Intramolecular Micelle Size of Polysoaps by Luminescence Quenching. Role of Solubilization

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Received March 25, 1993; Revised Manuscript Received June 11, 1993

ABSTRACT: The size of the hydrophobic microdomains of a hydrolyzed copolymer of maleic anhydride and hexyl vinyl ether has been measured in aqueous lithium chloride solutions by luminescence quenching using a single photon counting technique. Several probe-quencher combinations were employed, including pyrene with nonyl phenyl ketone, pyrene with benzophenone, and tris(2,2′-bipyridine)ruthenium(II) with 9-methylanthracene. The number of repeat units per microdomain averaged 45.6 and was insensitive to changes in the extent of micellization brought about by variations in pH and to changes in the probe-quencher systems. The extent of micellization was deduced from the maximum solubilization achievable with a number of water-insoluble organic compounds, including, among others, pyrene, nonyl phenyl ketone, and 9-methylanthracene, all of which were found to share a common pH dependence for their solubilization. However, an unexpected phenomenon occurred with benzophenone: its solubilization was found to be independent of pH, indicating that benzophenone was solubilized by micellized and unmicellized portions of the polymer alike. Ignoring this effect in the treatment of the luminescence quenching data would have resulted in an apparent decrease in microdomain size with increasing pH.

Introduction

Polysoaps are water-soluble polymers, usually polyelectrolytes, carrying hydrophobic side chains. Interest in this class of macromolecules has grown since their conception some 40 years ago.[1] In aqueous solution, polysoap molecules with sufficient hydrophobicity assume hypercoiled conformations due to the formation of intramolecular micelles. Like micelles formed by ordinary surfactants, these intramolecular microdomains solubilize water-insoluble organic molecules. However, unlike ordinary micelles, they need no critical concentration of polysoap for their formation.[1]

Insight into the nature of polysoap conformation received a massive boost with the advent of the fluorescence quenching technique,[2-5] which allowed, for the first time, the determination of what had only been suspected previously, namely that high polymeric polysoap molecules contain a large number of relatively small micelles,[6,7] with reported aggregation numbers ranging from 25 to 140.[8-10]

Most of the limited number of such studies carried out until now by this technique have employed the hydrolyzed alternating copolymers of maleic anhydride and alkyl vinyl ethers. Of special interest are the members of this family with alkyl groups containing from four to ten carbon atoms: they are micellized at low pH, but the extent of micellization diminishes with increasing pH as a result of the repulsion between ionized carboxylate groups. This behavior has been established through numerous studies involving potentiometric[11] and calorimetric[12] titrations, viscosimetry,[11] solubilization,[6] and fluorescence probes.[13,14] The fluorescence quenching studies indicate that the micelle sizes are independent of polysoap concentration[8] and degree of polymerization;[9] but some questions concerning the dependence of micelle size on the extent of micellization caused by pH changes remain. One paper reports that the micelle size stays constant,[8] while another paper reports that the micelle size decreases with diminishing extent of micellization.[9] The work reported here was undertaken in the hope of resolving this discrepancy and perhaps thereby gaining further insight into the application of the fluorescence quenching technique to the determination of microdomain size in polysoaps.

A hydrolyzed copolymer of maleic anhydride and hexyl vinyl ether (DP = 1100) in aqueous 0.1 M LiCl constituted the polysoap-solvent system. The structure of the repeat unit is shown in Figure 1. To explore possible effects of fluorescent probes and quencher, three combinations were employed, namely pyrene with nonyl phenyl ketone, pyrene with benzophenone, and a ruthenium complex with 9-methylanthracene. The time-resolved single photon counting method employed in the present study was a substantial improvement over the pulse-sampling technique used previously in this laboratory.[8] A further improvement consisted of determining solubilization using absorption spectroscopy instead of the previously used turbidity technique. Solubilization data give useful information concerning the extent of micellization as well as on the location of probe and quencher molecules, the latter of these factors furnishing a major clue for the achievement of our aims.

Experimental Section

The maleic anhydride-hexyl vinyl ether copolymer was our sample BRB#2, synthesized by B. Bhattacharia,[15] and characterized by Y. Zhong.[16] Its degree of polymerization was 1100 (expressed in repeat units). Polymer stock solutions were prepared by dissolving known weights of polymer, vacuum dried at 40 °C and 0.05 mmHg, in 2.5×10^{-3} M LiOH for 48-72 h at room temperature to a final pH between 2 and 3. It had been established previously that under these conditions the anhydride units were completely hydrolyzed. After dissolution more water was added to bring the concentration of these stock solutions to between 0.06 and 0.10 M. The stock solutions were stored at 4 °C and later, when needed, brought to the desired composition by the addition of, first, water, then LiOH or HCl, and, finally, LiCl solutions. The concentration of polymer throughout this paper is denoted by C_p and expressed in moles of repeat units per liter.

For solubilization determinations, saturated solutions of the solubilizates were prepared by adding excess amounts of large crystals to known volumes of polymer solution and slowly rotating the mixtures for 18-30 days at 23 °C in the dark. After filtration and centrifugation, the concentration of solubilizate was determined by absorption spectroscopy. Equilibrium was assumed to be reached when extending the mixing time produced no further changes. For all other experiments, the water-insoluble probes and quenchers were added to the polymer solutions from stock solutions in ethanol. Ethanol concentrations were kept constant

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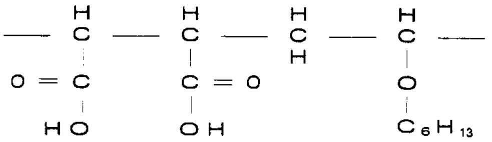

Figure 1. Structure of repeat unit of hydrolyzed copolymer of maleic anhydride and hexyl vinyl ether.

for any sample set by adding appropriate amounts of ethanol where necessary; however, the ethanol concentration was always kept below 2%, so that, as preliminary tests showed, it would have not effect on our experimental results.

Luminescence experiments were conducted using a Photochemical Research Associates (PRA) Model 3000 Fluorescence Lifetime Instrument (FLI), set up for single photon counting. The configuration and operation were similar to those described by Snyder et al.[17] Blanks, i.e., solutions without probe, were run under the same conditions as the samples and the counts subtracted from those of the corresponding sample decays to minimize the effects of scattered light. For pyrene, the excitation and emission wavelengths were 337 and 380 nm, respectively, and the ratio of pyrene to micelle concentrations was always less than 1/400. The corresponding values for the ruthenium probe were 452 and 630 nm and 1/130. Solutions were deoxygenated by purging with helium for 45–90 min immediately prior to measurement. Luminescence decay data were analyzed using software obtained from Photon Technologies International (PTI), which utilizes an iterative reconvolution procedure employing a weighted, nonlinear, least squares curve fitting method. The decay function used was[18]

$$I(t) = I(0)\exp[-A_2 t - A_3(1 - \exp(-A_4 t))] \tag{1}$$

where $I(t)$ is the luminescence intensity at time t after excitation. The criteria used to judge the goodness of fit were the reduced χ^2 value, the Durbin–Watson parameter, and the Runs Test statistic, in addition to plots of weighted residuals and the autocorrelation function. The coefficients of eq 1 are related to the molecular parameters of interest by means of the relations[18]

$$A_2 = k_0 + \frac{k_Q k_-}{k_Q + k_-}\frac{[Q_m]}{[M]} \tag{2}$$

$$A_3 = \left(\frac{k_Q}{k_Q + k_-}\right)^2 \frac{[Q_m]}{[M]} \tag{3}$$

$$A_4 = k_Q + k_- \tag{4}$$

where k_0 is the first-order rate constant for the luminescence decay of the probe in the absence of quencher, k_Q is the first-order rate constant for quenching inside a micelle, k_- is the exit rate constant for a quencher from a micelle, and $[Q_m]$ is the concentration in moles per liter of quencher solubilized in micelles, whose molar concentration is [M].

Equation 2 shows that if there is no movement of quencher in or out of a micelle during the excitation lifetime of the probe, i.e., if $k_- = 0$, the value of A_2 will not depend on the quencher concentration. Therefore, for determining whether there is such movement, it is important to determine whether A_2 varies with $[Q_m]$. It has been suggested[9] that it is necessary to measure the decay of a sample over a time scale of at least 7 times the fluorescence lifetime of the probe to obtain reliable values for A_2. We conducted preliminary experiments employing as the probe pyrene, with a lifetime in our micelles of approximately 400 ns in the absence of quencher, and ascertained that we obtained the same value for A_2 for a particular sample whether the time scale of the measurement was 1500 or 3000 ns. Consequently, to save significant amounts of time, we measured our sample decays over a time scale of 1500 ns.

Results and Discussion

Solubilization. The solubilization, S, expressed in moles of solubilizate per mole of polymer repeat unit, is shown as a function of pH for a number of organic compounds in Table I. The values of S were obtained from the measured solubilities in polymer solutions and in the 0.1 M LiCl solvent. The results obtained with the first five listed solubilizates are in line with expectations from previous work involving many distinct physical methods, all indicating that the extent of micellization decreases with increasing pH.[7–9,11,13,14] The behavior of benzophenone and its 4-bromo derivative, indicating that the solubilization appears to be independent of pH and, therefore, insensitive to the extent of micellization, is unexpected. Work is in progress aimed at understanding this apparent anomaly. However, whatever its cause, this behavior must be taken into account in the treatment of fluorescence quenching results involving benzophenone as the quencher.

Table I. Solubilization of Organic Compounds by Hexyl Polyacid: Dependence on pH

compd	pH	S ($\times 10^3$)	R^a
pyrene	3.7	4.36	
	3.9	4.43	
	4.2	4.38	
	4.5	4.18	
	5.7	2.68	0.64
9-methylanthracene	3.7	9.31	
	3.9	9.67	
	4.2	9.52	
	4.5	9.12	
	5.7	5.63	0.62
nonyl phenyl ketone	3.9	50.3	
	4.5	48.8	
	5.7	32.5	0.67
2-bromonaphthalene	3.9	72.9	
	4.5	70.6	
	5.7	44.1	0.62
biphenyl	4.5	12.8	
	5.7	8.0	0.63
benzophenone	4.5	20.1	
	5.7	19.8	0.99
4-bromobenzophenone	3.9	8.66	
	4.5	8.81	
	5.7	8.56	0.97

[a] R = ratio of S at pH 5.7 to S at pH 4.5.

Returning to the first five normally behaved solubilizates in Table I, values of the ratio, R, of the solubilization at pH 5.7 to that at pH 4.5, given in the last column, are seen to be in reasonable agreement, within the error limits of the experimental method, giving quantitative support to the conclusion that the observed pH dependence is a characteristic of the polyacid, namely its extent of micellization, Θ_m, and can be used to define this quantity. We note that at pH values of 4.2 and below, the values of S for pyrene and 9-methylanthracene are reasonably constant, and, therefore, we assume Θ_m to be unity in this pH region. Values of Θ_m at higher pH can then be calculated by averaging values of the ratio $S(\text{pH})/S(\text{pH} \le 4.2)$ of the first four compounds in Table I, where $S(\text{pH} \le 4.2)$ itself represents an average when more than one value in the indicated pH region is listed. The values of Θ_m so obtained are given in Table III. It should be noted that these results are consistent with corresponding values obtained from published potentiometric titration data[19] by a previously described method.[11]

If Poisson statistics is assumed for the distribution of quencher molecules among micelles,[20] the mass action law for the partitioning of quencher between the micellized polymer and the solvent medium may be expressed by

$$K = \frac{[Q_m]}{[Q_a][M]} \tag{5}$$

where $[Q_a]$ is the concentration, expressed in moles per liter, of quencher in the aqueous solvent medium.[21]

Table II. Parameters of Sample Run[a]

$[Q] \times 10^4$	$A_2 \times 10^{-6}$ (s^{-1})	A_3	$A_4 \times 10^{-7}$ (s^{-1})	$dA_2/d[Q_m] \times 10^{-8}$ ($M^{-1} s^{-1}$)	$dA_3/d[Q_m]$ (M^{-1})	$k_- \times 10^{-5}$ (s^{-1})	$k_Q \times 10^{-7}$ (s^{-1})	$[M] \times 10^4$	N
0.00	2.48								
1.62	2.58	0.230	1.83						
2.42	2.60	0.347	1.72						
3.23	2.66	0.464	1.78						
4.15	2.71	0.589	1.93						
			1.82[b]	5.40	1430	3.70	1.78	6.71	45.1

[a] $C_p = 0.0494$ M; pH = 5.7; $\theta_m = 0.613$; [pyrene] = 1.05×10^{-6} M; $[Q_m]/[Q] = 0.995$; quencher = nonyl phenyl ketone. [b] Average.

Table III. Micelle Size and Rate Constants

probe[a]	quencher[b]	pH	θ_m	$C_p \times 10^2$	$k_0 \times 10^{-6}$ (s^{-1})	$k_- \times 10^{-5}$ (s^{-1})	$k_Q \times 10^{-7}$ (s^{-1})	$[M] \times 10^4$	N
pyrene	NPK	4.5	0.963	2.14	2.52	4.64	1.82	4.44	46.4
		4.5	0.963	5.00	2.50	4.20	1.80	10.91	44.1
		5.7	0.613	4.94	2.49	4.53	1.84	6.53	46.3
		5.7	0.613	4.94	2.48	3.70	1.78	6.71	45.1
pyrene	BP	4.5	0.963	2.22	2.49	16.6	1.29	4.55	47.0
		4.5	0.963	4.09	2.52	14.2	1.22	8.32	47.6
		5.7	0.613	4.90	2.49	11.9	1.30	6.80	44.2
		5.7	0.613	4.92	2.50	13.3	1.36	6.44	46.8
$Ru(byp)_3^{2+}$	9-MeA	4.5	0.963	4.95	1.14	3.36	0.27	11.08	43.0
		5.7	0.613	5.38	1.20	2.27	0.33	7.33	45.0
									ave 45.6

[a] [Pyrene] = 1×10^{-6} M; $[Ru(bpy)_3^{2+}] = 1 \times 10^{-5}$ M. [b] NPK = nonyl phenyl ketone; BP = benzophenone; 9-MeA = 9-methylanthracene.

The micelle concentration, [M], is related to the micelle size, N, defined as the number of repeat units per micelle by the equation

$$N = \frac{\Theta_m C_p}{[M]} \tag{6}$$

Combination of eqs 5 and 6 leads to the working relation

$$K' = \frac{[Q_m]}{[Q_a]\Theta_m C_p} \tag{7}$$

where $K' = K/N$.[8] The values of K' may be evaluated at the solubility limit where $[Q_m]/\Theta_m C_p = S$ and $[Q_a]$ equals the solubility in 0.1 M LiCl, which was measured to be 7.16×10^{-6} M for nonyl phenyl ketone and 3.38×10^{-6} M for 9-methylanthracene, resulting in respective K' values for 7030 and 2810 M^{-1} for these quenchers. Equation 7 is also valid below the solubility limits of the quenchers and, combined with the appropriate stoichiometric relations, is used to calculate the values of $[Q_m]$ needed for the evaluation of the luminescence quenching data.

Because of the unusual solubilization behavior of benzophenone, some modification of this treatment is required. In this case, the measured value of S equals $[Q_p]/C_p$, where $[Q_p]$ represents the concentration of benzophenone solubilized by both micellized and unmicellized portions of the polymer. Because benzophenone appears to be uniformly solubilized by the entire macromolecule, whose micellized fraction is Θ_m, we assume that its concentration in the micelles is given by the relation $[Q_m] = \Theta_m[Q_p]$. With our measured value of 3.65×10^{-4} M for the solubility of benzophenone in 0.1 M LiCl, we obtain a value of 55.4 M^{-1} for K'.

Luminescence Quenching. The procedure used for obtaining the desired parameters is illustrated by means of a sample run presented in Table II. The data are obtained from five solutions differing only in their quencher concentrations, given in the first column. The next columns contain the parameters A_2, A_3, and A_4 obtained by fitting eq 1 to the instrumental decay data. The molecular parameters of interest, given in the bottom row, are derived by means of eqs 2–4 as follows. The value of k_- is obtained by means of the relation

$$k_- = \frac{D}{1 + D/A_4} \tag{8}$$

where D is defined by

$$D = \frac{dA_2/d[Q_m]}{dA_3/d[Q_m]} \tag{9}$$

The derivatives in eq 9 are also given in Table II. The value of k_Q is then obtained from eqs 4 and 8. Combining eqs 3, 4, and 8, we obtain, after some rearrangement, the micelle concentration, [M], in the form

$$[M] = \left(\frac{1}{dA_3/d[Q_m]}\right)\left(\frac{1}{[1 + (D/A_4)]^2}\right) \tag{10}$$

The micelle size, N, defined as the number of repeat units per micelle, is then obtained from 6.

Values of the various molecular parameters obtained in this manner for the three probe–quencher combinations under a variety of conditions are presented in Table III. Perhaps the most significant finding is that the micelle size, N, is essentially invariant to changes in the probe–quencher system and in the extent of micellization. The value of N is substantially larger than that found previously in this laboratory,[8] which we ascribe to unexpectedly serious incompatibilities in the combination of results from static and dynamic luminescence measurements necessitated in the previous work. This matter is treated in more detail elsewhere.[22] It is also noteworthy that the invariance of N with the extent of micellization is in disagreement with findings reported by others.[9] A possible reason for this apparent discrepancy will be discussed below.

The values of K_Q vary between the three probe–quencher systems but are essentially constant within each single system. This invariance with the extent of micellization lends further support to the finding that the micelle size is independent of Θ_m, since k_Q generally varies inversely with micelle size.[18] The large difference between the k_Q values of the pyrene–nonyl phenyl ketone and the $Ru(bpy)_3^{2+}$–9-methylanthracene systems is noteworthy, especially in consideration of their quenching behavior in homogeneous solution. Thus, we determined the second-order rate constant for the quenching of pyrene by nonyl phenyl ketone in heptane at 23 °C from a linear Stern–Volmer plot[23] to be 2.07×10^9 M^{-1} s^{-1}, while the corresponding value for the $Ru(bpy)_3{}^{2+}$–9-methylanthracene system in ethanol at 25 °C has been reported to be 4.2×10^9 M^{-1} s^{-1}.[3] The difference in quenching efficiencies is even greater than indicated by these numbers, since viscosities of heptane and ethanol at the indicated temperatures are 0.4 and 1.1 cP, respectively. The opposite order of the k_Q values in Table III may be explainable by the relative location of probes and quenchers in the micelles. For the pyrene–nonyl phenyl ketone system, both the probe and the quencher have the same solubilization behavior, suggesting that they are solubilized in the same region of the micelle, which would be expected to facilitate their rate of collision. For the $Ru(bpy)_3{}^{2+}$–9-methylanthracene system the cationic probe may be attracted to the anionic micelle surface, while the electroneutral quencher may be located closer to the hydrophobic center of the micelle.

Several conclusions may be drawn from the values of k_-. This quantity is generally defined and theoretically treated as the rate constant for the escape of a quencher molecule from a micelle;[18] nevertheless, it has been recognized that the measured effects giving rise to k_N could also be caused by movement between micelles of probe molecules.[9,24] However, examination of Table III shows that both the large difference between the k_- values of the first two systems, which differ only in the quenchers, and the rather small difference between the first and third systems, for which the polarity and solubility behavior are very similar for the quenchers but widely different for the probes, support the notion of k_- being predominantly, if not totally, attributable to movement of quencher. The relatively large k_- value of benzophenone may be due partly to its relatively large water solubility, but even more to its solubilization by both micellar and nonmicellar regions of the macromolecule, which should facilitate its direct movement between these regions.

To illustrate how it is possible to obtain an apparent decrease in the micelle size with decreasing extent of micellization, let us return briefly to the treatment of the pyrene–benzophenone system. If we had taken $[Q_m]$ equal to the entire solubilized benzophenone concentration, $[Q_p]$, the values for N at pH 4.5 would have been 45.3 and 45.8, i.e., very little changed from the values in Table III, while at pH 5.7 the values for N would have been 27.1 and 28.7, a substantial decrease from the values at the lower pH. It is quite likely that the cationic dodecylpyridinium ion, used by Binana-Limbelé et al. as a quencher for pyrene,[9] would be attracted to both hydrophobic and anionic groups of the copolymer of decyl vinyl ether and maleic acid used in their work and, therefore, bound to micellized as well as unmicellized residues. Had account been taken of this effect, their values of N would have been constant at a value close to 100 over the entire ionization range. It has been predicted that the aggregation number of spherical micelles should vary with the square of the number of carbon atoms in the alkyl chain of the surfactant.[25] The aggregation number ratio of 100/46 of the C_{10} and C_6 alkyl vinyl ether–maleic acid copolymers is not far from this prediction. It is noteworthy that the unusual treatment required for benzophenone is not necessary with micelles of simple surfactants, where the micelles are the sole solubilization loci.

Conclusions

Three probe–quencher combinations have resulted in identical values for the microdomain size of a hydrolyzed copolymer of hexyl vinyl ether and maleic anhydride, determined by single photon counting luminescence quenching. The microdomain size was also independent of the extent of micellization. Special account had to be taken of the unusual solubilization behavior of benzophenone in the treatment of the fluorescence quenching data resulting from its application as quencher. This finding illustrates the importance of considering the solubilization behavior of both probe and quencher in applying the fluorescence quenching technique to microdomain size determinations in polysoaps.

References and Notes

(1) Strauss, U. P.; Jackson, E. G. *J. Polym. Sci.* **1951**, *6*, 649.
(2) Turro, N. J.; Yekta, A. *J. Am. Chem. Soc.* **1978**, *110*, 5951.
(3) Almgren, M.; Lofroth, J.-E. *J. Colloid Interface Sci.* **1981**, *81*, 486.
(4) Thomas, J. K. *J. Phys. Chem.* **1987**, *91*, 267.
(5) Lianos, P.; Lang, J.; Zana, R. *J. Colloid Interface Sci.* **1983**, *91*, 276.
(6) Dubin, P. L.; Strauss, U. P. *Polyelectrolytes and Their Applications*; Reidel: Dordrecht, 1975; pp 3–13.
(7) Barbieri, B. W.; Strauss, U. P. *Macromolecules* **1985**, *18*, 411.
(8) Hsu, J.-L.; Strauss, U. P. *J. Phys. Chem.* **1987**, *91*, 6238.
(9) Binana-Limbelé, W.; Zana, R. *Macromolecules* **1990**, *23*, 2731.
(10) Olea, A. F.; Thomas, J. K. *Macromolecules* **1989**, *22*, 1165.
(11) Dubin, P.; Strauss, U. P. *J. Phys. Chem.* **1970**, *74*, 2824.
(12) Martin, P. J.; Strauss, U. P. *Biophys. Chem.* **1980**, *11*, 397.
(13) Strauss, U. P.; Vesnaver, G. *J. Phys. Chem.* **1975**, *79*, 2426.
(14) Strauss, U. P.; Schlesinger, M. S. *J. Phys. Chem.* **1978**, *82*, 1627.
(15) Bhattacharia, B. Unpublished results.
(16) Zhong, Y. Doctoral Dissertation, Rutgers University, 1990.
(17) Snyder, S. W.; Demas, J. N.; DeGraff, B. A. *Anal. Chem.* **1989**, *61*, 2704.
(18) Tachiya, M. *Chem. Phys. Lett.* **1975**, *33*, 289.
(19) Strauss, U. P.; Schlesinger, M. S. *J. Phys. Chem.* **1978**, *82*, 571.
(20) Almgren, M.; Grieser, F.; Thomas, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 279.
(21) Yekta, A.; Aikawa, M.; Turro, N. J. *Chem. Phys. Lett.* **1979**, *63*, 543.
(22) Strauss, U. P.; Zdanowicz, V. S.; Zhong, Y. To be published.
(23) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1983; pp 260–264.
(24) Almgren, M.; Lofroth, J.-E.; van Stam, J. *J. Phys. Chem.* **1986**, *90*, 4431.
(25) Borbèly, S.; Cser, L.; Ostanevich, Y. M.; Vass, S. *J. Phys. Chem.* **1989**, *93*, 7967.