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Amplified Fluorescence Quenching of Self-Assembled Polyelectrolyte−Dye Nanoparticles in Aqueous Solution

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Fluorescent polyelectrolytes (FPs) are under considerable investigation in view of their application as chemical and biological sensors as a result of their versatile intrinsic properties, such as optical, electronic, and mechanical, and their ubiquitous ability to self-assembly with oppositely charged molecules.[1–3] Recent interest has surrounded the reports on the development of conjugated FPs and their extremely high sensitivity to quenching by ionic quenchers at very low concentrations, which are termed as “amplified quenching” or “superquenching”.[4–8] Meanwhile, nonconjugated but “J” aggregated FPs were also observed to exhibit the amplified quenching effect.[9] Because of the hydrophilic charged side groups and hydrophobic main backbone groups, interchain aggregation and coil conformation of these polyelectrolytes are typically found in aqueous solution. It has been demonstrated that the aggregation structures have significantly enhanced the quenching efficiency.[5f,6a,b] However, the synthesis of the FPs is time-consuming, and their aggregation conformation exhibits a dynamic feature (easily rearranged by the variation of external conditions, e.g., pH, temperature, solvent, etc.). Self-assembled complexes have been of great interest because they can be easily obtained or facilely prepared, and they are quite stable and have the ability of self-organization when equilibrium condition is reached.[10] The self-assembled complexes could be good candidates for sensor applications. In the current work, we employed an ionic complex of self-assembled polyelectrolyte−dye to extend the studies of amplified fluorescence quenching. An anionic pyrene derivative of pyrenetetrasulfonic acid (PSA) was found to strongly bind with the cationic poly(allyamine hydrochloride) (PAH) to form complexes in aqueous solution. The polyelelectrolyte−dye complexes spontaneously self-aggregated into particles in dilute aqueous solution. In the particles, fluorescence from pyrene excimer is favored, and the excimer fluorescence can be efficiently and selectively quenched by ionic quencher of 9,10-anthraquinone-2,6-disulfonic acid (AQS^{2-}), while not for cationic quencher methyl viologen (MV^{2+}).

The self-assembly of PSA and PAH was investigated by UV–visible and fluorescence spectroscopy. Figure 1 shows the optical spectra of a solution of PSA (5 μM) in water titrated with PAH (range from 0 to 20 μM, here the concentration of PAH is given by the concentration of the polymer monomer). The absorption of pure PSA features the structured bands in the near-UV with three maxima at λ = 339, 355, and 375 nm (Figure 1a). As the amount of PAH increases, the intensity of absorbance peaks significantly decreases. In particular, when the ratio [PAH]/[PSA] = 20:5, a new absorbance peak appears at 365 nm. In this case, it should be noted that the charge ratio (φ) of PAH to PSA equals 1, namely, charge balance. The corresponding change of fluorescence spectra is displayed in Figure 1b. Pure PSA in water exhibits only the characteristic emission of monomer with maxima at 388 and 406 nm. The presence of PAH causes the reduction in the intensity of PSA monomer emission and induces the typical excimer formation at 500 nm. When $\varphi = 1$, the original monomer emission becomes

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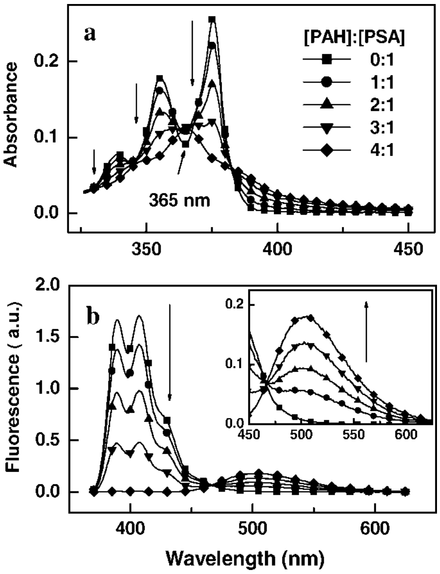

Figure 1. UV–vis absorption (a) and emission (b) spectra obtained by addition of PAH to PSA. The concentration of PSA is fixed at 5 μM, and the concentration of PAH varies from 0 to 20 μM. The excitation wavelength is 355 nm.

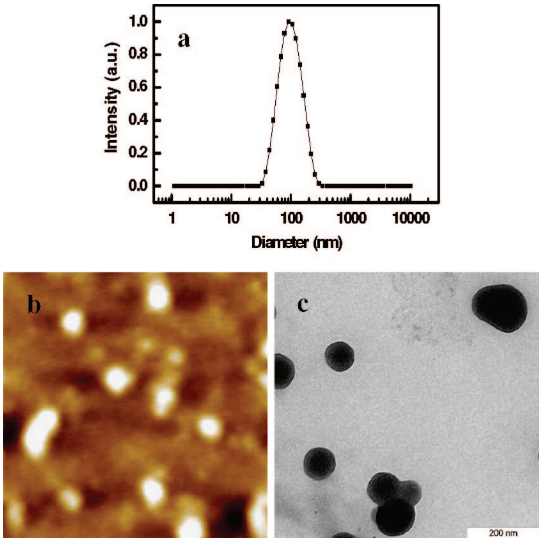

Figure 2. (a) Size distribution of the PAH:PSA particles determined by DLS at 25 °C. (b) AFM image performed in water with the size of 1 μm × 1 μm. (c) TEM image obtained from a drop of the PAH:PSA solution evaporating at room temperature.

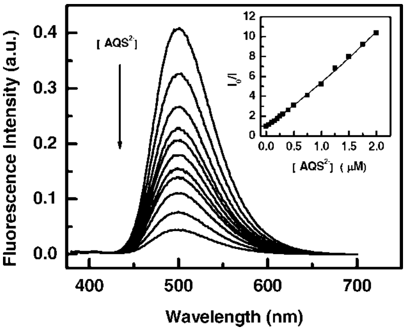

Figure 3. Emission spectra of the PAH:PSA nanocomplexes in the absence and presence of increasing concentration of AQS^{2-}. The Stern−Volmer plot of the fluorescence quenching is shown in the inset. The excitation wavelength is 365 nm.

very weak, nearly only excimer emission is present, and the ratio of fluorescence intensity between excimer (Ie) and monomer (Im) is 112.

The changes of absorption and fluorescence are believed to be due to formation of an ionic complex of polyelectrolyte−dye and aggregation of the complex in the aqueous solution. The self-aggregation of the PAH:PSA complexes was then investigated by light scattering measurement. The PSA dye alone is highly water-soluble, and the corresponding scattering intensity is very low. In contrast, the complexes of PAH:PSA show significantly increased scattering intensity. This clearly indicates the existence of aggregates in aqueous solution. As the charge ratio $\varphi = 1$, the complex of PAH:PSA was found to self-aggregate into particles. Figure 2a shows the size distribution of the aggregates determined by dynamic light scattering (DLS) from the solution of 5 μM PSA and 20 μM PAH. The broad peak corresponding to the hydrodynamic diameter (D_h) ranges from tens to hundreds of nanometers.

Atomic force microscopy (AFM), transmission electron microscopy (TEM), and confocal laser scanning microscope (CLSM) were also used to confirm the morphology of the polyelectrolyte−dye aggregates. Figure 2b shows an AFM image of the PAH:PSA complexes ([PSA] = 5 μM, and φ = 1) absorbed on the substrate of Si/PEI/PSS. The diameter of the spherical aggregates was observed at the range from 50 to 300 nm, which is close to the diameter determined by DLS. It should be pointed out that the height of the aggregates was only in the range of 5–15 nm, much smaller than the diameter, which must result from the deformation of the soft particles.[11] Figure 2c shows the TEM image of the PAH:PSA particles obtained from a drop of the PAH: PSA solution with [PSA] = 10 μM and [PAH] = 40 μM drying at room temperature. The CLSM image obtained from the solution of TRITC-PAH:PSA is shown in Figure S3, Supporting Information.

The aggregation of PSA and PAH ought to be driven by the electrostatic interaction of ion pairs of positively charged PAH and negatively charged PSA, consistent with previous works.[12] The flexibility of the polyelectrolyte should play an important role in the formation of the particles because formation of particles was not observed when using weakly flexible polyelectrolyte of poly(diallyldimethylammonium chloride) (PDDA) instead of PAH.

The fluorescence of pyrene excimer in the polyelectrolyte−dye nanoparticles was found to be efficiently quenched by the anionic electron acceptor AQS^{2-}. Figure 3 shows the fluorescence quenching of the nanoparticles. Here [PSA] = 5 μM and φ = 1, and [AQS^{2-}] varies in the range from 0 to 2 μM. Obviously, the excimer emission intensity (I) is

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reduced with increasing concentration of AQS^{2-}. The quenching behavior can simply be described by the Stern−Volmer equation of $I_0/I = 1 + K_{SV}[Q]$. A plot of I_0/I versus the concentration of AQS^{2-} is shown in the inset of Figure 3. At relatively low AQS^{2-} concentration, $[AQS^{2-}] < 0.5$ μM, a linear relationship is obtained with a slightly upward curvature at higher AQS^{2-} concentration. This behavior, referred to as superlinear, is usually observed at high quencher concentration and explained by the additional "sphere-of-action" quenching.[5b] The linear region provides the K_{SV} value of 4.8×10^6 M^{-1}. Cationic quencher of MV^{2+} was also used to quench the excimer emission, but weak quenching is observed, and the quenching constant of K_{SV} is only 1.9×10^2 M^{-1} (see Figure S4, Supporting Information). To clarify the quenching selectivity, the surface potential of the nanoparticles is the crucial point. The zeta potential results show that the PAH:PSA nanoparticles are positively charged (20 ± 4 mV), which indicates that the positively charged PAH dominates the surface of the particles. It is the reason that the anionic quenchers AQS^{2-} can easily bind to the ionic complex particles by electrostatic interaction and efficiently quench the fluorescence; however, the cationic quenchers MV^{2+} will be repulsed strongly.

Comparatively, fluorescence quenching of PSA monomer by MV^{2+} and AQS^{2-} were investigated (see Figures S5 and S6, Supporting Information). The quenching constants K_{SV} were calculated as 5.7×10^4 and 8.6×10^2 M^{-1} for MV^{2+} and AQS^{2-}, respectively. It is clearly seen that the quenching of monomer also has high selectivity, but the quenching efficiencies are much lower than the excimer quenched by AQS^{2-} in the particles of PAH:PSA complexes.

It is well know that fluorescence quenching is arisen from energy or electron transfer between donor and acceptor.[5e] Efficient energy transfer usually requires good spectra overlap between donor emission and acceptor absorption spectra. For the quencher of AQS^{2-}, its absorption spectra does not overlap with the emission spectra of PSA excimer (see Figure S7, Supporting Information). So the quenching of PAH:PSA complexes by AQS^{2-} could only be attributed to electron transfer. Although the detailed mechanism of amplified fluorescence quenching of the excimer emission is still under investigation, it is believed that the aggregation of the polyelectrolyte−dye complex plays an important role in the amplification. The aggregation of PAH:PSA complexes should lead to confinement of pyrene molecules because the formation of excimer is strongly dependent on the distance of the two neighboring pyrene molecules.[13,14] In this case, the nanoparticle formed by the PAH:PSA complexes can be regarded as a pseudo-conjugated system. In the particle, the excitons should be delocalized and can easily diffuse to the quencher association sites. Thus, the quencher can quench more excitons diffusing from the chromophores outside the quenching region and result in an amplified quenching sensitivity at the low quencher concentration (see Figure 4).

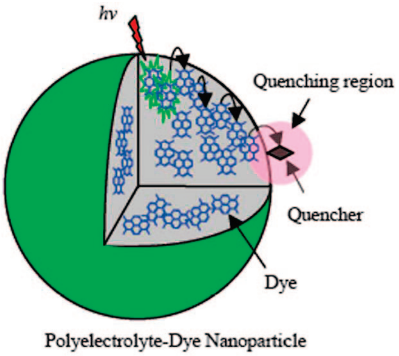

Figure 4. Graphic depicting the amplified fluorescence quenching processes in the polyelectrolyte−dye nanoparticle.

In conclusion, we have found that the ionic complexes of PAH:PSA can self-aggregate to nanoparticles in dilute aqueous solution, driven by the electrostatic interaction of ion pairs. During the spontaneous self-assembly, the confinement of pyrene molecules results in the fluorescence of excimer which can be efficiently and selectively quenched by anionic quencher AQS^{2-}. These observations open the opportunity for designing optical sensors by self-assembled ionic nanocomplexes of polyelectrolyte−dye.

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Supporting Information Available: The materials used and detailed experimental procedures, dependence of PSA monomer emission on the ratio of [PAH]/[PSA] (Figure S1), particle size distribution, emission with PAH $M_w = 15\,000$ (Figure S2), CLSM image of the particles (Figure S3), fluorescence quenching of excimer in the particles by MV^{2+} (Figure S4), quenching of PSA monomer by MV^{2+} (Figure S5), and AQS^{2-} (Figure S6) and optical spectra of PSA:PAH emission and AQS^{2-} absorption (Figure S7) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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