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References and Notes

(1) Gray, D. G. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1983**, *37*, 179.
(2) Gilbert, R. D.; Patton, P. A. *Prog. Polym. Sci.* **1983**, *9*, 115.
(3) Gray, D. G. *Faraday Discuss. Chem. Soc.* **1985**, *79*, 257.
(4) Werbowyj, R. S.; Gray, D. G. *Macromolecules* **1980**, *13*, 69.
(5) Werbowyj, R. S.; Gray, D. G. *Macromolecules* **1984**, *17*, 1512.
(6) Shimamura, K.; White, J. L.; Fellers, J. F. *J. Appl. Polym. Sci.* **1981**, *26*, 2165.
(7) Suto, S.; White, J. L.; Fellers, J. F. *Rheol. Acta* **1982**, *21*, 62.
(8) Bhadani, S. N.; Gray, D. G. *Mol. Cryst. Liq. Cryst.* **1984**, *102*, 255.
(9) Shannon, P. *Macromolecules* **1984**, *17*, 1873.
(10) Finkelmann, H.; Rehage, G. *Makromol. Chem., Rapid Commun.* **1980**, *1*, 733.
(11) Nishio, Y.; Yamane, T.; Takahashi, T. *J. Polym. Sci., Polym. Phys. Ed.* **1985**, *23*, 1043.
(12) Tsutsui, T.; Tanaka, R. *Polymer* **1980**, *21*, 1351.
(13) Krigbaum, W. R.; Cifferi, A.; Asrar, J.; Toriumi, H. *Mol. Cryst. Liq. Cryst.* **1981**, *76*, 79.
(14) Chiellini, E.; Galli, G. *Macromolecules* **1985**, *18*, 1652.
(15) Ito, K.; Kajiyama, T.; Takayanagi, M. *Polym. J. (Tokyo)* **1977**, *9*, 355.
(16) (a) Watanabe, J.; Sasaki, S.; Uematsu, I. *Polym. J. (Tokyo)* **1977**, *9*, 451. (b) Watanabe, J.; Naka, M.; Watanabe, J.; Watanabe, K.; Uematsu, I. *Polym. J. (Tokyo)* **1978**, *10*, 569.
(17) Samuels, R. J. *J. Polym. Sci., Part A-2* **1969**, *7*, 1197.
(18) Navard, P.; Haudin, J. M. *Polym. Sci. Technol.* **1985**, *28*, 389.
(19) de Vries, H. *Acta Crystallogr.* **1951**, *4*, 219.
(20) Bhadani, S. N.; Gray, D. G. *Mol. Cryst. Liq. Cryst.* **1983**, *99*, 29.
(21) Aspler, J. S.; Gray, D. G. *Polymer* **1982**, *23*, 43.
(22) Suto, S.; Kudo, M.; Karasawa, M. *J. Appl. Polym. Sci.* **1986**, *31*, 1327.

Synthesis and Characterization of Pyrene-Labeled (Hydroxypropyl)cellulose and Its Fluorescence in Solution

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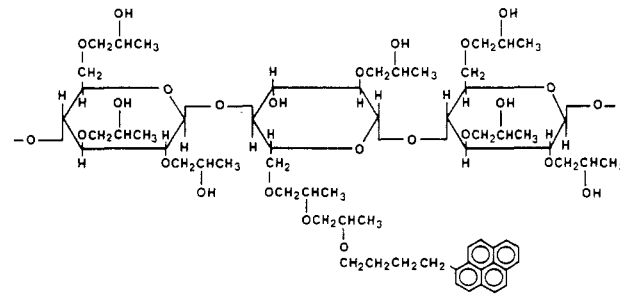
ABSTRACT: The fluorescence spectroscopy of pyrene-labeled (hydroxypropyl)cellulose was examined in solution in various alcohols and in water. The low relative excimer fluorescence intensity in alcohol solvents such as methanol is typical of a randomly coiled polymer containing a small fraction of pyrene groups. The very intense excimer intensity in water suggests that the polymer adopts a different conformation in aqueous solution, characterized by extensive ground-state dimerization and aggregation of the pyrene groups. There is also strong evidence for polymer–polymer association, even at very low polymer concentration, which also contributes to the extent of excimer emission. Other experiments involving fluorescence and UV absorption measurements on these samples also support this view.

(Hydroxypropyl)cellulose (HPC) is a polymer with many important industrial applications. Like (hydroxymethyl)cellulose and (hydroxyethyl)cellulose, HPC is a soluble derivative obtained by chemical functionalization of cellulose. Scientific interest in these polymers derives from the fact that they are semiflexible, nonionic, linear polymers[3] and that they form liquid crystalline states in the melt and in concentrated solutions.[4] A variety of their properties have been investigated, including dilute solution viscosity,[3,5] fluorescence depolarization in sheared solutions,[6a] and polymer interaction with surfactants in aqueous solutions.[6b]

Fluorescence labeling techniques have proved useful in studying a wide range of properties of various polymers and their mixtures. We anticipate that these techniques will also be useful in the study of HPC-containing systems. In this paper we describe the synthesis of pyrene-labeled HPC (HPC-Py), its characterization, and some of its fluorescence properties in dilute solution.

In order to carry out experiments in aqueous solution without concern for the possible loss of label through hydrolysis, we chose to attach the pyrene groups by means of an ether linkage. A convenient method involves treating a solution of HPC with a strong base to generate alkoxide sites, followed by reaction with 4-(1-pyrenyl)butyl tosylate. The pyrenes become attached, presumably, to oxypropyl pendant groups, leading to the kind of structure depicted below:



From our experiments, we show, for example, that the pyrene groups are attached to sites of considerable local flexibility. In alcohol solvents, the pyrene groups are predominantly unassociated, but form excimers by a dynamic mechanism after photoexcitation. In water, a different behavior is observed. The pyrenes stack or dimerize in a face-to-face configuration, so that the excimer and monomer emissions derive from different populations of

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pyrene groups. Various cosolvents or cosolutes can interact with HPC-Py to affect the extent of pyrene association in the ground state.

Experimental Section

Materials. (Hydroxypropyl)cellulose (HPC, Klucel L., Hercules Inc.) was purchased from Aldrich Chemical Co. The manufacturer's literature claims a molecular weight of 100 000. Other groups have reported measurements consistent with this value. One recent reference reports M(sedimentation) = 82 000;[8a] and another, 73 000 with M_n = 36 000.[8b] Spectral grade solvents were used. The distilled, deionized water had a conductance less than 5×10^{-7} Ω^{-1} cm^{-1}. 4-(1-Pyrenyl)butanol[9] was prepared by reduction of 4-(1-pyrenyl)butyric acid with lithium aluminum hydride in tetrahydrofuran (THF) and was recrystallized from ethyl acetate/hexane (2:1 (v/v)). Dimethylformamide (DMF) was dried by reflux over calcium hydride, followed by distillation at reduced pressure.

Synthesis. 4-(1-Pyrenyl)butyl Tosylate (2).[10] To a solution of 4-(1-pyrenyl)butanol (1.0 g, 3.65 mmol) in chloroform (10 mL, flushed through alumina) were added first pyridine (0.58 g, 7.3 mmol) and then p-toluenesulfonyl chloride (1.04 g, 5.48 mmol) in small portions over a period of 5 min. The mixture was stirred at ca. 22 °C in the dark under nitrogen for 1.5 h. The reaction mixture, diluted to 20 mL with diethyl ether, was extracted successively with 10% aqueous HCl (twice), water (twice), 5% aqueous sodium bicarbonate solution (twice), water (once), and saturated brine (twice). The organic layer was dried over $MgSO_4$ and evaporated to yield an oil. Crystallization from ethyl acetate/hexane (25 mL, 1:2 (v/v)) yielded **2** (1.34 g, 86%): mp 91–92 °C; λ_{max} (ϵ, THF) 343 nm (38 500), 327 (25 000); ^{1}H NMR ($CDCl_3$, δ) 1.6–2.0 (m, 4 H), 2.22 (s, 3 H), 3.27 (br t, 2 H), 4.06 (br t, 2 H), 7.1–8.3 (m, 13 H).

Pyrene-Labeled (Hydroxypropyl)cellulose (HPC-Py). To prepare the sample of higher pyrene content (HPC-Py/26), a solution of 4-(1-pyrenyl)butyl tosylate (250 mg, 5.8 mmol) in dry DMF (1.0 mL) was added to a solution of HPC (2.0 g) in dry DMF (15 mL). The HPC had previously been dried by azeotropic distillation of water from solutions of the polymer in toluene (three times). The mixture was stirred at ca. 22 °C for 2 h. Then sodium hydride (100 mg, 60% dispersion in oil, washed twice with dry hexanes) in DMF (0.5 mL) was added, and the mixture was allowed to stand overnight. Excess base was neutralized with diluted acetic acid (3 mL, 3:1 (v/v) with water).

Solvents were removed at 55 °C at reduced pressure. The residual material was dissolved in THF (20 mL). Addition of hexanes led to the precipitation of the polymer, which was separated by decanting off the supernate. This reprecipitation was repeated three times. The final product was dried in vacuo at 65 °C for 24 h to yield 0.95 g of a white amorphous solid. GPC analysis indicated that all Py groups in the sample were attached to the polymer chain. By UV analysis, using 4-(1-pyrenyl)butanol as a model, the pyrene content was calculated to be 1.23×10^{-4} mol/g polymer. This corresponds to 1 pyrene per 26 glucose units in a polymer of M_n = 36 000. The denominator in HPC-Py/26 represents the average number of glucose units per pyrene group in the polymer.

A sample of lower Py content (HPC-Py/216) was prepared similarly, using 2.0 g of HPC, 30 mg of 4-(1-pyrenyl)butyl tosylate (0.07 mmol), and NaH (100 mg). The pyrene content in the final polymer (1.9 g) was calculated to be 1.37×10^{-5} mol/g, equivalent to 1 Py per 216 glucose units for a polymer of $M_n \approx$ 36 000.

Instrumentation. ^{1}H NMR spectra were recorded at 80 MHz with a Bruker WP-80 spectrometer. Spectra were run in chloroform-d containing 0.1% Me_4Si as an internal standard. UV–visible spectra were recorded either with a Hewlett-Packard 8450A diode array spectrometer or with a Shimadzu Model 200S spectrometer. Steady-state fluorescence spectra were run on a Hitachi Model 4000 fluorescence spectrometer. Fluorescence decay measurements were made with a time-correlated single-photon-counting instrument purchased from Photochemical Research Associates (London, Ontario, Canada). Molecular weights and molecular weight distributions were estimated by gel permeation chromatography (GPC), using a Hewlett-Packard 1090 instrument equipped with a 1037A refractive index detector and a 8194A diode array UV–visible detector set at 278, 316, 328, and 344 nm. The columns used were PL-gel 10^5, 10^4, 10^3, 500, and 300 Å. Spectral grade THF (BDH) was used as the solvent with a flow rate of 1.0 mL/min.

Fluorescence Measurements. Spectra were fully corrected. The excitation slits were set at 5 nm. Emission slits were normally set at 5 nm, although some experiments were run with 3-nm slits. Because the excitation spectra of the pyrene monomer and excimer emission were different for the polymer samples in aqueous solution, the excitation wavelength had to be selected carefully. A wavelength of 330 nm was chosen for most experiments, since it is 2 nm to lower wavelength of a maximum in the excimer excitation spectrum and 2 nm to longer wavelength from the corresponding maximum in the monomer spectrum. For solutions in alcohol solvents and for quantum yield measurements in mixed solvents, the excitation wavelength was the peak maximum in the UV spectrum closest to 330 nm. All measurements were made at 20 °C. Samples were degassed by vigorous bubbling of solvent-saturated argon for 30 s immediately prior to the measurement. It was found that continued bubbling for longer periods of time made no difference, whereas insufficient degassing, particularly for methanol solutions, led to a reduced fluorescence intensity and in some cases to changes in the excimer/monomer fluorescence intensity ratio (I_E/I_M). In aqueous solution, emission intensities were unaffected by degassing. Most of these experiments were carried out on air-saturated solutions.

Peak heights and peak areas were proportional. Quantum yields were calculated by integration of peak areas after conversion of the spectra of wavenumber units, using quinine sulfate as a standard. Beer's law corrections were applied as necessary for optical density changes, whereas index of refraction corrections were negligible. In aqueous solutions, these values are apparent quantum yields, because monomer and excimer emission derive from different species that absorb light at very similar wavelengths.

Samples for Spectroscopic Analysis. HPC-Py solutions were prepared at room temperature (19 °C) by allowing the polymer to swell and then dissolve in excess solvent. Dissolution in methanol, ethanol, and ethoxyethanol was rapid. In water, glycerol, and diethylene glycol, dissolution was slow. The mixtures were allowed to stand typically 24 h before they were diluted to a known total volume. For the HPC-Py/26 sample, the solutions for spectroscopic analysis contained approximately 5 mg of polymer in 100 mL of solvent. For the sample of lower Py content, 40 mg in 100 mL was used.

Results

Synthesis and Characterization of HPC-Py. Linkage of the pyrene chromophore to HPC was achieved by reaction of the sodium alkoxide derivative of HPC with 4-(1-pyrenyl)butyl tosylate, prepared from the corresponding alcohol. Because of the tendency of HPC alkoxides to form thick gels in DMF, it was necessary to invert the normal order of adding reagents in the Williamson ether synthesis. Instead of adding the tosylate to the preformed alkoxide, the tosylate was mixed first with a solution of carefully dried HPC in DMF. When this solution became clear, indicating that all the tosylate had dissolved, then the sodium hydride was added. Under these circumstances, the diffusion distance of the tosylate to the reactive sites on the polymer was small, and the reaction proceeded in high yield. The extent of pyrene incorporation into the polymer was controlled through the ratio of the reactants.

The purity of the HPC-Py was an important aspect of these experiments. Through the use of tandem UV–visible and refractive index detectors for the GPC analysis, it was established that the pyrene groups were covalently linked to the polymer, that after several reprecipitations the sample contained less than 0.1% low molecular weight pyrene impurities, and that the chemical transformation did not alter the molecular weight or the (broad) molecular weight distribution of the polymer.

The concentration of pyrene chromophores incorporated into the polymer was determined by UV absorption

Table I
Peak-to-Valley Ratios for Pyrene Absorption and Excitation Spectra

sample	OD(343)/OD(334)	$I(343)/I(334)$ λ_{em} = 379	λ_{em} = 489	I_E/I_M
		In Water		
$Py(CH_2)_4OH$	2.0	2.45		
HPC-Py/216	2.16	2.34	1.75	0.10
HPC-Py/26[a]	1.89	2.12	1.75	2.01
		In Methanol		
$Py(CH_2)_4OH$	2.42	2.82		
HPC-Py/216	3.50	2.80		0.04
HPC-Py/26	3.20	2.71	2.44	0.31

[a] At 100 ppm polymer concentration.

measurements in methanol, using 4-(1-pyrenyl)butanol (λ_{max} = 343 nm, ϵ = 45 000) as a model compound. The M_n of Klucel L has been reported in the literature to be 36 000. Using this value in conjunction with the measured incorporation of pyrene groups into the HPC polymer, we estimate that HPC-Py/26 contains on average 1 pyrene per 26 glucose units or 4 Py per chain and that HPC-Py/216 contains 1 pyrene per 216 glucose units or 0.5 Py per chain.

In aqueous solution, HPC-Py/26 exhibits a strong hypochromic effect. The extinction coefficient for the pyrene groups attached to HPC-Py/26 decreases from its (assumed) value of 45 000 in methanol to 25 000 in water. This change is accompanied by a shift in the wavelength of maximum absorption from 343 nm in methanol to 347 nm in water. Aqueous solutions of HPC-Py/216 also exhibit a hypochromic effect, but it is much weaker in magnitude. Here the extinction coeficient of the pyrene chromophore decreases to 40 000.

There is also some peak broadening that occurs in the UV and excitation spectra of aqueous solutions of HPC-Py.[11] A qualitative indication of broadening is the ratio of peak-to-valley intensities. These values are presented in Table I.

Fluorescence Spectra of HPC-Py. Emission and excitation spectra were measured in various alcohol solvents and in water. Salient features of these spectra are reported in this section.

Alcohol Solvents. In methanol, HPC-Py/26 shows an emission due to locally excited pyrene chromophores (intensity I_M, "monomer" emission), with the (0,0) band located at 379 nm and a broad emission centered at 485 nm due to pyrene excimer (intensity I_E). The relative intensities of the two emissions, I_E/I_M, is sensitive to a change of solvents; but in methanol and in ethanol, it did not change measurably over a range of concentrations of HPC-Py/26 from 10 ppm ([Py] = 1.2×10^{-6} M) to 180 ppm. Identical excitation spectra were obtained for emission monitored at 379 nm and at 485 nm, and the maxima correspond with those in the UV absorption spectra.

In other alcohol solvents, the general features of the steady-state fluorescence spectra resemble those in methanol. Changes in I_E/I_M occur. Not surprisingly, this ratio decreases in solvents of higher viscosity. The excimer emission is barely detectable in diethylene glycol. Curiously, in glycerol one can observe a much larger amount of excimer than in diethylene glycol. There are other peculiar features in glycerol, particularly in the excitation spectra, and these become more pronounced for solutions of HPC-Py/26 in water.

The fluorescence spectra in alcohol solutions of HPC-Py/216 present features similar to those of HPC-Py/26,

Table II
Values of the Pyrene Excimer-to-Monomer Fluorescence Intensities for HPC-Py/216 and HPC-Py/26 in Water and Alcohols

solvent	I_E/I_M HPC-Py/216	HPC-Py/26
water	0.13	1.60
methanol	0.05	0.31
ethanol	0.05	0.31
ethoxyethanol	<0.02	0.23
diethylene glycol	<0.02	0.10
glycerol	<0.02	0.18

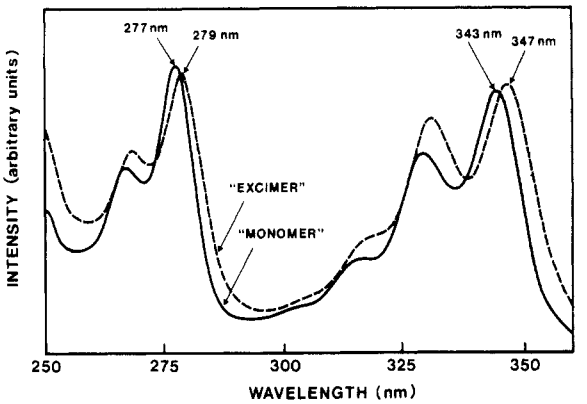

Figure 1. Normalized excitation spectra of HPC-Py/26 in water monitored at 379 nm (monomer) and at 489 nm (excimer).

but the relative amount of excimer with respect to monomer is smaller. In ethoxyethanol, diethylene glycol, and glycerol, no excimer at all can be detected (see Table II).

In water the emission of HPC-Py/26 presents a relatively weak emission due to locally excited pyrene and a strong excimer band with a peak at 489 nm. Excitation spectra for the monomer and for the excimer are clearly different (Figure 1). The general features of the spectra are similar, but the former is blue-shifted by about 4 nm. It is the latter that corresponds to the UV absorption spectrum of the polymer in water solution. These observations all indicate that the excimer originates from pairs or aggregates of pyrene groups that exist prior to excitation. Exciting these samples at 360 nm, or in the far red end of the absorption spectrum (370 or 375 nm), leads to enhanced excimer intensity relative to monomer emission, but the shape and peak position of the excimer band are not changed. A strong hypochromic effect seen in the UV spectra of these samples suggests a face-to-face configuration of pyrene groups within the aggregates. Over a time scale of weeks, neither the UV spectra nor the fluorescence spectra of stock solutions stored in the dark at room temperature (15 °C) underwent any noticeable changes.

In contrast, HPC-Py/216 in water exhibits a strong pyrene monomer emission due to isolated pyene groups and a much weaker excimer band at 489 nm. Because of the lower pyrene content of this polymer, it was necessary to study solutions of higher polymer concentration than in the case of HPC-Py/26. In fact, the I_E/I_M ratio increased from 0.08 to 0.13 when the polymer concentration was increased from 40 to 400 ppm.

The most important question about the intense excimer in HPC-Py/26 is whether it is intramolecular or intermolecular in origin. Over a limited concentration range, a plot of I_E/I_M is linear in [Py], with a very mild slope, suggesting that most of the excimer is formed intramolecularly. In order to examine this behavior in more detail, we carried out two sets of experiments, with results reported in Figure 2. In the first experiment we looked at

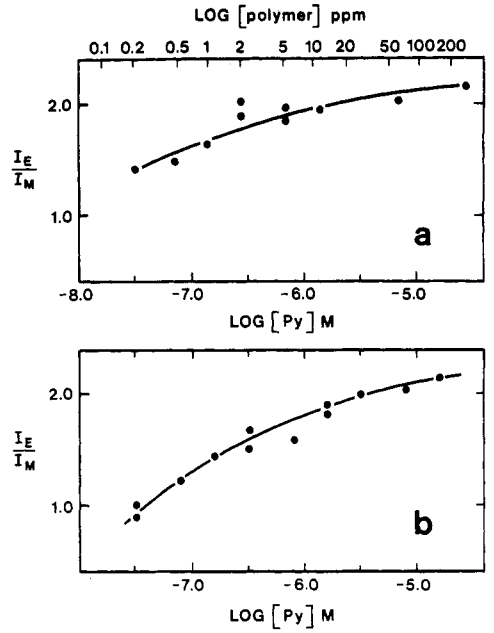

Figure 2. Plots of I_E/I_M as a function of log [HPC-Py/26]: (a) in water solution; (b) in water containing a unlabeled HPC. Here the solutions contained a constant amount (130 mg/L) of HPC polymer.

I_E/I_M over as wide as possible a range of polymer concentration. This range was limited at the dilute end (ca. 0.2 ppm polymer) by our ability to measure fluorescence intensities reliably and at the concentrated end by the formation of turbid solutions at polymer concentrations much above 400 ppm. As shown in semilogarithmic form in the top part of Figure 2, I_E/I_M varies from a value just over 2.0 at the high-concentration limit to a value of 1.4 at 0.2 ppm polymer concentration.

The second set of experiments consisted of competition experiments in which HPC-Py/26 was mixed with unlabeled HPC. The total polymer concentration was maintained at 130 ppm, but the labeled polymer was varied in concentration from 0.2 to 130 ppm. After the polymer solutions were mixed, the samples were allowed to stand for 3–4 h to ensure equilibration. These results are shown in Figure 2b. The changes in I_E/I_M are somewhat more pronounced than in Figure 2a. At the lowest measurable concentration of labeled polymer, the I_E/I_M value is close to 1.0. Both plots are curved downward, implying that the intensity ratio decreases even further at lower concentration of labeled chains. Nevertheless, the values of I_E/I_M reported in Figure 2 are significantly larger than the value of 0.3 found for the same polymer in methanol and ethanol solutions.

Fluorescence Decay Measurements. Fluorescence decay measurements on methanol solutions of HPC-Py/26 showed a nonexponential decay for the monomer emission [$I_M(t)$] which could be fit to a sum of two exponential terms with decay times of 19 and 114 ns (Table III). The excimer profile showed both a growing-in and a decaying component (11 and 67 ns, respectively). Its decay parameters are substantially different from those of the monomer. These results are consistent with dynamic formation of excimer, with little preassociation of ground-state pyrene groups. The complexity of the fluorescence decay reflects the distribution of pyrene separations on the polymer and the fact that various polymer chains in the sample contain different numbers of pyrenes. In addition, the pyrene groups can be attached to chemically different positions on the glucose rings of the polymer chains.

Table III
Fluorescence Decay Measurements[a,b]

	monomer		excimer	
sample	τ, ns	prefactor	τ, ns	prefactor
HPC-Py/26 in H_2O	$\tau_1 = 6$	$a_1 = 0.350$	$\tau = 70$	
	$\tau_2 = 33$	$a_2 = 0.350$		
	$\tau_3 = 106$	$a_3 = 0.300$		
	$\langle\tau\rangle = 83$			
HPC-Py/216 in H_2O	$\tau_1 = 28$	$a_1 = 0.170$	$\tau_1 = 3.5$	$a_1 = 0.643$
	$\tau_2 = 116$	$a_2 = 0.830$	$\tau_2 = 84$	$a_2 = 0.357$
	$\langle\tau\rangle = 112$		$\langle\tau\rangle = 78$	
HPC-Py/26 in MeOH	$\tau_1 = 19$	$a_1 = 0.270$	$\tau_1 = 10.8$	$a_1 = -0.70$
	$\tau_2 = 114$	$a_2 = 0.830$	$\tau_2 = 67$	$a_2 = 1.00$
	$\langle\tau\rangle = 108$			

[a] Intensities were fit to a sum of exponentials: $I(T) = \sum a_i \exp(-t/\tau_i)$. Fits to three exponential terms were employed when fits to two exponential terms were clearly inadequate. [b] The angular brackets indicate mean lifetimes calculated from the data.

The fluorescence decay measurements on HPC-Py in water are summarized in Table III. The most important observation from these data is that no rising component can be detected in the excimer profile. This result implies either that all but a very small fraction of the excimer emission arises from ground-state aggregates of pyrene groups or that the excimer forms faster than resolution (2–3 ns) of the measurement. This situation is quite different from that in methanol, where most of the excimer is formed in a dynamic process. In methanol, the fact that a_1 is less than $-a_2$ indicates that some excimer is formed either very quickly or from ground-state association complexes.

The excimer decay for the HPC/26 sample can be fit reasonably well to a single-exponential curve ($\tau = 70$ ns, $\chi^2 = 1.4$). By contrast, the excimer emission in the HPC-Py/216 sample shows two very well resolved components, a short component (3.5 ns, 7% of the total intensity) and a longer component (84 ns, 93% of the intensity, $\chi^2 = 1.00$). The differences in the decay times of the long components are not necessarily inconsistent, but we have no simple explanation for the short component in the HPC-Py/216 excimer decay.

The monomer decays from all of the samples are nonexponential. For convenience, we report their shapes as fits to a sum of exponential terms. If the polymers in aqueous solution could be described simply in terms of aggregated pyrenes that gave excimer emission and isolated pyrene groups that gave only monomer emission, one might anticipate similar mean decay times from the monomer emission in HPC-Py/26 and HPC-Py/216. The fact that the $\langle\tau\rangle$ value is significantly shorter for the polymer containing more pyrene groups suggests that at least some of these groups form excimers in a dynamic process.

Fluorescence in Mixed Solvents. To investigate further the difference in behavior of HPC-Py in water and in alcohols, the UV absorption and fluorescence spectra of the polymer were measured in water/methanol mixtures. Addition of methanol to an HPC-Py/26 solution in water resulted in a large increase of the optical density of the solution, reflecting a correspondingly large increase in the extinction coefficient of the pyrene chromophore. This change is accompanied by a 4-nm red shift of the (0,0) band in the UV spectrum. In the fluorescence spectrum, the addition of methanol resulted in a decrease of the excimer emission intensity and an increase in I_M. A plot of I_E/I_M vs. solvent composition is shown in Figure 3. With increasing methanol concentration, there is also a

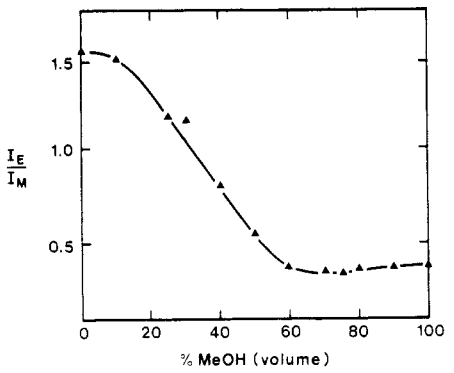

Figure 3. Plot of I_E/I_M for HPC-Py/26 as a function of the water/methanol composition (v/v).

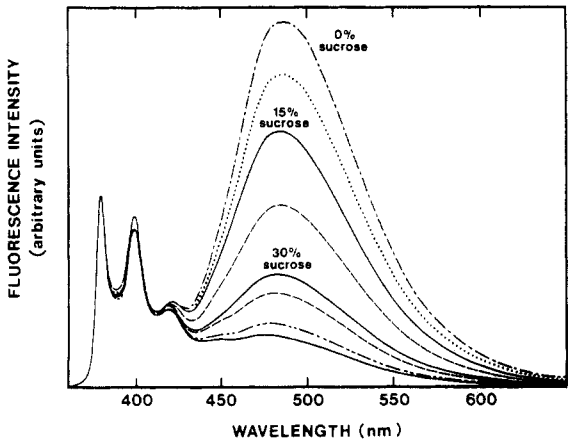

Figure 4. Fluorescence spectra of HPC-Py/26 (0.3 mg/mL) in aqueous solution for varying mixtures of sucrose and water. The sucrose concentration varies from 0% to 60% by weight (λ_{exc} = 330 nm).

small shift to the blue in the position of the excimer maximum.

We were curious to see if solution viscosity had any effect on I_E/I_M in water. This led us to prepare mixtures of sucrose and water as solvent media and examine the behavior of HPC-Py/26. Interesting results were obtained; but as we show below, these are caused by changes in the solvency of the medium rather than viscosity. Two changes occur in aqueous sucrose. First, the extinction coefficient of the pyrene groups increases in solutions containing high concentrations of sucrose. In addition, there are large decreases in the excimer fluorescence intensity and corresponding increases in the monomer intensity. There are no changes in the shape of the monomer band, but the excimer peak shifts to 480 nm at 60% sucrose (by weight). Spectra are shown in Figure 4. In Figure 5 are shown both the changes in I_E/I_M that occur as well as the dependence of solution viscosity on sucrose concentration. Interestingly, the decrease in I_E/I_M does not correspond to the increase in solution viscosity. Rather, it seems that the polymer experiences a change in the solvency of the medium which leads to the break up of the associated pyrenes found in pure water. Supporting this idea is the finding that at 60% sucrose, the monomer and excimer have identical excitation spectra.

Discussion

Pure Solvents. There are no surprising aspects to the behavior of HPC-Py in alcohol solvents. There is little evidence for significant ground-state association of pyrene groups. Upon irradiation, a fraction of the excited pyrenes form intramolecular excimers by diffusion and encounter with other pyrenes. The extent of excimer formation

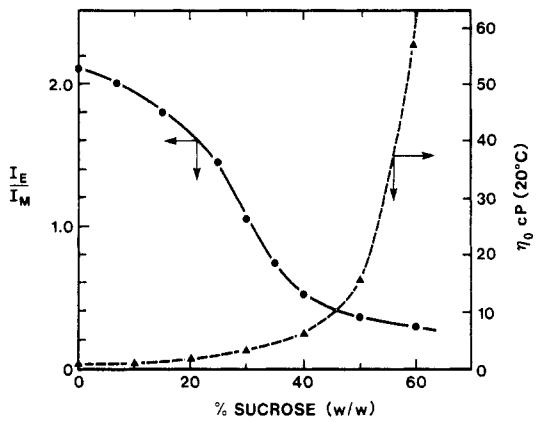

Figure 5. Plot of I_E/I_M for HPC-Py/26 (0.1 mg/mL) in aqueous sucrose solutions and of bulk solution viscosities (in cP at 20 °C) as a function of the weight percent sucrose in water.

depends upon factors such as the flexibility of the polymer, the solvent viscosity, and the quality of the solvent for the polymer.

The situation in water is quite different. Evidence from three sources points to the fact that the pyrene groups exist in the form of intramolecular dimers and aggregates, even at very low levels of pyrene substitution. These observations include the high intensity of the excimer emission, the different excitation spectra for the monomer and excimer emissions, and the strong hypochromicity in aqueous solution.

We originally considered the possibility that HPC might form helices in water and that the pitch might vary to allow interaction of pyrene groups that are still remote along the chain contour. A strong interaction of either the isolated pyrenes or the dimer sites with the helix ought to give rise to a detectable signal in circular dichroism measurements. The fact that no signal could be detected, neither in methanol nor in water, is inconsistent with a long-range chiral conformation of the macromolecule.

These conclusions are in accord with previous studies on dilute HPC solutions. One of the most interesting features of HPC is its wide range of solubilities. Normally, polymers are soluble in liquids whose Hildebrand solubility parameter (δ_H) is within ±3 of that of the polymer. HPC is soluble in solvents ranging from water (δ_H = 23.4) to piperidine (δ_H = 8.7) and in solvents of very different hydrogen-bonding ability (e.g., chloroform, tetrahydrofuran, dimethylformamide, acetone, alcohols, and water). Viscosity and surface tension measurements[14] have established a solubility parameter value for HPC of 10.7 and have shown that the range of solubilities is significantly more narrow in solvents of poor hydrogen-bonding ability. The solubility of HPC in these solvents has been explained by Samuels[15] in terms of a structure in which the OH groups of HPC are involved in extensive hydrogen bonding.

Among alcohol solvents, HPC has the highest limiting viscosity number in *tert*-butyl alcohol (δ_H = 10.5) and somewhat smaller but similar values in 1-butanol (δ_H = 11.3), ethanol (δ_H = 12.5), and methanol (δ_H = 14.3).[14] These should by considered good solvents for the polymer. Our results indicate no tendency for HPC to associate in dilute solution in methanol and ethanol. Taken together, these results indicate that alcohols and other solvents with solubility parameters in the neighborhood of δ_H = 11 are normal solvents for HPC. Thus it is the solubility in water that is unusual.

Our results in water, Figure 2, indicate quite clearly that one of the peculiar features of aqueous HPC solutions is the tendency of the polymers to associate, even at very low

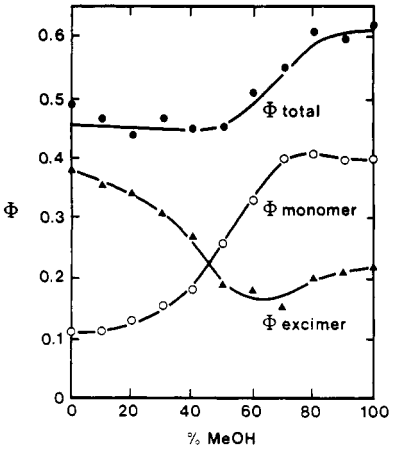

Figure 6. Total fluorescence quantum yields as a function of solvent composition in water/methanol mixture for samples of HPC-Py/26 (52 mg/L). Also plotted are the fractional contributions of monomer and excimer emission to that total (see text).

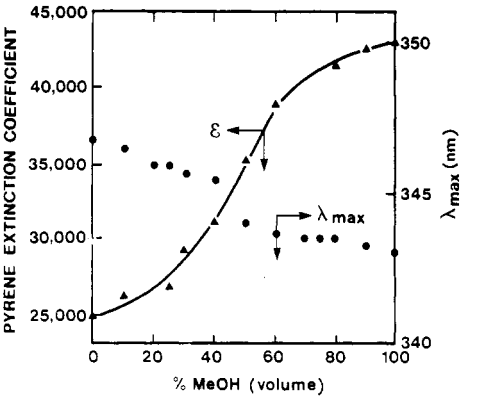

Figure 7. Molar decadic extinction coefficients ϵ and wavelengths of maximum absorbance of the pyrene chromophore in HPC-Py/26 as a function of solvent composition in water/methanol mixtures. In these experiments, the polymer concentration was approximately 0.05 mg/mL.

polymer concentration. There are two aspects of this behavior that we would like to comment upon. First, there is a much milder decrease in I_E/I_M upon dilution with water than upon dilution with unlabeled polymer solution. This result suggests that aggregation is a property of the polymer itself and is not brought about by the presence of the pyrene groups. The pyrene excimer seems to serve only as a very sensitive sensor of the association.

The second point concerns the magnitude of I_E/I_M at the lowest concentrations of polymer. This value is still much larger than the value of 0.3 found in methanol and ethanol solution. Although we have no other evidence to support our conjecture, we believe that a significant portion of the intense excimer emission is of intramolecular origin. This in turn would imply that HPC assumes a different conformation in water than in alcohol solvents.

Mixed Solvents. Some aspects of the peculiar behavior of HPC-Py in water may be understood through a careful analysis of the changes in the pyrene fluorescence upon addition of methanol to aqueous HPC-Py solutions. Since the monomer and excimer emissions derive from different ground-state species, it is useful to examine separately the changes in the excimer and monomer emission intensities as a function of the methanol/water ratio.

Our approach was to determine the total quantum yield of emission for (rigorously degassed) samples of 52 ppm polymer concentration as a function of solvent composition. Intensities were apportioned to monomer and to excimer emission in proportion to their relative areas after conversion of the spectra to wavenumber units. The results are presented in Figure 6. The total quantum yield is about 0.5 in water and about 0.6 in methanol. In fact, the quantum yield remains constant until the solvent composition exceeds 50% methanol and then undergoes a pronounced increase in the range 50–80% methanol. This increase corresponds to a very large increase in the quantum yield of monomer emission. In the region 0–50% methanol, the constant total quantum yield derives from a gradual decrease in the excimer intensity, offset by a corresponding increase in the monomer intensity. The picture that emerges from these results is that methanol disrupts the forces that lead to polymer association and to conformational differences in water. The total quantum yield changes only slightly, but there is an increasing importance of excimer emission in solvent of increasing aqueous content.

HPC is a very hydrophobic polymer.[6,14] The driving force for association in water may be some combination of hydrophobic interactions and highly cooperative intermolecular hydrogen bonding. The role of methanol may be to disrupt the hydrophobic interactions while at the same time still satisfying the hydrogen-bonding requirements of the polymer. In terms of the spectroscopy we observe, we believe that in highly aqueous media, addition of methanol also causes breakup of the pyrene dimer sites, leading to an increase in I_M and a corresponding decrease in I_E. At very high methanol compositions, there is also an upturn (Figure 6) in the amount of excimer. Here the polymer is no longer associated, and dynamic intramolecular excimer formation from locally excited pyrene is the predominant mechanism of excimer formation.

This picture of polymer dissociation and unfolding, accompanied by methanol-induced disruption of the pyrene dimer sites, is supported by the UV absorption data for the polymer in mixed solvents, as shown in Figure 7. There is a cooperative change in the extinction coefficient and a small shift in the wavelength of maximum absorption. It is interesting to note that the inflection point in the plot of ϵ vs. solvent composition corresponds more to that of I_M than to that of I_E or I_E/I_M.

On this basis we can now interpret our results on the fluorescence of aqueous HPC/26 solutions in the presence of sucrose. We knew from diffusion studies of sucrose in water in the presence and absence of HPC[16] that there is no specific interaction between these molecules. Originally we considered the possibility that the viscosity of the sucrose solution might play an important role in these experiments. Examination of Figure 4 indicates, however, that the changes in viscosity occur in quite a different sucrose concentration domain than the changes in I_E/I_M. The experiments in aqueous sucrose mixtures provide further evidence that the changes in I_E/I_M reflect the solvency of the medium for the polymer.

Summary

The fluorescence spectroscopy of pyrene-labeled (hydroxypropyl)cellulose samples was examined in solution in various alcohols and in water. The low relative excimer fluorescence intensity in alcohol solvents such as methanol is typical of a randomly coiled polymer containing a small fraction of pyrene groups. The very intense excimer intensity in water derives from associated polymer molecules, even at very low polymer concentration, plus a possible intramolecular contribution. Polymer association is a property of the polymer and is not induced only by the presence of the pyrene groups. The excimer emission derives from ground-state dimers and higher aggregates

of pyrene groups. A very strong hypochromic effect suggests that in these species the pyrenes are stacked face-to-face.

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Registry No. HPC, 9004-64-2; H_2O, 7732-18-5; MeOH, 67-56-1; $MeCH_2OH$, 64-17-5; $MeCH_2OCH_2CH_2OH$, 110-80-5; $HO(CH_2)_2O(CH_2)_2OH$, 111-46-6; $HOCH_2CH(OH)CH_2OH$, 56-81-5.

References and Notes

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(2) Permanent address: Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1.
(3) Wirick, M. G.; Waldman, M. H. *J. Appl. Polym. Sci.* **1970**, *14*, 579.
(4) Gray, D. G. *J. Appl. Polym. Sci., Polym. Symp.* **1983**, *37*, 179.
(5) Flory, P. J.; Spurr, O. K.; Carpenter, D. K. *J. Polym. Sci.* **1958**, *28*, 231.
(6) (a) Claesson, S.; Odani, H. *Discuss. Faraday Soc.* **1970**, *49*, 268. (b) Winnik, F. M.; Winnik, M. A.; Tazuke, S. *J. Chem. Phys.*, in press.
(7) (a) Winnik, M. A., Ed. *Photophysical* and *Photochemical Tools in Polymer Science*; D. Reidel: Dodrecht, Holland, **1986**. (b) For another application of pyrene-labeling to study a water-soluble polymer, see: Turro, N. J.; Arora, K. S. *Polymer* **1986**, *27*, 783.
(8) (a) Webowyj, R. S.; Gray, D. G. *Macromolecules* **1984**, *17*, 1512. (b) Nystrom, B.; Bergman, R. *Eur. Polym. J.* **1978**, *14*, 431.
(9) Schnieders, C.; Müllen, K.; Huber, W. *Tetrahedron* **1984**, *40*, 1701.
(10) Best yield and highest purity were obtained when the conditions described in the following reference were observed meticulously: Kabalka, G. W.; Varma, M.; Varma, R. S.; Srivastava, P. C.; Knapp, F. F., Jr. *J. Org. Chem.* **1986**, *51* 2386.
(11) This effect and the observation of other changes in the absorption spectra of fluorescent labels induced by intra- and intermolecular polymer interactions have been reported by Suzuki[12] and by Herkstroeter.[13]
(12) Suzuki, Y.; Tazuke, S. *Macromolecules* **1980**, *13*, 25; **1981**, *14*, 1742.
(13) Herkstroeter, W. G.; Martic, P. A.; Hartman, S. E.; Williams, J. L. R.; Farid, S. *J. Polym. Sci., Polym. Chem. Ed.* **1983**, *21*, 2473.
(14) Roberts, G. A. F.; Thomas, J. M. *Polymer* **1978**, *19*, 459.
(15) Samuels, R. G. *J. Polym. Sci.* **1969**, *7*, 1197.
(16) Nystrom, B.; Roots, J. *Eur. Polym. J.* **1980**, *16*, 201.

Cooperative Binding of Sodium Myristate to Amylose

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ABSTRACT: The binding of sodium myristate to amylose has been studied by a surface tension method. Surface tensions of myristate solutions were compared with those of systems containing amylose. Slow adsorption of amylose–myristate complex at the air–water interface has rendered possible the use of surface tension for estimating mean activities of sodium myristate in the presence of amylose and the construction of a binding isotherm. Information has also been gained on the "surface activity" of complexes. Comparison of the isotherm with optical rotation studies has revealed that a relatively low level of binding can fully induce a conformational transition in the amylose to a helical state. Moreover, this state remains unaffected by the subsequent binding at free myristate approaching the critical micelle concentration. Optical rotation studies together with measurements of surface activity of the complex provide supportive evidence for more than one type of binding process. A cooperative mode of binding is inferred for low free myristate with selective binding to the helical conformer. The cooperative constants and saturation binding for this mode, estimated on linear Ising theory, conform to a model of interrupted but extensive end-to-end packing of extended myristate molecules in the cavity of an amylose helix with six residues per turn. The secondary binding is also cooperative, reflecting lateral hydrophobic attraction between adsorbed myristate molecules and free energies of binding approaching that of micellization.

1. Introduction

Amylose can interact strongly with many polar and nonpolar compounds, including lipids and emulsifiers.[1-5] At the level of secondary structure, the interaction is believed to involve complexed lipid molecules located within single-helical conformations of the amylose. There is strong evidence for this picture from X-ray diffraction studies in the solid state[5-7] and also complementary support from structural studies on aqueous solutions.[8-10]

Research on amylose–lipid interactions has been directed mainly to the structure of the complexes and less to their thermodynamic properties. Differential scanning calorimetry[9,11-13] has been used to characterize the melting of complexes. However, there are only a few quantitative studies on the binding of lipid molecules to the amylose.[14,15] Free energies of amylose–lipid interactions are thus largely uncharacterized, and their full scope may not be fully appreciated.

The subject of this paper is the measurement of binding of sodium myristate to amylose. The conditions chosen closely match those of earlier structural studies[9,10] on the conformational behavior of amylose–myristate systems by optical rotation and NMR methods.

The present objective of complementing studies of secondary structure with thermodynamic measurements has dictated the choice of systems dilute in amylose. Adsorption studies are then not straightforward (in the absence of an established myristate-specific potentiometric method) because of the enhanced difficulty of separating amylose–myristate complex from its surrounding solution and competing adsorption of myristate onto glassware, dialysis tubing, etc. It was found that a surface tension method could be utilized, providing information not only on the binding of myristate to amylose but also on the surface activity of the complexes formed.

2. Experimental Section

Lipid-free amylose (type III, DP = 970) was obtained from Sigma Chemical Co. Ltd. Sodium myristate was prepared from pure myristic acid (BDH) by refluxing with excess sodium hy-

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