

Spectroscopic studies of fluorescent perylene dyes

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Abstract—The lowest electronic transition of the fluorescent perylene dye bis-(3,5-di-*tert*-butylphenyl)-perylene-3,4:9,10-bis(carboximide) has been investigated by means of linear dichroism (LD), circular dichroism (CD), and by steady-state and time-resolved fluorescence spectroscopic techniques. The absorption and emission transition dipoles were found to be parallel with one direction along the whole vibronic progression of the spectra and thus correspond to a single electronic transition. A Förster radius of 54.6 Å was found for the dye in ethanol at 298 K. LD and absorption spectra obtained for the two stereoisomers of the dye when solubilized in a lyotropic liquid crystalline phase and in unilamellar vesicles suggest that the dyes form aggregates, presumably of J type. CD experiments suggest that these are linear J aggregates.

INTRODUCTION

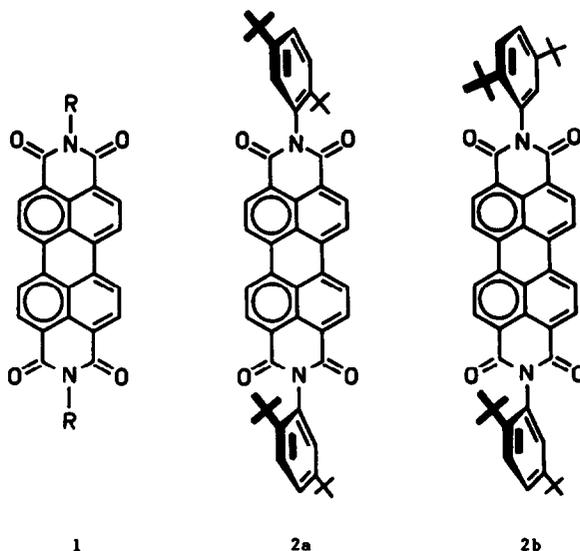
PERYLENE DYES, perylene-3,4:9,10-tetracarboxylic bisimides (**1**) have unusually high light and chemical stabilities and are used as technical pigments [1]. Their strong fluorescence in solution was reported much later than their innovation [2] mainly due to the bad solubility of these dyes. This problem could be overcome by the introduction of *tert*-butyl groups to aromatic substituents [3]. Among these, dye **2** is of interest for practical applications [4, 5] and can be obtained in both stereoisomers [5]. These dyes can be used as laser dyes [6–8], for fluorescent solar concentrators [9], highly sensitive analysis methods [10, 11] and fluorescence immunoassays [18]. It is therefore of interest to obtain more information about the chromophore and its intermolecular interactions.

EXPERIMENTAL

The dyes **2a** and **2b** were prepared and purified according to the literature [5]. 1,2-Dioleoyl-*sn*-glycero-phosphocholine (DOPC) was purchased from Avanti Polar Lipids Inc. (U.S.A.). Details concerning the preparation of unilamellar vesicles are given in [12]. The lyotropic nematic phase was composed of potassium laurate, KCl and D₂O as it described in Ref. [13]. The macroscopical alignment of the liquid crystal was checked by observing the samples when placed between two crossed polarizers.

The steady-state fluorescence spectra and anisotropies were obtained using a SPEX Fluorolog 112 instrument (SPEX Ind., New Jersey, U.S.A.), equipped with Glan-Thompson polarizers. The spectral bandwidths were 5.6 and 2.7 nm for the excitation and emission monochromators, respectively. Further details concerning correction factors and the calculation of fluorescence anisotropies are given in Refs [13] and [14]. A PRA 3000 system (Photophysical Research Ass. Inc., Canada) was used for single-photon counting measurements of the fluorescence decay. The excitation source was a thyratron-gated flash lamp (model 510C, PRA) filled with deuterium gas and operated at about 30 kHz. The excitation and emission wavelengths were selected by interference filters (Omega/Saven AB, Sweden) centred at 479 and 550 nm. The fluorescence emission was also observed above 550 nm through a long pass filter Schott KV 550 (Schott, F.R.G.). The maximum absorbance of all samples was kept below 0.08 which corresponds to a total concentration of less than 10⁻⁶ M.

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Linear dichroism (LD) and circular dichroism (CD) were recorded on a JASCO J-600 (Japan). The absorption spectra were recorded on a Varian Cary 219 (U.S.A.) absorption spectrometer supplemented with sheet polarizers (HNP'B, Polarizers Ltd, U.K.).

RESULTS AND DISCUSSION

The absorption and fluorescence spectra of **2a** and **2b** are identical within experimental accuracy and furthermore, regarding their shape, similar to the spectra of perylene as can be seen in Fig. 1. However, compared to the perylene spectra they are red-shifted by about 100 nm.

The fluorescence anisotropies of both isomers in propylene glycol were studied as a function of temperature and a typical result is displayed in Fig. 1. Clearly, both excitation and emission anisotropies are the same in the 440–640 nm wavelength region. Thus, the first electronic transition with its vibronic bands contains one direction of the electronic transition dipole moment. This is in accordance with the results found for perylene. With decreasing temperature the anisotropies increase below 253 K and reach a plateau value of 0.378. This limiting anisotropy is slightly higher than that previously found for perylene and its alkyl derivatives, but it is the same value as found for the perylenoyl derivatives [14]. The limiting anisotropy was calculated as a mean value of the anisotropies determined in the range 430–650 nm. The value is reproducible within ± 0.003 . The

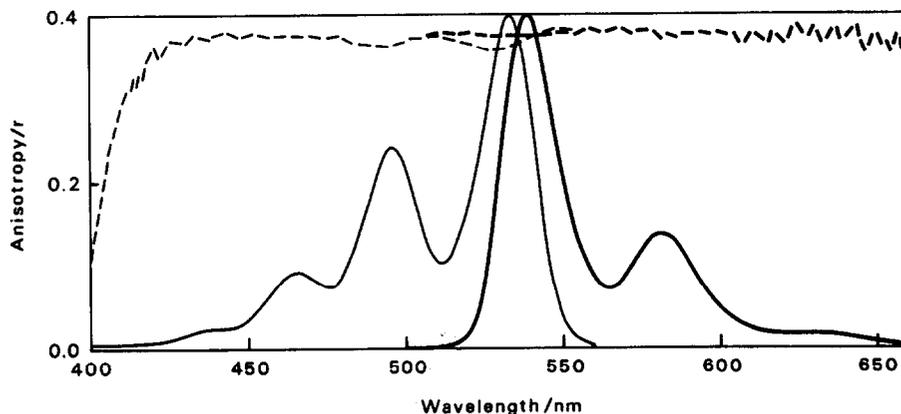


Fig. 1. Absorption (—) and fluorescence spectra (---) of **2a** and **2b** in propylene glycol. The excitation (---) and emission (---) anisotropies were determined at 235 K. The excitation and emission wavelengths were 533 nm and 540 nm, respectively.

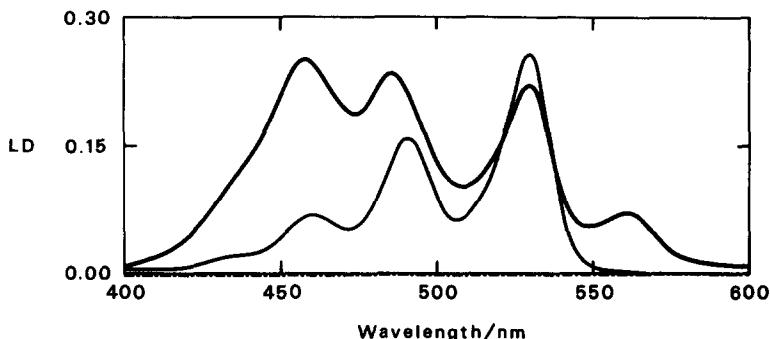


Fig. 2. Linear dichroism (LD) spectra of the perylene dyes **2a** (—) and **2b** (---) when solubilized in a lyotropic nematic liquid crystal which is macroscopically oriented.

value of 0.378 which we obtained is close to the theoretical maximum of 0.4 expected for parallel absorption and emission dipoles (for perpendicular vectors one would expect a value of -0.2). It can thus be concluded that these dipoles are nearly parallel for both isomers.

The anisotropy of **2a** and **2b** decreases monotonically from the maximum value of 0.378 below 245 K to 0.336 at 293 K. Hence, at the lower temperatures the rotational motions are negligible on the timescale of fluorescence. We estimate a mean rotational rate of $3.3 \times 10^7 \text{ s}^{-1}$ at 293 K. The corresponding rates [15] of perylene and 2,5,8,11-tetra-tert-butylperylene are 1.3×10^9 and $1.4 \times 10^8 \text{ s}^{-1}$. The much slower rotational rate of the isomers is qualitatively explained by the large difference in molecular volume.

The time-resolved fluorescence of **2a** and **2b** in 1,2-propanediol and methanol show mono-exponential decays with one and the same fluorescence lifetime of $3.8 \pm 0.1 \text{ ns}$. The fluorescence quantum yield (ϕ_f) in methanol is very close to one (cf. $\phi_f = 99\%$ reported in Ref. [7]). Thus, the radiative lifetime of **2a** and **2b** is also 3.8 ns. As a comparison we note that the radiative and the measured lifetimes of perylene are 6.0 ns and 5.2 ns in ethanol [13]. The slightly longer radiative lifetime of perylene is compatible with its lower molar absorptivity ($\epsilon_{\text{max}} \approx 40\,000 \text{ M}^{-1} \text{ cm}^{-1}$) for the first electronic transition as compared to **2a** and **2b** ($\epsilon_{\text{max}} \approx 95\,000 \text{ M}^{-1} \text{ cm}^{-1}$). The Förster radius (R_0) of the isomers in ethanol has also been determined according to Ref. [12], yielding $R_0 = 54.6 \pm 1 \text{ \AA}$ at 298 K. This number is similar to the typical R_0 values of rhodamine dyes, but smaller than the values found for perylene and its alkyl derivatives. For example, 2,5,8,11-tetra-tert-butylperylene has a Förster radius of 33 Å [12].

Notice that **2a** and **2b** differ only with respect to a rotation of the di-tert-butyl-phenyl ring about an N–C bond. No spectroscopic differences could be detected within the accuracy of these experiments. This is in agreement with previous IR and UV–vis. studies concerning peak positions, line shape, molar absorptivity and fluorescence quantum yields [5].

The linear dichroism (LD) of **2a** and **2b** solubilized in a lyotropic nematic liquid crystal was also investigated. This liquid crystalline phase consists of long rod-like micelles that macroscopically orient in a strong magnetic field (5.9 T). The long axis of the aggregates align parallel to the magnetic field. The LD recorded with the linear polarizations of light parallel and perpendicular to the aggregates is shown in Fig. 2. The LD spectrum of **2b** has the same shape as the absorption spectrum $A(\lambda)$ in the 430–530 nm wavelength region. Hence, $\text{LD}(\lambda)/A(\lambda)$ is a constant which shows that this spectral region contains one direction of the electronic transition dipole moment. The average orientation of the electronic transition dipoles can be calculated using an order parameter [13] of $S = 0.07$. This value is compatible with a low orientation and indicates that the long axis of the dye tends to orient parallel with the rod-like micellar aggregates, provided that the transition dipole moment is long-axis-polarized as in perylene. However from this experiment we cannot draw any conclusion as to whether the direction of the electronic transition dipole is parallel to the long or short axis of the molecular plane. For assigning this polarization one needs independent information about the orientation distribution of the molecules

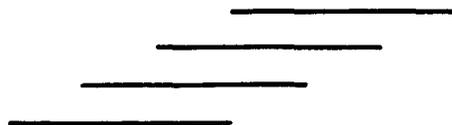


Fig. 3. Schematic linear orientation of dye molecules in J aggregates.

with respect to the long axis of the system. Taken together, the LD and the fluorescence anisotropy experiments show that the absorption spectrum between 420 and 550 nm is the lowest and a purely polarized electronic transition.

For **2a** the absorption and the LD spectra are more complicated. Here, $LD(\lambda)/A(\lambda)$ is no longer constant with wavelength which strongly suggests that different kinds of absorbing species are present. The new band observed at 560 nm in both absorption and LD spectra indicates that aggregated forms of dyes are present.

These spectral changes are typical of the formation of J type aggregates [16]. It is remarkable that the formation occurs even at concentrations of 10^{-6} – 10^{-7} mol dm $^{-3}$ (but see Ref. [17]).

In order to find out whether the isomers mutually interact also in other aggregates of amphiphiles, we have solubilized them at different molar ratios in unilamellar vesicles of 1,2-dioleoyl-*sn*-glycero-phosphocholine (DOPC). At molar ratios of $[DOPC]/[dye] \approx 100$ – 1000 we observe absorption spectra for both dyes, where the 560 nm band is also present. The spectral shape is actually similar to that of the LD spectrum of **2a** shown in Fig. 2. The shape of the fluorescent spectra changes so that more intensity is emitted at $\lambda_{em} \approx 560$ nm with increasing molar ratio of $[DOPC]/[dye]$. Thereby, the fluorescence spectra appear broadened. For such samples the excitation anisotropy (monitored at 536 nm) is constant with wavelength, while the emission anisotropy (monitored at 536 nm) strongly decreases at wavelengths longer than 560 nm. We have also investigated the emission anisotropy when we excite at 490 nm where there is overlap between the different spectral species. The shape of the emission anisotropy is found to be identical with that observed at $\lambda_{exc} = 530$ nm, but the magnitude of the anisotropy is about 0.03 lower. The change in spectral shape of the fluorescence spectra and the decreasing emission anisotropy observed at $\lambda_{em} = 560$ nm are compatible with energy transfer to and from the monomer absorbing at 560 nm.

Candidates for the new absorbing species are dimers, trimers and J type aggregates which may give rise to absorption bands on the red and blue sides of the main vibronic band at 530 nm. Additional information about the structure of such aggregates can be extracted from CD spectroscopy. We find no detectable CD effects in the 400–600 nm region. These results are compatible with a coplanar aggregation of the dyes where the transition dipoles are parallel, as is schematically illustrated in Fig. 3. If the aggregates are of J type these can be linear (cf. Fig. 3) or helical. In order to find support for the helical structure, we have studied the CD of the isomers when solubilized in lipid vesicles which consist of optical active lipid molecules. The lipid matrix should induce a preference of one enantiomer of the two possible helices, and a CD effect of the absorption of the J aggregates might be expected. No CD effect was observed and this indicates that the J aggregates are linear as given in Fig. 3. Notice however, that this does not exclude that the aggregates are dimers or trimers.

CONCLUSIONS

The single electronic transition involving the long wavelength absorption of the perylene dyes and the parallel transition moment in absorption and emission make the perylene dyes an ideal probe for the investigation of orientation phenomena. The orientation can be controlled by the substituents at the terminal nitrogens. The results are important for example in the development of immunoliposomes [18, 19]. These results will be reported elsewhere.

A further interesting result is that **2a** forms aggregates more easily than does **2b**. One can compare this effect with the lower solubility of **2a** in organic solvent compared to **2b**, both in solvents with high and low polarity [5]. This indicates a higher crystal lattice energy of **2a** compared to **2b**, because specific solvent effects can be excluded by the use of very different types of solvents. The lattice energy cannot be estimated by comparing the melting points, for the dyes decompose at temperatures above 360°C before melting. The higher lattice energy of **2a** corresponds to a more economic and simple packing of these molecules. A reason for this might be the point symmetric oriented tert-butyl groups in the molecule which gives the one side of the chromophore the same shape as the other one and provides a simple stacking structure.

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