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Solvent-dependent aggregation of a water-soluble poly(fluorene) controls energy transfer to chromophore-labeled DNA

Shu Wang and Guillermo C. Bazan*

Departments of Material and Chemistry & Biochemistry, Institute for Polymers and Organic Solids, University of California, Santa Barbara CA 93106, USA. E-mail: bazan@chem.ucsb.edu

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The solvent-dependent aggregation properties of a water-soluble cationic poly(fluorene) were studied and used to control fluorescence resonance energy transfer to Texas Red-labeled DNA.

Conjugated polymers have established themselves as useful materials in optoelectronic applications such as light-emitting diodes (LEDs),[1] field effect transistors (FETs),[2] photovoltaic devices[3] and chemical and biological sensors.[4] Their electrical and optical properties are controlled by molecular conformations and supramolecular assembly.[5] The aggregation of conjugated polymers in organic solvents has been extensively studied to obtain insight into how the interchain arrangement can be optimized for use in optoelectronic devices.[6] Recently, we reported novel fluorescent biosensors based on water-soluble light-harvesting conjugated polymers to identify DNA and RNA in aqueous media.[7] To obtain insight into the signal transduction mechanism by these polymers one needs to understand their aggregation in solution. Although studies on the aggregation of some water-soluble conjugated polymer with ionic substitutes (amine or sulfonate groups) have appeared,[8] there are only a few reports[9] on the use of solvophobic interactions to tune optical properties. In this contribution, we report the different aggregation tendencies of water-soluble cationic poly[9,9-bis(6′-N,N,N-trimethylammonium-hexyl)fluorene diiodide] (poly**1**) in aqueous solutions with varying amounts of THF and their influence on the fluorescence resonance energy transfer (FRET) of poly**1** to Texas Red dye-labeled DNA.

Poly **1** is a rigid-rod like molecule with the structure shown in Scheme 1. The backbone and alkyl side chain are hydrophobic moieties, while the cationic charged quaternary amines control electrostatic interactions. Dissociation of the charged ionogenic groups requires polar solvents, while the hydrophobic segments are better accommodated in non-polar solvents. The resulting amphiphilic characteristics lead to different aggregation structures in different solvents.[8b–c]

To obtain better understanding of aggregation as a function of solvent polarity, we examined poly**1** in water with varying amounts of THF using fluorescence spectroscopy. As shown in Fig. 1, the emission intensity of poly**1** increases gradually with the addition of THF. The shapes of the spectra show negligible change. Highest emission intensity is observed with a 60:40 (v/v) THF:water ratio. However, the emission intensity begins to decrease when THF content is higher than 80%. Additionally, the emission maxima of poly**1** (419 nm in water) blue shifts to 414 nm when the THF content is between 30% to 80% (v/v). When the THF/H_2O composition is 90:10, the emission red-shifts to 423 nm. These results suggest the presence of two different aggregation states. We propose that the aggregation in water is dominated by the interchain hydrophobic interactions, which lead to lower emission intensities due to π–π interactions. Adding THF to an aqueous poly**1** solution breaks the aggregates. Reduced interchain contacts lead to reduced self-quenching (higher emission intensities) and higher emission frequencies. When THF is higher than 80% a new aggregate structure forms, which is dominated by the electrostatic interactions of charged quaternary amine groups and charge compensating iodide anions.

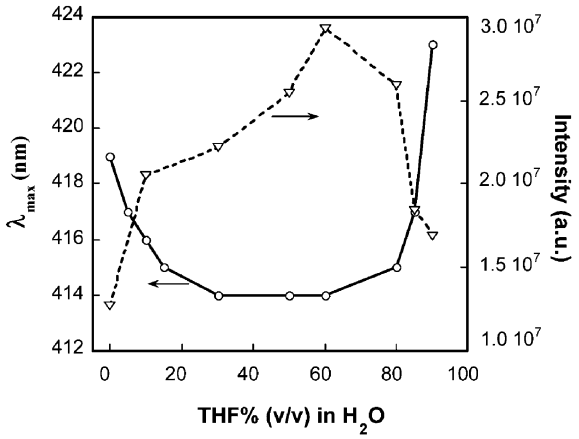
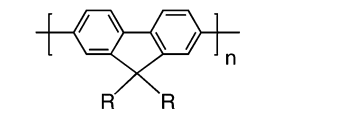


Fig. 1 Fluorescence intensity and emission maxima of poly**1** as a function of THF content in water ([poly**1**] = 2.0×10^{-6} M, λ_{exc} = 380 nm).

^{1}H-NMR spectroscopy can be used to study polymer conformations and gives rich information about structural properties.[10] The characteristic signals of the aromatic protons in the poly**1** backbone (δ = 7.7–8.2 ppm) are broadened into the baseline in D_2O. In 60% THF-d_8/D_2O (v/v) and 90% THF-d_8/D_2O (v/v) all the proton signals can be clearly detected. Therefore, in pure D_2O, the hydrophobic backbone of poly**1** forms a tightly packed core in which the chains experience little tumbling motion within the timescale of the NMR experiment.[11,12] This observation implies a tighter aggregation in D_2O, compared to in 60% THF-d_8/D_2O (v/v) and 90% THF-d_8/D_2O (v/v). When comparing the chemical shifts in 90% THF-d_8/D_2O (v/v) one observes a 0.25 ppm shift toward lower field relative to 60% THF-d_8/D_2O (v/v). This change indicates stronger π-stacking interactions.[6d]

Two aggregation models of poly**1** are proposed based on the fluorescence and ^{1}H-NMR spectroscopy results measurements, as shown in Scheme 2. Singe chain behavior, or weak aggregation, occurs when the THF content is in the range from 30% to 80% (Scheme 2A). These solvent mixtures allow for solvation of both components of the polymer structure. Poly**1** in pure water forms very tight aggregates, with chains coming together and forming an inner core (Scheme 2B). When the THF content is higher than 80% the ionic interactions of charged groups with the non-polar medium lead to burying these groups within a new aggregate structure (Scheme 2C).



R = $CH_2CH_2CH_2CH_2CH_2CH_2\overset{\oplus}{N}Me_3\ \overset{\ominus}{I}$

Poly**1**

Scheme 1 The chemical structure of cationic polyfluorene.

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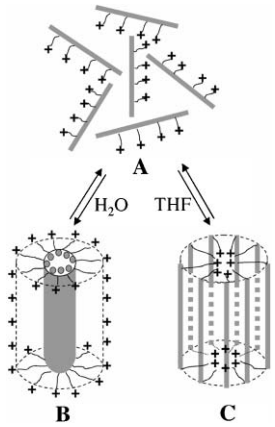

Scheme 2 The proposed aggregation modes of cationic poly**1** in water with different THF content.

To examine the effect of aggregation on the FRET efficiency of poly**1**, we chose Texas Red-labeled single stranded DNA (5′-Texas Red-ATCTTGACTATGTGGGTGCT-3′, ssDNA-TR) as an acceptor. For these experiments we excited at 380 nm where poly**1** absorbs strongly, while Texas Red shows negligible absorption. Upon addition of ssDNA-TR to a solution of poly**1** in water, the emission intensity of poly**1** decreases and the emission of the TR dye appears (Fig. 2). Similar observations were made for poly**1** in 60% THF/H_2O (v/v), however the emission intensity of TR resulting from FRET is approximately three fold greater than that in pure water. In 90% THF/H_2O (v/v) the emission from the acceptor is weakest. Thus, the best FRET from poly**1** to TR is obtained in 60% THF/H_2O (v/v).

The FRET dependence in Fig. 2 can be correlated to the aggregation models in Scheme 2. The availability of weakly aggregated poly**1** in 60% THF/H_2O (v/v) allows for quick complexation with the oppositely charged ssDNA-TR. Increased poly**1**/ssDNA-TR contact, together with the higher quantum efficiency in this solvent medium, yields more efficient FRET. In water and in a 90:10 THF/ H_2O mixture the chains are buried within the supramolecular structures and are not as available for complexation with DNA. Less efficient contacts increase the average poly**1**/Texas Red distance and reduce FRET efficiencies.[13] We note that the FRET measurements were made immediately upon addition of DNA and are responsive to the instantaneous macromolecular arrangemts. Allowing the solutions to stand results in redistribution of charged polyelectrolytes. Under some circumstances this leads to precipitation, as documented with other polyelectrolyte systems.[14]

In summary, ^{1}H-NMR and fluorescence spectroscopies reveal that poly**1** displays two types of aggregate structures, depending on the solvent polarity. These structures influence the FRET efficiency to Texas Red-labeled DNA. We recognize that other supramolecular structures can be considered to illustrate the concept in Scheme 2. For example, lamellar arrangements of polymer chains can be envisioned.[6d] Despite these structural uncertainties, the overall aggregation driving force is the amphiphilic nature of the poly**1** structure.

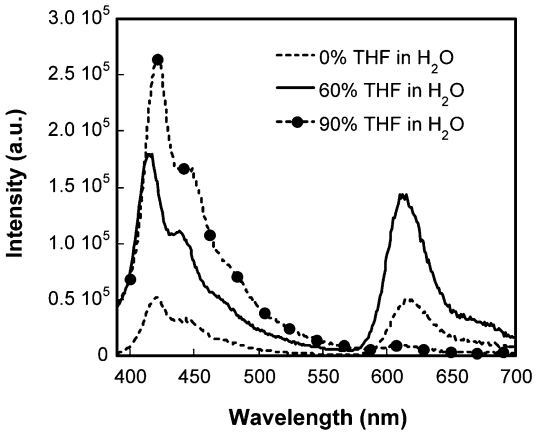

Fig. 2 Fluroescence spectra of poly**1** in the presence of ssDNA-TR ([poly**1**] = 2.0×10^{-6} M; [DNA] = 5.0×10^{-8} M; λ_{exc} = 380 nm).

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