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ARTICLES

Femtosecond Fluorescence Upconversion Study of the Rotations of Perylene and Tetracene in Hexadecane

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The rotational relaxation of perylene and tetracene in hexadecane has been studied at several temperatures by femtosecond fluorescence upconversion. The initial emission anisotropy values of both D_{2h} symmetric fluorophores are now found to be very close to the theoretical value of 0.4, and the anisotropy decay observed with subpicosecond time resolution can no longer be described as a biexponential. The traditional in-plane and out-of-plane rotational diffusion time constants of perylene in hexadecane are verified to be linearly dependent on viscosity/temperature. Moreover, a third, ultrafast rotation, empirically fit to a time constant of ∼ 450 fs for perylene and ∼600 fs for tetracene, has been found. It is independent of temperature and viscosity, and the amplitude ("β_{fast}") explains the previously described "r_0 defect ". Since β_{fast} is ∼0.03 for tetracene vs ∼0.05 for perylene, we presently suggest that "in-plane" femtosecond libration of the molecules within a solvent pocket yields the observed anisotropy.

1. Introduction

The rotational relaxation of molecules in solution depends strongly upon details of their interaction with the surrounding solvent.[1] As a potentially great source of information on solute−solvent interactions, studies of rotational diffusion can reveal not only local "microviscosity" but also the details of solvent organization, such as microheterogeneity or orientational order.[2−4] Diffusion coefficients for rigid molecules of various shapes may be calculated from hydrodynamic theory. For fluorescent probes with appropriate symmetry, the anisotropic rotational diffusion theories can be simplified considerably.[5,6] For example, "rodlike" probes with excitation and emission dipoles held closely to the long axis become insensitive to rotation about that axis; "disklike" probes with D_{4h} or higher symmetry are reporters of "out of plane" rotation alone. Experimentally, time-resolved fluorescence anisotropy, providing the angular autocorrelation function of the emission transition dipole, is a powerful measurement technique to extract diffusion coefficients.

Perylene is a good model system for rotational diffusion studies, since two relaxation times (dominated by "in-plane" and "out of plane" rotation) may be reliably determined under most experimental conditions.[7−13] A number of studies of perylene rotations have been reported in both isotropic and ordered (e.g., membrane bilayer) surroundings.[14−17] In viscous solvents, the Brownian rotations "in plane" have typically been about 10-fold faster than those "out of plane".[2] In both viscous and glassy solvents, the apparent initial anisotropy r_0 has not been seen to exceed 0.35, while the theoretical value for parallel excitation and emission dipoles is 0.4. This theoretical value is, however, closely approached for 3,9-dibromoperylene in glycerol.[11]

Potential explanations for perylene's "r_0 defect" can be separated into four categories. First, inadequate time resolution: Since the temporal resolution of transient signals is limited by the detector's "impulse response function" (or, equivalently, by limited bandwidth in the frequency domain), a rapid true rotational depolarization may occur after excitation yet prior to effective measurement, which means a depolarization has completed within about 20 ps (or less than microchannel plate-limited response time). Second, solvent relaxation: The initial excited state of the probe couples electrostatically with local solvent, and the resulting "relaxed" state may have an emission transition moment displaced slightly from the initial position. Hence, the time scale of this pseudorotation should match that of spectral evolution. This is analogous to "level crossing" kinetics.[18] Third, incomplete spectral separation between molecular transitions that yield long and short axis (perpendicular) transition moments, respectively: This is especially likely if the excited molecule can break its D_{2h} symmetry.[20] Fourth, local thermalization: The energy of the Stokes shift (between excitation and emission wavelengths) corresponds to ∼ 9.6 kcal/mol.[21] Depending upon the excess energy above the origin and thermal conduction properties of the solvent, a vibrationally "hot" solute molecule might temporarily be surrounded in solvent molecules with temperature tens of degrees above ambient, yielding lower effective viscosity for perhaps a few tens of femtoseconds.

To discern among those mechanisms, in this paper we present femtosecond time-resolved measurements of perylene and tetracene rotation in hexadecane at different temperatures. A third rotation time, empirically fit to a time constant of ∼450fs for perylene and ∼600 fs for tetracene, has been found. A mechanism of "in-plane" femtosecond libration of the molecules

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within a solvent pocket appears to best explain these results and prior observations of a "r_0 defect".

2. Experimental Section

The time-resolved emission anisotropy decay measurements were carried out using an ultraviolet femtosecond upconversion system. A mode-locked Ti:sapphire laser (Tsunami, Spectra-Physics) pumped by an argon ion laser (Beamlock 2060, Spectra-Physics) was used to generate a 600 mW pulse train with a typical pulse duration of 120 fs at a repetition rate of 80 MHz. The output wavelength can be tuned from 780 to 925 nm and converted to visible (150 mW) or ultraviolet (50 mW) in a harmonic generator (GWU, Spectra-Physics). The second (VIS) or third harmonic (UV) pulse was focused into a free-standing jet (1 mm thickness) of sample solution for photoexcitation. The residual fundamental pulse was retroreflected from a cube corner on a computer-controlled stage, and this variably delayed pulse was used as a gate pulse for the upconversion process. The fluorescence emission was collected and focused into a 0.3 mm thick BBO mixing crystal. A band-pass filter preceded the collecting lens to remove any remanent excitation, and the fluorescence polarization (parallel and perpendicular) was selected by rotating a zero-order broad-band half-wave plate behind the collecting lens. The upconversion signal was produced via type I collinear sum frequency generation with the gate pulse in the crystal, separated from the strong background using a band-pass filter and monochromator, and finally detected by a "solar blind" photomultiplier (dark counts < 1 cps) with a single photon counter (Ortec 994, EG&G). The "lamp" (or apparatus) function was determined by measuring the cross-correlation either between the vis (or UV) laser and infrared laser or between UV-generated Raman scattering in water and the infrared laser. In both ways, the lamp (instrument response) function was found to be around 390 fs (fwhm), with a timing jitter of less than 30 fs. Instrument calibration with the linear fluorophore *p*-terphenyl yielded an initial anisotropy of 0.40 ± 0.01 and a single rotational correlation time of 41 ps in cyclohexane (both from Sigma/Aldrich).

Steady-state absorption and fluorescence spectra were recorded with a diode array spectrophotometer (HP 8452A) and Fluorolog-3 spectrophotofluorometer (SPEX), respectively.

Perylene (99+%) and tetracene (2,3-benzanthracene, 98%) were purchased from Aldrich Chemical Co. Hexadecane (anhydrous, >99%) was also from Aldrich. They were used without further purification. A typical concentration in hexadecane solution was $(1-3) \times 10^{-3}$ M. A fresh sample solution was prepared for each time-resolved measurement. The absorption spectra at these millimolar concentrations did not differ from those at 10^{-5} M.

3. Results and Discussion

3.1. Anisotropic Rotation. When a fluorophore is excited with a pulse of horizontally polarized light, the collinear time-resolved anisotropy decay $r(t)$ is determined by measuring the decay of the horizontally (i.e. parallel $I_{\|}$) and vertically (i.e. perpendicular $I_{\perp}$) polarized components of the emission, which is given by[6]

$$r(t) = \frac{I_{\|}(t) - I_{\perp}(t)}{3I_{\mathrm{M}}(t)} = \frac{I_{\|}(t) - I_{\perp}(t)}{I_{\|}(t) + 2I_{\perp}(t)} \qquad (1)$$

where $I_{\mathrm{M}}(t)$ is the total (magic angle) intensity decay.[19] In practice, all the multiexponential intensity decay functions are convolved with the lamp function $L(t)$ to fit the experimental $I(t)$ functions.

Generally, $r(t)$ can be theoretically described as a multiexponential decay,

$$r(t) = r_0 \sum_j g_j \exp(-t/\phi_j) = \sum_j \beta_j \exp(-t/\phi_j) \qquad (2)$$

where $r_0 = \Sigma_j \beta_j$ is the limiting anisotropy in the absence of rotational diffusion or energy transfer, and it is given for most fluorophores by

$$r_0 = \frac{2}{5}\left[\frac{3\cos^2\theta - 1}{2}\right] \qquad (3)$$

θ is the displacement angle between the absorption and emission dipoles, which can vary from 0 to 90°. Thus, r_0 must lie within the range -0.20 to 0.40 for an isotropic solution with single-photon excitation.

Assuming perylene rotates like an oblate ellipsoid, its hydrodynamics normally can be described using only two diffusion coefficients $D_{\|}$ (in plane) and $D_{\perp}$ (out-of-plane), respectively.

$$r(t) = \pm 0.3 \exp(-4D_{\|}t - 2D_{\perp}t) + 0.1 \exp(-6D_{\perp}t) \qquad (4)$$

where the plus and minus correspond to selective excitation from S0 to S1 and S*n*, respectively. For perylene, well-separated S0 → S1 and S0 → S*n* transitions are oriented along the long and short axes, respectively. Therefore, r_0 is predicted to be near 0.4 for the S0 → S1 transition. For S0 → S*n*, rapid internal conversion yields an emission dipole along the long axis prior to any rotations, so r_0 approaches -0.20. Prior measurements of perylene, however, failed to approach those limiting values.

In this paper, we have instead described the anisotropy decay of perylene with three correlation times,

$$r(t) = \beta_1 \exp(-t/\phi_1) + \beta_2 \exp(-t/\phi_2) + \beta_{\mathrm{fast}} \exp(-t/\phi_3) \qquad (5)$$

The amplitudes β_1 and β_2 depend on the angles between the absorption and emission transition moments found after about 1 ps, and the two rotational correlation times ϕ_1 and ϕ_2 are determined by the two diffusion coefficients $D_{\|}$ and $D_{\perp}$,

$$\phi_1 = (4D_{\|} + 2D_{\perp})^{-1} \quad \phi_2 = (6D_{\perp})^{-1} \qquad (6)$$

Further, these first two (hydrodynamic) rotational correlation times are functions of the solvent viscosity (η), solute volume (V), and temperature (T):

$$\phi = C\frac{\eta V}{RT} \qquad (7)$$

Here R is the gas constant.

The third amplitude β_{fast} and its correlation time ϕ_3 are used empirically to fit the ultrafast rotation (or libration), although we recognize this motion is likely nonexponential.

3.2. Steady-State Fluorescence. Figure 1A,B shows the absorption and emission spectra of perylene and tetracene in hexadecane, respectively. The molecular structures are also depicted in the inset. Both perylene and tetracene exhibit three absorption bands in the visible and ultraviolet range. For perylene, the lowest absorption band is around 410 nm, assigned to the S0 → S1 transition (transition dipole moment along the long axis). The second band is at 254 nm for the S0 → Sn transitions (along the short axis). The shortest band around 220 nm is a mixture of excited states whose dipoles can lie along

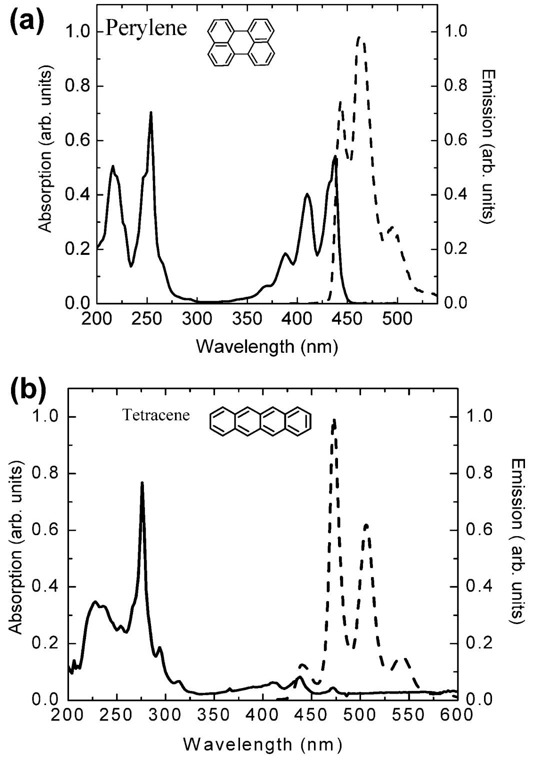

Figure 1. Ultraviolet−visible absorption spectrum (solid line) and fluorescence spectrum (dashed line) of perylene (A) and tetracene (B) in hexadecane with 1 mM solution. Both molecular structures are depicted in the insets.

either axis. For tetracene,[19,20] the strongest band is peaked around 273 nm, which is assigned to the S0 → ^1B_b transition, whose moment is parallel to the long axis of the molecule. A weak absorption showing clear vibrational structure around 410 nm is due to the S0 → ^1L_a transition. (Parallel to the short axis). Near 410 nm, both D_{2h} symmetric fluorophores can be excited to yield different correlation times.

3.3. Anisotropy Decay of Perylene. Prior measurements of perylene rotation in various solvents yielded biexponential decay of the fluorescence emission anisotropy.[8] Due to the "r_0 defect", the expected β_1 value 0.3 was never achieved. When we first performed upconversion studies on the same molecule in hexadecane in a wide time range (200 ps) with 1 ps steps, the same sort of result was found. However, after we improved the scan time step to 20 fs, this biexponential fit became unacceptable. Figures 2 and 3 show the fluorescence intensity (parallel, upper curve; perpendicular, lower curve) decay on different time scales. The insets are the time-resolved anisotropy with the biexponential model (dashed line) and the triexponential model (solid line) fit, respectively. The sample was excited at 410 nm. After analysis with the triexponential model (eq 5) convolved with the nearly Gaussian lamp function (solid curve), normal in-plane and out-of-plane decay time constants have been calculated, $\beta_1 = 0.24$, $\phi_1 = 21$ ps and $\beta_2 = 0.1$, $\phi_2 = 130$ ps. One very fast component ($\phi_3 = 450$ fs) with the amplitude of $\beta_{\text{fast}} = 0.05$ was found. Therefore, $r_0 = 0.39 \pm 0.02$, a value comparable to the maximum measured for DPH and other "rodlike" probes, was obtained. We questioned the origin of this key ∼450 fs reorientation term. The large measured r_0 makes state mixing (reason 3 above) unlikely, and the lack of spectral relaxation in a nonpolar solvent (hexadecane) makes solvent relaxation (reason 2) unlikely. Excess thermal energy (reason 4) concepts, regardless of precise theory predictions, demand that excitation of S0 → S*n* should yield both a faster relaxation and a larger associated β than S0 → S1. In our experiments, excitation of S0 → S*n* at 260 nm yielded an apparent r_0 of −0.18, a β_{fast} of −0.05, and nearly the same correlation time of 450 fs, which implies that the fourth reason is unlikely too. The remanent "negative r_0 defect" of 0.02 can be reconciled by the overlap between two S*n* states (or S2 and S1 tail), the weaker of which is long axis polarized. We deduce this from measurements of r_0 in the excitation range 258−275 nm, as shown in Table 1. The mixing of these states varies slowly, and we could not fully escape the consequent overlap.

TABLE 1: Dependence of Initial Anisotropy Values with Excitation Wavelength (at S2 State)

	excitation (nm)				
	260	262	267	269	271
r_0	−0.18	−0.18	−0.16	−0.15	−0.12

Having established the presence of a rotational depolarization responsible for the "r_0 defect", we sought to further characterize the rotation. Given the disklike rotation of perylene, it is anticipated that the rapid mode might be quasiballistic.[16] Such

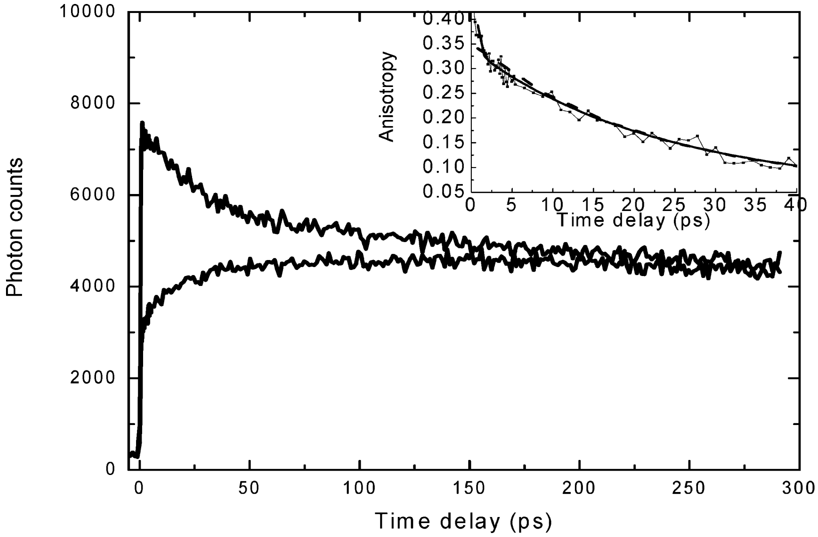

Figure 2. Fluorescence intensity decay for perylene in hexadecane at 476 nm after excitation at 410 nm within 300 ps: top curve, parallel; bottom curve, perpendicular. The anisotropy decay is shown in the inset along with the fit (solid line) $r(t) = 0.24 \exp(-t/21) + 0.1 \exp(-t/130) + 0.05 \exp(-t/0.45)$, and the fit (dashed line) $r(t) = 0.256 \exp(-t/19) + 0.1 \exp(-t/126)$.

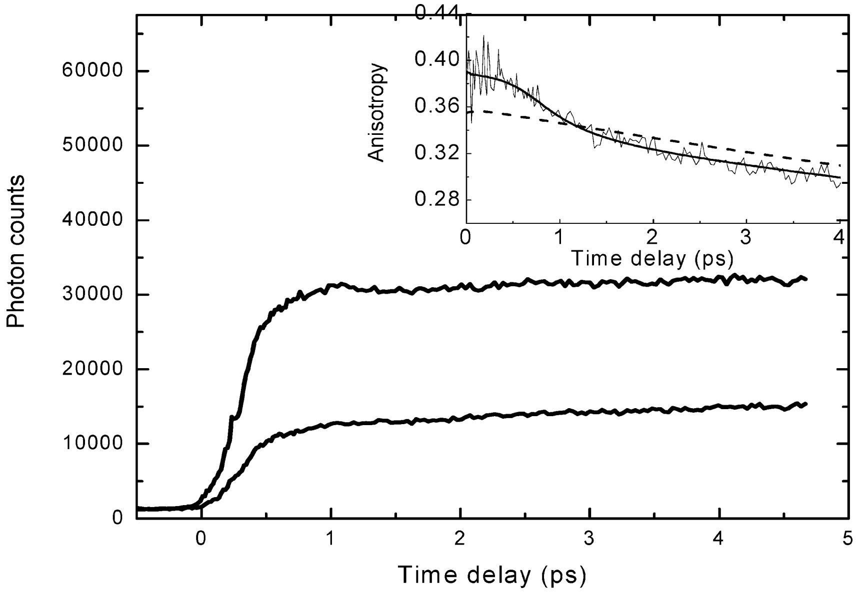

Figure 3. Fluorescence intensity decay for perylene in hexadecane at 476 nm after excitation at 410 nm within 4 ps: top curve, parallel; bottom curve, perpendicular. The anisotropy decay is shown in the inset with two fits.

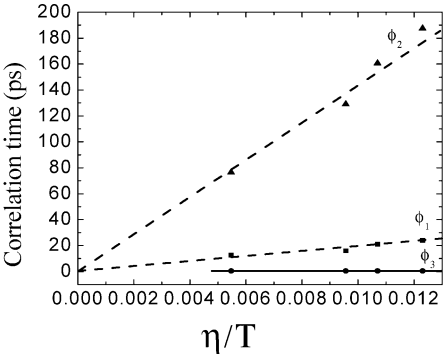

Figure 4. Correlation times vs viscosity/temperature for perylene in hexadecane. Linear fits are shown as solid lines.

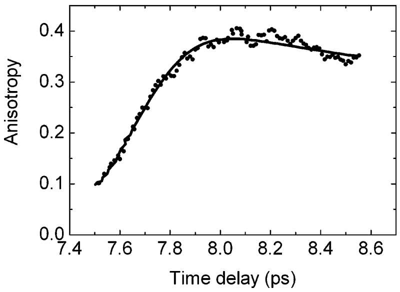

Figure 5. Typical anisotropy decay for tetracene in hexadecane in the initial 1 ps after excitation at 410 nm. The solid black line is a convolved fit using the multiexponential eq 5.

a "slipping" in plane rotation might occur with little or no solvent friction, at least within the first 1 ps. To investigate the coupling of the ultrafast rotation to solvent, we carried out measurements for both perylene and tetracene in hexadecane at several temperatures.

Figure 4 shows three correlation times vs viscosity at several temperatures for perylene in hexadecane. The sample was excited at the wavelength of 410 nm. The normal in- and out-of-plane rotational diffusion time constants ϕ_1 and ϕ_2 are found to be linearly dependent on viscosity/temperature as expected. The ultrafast correlation time $\phi_3 = 450$ fs is essentially unchanged, however, although viscosity changes radically. This suggests either quasiballistic motion or some sort of "internal rotation" of the transition. Similarly, a comparable ultrafast rotation with $\phi_3 = 600$ fs does not vary with changing viscosity/temperature for tetracene solution in hexadecane. Figure 5 shows a typical time-resolved fluorescence anisotropy decay of tetracene in hexadecane at 32 °C in the initial 1 ps window, with $r_0(t) = 0.27\ \exp(-t/10) + 0.08\ \exp(-t/160) + 0.03\ \exp(-t/0.6)$ and $r_0 = 0.38 \pm 0.02$. The excitation wavelength is also 410 nm. The axis shift is a result from the different zero time delay between the lamp function and fluorescence. Interestingly, β_{fast} is 0.05 for perylene and 0.03 for tetracene, which suggests the previously described "r_0 defect " depends on solute shape, as expected in solvent pockets.[12,21]

In conclusion, the initial emission anisotropy values of both fluorophores are very close to the theoretical value of 0.4. The two slower rotational correlation times are in accord with "in-plane" and "out-of-plane" diffusive reorientation, and they vary monotonically with the viscosity and temperature of solution. The ratio of diffusion constants for the axes perpendicular and parallel to the plane of perylene molecular, $D_{\|}/D_{\perp}$, is about 8.5–11.2 for perylene in hexadecane, as expected.[8] The most important thing is that the ultrafast rotation rate ($1/\phi_3$) is nearly unchanged (empirically fit to a time constant of ~450 fs for perylene and ~600 fs for tetracene), although viscosity changes 30-fold. The amplitude β_{fast} we measured explains the previously described "r_0 defect ". The fact that β_{fast} alternated sign when UV excited, but maintained essentially the same magnitude, requires a dominant "in-plane" nature to the libration, since isotropic libration would have yielded a negative magnitude half the positive. We cannot, however, rule out some "out-of-plane" contribution to this term.

It is, of course, impossible to distinguish rotation of the molecule from rotation of the oscillator using emission anisotropy. Nevertheless, the combination of S1 and S*n* data and the variety of temperatures strongly suggests librations. Clearly, quantification of β_{fast} yields additional insights beyond those available from measurements of the rate alone. β_{fast} is ~0.05 for perylene vs ~0.03 for the longer, thinner tetracene molecule; this implies that "in-plane" femtosecond libration of the molecules within a solvent pocket is responsible for the observed anisotropy transient. This fit is, of course, only a first-order approach for the most rapid rotations of the solutes; hence, molecular dynamics simulations are currently being carried out in our laboratory to better understand this phenomenon.[29] The simulations encompass both hydrophobic and hydrogen bonding solvents such as ethylene glycol; we found this libration is also present in the latter (data not shown).[26] We have seen similar librations for Trp analogues in solution,[24,25] and we expect to find related (but differently constrained) librations in our studies of peptide and protein dynamics,[27,28] leading to more appropriate models.

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