 by a break-point in the straights) the micelles become saturated —

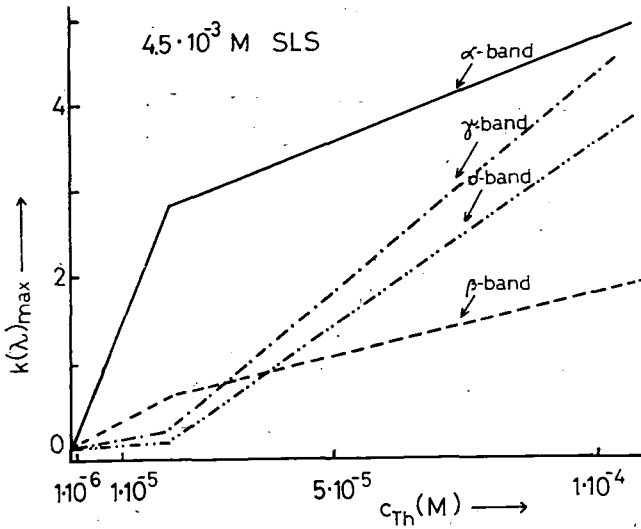


Fig. 2.

the ratio of the monomers and the dimers decreases — and simultaneously aggregates and dye-detergent complexes are formed.

The break-point of the α -band maximum appears at higher dye concentration. Namely, in solutions containing more detergent, the number of micelles increases thus more and more dye molecules are necessary for obtaining the same local concentration in the micelles.

It can be shown by measuring the degree of fluorescence polarization that the absorption and the polarization characteristics of the system examined are determined by the local concentration.

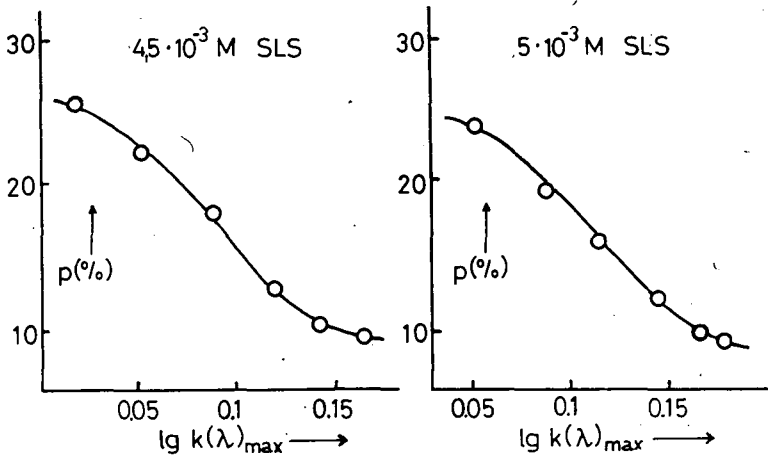


Fig. 3.

Fig. 3 contains the degrees of fluorescence polarization measured at $4.5 \cdot 10^{-3}$ M and at $5 \cdot 10^{-3}$ M detergent concentration as a function of $\log k(\lambda)_{\max}$. The curves are typical concentrational ones. The supposition that the concentrational depolarization in micellar systems can be described by the theory of Förster-Ore [11–12],

permits to calculate the local concentration of the dye molecules present in the micelles. According to this theory the point at $p/p_0 = 0.55$ lies at a given c_0 concentration proportional to the inverse of the cube of the energy transfer constant R_0 [13];

$$c_0 = \frac{3}{4} \frac{1}{N'R_0^3} \quad (1)$$

Calculating with $R_0 = 46 \text{ \AA}$ (obtained from the overlap of absorption and fluorescence spectra of the systems), $c_0 = 4 \cdot 10^{-3}$ M.

Knowing the values of $k(\lambda)_{\max}$ and that $k(\lambda)_{\max} = Kc_{loc}$ pertaining to $c_0 = c_{loc}$ the value of the K constant can be determined and the local dye concentration for another solution can be calculated.

In the table the calculated values of the local concentration are given in the concentration range of $1 \cdot 10^6$ M– $2 \cdot 10^{-4}$ M. It can be seen from the data that the local concentrations are higher by 2 to 3 orders of magnitude than the bulk dye concentration.

The local concentrations given in the table correspond to the average distances from 10 to 60 \AA between the dye molecules. These values are similar to the mean distances of the real pigments present in the chloroplasts.

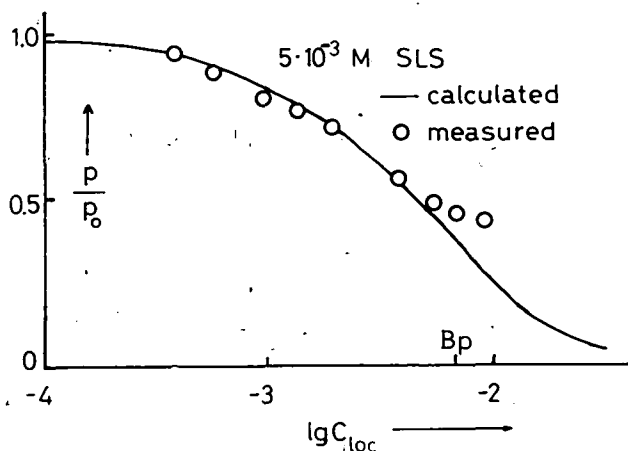


Fig. 4.

Fig. 4 shows the calculated (solid line) and the measured (circles) values of the relative degree of fluorescence polarization (p/p_0) as a function of the logarithmic of the local concentration. The calculation was carried out by the Förster-Ore theory.

It can be seen from the figure that at lower dye concentrations the calculated values and the measured ones of p/p_0 are in a good accordance. At higher concentrations, from the point B_p — corresponding to the local concentration pertaining to the break points in the Fig. 2 — the deviations between the measured and the calculated values are more significant. These deviations, however, can be interpreted by the formation of aggregates and their participation in the energy transfer.

Conclusions

The presented results of fluorescence polarization measurements prove that the structure and the dimensions of the chloroplasts are similar — in certain detergent concentration range — to those found in dye-detergent systems. The excitation energy transfer between the dye molecules present in the micelles can be described by the Förster's theory. Useful information concerning the photophysical processes of the photosynthesis may be expected from the results of further investigations of the same kind of dye-detergent systems.

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The authors are indebted to Prof. L. SZALAY, Director of the Institute of Biophysics for the helpful discussions and for the valuable advices during the preparation of present paper.

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СТЕПЕНЬ ПОЛЯРИЗАЦИИ ЛЮМИНЕСЦЕНЦИИ МОДЕЛЬНОЙ СИСТЕМЫ КРАСИТЕЛЯ-ДЕТЕРГЕНТА

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Установили, что спектры поглощения и степень поляризации люминесценции растворов тионина в натрийлаурил-сульфате зависят от локальной концентрации тионина в мицеллах. На основе полученных кривых концентрационной деполяризации по теории Фёрстера-рассчитали, что локальная концентрация тионина в мицеллах на 2—3 порядка больше чем введенной в раствор концентрации красителя и достигает концентрации пигмента в мицеллах, характерной для хлоропластов. Перенос энергии между молекулами тионина происходит в соответствии с теорией Фёрстера.