Determination of the Directions of the Transition Dipoles in Tetrabutylperylene in Stretched Polymers

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The directions of the transition dipole moments of 2,5,8,11,-tetra-butylperylene were determined from angle-resolved fluorescence depolarization experiments on molecules embedded in a stretched anhydrous nitrocellulose matrix. The absorption transition moments lies almost parallel to the elongated axis of the molecule, but the emission transition moment makes an angle of 20° with the axis. The orientational distribution of the molecules in the polymer indicates significant deviations from a circular form.

INTRODUCTION

Perylene has been widely used as a fluorescence probe in studies of orientational order and rotational dynamics as well as energy-transfer processes in lipid bilayer systems [1–7]. Its popularity is derived from its strong absorption bands in two distinct wavelength regions (see Fig. 1), its high quantum yield, and its approximate disk-like shape. The extraction of information from fluorescence depolarization experiments on perylene is hampered by the fact that its location within the lipid bilayer is not well defined. Moreover, the description of its motion requires the use of extended models which take into account its disk-like form. Since the transition moments lie in the molecular plane, rotational motions about the normal to the plane as well as the tumbling of the normal within the bilayer structure will contribute to the depolarization of the fluorescence. Furthermore, any deviations from a disk-like shape will introduce additional complications in the description of its
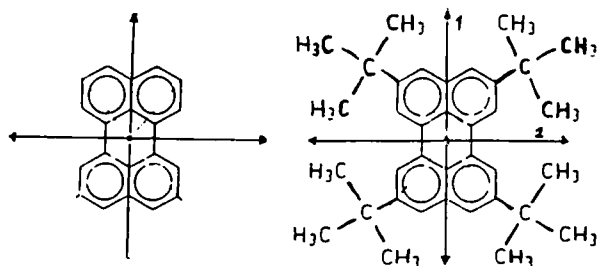

Fig. 1. Structures of perylene (left) and TBpe (right). The suggested symmetry axes are given.

orientational order and motion in the bilayer. The information about the dynamic behavior of the perylene molecule can be extracted from depolarization experiments only if the directions of the dipole transition moments within the molecular frame are known.

The problems associated with perylene as a probe for bilayer systems can be overcome to a large extent by using its tetrabutyl derivative, 2,5,8,11-tetra-t-butyl-perylene (TBpe) [8]. The addition of the butyl groups (see Fig. 1) is thought to stabilize the molecule in a

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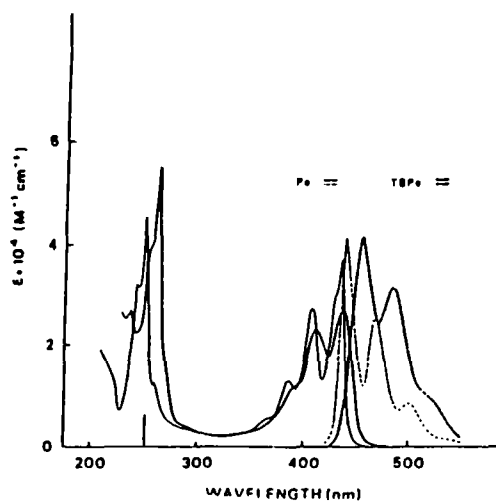
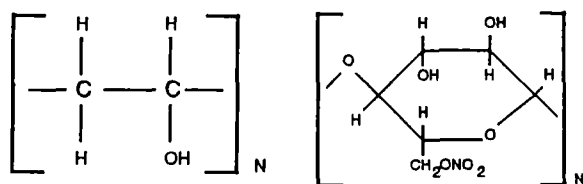Fig. 2. Absorbance, excitation, and emission spectra of perylene and TBpe.

Fig. 3. Structures of PVA (left) and NC (right).

planar configuration and, furthermore, increases its solubility in the lipid matrix. The TBpe molecules appear to be located between the tails of the lipid molecules [8,9].

In view of the potential of TBpe molecules as probes for lipid systems, we have undertaken a study of the directions of transition moments in the molecular frame. In addition, we investigated any possible deviations from the disk-like shape. To this end TBpe was embedded in two polymer matrices: anhydrous nitrocellulose (NC) and anhydrous polyvinylalcohol (PVA) (Fig. 3), where the rotational motions are expected to be quenched. We find that the absorption transition moment at 420 nm lies almost parallel to symmetry axis 1 in Fig. 1, but the emission transition moment makes an angle of 20° with that axis. The extracted orientational distribution of the molecules in NC indicates significant deviations from a circular form.

MATERIALS AND METHODS

TBpe was the kind gift of Dr. L. B. Å. Johansson, University of Umeå, Umeå, Sweden, and used without

further purification. Dimethyl sulfoxide (DMSO) of analytical-grade purity was purchased from J. T. Baker Chemicals B.V. and used as obtained.

One hundred percent hydrolyzed PVA with an average molecular weight of 100 kD was obtained from Aldrich-Chemie and purified as described previously [10]. NC was purchased from BDH and purified before use to remove all traces of acid. The NC was stirred overnight in a 0.01 M aqueous NaOH solution. The mixture was rinsed several times with water and then dried at room temperature under a nitrogen stream.

The PVA films were prepared by the addition of 1 g of PVA to 8 ml of DMSO and mixing at 80°C. TBpe was added to the resulting solution. NC films were prepared by dissolving 1 g of NC in 5 ml of DMSO and mixing at room temperature. Again, the TBpe was added to the solution. The PVA or NC films were cast by pouring the corresponding mixture on glass plates and letting them dry under a nitrogen atmosphere for about 15 h. The optical density of the films was kept below 0.1, to avoid reabsorption. The final concentrations of TBpe were $5 \cdot 10^{-7}$ to $3 \cdot 10^{-6}$ mol/g. The films were stretched up to four times their original length. The PVA films were stretched at 80 to 100°C. It was necessary to equilibrate the NC films in a DMSO-saturated atmosphere for 2 h prior to stretching.

The stretched or unstretched films were placed between quartz plates, using ultrapure glycerol ($n = 1.52$) to ensure optical contact. The samples were glued around the edges and masked with black tape, leaving a spot 2 mm in diameter for illumination.

The absorption spectra of the films were measured on a SLM spectrophotometer (Aminco-DW2000). The steady-state excitation and emission spectra were measured with a SPF 500 SLM-Aminco spectrofluorimeter. A special film holder was placed in the sample chamber, with the film surface making an angle of 45° with the incident and emitted beams. The steady-state fluorescence anisotropy [$r = (A-1)/(A+2)$, with $A = G^*(I_{VV}/I_{VH})$ was measured on a home-built angle-resolved setup described previously [11]. The correction factor $G = I_{HH}/I_{HV}$ for the detection channel was determined using a dilute TBpe–DMSO solution. The time-resolved fluorescence experiments were carried out in Daresbury, UK, using synchrotron radiation as a tunable light source with high-repetition frequency as described elsewhere [11]. The excitation wavelength was selected using a 0.1- to 3-nm bandwidth monochromator. The emission wavelength was selected using interference and cutoff filters so as to suppress stray light. The decay curves were analyzed with a deconvolution algorithm using a nonlinear least-squares Marquardt procedure.
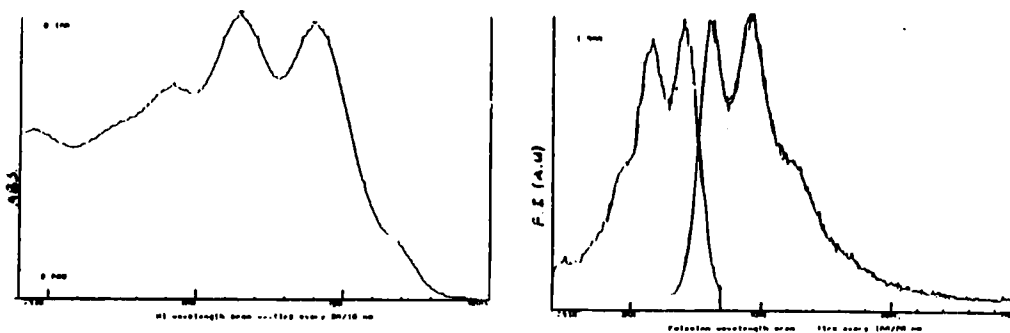
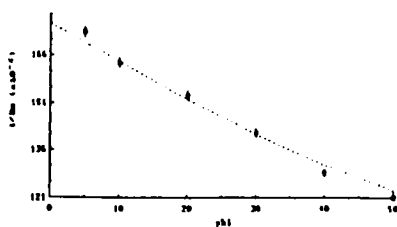Fig. 4. Absorbance, excitations, and emission spectra of TBpe in stretched PVA.

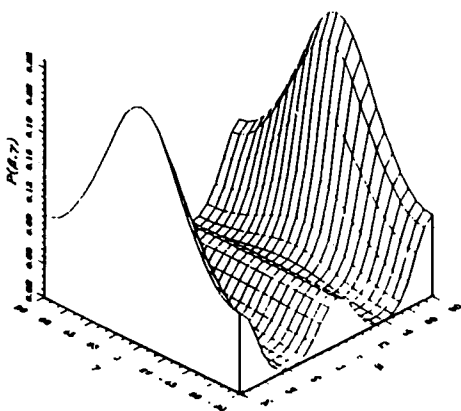Fig. 5. Typical angle-resolved fluorescence depolarization curve.

Fig. 6. Distribution function of TBpe in 2.8 × stretched NC film.

RESULTS AND DISCUSSION

The films of TBpe in NC and PVA matrices were optically homogeneous and yielded absorbance spectra in agreement with those measured from solutions with the exception of the stretched PVA films. The excitation spectra match the absorbance and were found to be independent of the emission wavelength used. Moreover, the emission spectra of TBpe in the stretched NC and

unstretched NC and PVA films also corresponded closely with the ones from solutions. However, all these film spectra were red-shifted by 4 to 7 nm relative to those in solution (Figs. 2 and 4).

TBpe in stretched PVA film exhibited an anomalous behavior in that changes in the absorbance, excitation, and emission spectra were observed (Fig. 4). We believe that these changes arise from the partial aggregation of the TBpe molecules into nonfluorescent forms. Consequently the TBpe–PVA system is not considered further.

A steady-state anisotropy of 0.36 ± 0.01 was found across the whole emission band for the unstretched TBpe–NC films in the excitation region 360–450 nm. This value is in good agreement with literature values [13]. The excitation of the TBpe molecules in their absorption band around 260 nm was seriously hampered by the strong absorbance of NC.

The time-resolved fluorescence anisotropy of TBpe in all the films exhibited no decay on the time scale of the fluorescence lifetime, with a constant value of 0.36 ± 0.01, as found above. This corroborates our assumption that the rotational motions of the TBpe molecules in the films are quenched. The fluorescence intensity decays exhibited a complex behavior in the films and were best described using three exponential decay components. The average lifetime was found to be 2.50 ± 0.02 ns, compared to 4.6 ± 0.1 ns in solution [9]. The short-lifetime components are most probably due to the trapping of TBpe fluorescence by the NC polymer chains. However, this process does not affect the anisotropy measurements.

Angle-resolved fluorescence depolarization measurements were carried out on NC films stretched to 2.5 times their original lengths (Fig. 5). Polarization ratios were measured for 60 distinct combinations of the angles of incidence and emission. The experimental depolari-

zation ratios are determined by five independent parameters reflecting the orientational order of the molecules and directions of the transition dipoles in the molecular frame. The orientational order of the molecules is taken to be described in terms of the orienting potential $U(\beta,\gamma)$

$$U(\beta,\gamma) = -kT \{\lambda_2 P_2 (\cos\beta) + \lambda_4 P_4 (\cos\beta) + \epsilon \sin^2\beta\cos2\gamma\}$$

Here P_L are the Legendre polynomials of rank L and the angles β and γ are the Euler angles [11] characterizing the orientation of the molecules in the frame of the stretched film. The orientational distribution of the molecules is given by the Boltzmann distribution corresponding to this potential (Fig. 6). The choice of this potential is based on the maximum entropy method [14] and reflects the fact that only the order parameters $\langle P_2 \rangle$, $\langle P_4 \rangle$, and $\langle \sin^2\beta \cos2\gamma \rangle$ of the in-plane symmetry axes of the molecules can be determined in a model-independent way [11]. In addition, we assume that both the absorption and the emission transition dipole moments lie in the plane of the TBpe molecule. Consequently the orientation of each dipole relative to the in-plane symmetry axis is characterized by a single-angle β_m or β_n, where the suffix denotes the absorption and emission dipoles, respectively.

Analysis of the experimental data along the lines described by us earlier [12] shows that TBpe in NC matrices has two preferential orientations along the stretch direction of the film. This finding is a strong indication that TBpe does not behave as a circular disk. The absorption transition moment makes an angle of 7° with molecular symmetry axis 1 shown in Fig. 1, while the emission transition moment makes an angle of 20° with the same axis. The difference angle between the absorption and the emission transition dipole of 13° corresponds with the value found for the steady-state anisotropy.

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