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Superquenching in Cyanine Pendant Poly(L-lysine) Dyes: Dependence on Molecular Weight, Solvent, and Aggregation

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Abstract: A series of poly(L-lysines) ranging in number of repeat units (N_{PRU}) from 6 to 900 has been synthesized and the photophysics of the series and monomer cyanine dye have been studied in solution. In water or aqueous dimethyl sulfoxide, the oligomers and polymers exhibit high sensitivity to fluorescence quenching by oppositely charged electron acceptors; in this study, 9,10-anthraquinone-2,6-disulfonate was used as a quencher for the cationic fluorescent polyelectrolytes. Quenching constants (K_{SV}) measured in 50:50 (v/v) dimethyl sulfoxide−water increase monotonically with increase in N_{PRU} ranging from 630 M^{-1} for monomer to 1.2×10^9 M^{-1} for dilute solutions of the polymer having $N_{PRU} \sim 900$. The polymers having $N_{PRU} > 100$ exhibit predominantly J-aggregate absorption and fluorescence and enhanced susceptibility to quenching. For the polymers exhibiting strong J-aggregation, the effective exciton domain quenched by a single quencher reaches ~100 PRU. The results of this study permit a semiquantitative analysis of superquenching of fluorescent polyelectrolytes in solution and the factors that control it.

Introduction

Recent studies have shown that a number of conjugated polymers exhibit efficient quenching by small molecules capable of associating with the polymer in solution and thin films.[1−10] The high quenching sensitivity is attributed to a combination of energy and exciton migration within the excited polymer such that nonradiative decay at one or more trap sites can effectively compete with photoluminescence.[9−11] Recently we and others reported dramatic enhancement of quenching when conjugated polyelectrolytes in solution are exposed to oppositely charged electron or energy acceptors.[2,4−8] It has been proposed that the enhanced quenching, as indicated by a large measured Stern−Volmer quenching constant, K_{SV}, is due to the product of a moderately strong, but not specific association constant between quencher and polyelectrolyte with the amplification provided by exciton/energy migration through the polymer.[12] Very recently we showed that a second class of fluorescent polyelectrolyte, containing formally nonconjugated chromophores pendant on each repeat unit of a synthetic polypeptide, also exhibits a remarkable “superquenching” sensitivity to oppositely charged energy and electron acceptors.[13] The polymer used in these studies, **1**, was shown to exist in aqueous solution, with the chromophores in a “J”-aggregate array.[13,14] The polymer used in these initial studies had ~250 polymer repeat units (N_{PRU}); since poly(L-lysine) can be obtained in a wide range of molecular weights it is possible to synthesize **1** with a corresponding wide range of N_{PRU}/molecule and to investigate the polymer photophysics and susceptibility to quenching as a function of molecular weight, solvent, and state of aggregation. In the present paper, we report the results of this study. The results provide a remarkable picture of the role of aggregation and polymer molecular weight on quenching sensitivity and provide insights in determining how to construct systems exhibiting maximum quenching sensitivity.

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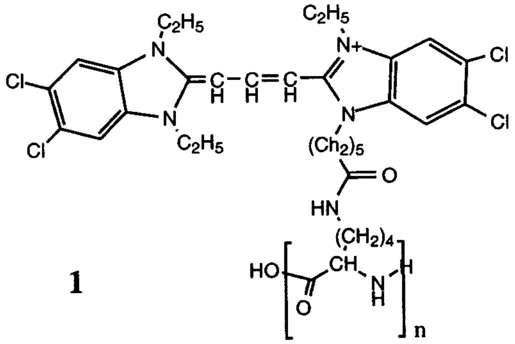

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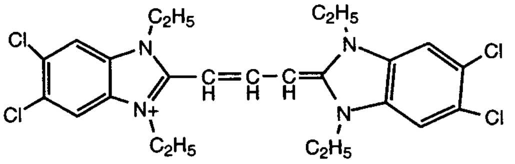

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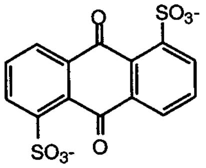

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Experimental Section

Materials. The 5,6-dichloro-1-ethyl-2-methylbenzimidazole was a gift from the Eastman Kodak Co. Research Laboratories and was also purchased from TCI America. The reagents employed in the two-step dye polymer synthesis, 2-(2-acetanilodovinyl)-1,3-diethyl-5,6-dichlorobenzimidazolium tosylate and 3-(5-*p*-nitrophenyloxycarbonyl)-5,6-dichloro-1-ethyl-2-methylbenzimidazolium iodide/trifluoroacetate (PNP ester), were prepared following the procedures of Roberts et al.[15] Anhydrous dimethyl sulfoxide (DMSO), triethylamine (stored over KOH), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were purchased from Aldrich. The eight poly(L-lysine) hydrobromide samples were purchased from Sigma with molecular weights determined by viscometry except for MW 500−2000, which was determined by capillary electrophoresis. The reported molecular weight and N_{PRU} for each poly(L-lysine) sample are as follows: 500−2000 (5−6), 2900 (14), 6900 (33), 10,700 (51), 13 200 (63), 23 400 (112), 84 000 (404), and 189 400 (906). The cyanine polymer prepared from the 50 000 MW poly(L-lysine) ($N_{\mathrm{PRU}} = 250$) was a sample of the polymer prepared in the previous study.[14] (The polymers are referred to by the number of repeat units, e.g., **1**-(250) for the polymer having 250 repeat units.) 9,10-Anthraquinone-2,6-disulfonic acid disodium salt was purchased from Aldrich and used as received. All reagents and solvents were reagent grade and were used as received.

Dye Polymer Synthesis. A general method similar to the two-step procedure described for the dye-capping of MW 50 000 poly(L-lysine) hydrochloride was used.[14] The experimental procedure for the dye-capping of poly(L-lysine) hydrobromide (MW 84 000, $N_{\mathrm{PRU}} = 404$) is described. A solution of 104 mg of poly(L-lysine) hydrobromide in 6 mL of dry DMSO was stirred under Ar. To the mixture was added 0.11 mL of triethylamine and 355 mg of PNP ester. After stirring for 3 h, 25 mg of poly(L-lysine) and 0.11 mL of triethylamine were added to the solution. The reaction of poly(L-lysine) and PNP ester was followed by ^{1}H NMR (Bruker ARX 500-MHZ spectrometer with DMSO-d_6 as solvent) to ensure that >95% of the PNP ester had reacted. The disappearance of the aryl protons in the *p*-nitrophenyl of the PNP ester and the appearance of the aryl protons in the *p*-nitrophenolate ion were used to monitor the reaction. Additional amounts of poly(L-lysine) hydrobromide were added, as necessary, to ensure that >95% of the PNP had reacted. After stirring 20 h at 25° C, 1.6 g (2.8 mmol) of 2-(2-acetanilidovinyl)-1,3-diethyl-5,6-dichlorobenzimidazolium tosylate and 0.17 mL of DBU were added to the solution. After stirring for 6 h, the dark red slurry was added dropwise to 100 mL of rapidly stirred CH_3OH. The suspension was stirred for 30 min and then centrifuged. After the liquid was decanted from the red solid, the solid was washed with CH_3OH (3 × 10 mL) and dried at 25° C under vacuum to give 0.8 g of dye polymer.

Measurements. Fluorescence lifetime measurements were made using the time-correlated single photon counting method with an ultrafast dye laser system. It included a frequency-doubled, mode-locked Coherent Nd:YAG laser, which synchronously pumped a cavity-dumped Coherent 700 dye laser. The pulse width was ~7 ps, and the average power was ~100 μW. Fluorescence emission was detected at the magic angle using a single-grating monochromator and a microchannel plate photomultiplier. The instrument response time was ~35−50 ps, as verified by scattering from Ludox AS-40. The spectrometer was controlled by software based on the LabView program from National Instruments. The dye laser was tuned to the desired excitation wavelength. Measurements were made on air-saturated samples at room temperature.

UV/visible absorption spectra were obtained on a Perkin-Elmer Lambda Bio-40 spectrophotometer. Steady-state photoluminescence studies were carried out using a Spex Flurolog spectrofluorometer with a Spex DM3000f spectroscopy computer. Fluorescence quantum yields of the solutions were determined by comparison with **1**-(250) in 50% v/v of DMSO/H_2O ($\Phi_f = 0.02$) and in H_2O ($\Phi_f = 0.01$).

The cyanine dye polymers and monomer fluorescence quenching protocol was as follows: The dye polymers or monomer (1.5 mg) was added to *N,N*-dimethylformamide (DMF; 1 mL) in a vial, the vial was left closed at room temperature for 5 h to ensure that the dye was dissolved, and the resulting dye solution labeled A was stored in a refrigerator at 0−4 °C. This solution may be stored indefinitely. To carry out a quenching study, (1) dye solution A (20 μL) was added into deionized water or DMSO−water (1 mL) in a vial with stirring to yield dye solution B. (2) Dye solution B was further diluted ~100 times with the solvent to give dye solution C with an optical density of ~0.05 at the $\lambda_{\max}$ (from the UV/visible absorption spectrum). (3) The emission spectra were acquired using excitation at 530 or 500 nm and recorded immediately before and (4) after addition of an aliquot of quencher solution into dye solution C. The emission intensity ratio (I_0/I) at $\lambda_{\max}$ before (I_0) and after (I) addition of an aliquot of quencher solution was used to derive the quenching constant from a Stern−Volmer plot of the data. Steps 2−4 were repeated 7−10 times at different quencher concentrations.

Results and Discussion

For this study, we chose a series of starting poly(L-lysines) encompassing a PRU range from 5−6 to 906. The monomeric cyanine dye **2** was also studied as a reference. For convenience the polymers are referred to according to the N_{PRU} as **1**-(5−6), **1**-(250), **1**-(906), etc. Since our principal interest in the polymers is in biosensing applications involving aqueous, or partially aqueous, media or aqueous solution−solid interfaces, we have focused our studies on aqueous or aqueous−organic solutions. However, we have carried out limited studies of some of the polymers in a range of organic solvents. As might be anticipated, we find that for a given polymer, the tendency to form "J"-aggregates decreases as the solvent is changed from strongly associating hydrogen-bonding solvents such as water, or alcohols, to less polar or non-hydrogen bond forming organics. Thus,

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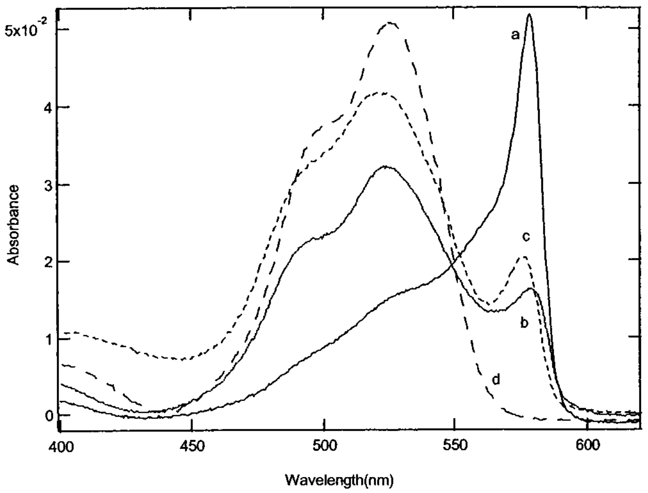

Figure 1. Absorption spectra of polymer **1**-(906) in different solvents: (a) 50% DMSO−water, (b) ethanol, (c) acetonitrile, and (d) methylene chloride.

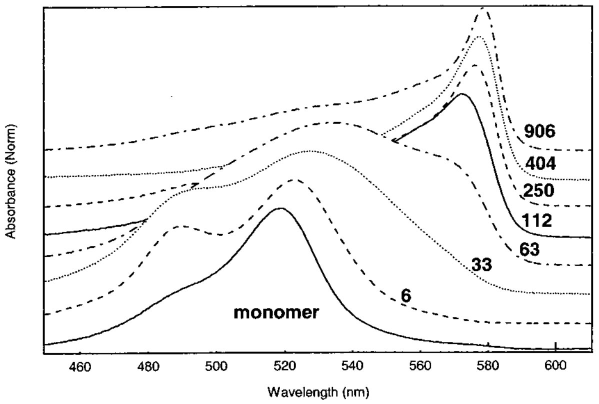

Figure 2. Absorption spectra of polymers **1** and monomer **2** in 50% DMSO−water.

as shown in Figure 1, **1**-(906) exhibits predominantly J-aggregate absorption in 50:50 water−DMSO (similar spectra (not shown) are obtained in pure water). In ethanol and acetonitrile the J-aggregate is still observable but the principal absorption is centered near 520 nm, in the same region where the monomer absorbs, while in DMF, methylene chloride (Figure 1), or chloroform no J-aggregate is discernible. As we examine the series of different molecular weight polymers in 50:50 water−DMSO or pure water, it is found that there is a clear molecular weight threshold for formation of J-aggregate. Thus, as shown in Figure 2, absorption spectra show no evidence for J-aggregate for monomer **2** and polymers **1**-(5−6), and **1**-(33) (although note that **1**-(33) gives fluorescence primarily from a red-shifted (from monomer) J-aggregate (see Figure 3). The beginnings of J-aggregate (as a small shoulder) are discernible for **1**-(63), and J-aggregate is dominant for the higher polymers in the series. The sharp J-aggregate band near 580 nm becomes sharper and shows a small red shift as the molecular weight of the polymer is increased. The molar extinction coefficient in terms of ϵ/PRU is in the range of 2.5×10^5 for the fully J-aggregated polymers. In terms of ϵ/polymer molecule, this translates to 2.3×10^8 for **1**-(906); the ability of the polymer to harvest light effectively is demonstrated by the fact that a readily discernible color is observed in a cuvette containing a 0.1 nM solution of polymers such as **1**-(250) or **1**-(906).

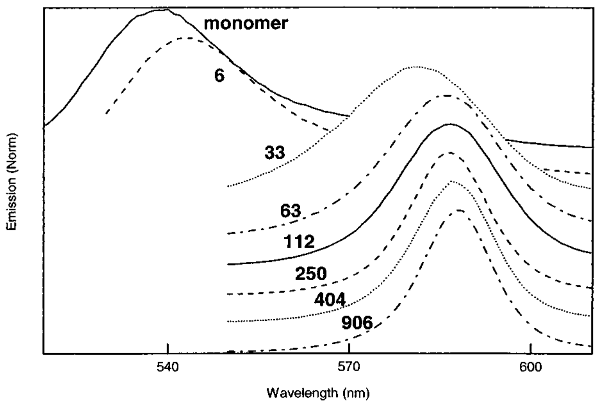

Figure 3. Fluorescence spectra (normalized) for polymers **1** and monomer **2** in 50% DMSO−water.

Table 1. Fluorescence Quantum yields for Cyanine Dye Polymers at 22 °C

MW of starting polylysine	PRU	ϕ (%) in DMSO–H_2O (50%)[a]	ϕ (%) in H_2O	lifetime[b]	χ^2
189000	906	2.6	0.86	9 ps (95.0%), 57 ps (4.4%)	1.23
84000	404	2.5	0.70		
50000	250	2.0	1.0	9 ps (91.3%), 57 ps (8%)	1.37
23000	112	2.6	0.76		
13200	63	2.0	1.4		
6900	33	1.2	1.3		
500−2000	5−6	2.7	2.1	60 ps (51.6%), 0.26 ns (34.2%), 0.85 ns (13.5%)	1.37
monomer **2**	1	1.7	0.3[a]	0.15 ns (85.7%), 0.65 ns (14.3%)	0.92

[a] ϕ is the quantum yield with 5% error; the quantum yield of monomer in water is low due to the presence of J-aggregates. [b] The short lifetime of less than 60 ps (instrument response) is not precise, only the order of magnitude can be approximated; the long lifetime has an error of ±1 at the second significant number.

The fluorescence of the polymers generally follows the absorption with a few notable exceptions. For the series of solvents indicated above, the J-aggregate fluorescence dominates for all solutions where any J-aggregate can be detected in the absorption. Thus, even in solutions such as ethanol or acetonitrile, where the J-aggregate component of the polymer, as indicated by absorption, is relatively slight, the only fluorescence detected is that from the J-aggregate. Similarly, as shown in Figure 3, the fluorescence from all of the polymers except **1**-(5−6) is dominated by the J-aggregate and no component corresponding to the monomer or small aggregate is detected. Here again, as noted for the absorption spectra, the fluorescence from J-aggregate sharpens and shows a small red shift as the molecular weight of the polymer increases. Fluorescence quantum yields and lifetimes in water and water−DMSO are listed in Table 1. In general, the quantum efficiencies are low. There is no discernible concentration effect on absorption, emission, or fluorescence quantum efficiency for the water−DMSO solutions of the polymers in the range employed for the quenching study ([PRU], (8−20) × 10^{-8} M). For aqueous solutions of **1**-(906), there is no change in the absorption spectrum over a range from 4 to 40 °C and only a small decrease in fluorescence. The fluorescence lifetimes for the J-aggregates of polymers **1**-(906) and **1**-(250) were measured by time-

Table 2. Fluorescence Quenching of Cyanine Dye Polymers by 9,10-Anthraquinone-2,6-Disulfonate at 22 °C

MW of starting polylysine	PRU	K_{SV} (M^{-1}) in DMSO–H_2O (50%)[a]	in H_2O	in CH_2Cl_2
189000	906	5.2×10^8	4.6×10^8	1.5×10^7
84000	404	4.8×10^8		
50000	250	4.6×10^8	4.8×10^8	
23000	112	1.6×10^8		
13200	63	7.5×10^7	5.5×10^7	
6900	33	3.4×10^7	4.3×10^7	2.7×10^7
500−2000	5−6	1.0×10^6		
monomer **2**	1	630[b]		

[a] The error in K_{SV} is ±5%. All quenching studies reported here were carried out with [PRU] ~ 2×10^{-7} M. [b] The quenching constant of monomer in DMSO is about 55 M^{-1}.

correlated photon counting to both be around 9 ps (Table 1). Interestingly, these values are very close to that found for the J-aggregate of **1**-(250) when coated on a slide with an outer layer of RDS Laponite clay.[14] The lifetime for monomer **2** has been measured and found to be much longer and biexponential (Table 1).

The quenching of the series of cyanine−poly(L-lysine) oligomers **1** and monomer **2** has been investigated using the dianionic electron acceptor 9,10-anthraquinone-2,6-disulfonate (AQS^{2-}) (**3**) in water and in water−DMSO (50:50 v/v). In general, quenching of the series **1** and **2** by **3** was found to give linear Stern−Volmer plots (ϕ_0/ϕ vs [**3**]) and the quenching constants, K_{SV} (slope of these plots), were found to increase monotonically with increasing molecular weight (or number of PRU) as shown in Table 2. The results reported in Table 2 were all obtained with dilute solutions ([PRU] ~ 2×10^{-7} M) of comparable optical density. It was found for polymers **1**-(250), **1**-(404), and **1**-(906) that K_{SV} increases with decrease in [polymer]. Thus, at a concentration of PRU ~ 8×10^{-8} M, the K_{SV} values for the three highest molecular weight polymers are 6.6×10^8, 1×10^9, and 1.2×10^9 M^{-1}, respectively, slightly higher than those reported in Table 2. The K_{SV} for monomer in

$$C^+ + AQS^{2-} \overset{K_1}{\rightleftharpoons} [C^+, AQS^{2-}]^- \qquad (1)$$

H_2O−DMSO is 630 M^{-1}; interestingly the K_{SV} for monomer in pure DMSO is 55 M^{-1}, or more than 1 order of magnitude lower.[16] For monomer, assuming no dynamic quenching or aggregation, it can be shown that K_{SV} should be identical to the value of the equilibrium constant for the association shown in eq 1, where C is the cyanine monomer **2** and AQS^{2-} is quencher **3**. It is also assumed that the complex, $[C^+, AQS^{2-}]^-$, is nonemissive. From a treatment similar to that used by Weller[17] for determining the free energy due to organic ion attraction in organic solvent (assuming an ion separation of 0.65 nm), we estimate the free energy of attraction between monomeric **2** and **3** should be ~0.12 eV in pure water and ~0.21 eV in DMSO, corresponding to estimates for K_1 of 10^2 and 3550 M^{-1}, respectively. The measured value in H_2O−DMSO is rather close to what might be estimated for this solvent mixture (the lower dielectric constant for the mixture (compared to pure water) should increase slightly the free energy of attraction and, hence, K_1). The lower value for pure DMSO is less easy to understand.[16]

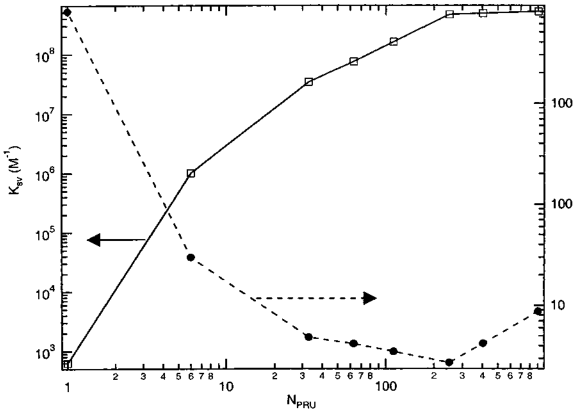

Figure 4. K_{SV} (left axis) and Q/P_{50} (right axis) as a function of N_{PRU} for polymer **1** in 50% DMSO−water.

Table 3. Amplification Factor, Q/P at 50% Quench, and PRU/Q at 50% Quench

MW of starting polylysine	PRU	amplification relative to quenching of monomer[a]	Q/P at 50% quenching	PRU/Q at 50% quenching
189000	906	8.2×10^5	9.0	104
84000	404	7.6×10^5	4.4	96
50000	250	7.3×10^5	2.6	92
23000	112	2.5×10^5	3.4	32
13200	63	1.2×10^5	4.2	15
6900	33	5.4×10^4	4.3	6.8
500−2000	5~6	1.5×10^3	30	0.1
monomer **2**	1	1	7900	0.00013

[a] These parameters derived from data in Table 2; similar error ranges can be estimated.

There is a sharp increase in K_{SV} for the smallest oligomer (MW of starting poly(L-lysine) 500−2000; mass spectral analysis of the dye-derivatized sample indicate N_{PRU} ~ 6 predominant) to 1×10^6 M^{-1}, or more than a factor of 10^3 compared to monomer. From a similar analysis of the Coulombic attraction, it appears that much of this increase can be accounted for in terms of increased attraction between the more highly charged oligomer and the quencher. Interestingly it has been observed that a similar-sized oligomer of the anionic conjugated polymer used in previous studies exhibits a similar K_{SV} value for quenching by the dicationic electron acceptor methyl viologen.[18]

For the series beyond **1**-(5−6), the monotonic increase in K_{SV} is not continuous, as indicated by the plot in Figure 4. While there is a large increase in K_{SV} in going from monomer to the lower molecular weight oligomer, as the molecular weight of the plymer increases, there is a continuing increase in both K_{SV} and the amplification factor (Tables 2 and 3). An evident near-saturation is reached for the highest molecular weight polymers. To analyze the quenching and the extent of amplification due to “communication” between the chromophores, we introduce three parameters listed in Table 3 for each of the polymers. The first is the amplification factor, which is simply the ratio of K_{SV} for the specific polymer divided by K_{SV} for the monomer.

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(16) A value somewhat higher than that for the DMSO−water mixture would be anticipated.[20] The fact that the value for the DMSO−water mixture is higher might be attributed to an enhancement of binding in this mixture from a hydrophobic contribution.

(17) Weller, A. Z. *Phys. Chem. N. F.* **1982**, *133*, 93−98.

(18) Bazan, G. C., private communication.

Two additional parameters are instructive: the number of quenchers per polymer (Q/P) at 50% quenching and the number of polymer repeat units per quencher (PRU/Q) at 50% quenching. The value of $(PRU/Q)_{50}$ scales with K_{SV}: $(PRU/Q)_{50} = [PRU]K_{SV}$. The $(Q/P)_{50}$ parameter is shown graphically in Figure 4. As shown in Table 3, the number of quenchers per polymer chain at 50% quenching decreases rapidly as a function of polymer molecular weight and then increases slightly beyond the minimum at $N_{PRU} \sim 250$. There is a clear decrease in this number $(Q/P)_{50}$ as the polymer goes from minimal ($N_{PRU} \sim 63$) to predominantly J-aggregate ($N_{PRU} \sim 110$). It is clear that there is amplified quenching due to "communication" within the polymer even for **1**-(33) where barely detectable J-aggregation occurs. This is also evident when the $(PRU/Q)_{50}$ values are examined; for **1**-(33), one quencher can quench effectively several PRUs. A similar conclusion that the quenching "saturates" once a good J-aggregate is formed is reached by examining the $(PRU/Q)_{50}$ values. These saturate around 100 repeat units per quencher once the polymer is effectively entirely in the J-aggregate structure.

The amplified quenching of the polymer compared to monomer is indicated both in terms of the simple amplification factor listed in Table 3 and in the calculated number of quenchers per polymer that quench 50% of the fluorescence. For all polymers beyond **1**-(5−6), it is clear that one quencher can extinguish the excitation delivered to several different sites within the polymer. The smallest values of Q/P at 50% quenching are in the range 2.5−3 for polymers **1**-(250) and **1**-(404) at the lowest concentrations of polymer used and where the polymer is strongly J-aggregated. The already low values for polymers **1**-(33) and **1**-(63) where J-aggregate is prominent in fluorescence but not absorption suggest as mentioned earlier that a small component of J-aggregate may act as a trap to collect excitation delivered to nonaggregate sites. However, the fact that these polymers are quenched with very low numbers of quenchers indicates that either the quencher may bind selectively to a J-aggregate site or the association of a quencher must be near enough to a J-aggregate domain so that a quencher can effectively "harvest" excitation delivered to a large number of PRU.

Additional evidence that J-aggregation of the cyanine dyes is important in enhancing quenching by **3** is provided by a comparison of the quenching behavior of polymers **1**-(906) and **1**-(33) in methylene chloride and H_2O−DMSO. There is no J-aggregate detectable in CH_2Cl_2 for absorption or fluorescence for either polymer. In contrast, H_2O−DMSO absorption spectra suggest that **1**-(906) is largely J-aggregate and **1**-(33) shows no evidence of J-aggregation, even though the fluorescence is dominated by the J-aggregate. For **1**-(33), the K_{SV} values in CH_2Cl_2 and H_2O−DMSO are 2.7×10^7 and 3.4×10^7 M^{-1}, respectively. For **1**-(906), the K_{SV} values in CH_2Cl_2 and H_2O−DMSO are 1.5×10^7 and 5.2×10^8 M^{-1}, respectively. The comparable values of K_{SV} for the two polymers in CH_2Cl_2 where there is no J-aggregate for either (that for **1**-(906) is actually slightly smaller than the value for **1**-(33)) suggest that that in the absence of J-aggregation there may be little or no increase in quenching efficiency as a function of molecular weight beyond a given size. The moderate increase in K_{SV} for **1**-(906) provides some idea of the magnitude of enhancement of quenching due to J-aggregation. The role of the J-aggregate is also exemplified by comparing the Q/P and PRU/Q values for the two polymers in methylene chloride. For **1**-(33), these are 6.11 and 5.4, respectively. Thus, for this polymer, the ratio of PRU/Q is relatively small but nonetheless greater than unity, indicating that some "communication" occurs, albeit less than where J-aggregate is present, even as a minor component. The change in these parameters is even more dramatic for polymer **1**-(906); here for the methylene chloride solutions, the Q/P ratio is 301, or put another way, the PRU/Q at 50% quenching is 3. While there is evidently some amplification when there is a weak aggregation of the polymer as evidenced by the broadened (compared to monomer) absorption and fluorescence for the polymers in methylene chloride, the major source of amplification beyond the enhanced binding of the quencher to polymer clearly comes from "communication" within the J-aggregate.

We note that the J-aggregate quenching "saturates" at ∼100 PRU/Q. If excited-state delocalization extended over an entire polymer chain, it might be anticipated that one quencher could quench a single polymer molecule, regardless of the polymer size and the value for Q/P_{50} might be less than unity. One possible reason for the saturation at lower values of PRU/Q_{50} may be that the synthetic procedure may result in "defects" (most probably an underivatized or "half"-derivatized residue on the polylysine) that confine the excitation to smaller domains. Since the available characterization tools cannot detect structural defects at levels below a few percent for the highest polymers, we cannot rule this out as an explanation. However, comparison of the present results with other studies of J-aggregates not polymer-derived suggests somewhat similar domain sizes for effective J-aggregate delocalization in several cases. Thus, while studies of a cyanine J-aggregate quenching by energy acceptors in a solid (Langmuir−Blodgett) film indicate that incorporation of an energy acceptor in the same layer at a level of 1 quencher/12 000 monomers in the aggregate results in 50% quenching,[19,20] for incorporation of the same energy acceptor in an adjacent layer, a more than 10-fold reduction in the number of monomers quenched (to ∼300) is observed.[21] An investigation of quenching of a similar cyanine J-aggregate Langmuir−Blodgett film by an electron acceptor in an adjacent layer (amphiphilic viologen) indicated 50% quenching of the J-aggregate fluorescence occurs at a level of 1 quencher/44 molecules of the cyanine,[22] a value about half that found in this study. Investigations with electron acceptors in Langmuir−Blodgett films of squaraine dyes suggest that quenching occurs at similar levels (1 "trap" per 25 aggregated molecules).[23] These values are also consistent with the exciton migration distance of ∼50 nm estimated from NSOM studies of pseudoisocyanine aggregates grown in poly(vinyl sulfate) thin films.[24] Other optical studies of J-aggregate excitons suggest that the exciton domain may be small, even at low temperatures.[25−28] These previous studies were all carried

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out in rigid solid media, and it is not clear to what extent the solution-phase polymer J-aggregates can be compared with them. It is also possible that in fluid media the conformations of polymers **1** are mobile and more random than in a crystal or solid and that imperfections (synthetic, as mentioned above, or conformational) might limit the size of J-aggregate domains and the effective exciton range. Interestingly, studies of liposomes containing J-aggregate cyanine dyes give a very similar number to the solid-phase results, and it would be reasonable to expect that a liposome in water might be somewhat comparable to a semiorganized J-aggregate polymer.[29] As noted earlier,[13] for **1**-(250) adsorption of the polymer onto an anionic clay particle increases the quenching constant, suggesting that as the polymer is immobilized and concentrated, enhanced quenching might be facilitated by a variety of factors.

The results obtained in the present study provide a clear picture of the factors that lead to superquenching for fluorescent polyelectrolytes in aqueous solution. The ability of polyelectrolytes to behave like a micelle in "collecting" oppositely charged or hydrophobic quenchers provides for enhanced association and hence enhanced quenching even for relatively small oligomers. From our studies of the cyanine dye pendant polylysine and previous investigations of other fluorescent polyelectrolytes, we can infer that this "collection" contribution to K_{SV} may level off at $\sim 10^7$ M^{-1}. Additionally, as shown in the present study, an enhanced sensitivity to quenching can be obtained where the chromophores of the fluorescent polyelectrolyte are linked via conjugation or via association in an aggregated state. From the foregoing discussion and our results, it appears that for J-aggregates and electron-transfer quenching this amplification may provide for a factor of 10^2 so that quenching constants of $K_{SV} \sim 10^9$ M^{-1} may be obtained for optimized systems. The observation that energy-transfer quenchers may access larger effective exciton domains suggests that even higher K_{SV} values (perhaps as high as 10^{11} M^{-1}) might be attainable with quenchers or quencher conjugates based on this type of quenching. For conjugated polyelectrolytes with effective coupling throughout the chain, it may be reasonable that the limiting $K_{SV} \sim [10^7(N_{PRU})]$ and thus even higher K_{SV}s may be anticipated. For $K_{SV} \sim 10^9$ M^{-1} such as observed for the present polymers with $N_{PRU} = 250$, 404, and 906, and **3** as quencher, quencher–analyte concentrations of 50 pM (5% quenching) (or, for example, 50 fmol in a 1-mL sample) can be detected. If employed as a fluorescence turn on sensor with a receptor having multiple binding sites, the sensitivity can be proportionally higher. If K_{SV} can be increased by 1–2 orders of magnitude, as appears reasonable, the sensitivity can be pushed to subpicomolar levels.

The present results suggest that solution-phase dye pendant polymers form J-aggregates exhibiting exciton delocalization extending over comparable domain sizes to those observed in previous studies of crystalline and thin-film J-aggregates of the same and similar monomers. The quenching constants obtained in this study for the higher molecular weight polymers in their J-aggregated state are, to our knowledge, the largest observed to date.

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