Cooperative Conformational Transitions in Phenylene Ethynylene Oligomers: Chain-Length Dependence

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Abstract: Fluorescence quenching has been used to study the cooperative conformational transition in a series of oligo(phenylene ethynylene)s having tri(ethylene glycol) monomethyl ether side chains. In nonpolar solvents such as chloroform, the intensity of fluorescence emission from the backbone chromophore increases smoothly as the chain lengthens from the dimer through the octadecamer. In polar solvents such as acetonitrile, on the other hand, chains having more than eight units exhibit fluorescence quenching concomitant with the growth of an intramolecular excimer-like band. This observation is consistent with π-stacking of aromatic rings for chains that are long enough to fold back on themselves, driven by solvophobic interactions. Titration experiments in which the solvent composition was gradually changed from pure acetonitrile to pure chloroform showed sigmoidal curves characteristic of a cooperative transition. These data were analyzed using a two-state approximation and a model in which the free energy difference between conformational states depends linearly on solvent composition. The stability of the ordered state was found to increase linearly with chain length, suggestive of a regularly repeating conformation such as a helix. Therefore the data were fit to a two-state helix−coil equilibrium model where good agreement was observed. The parameters obtained from this analysis revealed a highly cooperative transition driven to fold by strongly interacting monomer pairs.

Introduction

Many biological and synthetic macromolecules undergo conformational changes between ordered and disordered states in a highly cooperative manner.[1] Examples include the coil−globule transition in synthetic macromolecules,[2] the helix−coil transition in peptides[3] and nucleic acids,[4] the β-sheet to coil transition in peptides,[5,6] and the denaturation of proteins[1] and RNA.[7] We recently discovered that certain phenylene ethynylene oligomers undergo a cooperative conformational transition in solution from a random state to a putative helical structure that involves π-stacked aromatic residues (Figure 1).[8] Here we report the chain length dependence of this process, and we compare the observed behavior to that of other oligomers and polymers displaying conformational transitions.

Cooperative collapse of random-coil homopolymers to compact globular conformations has been experimentally observed for a wide variety of synthetic backbones.[2] The formation of these compact structures is driven by attractive interactions between polymer segments as the quality of the solvent decreases or as the temperature is changed.[9] This transition is generally described as a second-order process, although recent simulations have shown that first-order (two-state) transitions

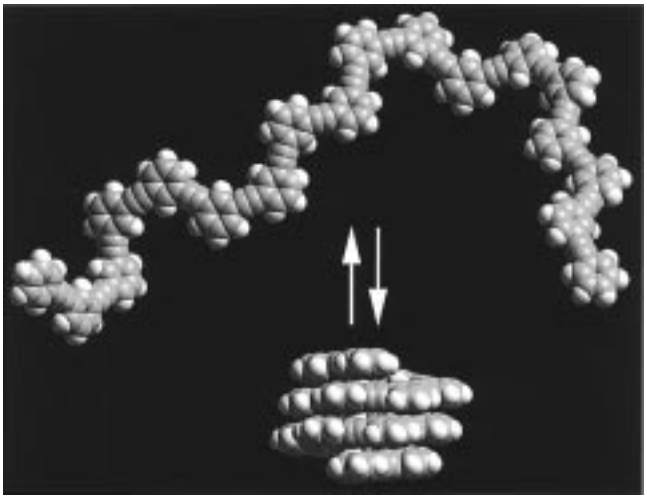

Figure 1. A space-filling model showing the proposed conformational equilibrium for a phenylene ethynylene oligomer of length $n = 18$. For clarity, side chains have been removed.

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Table 1. Cooperativity of the Helix−Coil Transition for Selected Biopolymers

polymer or oligomer	solvent	σ	ΔG_{nuc} (kcal mol^{-1})[a]	ref
poly-γ-benzyl-L-glutamate	ethylene dichloride:dichloroacetic acid (30:70)	2×10^{-4}	5.0	23
Ac−Y(AEAAKA)$_k$F−NH$_2$[b]	aqueous buffer (pH 7.0)	3×10^{-3}	3.5	24
single-stranded oligo(adenylic acid)	aqueous buffer (pH 7.3)	6×10^{-1}	0.3	25

[a] At 300 K. [b] A, L-alanine; E, L-glutamic acid; F, L-phenylalanine; K, L-lysine; Y, L-tyrosine; k = number of repeats.

are possible for stiff chains.[10] Recent calorimetric studies on poly(*N*-isopropylacrylamide) indicated that the coil-globule transition for low molecular weight polymers follows a two-state process.[11,12] Conformational transitions of this type have thus often been compared to the denaturation of small proteins, a process that also tends to follow a cooperative, two-state transition model (eq 1).[13−15] Unlike proteins, however, homopolymers do not generally collapse to a unique conformational state.[16] In fact, the size of the ensemble of compact conformations grows exponentially with chain length.[17,18]

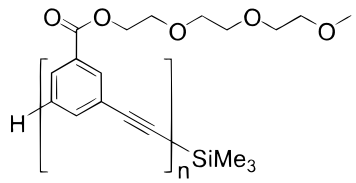
$$\text{denatured} \overset{K_{eq}}{\rightleftharpoons} \text{native} \qquad (1)$$

Cooperative helix−coil transitions have been extensively documented for peptides[3] and nucleic acids.[4] According to the helix−coil model,[19] the energy associated with helix formation is divided into a nucleation term and a helix propagation term.[20] In forming the initial turn of the helix, the first n_0 residues must be fixed without any gain in stability from monomer−monomer interactions. This is the helix nucleation event that has associated with it a free energy change denoted ΔG_{nuc}. Addition of a monomer to an already existing helix involves a free energy change, ΔG_s, which takes into account the enthalpic stabilization due to monomer−monomer interactions, the negative entropy of fixing the backbone conformation, and the change in free energy of the side chain. ΔG_s is associated with an equilibrium constant, s, called the helix propagation constant ($\Delta G_s = -RT \ln s$). The overall equilibrium constant for the formation of the helix is thus given by eq 2

$$K_{eq} = \sigma s^{n-n_0} \qquad (2)$$

where n is the number of monomers in the helical segment and $\Delta G_{nuc} = -RT \ln \sigma$. The magnitude of σ quantifies the degree of cooperativity (the smaller the value of σ, the stronger the cooperativity; a σ value of 1 corresponds to a noncooperative transition).[21] Table 1 summarizes experimentally measured σ values of selected biopolymers. It can be seen that the helix−coil transition in peptides is highly cooperative while single-stranded nucleic acid homopolymers (e.g., oligo(adenylic acid)) transform from coil to helix in a noncooperative manner. The bimolecular character of double-stranded nucleic acids introduces additional complications to the helix−coil transition.[22] In particular, duplex DNA exhibits concentration-dependent equilibria and can form intermediates involving internal loops. Nonetheless, the order−disorder process generally shows high cooperativity and two-state character.[4,22]

Solvent plays a critical role in the formation and stability of macromolecular structures.[26] Although the α-helix is the most abundant element of secondary structure in native proteins, many helix-forming sequences are only marginally stable in water.[27,28] However, solvents such as 2,2,2-trifluoroethanol (TFE) are known to stabilize the α-helical conformation of short peptides. Examples have been reported where aqueous solutions of peptide sequences undergo cooperative transitions to their α-helical state upon titration with TFE.[30] In these cases, helix stability has been shown to follow a linear free energy dependence on TFE composition, analogous to the relationship that is often used to describe the solvent denaturation of proteins by reagents such as urea or guanidinium salts.[31] The solvent environment can also influence the conformational behavior of nucleic acids.[32,33] Compared to proteins, however, cosolvents cause relatively minor changes to the stability of duplex DNA in aqueous solution.[33] This behavior may reflect a key difference between protein and nucleic acid solution structures: the stacking and pairing of nucleotide bases leads to highly complementary and far stronger interactions than exhibited by amino acid residues of peptides.[7]



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n = 2, 4, 6, 8, 10, 12, 14, 16, 18

There has recently been a growing interest in the development of nonbiological oligomers and polymers that exhibit a well-defined solution conformation.[8,34−38] We were thus intrigued

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by the observation that oligo(phenylene ethynylene)s (**1**) containing as few as 18 repeat units undergo a cooperative conformational transition upon changes in solvent composition or temperature.[8] A helical structure was inferred on the basis of NMR and UV spectroscopic data as well as molecular modeling calculations. To gain a better understanding of the conformational behavior of these oligomers, we report here the chain-length dependence of this transition as monitored by fluorescence spectroscopy. The data are analyzed using a two-state approximation and a model in which the free energy difference between conformational states depends linearly on solvent composition. In acetonitrile at 23 °C, we find that the stability of the ordered conformation increases by about 0.7 kcal mol^{-1} with each additional residue. Since the ordered conformational state is postulated to have a helical structure, we compare our results to the behavior predicted by the helix−coil transition model, to which very good agreement is observed.

Experimental Methods and Data Analysis

Oligomer Synthesis and Purification. The synthesis of the phenylene ethynylene oligomers **1** was performed in solution using a divergent/convergent oligomer growth strategy.[39] All compounds were purified by silica gel column chromatography. Characterization of intermediates leading to **1** ($n = 2$) included the methods of ^{1}H and ^{13}C NMR, mass spectrometry (EI), and combustion analysis (see Supporting Information). Oligomers **1** ($n = 4$ through $n = 18$) were characterized by ^{1}H NMR and by MALDI-TOF mass spectrometry using indoleacrylic acid as the matrix. In all cases the sodium-attached molecular ion was observed. The purity of oligomers **1** ($n = 2-18$) was determined by a size-exclusion chromatography (SEC) and high-pressure liquid chromatography (HPLC). SEC was performed using a Waters 510 pump, Waters 996 photodiode array detector, and a series of three Waters styragel HR 4E 7.8 × 300 columns calibrated with narrow molecular weight polystyrene standards. HPLC was performed with a Rainin binary gradient system equipped with two SD-200 pumps, a Si 80-125-CS analytical column (4.6 × 250 mm), and a UV detector operating at 275 nm. All compounds were greater than 99% pure based on peak areas (see Supporting Information).

Fluorescence and Absorption Measurements. Fluorescence and excitation spectra were recorded on a Photon Technology International (PTI) QM-1 fluorimeter using a 1-cm quartz cuvette in the right angle geometry at room temperature (23 °C). The fluorescence and excitation spectra were corrected for the wavelength dependence of detector sensitivity and excitation source output. The UV absorption spectra were recorded on a Shimadzu (model UV-160A) spectrophotometer using 1-cm quartz cells. The absorbance of the solutions for the fluorescence measurements (Figures 2 and 3) was approximately 0.1 at 289 nm (the excitation wavelength).

For the titration experiments, two stock solutions (100 mL each) of the appropriate oligomer in spectrophotometric grade $CHCl_3$ and CH_3CN were prepared. The OD_{289} of each solution was ≈0.1 for the fluorescence titrations (Figure 4) and ≈0.75 for the UV titrations (Figure 7, top). Mixed solvent compositions from 0 to 100% $CHCl_3$ were prepared by adding the appropriate volume of the $CHCl_3$ solution into a volumetric flask and then diluting to a total volume of 5 mL with the CH_3CN solution. The desired spectral property (fluorescence intensity at 350 nm or A_{305}/A_{289}) and OD_{289} were measured for each sample. For each solvent composition, the data shown is an average of two different solutions that gave almost identical values (±0.5%). The fluorescence data in Figure 4 were normalized by scaling the fluorescence intensity at 350 nm based on a constant optical density (OD) at 289 nm (OD = 0.1). Plots of fraction folded verses solvent composition were obtained from normalized fluorescence intensity data using eq 3

$$f_U = \frac{I_F - I}{I_F - I_U} \tag{3}$$

(f_U is the mole fraction of oligomers in the unfolded state, I is the fluorescent intensity at 350 nm for an intermediate solvent composition, and I_U and I_F are the intensity values characteristic of the fully unfolded and folded states, respectively).[40] The baseline values (I_U and I_F) were treated as linear functions of solvent composition in order to compensate for drift in the pre- and post-transition baselines.[40] Equilibrium constants, K_{eq}, and corresponding free energy changes, ΔG, for the conformational transition at any point along the titration curve were obtained by the standard relationships $K_{eq} = f_F/f_U$ and $\Delta G = -RT \ln K_{eq}$, where $f_F = 1 - f_U$ is the mole fraction of oligomers in the folded state. By analogy to the solvent denaturation of proteins and peptide secondary structures, the free energy change between these conformational states was assumed to depend linearly on solvent composition (eq 4).[30,31,40] $\Delta G(CH_3CN)$ in eq 4 represents the free energy difference between the ordered and disordered conformations in pure acetonitrile, and the value of m describes how rapidly the free energy of the transition changes with solvent composition. The intercept of a plot of ΔG verses composition thus provides $\Delta G(CH_3CN)$. The concentration of denaturant required to reach the midpoint of the transition (i.e., $[CHCl_3]_{1/2}$) is given by $\Delta G(CH_3CN)/m$.

$$\Delta G = \Delta G(CH_3CN) - m[CHCl_3] \tag{4}$$

The fluorescence data were fit to a two-state model of helix−coil equilibria.[41] In accord with eq 3, the fluorescence intensity signal was taken to have the form $I = I_F + (I_U - I_F)f_U$. For a two-state model, $f_U = [1+K_{eq}]^{-1}$ where K_{eq} is given in eq 2. ΔG_s and ΔG_{nuc} were each assumed to be a linear function of the concentration of $CHCl_3$: $\ln(s) = a_s[CHCl_3] + b_s$; $\ln(\sigma) = a_{nuc}[CHCl_3] + b_{nuc}$. I_F and I_U were taken to be the normalized fluorescence intensities of the octadecamer ($n = 18$) in 0% $CHCl_3$ and 100% $CHCl_3$, respectively. After equally weighting each data set ($n = 10$, 12, 14, 16, and 18), the four variable parameters were determined by a nonlinear least-squares fit.

Results

Figures 2 and 3 show fluorescence spectra of the series of phenylene ethynylene oligomers (dimer through the octadecamer) in chloroform and acetonitrile, respectively. The end groups for oligomers **1** were selected because they do not produce interfering fluorescence nor do they cause fluorescence quenching, unlike the end groups of the oligomers used in our earlier study.[8] In chloroform, the normalized fluorescence intensity at 350 nm increases smoothly as the chain lengthens. In acetonitrile, on the other hand, the fluorescence emission at 350 nm intensifies only in going from the dimer to the octamer. For the decamer in acetonitrile, emission at 350 nm is partially quenched and the fluorescence band begins to broaden. Oligomers longer than the decamer show very little emission at 350 nm in acetonitrile, but they exhibit a new fluorescence band at 420 nm. The broad, featureless appearance of this red-shifted band is reminiscent of excimer fluorescence, as might be expected for π-stacked aromatic rings.[42] Because this π-stacked structure is likely to be present in the ground state (i.e., there is preassociation), we refer to this as excimer-like emission. Using mixed solvents, it is possible to find compositions in which emission bands at both 350 and 420 nm are present. To

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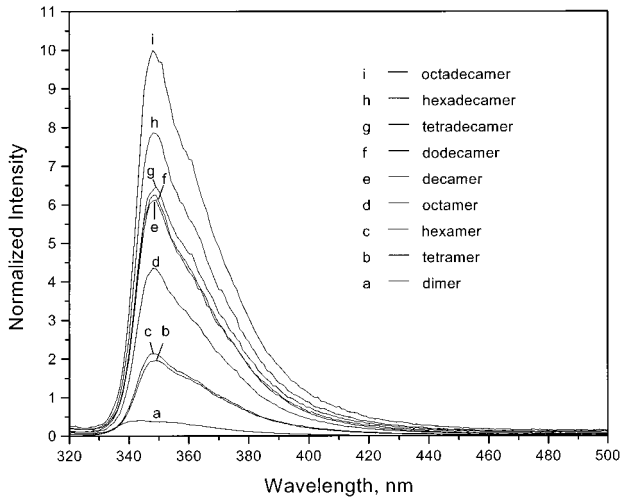

Figure 2. Fluorescence spectra of the phenylene ethynylene oligomers ($n = 2-18$) in chloroform using an excitation wavelength of 289 nm. The spectra were normalized to a constant oligomer molarity as determined by optical density measurements (dimer OD $\approx$ 0.1).

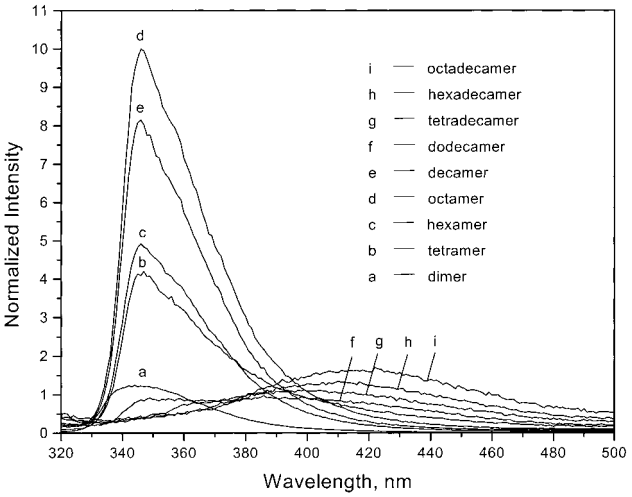

Figure 3. Fluorescence spectra of the phenylene ethynylene oligomers ($n = 2-18$) in acetonitrile using an excitation wavelength of 289 nm. The spectra were normalized to a constant oligomer molarity as determined by optical density measurements (dimer OD $\approx$ 0.1).

investigate the possibility of preassociation, excitation spectra were collected on the octadecamer in 55 vol % $CHCl_3$ in CH_3CN, a solvent mixture that exhibited two emission bands (see Supporting Information). When monitoring emission from the 350 nm band, the excitation spectrum was nearly identical to the absorption spectrum obtained in pure chloroform. When monitoring emission from the 420 nm band, the excitation spectrum approximated the absorption spectrum obtained in pure acetonitrile.

Figure 4 shows a plot of the normalized fluorescence emission at 350 nm as a function of the volume percent chloroform in acetonitrile (oligomers $n = 8-18$). These curves are presumed to be equilibrium measurements since the observations were independent of time and since representative data were shown to be reversible. They also reflect purely intramolecular effects as evidenced by the ratio of emission intensities (350 and 420 nm bands) being independent of concentration over a 100-fold dilution (e.g., octadecamer in 55% $CHCl_3$ in CH_3CN; the lowest concentration examined was 3.4×10^{-8} M). From Figure 4 it can be seen that the shortest chain (i.e., the octamer) does not undergo any apparent change as the solvent is switched from

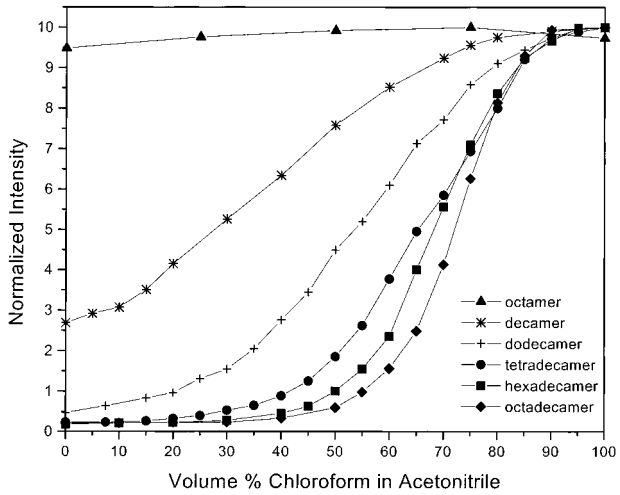

Figure 4. Plot of normalized fluorescence intensity at 350 nm vs the volume percent of chloroform in acetonitrile ($n = 8-18$). All spectra were normalized to a constant optical density of 0.1 at 288 nm.

Table 2. Solvent-Induced Unfolding of Phenylene Ethynylene Oligomers

chain length	$[CHCl_3]_{1/2}$ (vol %)	m (cal mol^{-1})	$\Delta G(CH_3CN)$ (kcal mol^{-1})
12-mer[a]	56	54 ± 2	-3.0 ± 0.1
14-mer[a]	64	68 ± 2	-4.3 ± 0.1
16-mer[a]	69	84 ± 2	-5.8 ± 0.1
18-mer[a]	72	99 ± 4	-7.1 ± 0.3
18-mer[b]	65	101 ± 4	-6.5 ± 0.2

[a] Determined by fluorescence quenching. [b] Determined by UV absorption ratios.

acetonitrile to chloroform. Longer chains exhibit distinct sigmoidal curves—the hallmark of cooperative phenomena.[1] At low chloroform compositions, the longest oligomers show complete quenching of 350 nm fluorescence. At high chloroform compositions, their fluorescence emission has totally returned. The sharpness of the transition increases as the chain length increases. Moreover, the amount of chloroform needed to reach the midpoint of this transition ($[CHCl_3]_{1/2}$) increases as the chain lengthens (Table 2).

To the degree that fluorescence quenching at 350 nm faithfully represents the conformational state of these oligomers, it is possible to transform the data in Figure 4 to a plot of fraction unfolded vs solvent composition using eq 3 (Figure 5a). In using eq 3, the oligomers are assumed to either be fully folded or fully unfolded, and the extent of fluorescence quenching at 350 nm is assumed to be proportional to the mole fraction of folded oligomers. The resulting titration curves are shown (Figure 5, top) for those oligomer lengths that exhibited well-defined pre- and post-transitional baselines (i.e., $n = 12-18$).

Using the data in Figure 5a and the relationship given in eq 4, it is possible to estimate the difference in free energy between the ordered and disordered conformations in pure acetonitrile, $\Delta G(CH_3CN)$. This analysis closely follows procedures commonly used to determine the stability of folded proteins and secondary structures from solvent denaturation curves.[30,31,40] Schellman has provided theoretical justification for the general use of eq 4 to describe the conformational disordering in macromolecules by selective solvation.[43] Using this approach, free energy verses solvent composition plots are shown in Figure 5b and the results are summarized in Table 2. It can clearly be seen that the m values increase as the chain lengthens, reflecting

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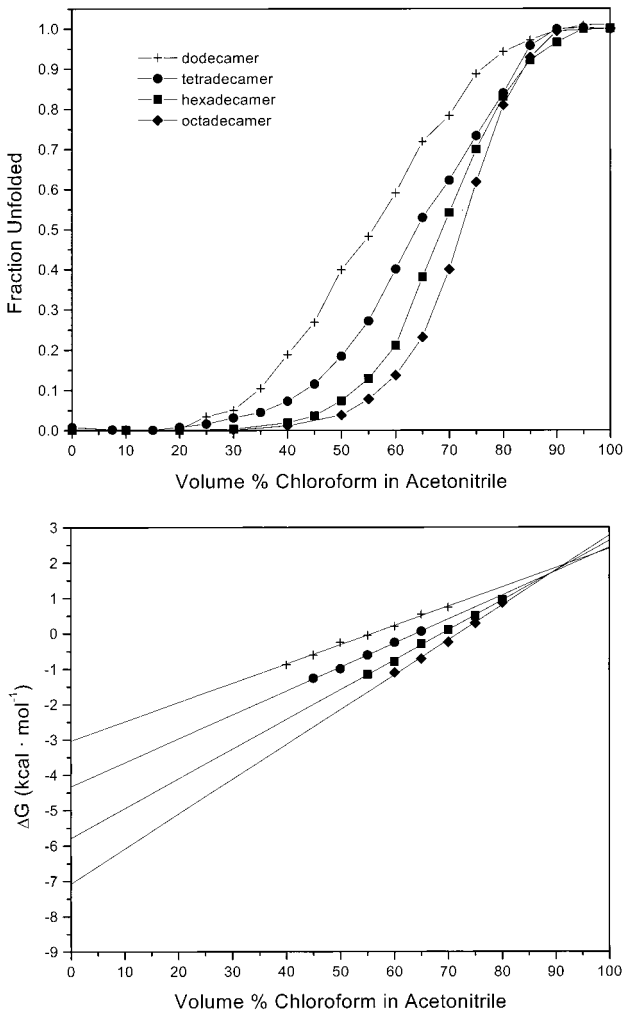

Figure 5. (Top) Titration curves (fraction unfolded vs solvent composition) for the dodecamer through the octadecamer using eq 3 and the data shown in Figure 4. (Bottom) Plot of ΔG vs volume percent of chloroform in acetonitrile. The values of free energy were obtained from the data in the upper plot ($\Delta G = -RT \ln(f_F/f_U)$). Linear extrapolation of these data to 0% chloroform gives the values of $\Delta G(CH_3CN)$. Only those data in the transition regions of the curves can reliably be used for the free energy extrapolation.[40]

the increasing sharpness of the transition. The stability of the ordered conformation also increases as the chain grows. Figure 6 shows a plot of $\Delta G(CH_3CN)$ as a function of chain length for the dodecamer through the octadecamer. It is evident from this graph that a linear relationship exists between oligomer length and conformational stability.

Experiments were conducted to address the validity of the data treatment used above, especially with regard to the two-state approximation (eq 3). Close examination of the fluorescence spectra showed no strong evidence for conformational intermediates in any of the solvent mixtures. Specifically, spectra at intermediate solvent compositions were fairly well approximated by a linear combination of the two spectra from the limiting solvents (see Supporting Information). The only noticeable difference was a small (up to ca. 10 nm) red shift in the excimer-like band of the simulated spectra compared to the actual data. The close agreement between the actual and simulated spectra can be interpreted in more than one way. As already discussed, it may mean that partially folded structures are not highly populated, as expected for a transition with two-state character. Alternatively, there may simply be insufficient resolution in the fluorescence spectra to distinguish between

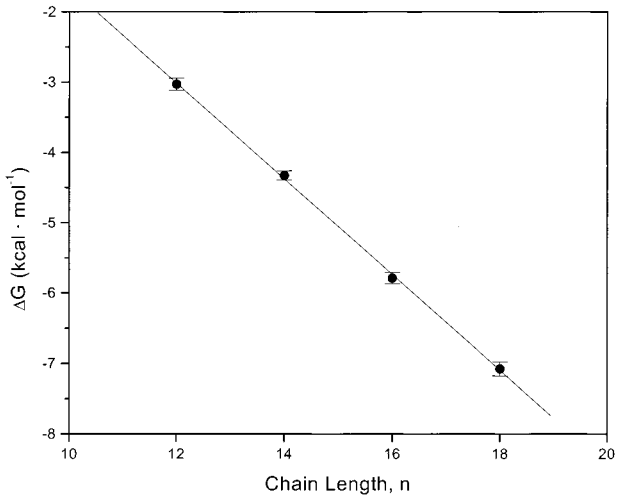

Figure 6. Plot of $\Delta G(CH_3CN)$ vs chain length for the dodecamer through the octadecamer. The linear equation used to fit these data is given by $\Delta G(CH_3CN) = 5.1 - 0.68n$ (kcal mol^{-1}).

partially folded and fully folded forms. The possibility of insufficient resolution is plausible and must be considered more thoroughly. In particular, should partially folded conformations exist in which two or more residues are π-stacked, an excimer-like band would already likely be present. Furthermore, efficient intramolecular resonance energy transfer to the excimer would quench the 350 nm fluorescence from the unfolded part of the molecule.[42] Thus only excimer-like emission might be observed from partially folded chains. The fluorescence spectra in such a case would not provide any information about partially folded conformations. Moreover, since quenching at 350 nm for these partially folded structures could be extensive, the sharpness and stability of the titration curves would be exaggerated relative to the actual transition. Given these possibilities, it thus became apparent that the conformational transition as measured by fluorescence must be corroborated by an alternative method.

Previously we showed that the ratio of UV absorption at 305 and 289 nm is sensitive to backbone conformation.[8] This provides an opportunity to compare the transition as measured by two independent techniques. The results are shown in Figure 7, where it can be seen that there is reasonable agreement between the two methods. In particular, the sharpness of the transition is identical within error when monitored by UV or fluorescence (plots of ΔG vs solvent composition have identical m values, Figure 7c). However, the midpoint of the transition is shifted to slightly higher percent chloroform for the fluorescence quenching method. Thus, the value of $\Delta G(CH_3CN)$ obtained by fluorescence is somewhat more negative than that obtained by UV, indicating a greater stability of the folded conformation as measured by fluorescence (Table 2). This may be because the UV measurements are less sensitive than the fluorescence measurements, or because of the occurrence of partially folded intermediates whose 350 nm fluorescence is quenched (vide supra). In either case, it is important to note that the values of $\Delta G(CH_3CN)$ measured by two independent methods are in reasonable agreement (Table 2).

Discussion

We established that at the concentrations typical of the fluorescence measurements, nonaggregated, *unimolecular* species predominate. This was confirmed by noting that fluorescence spectra from the 18-mer did not change shape over a 100-fold variation in concentration. The lack of aggregation at these

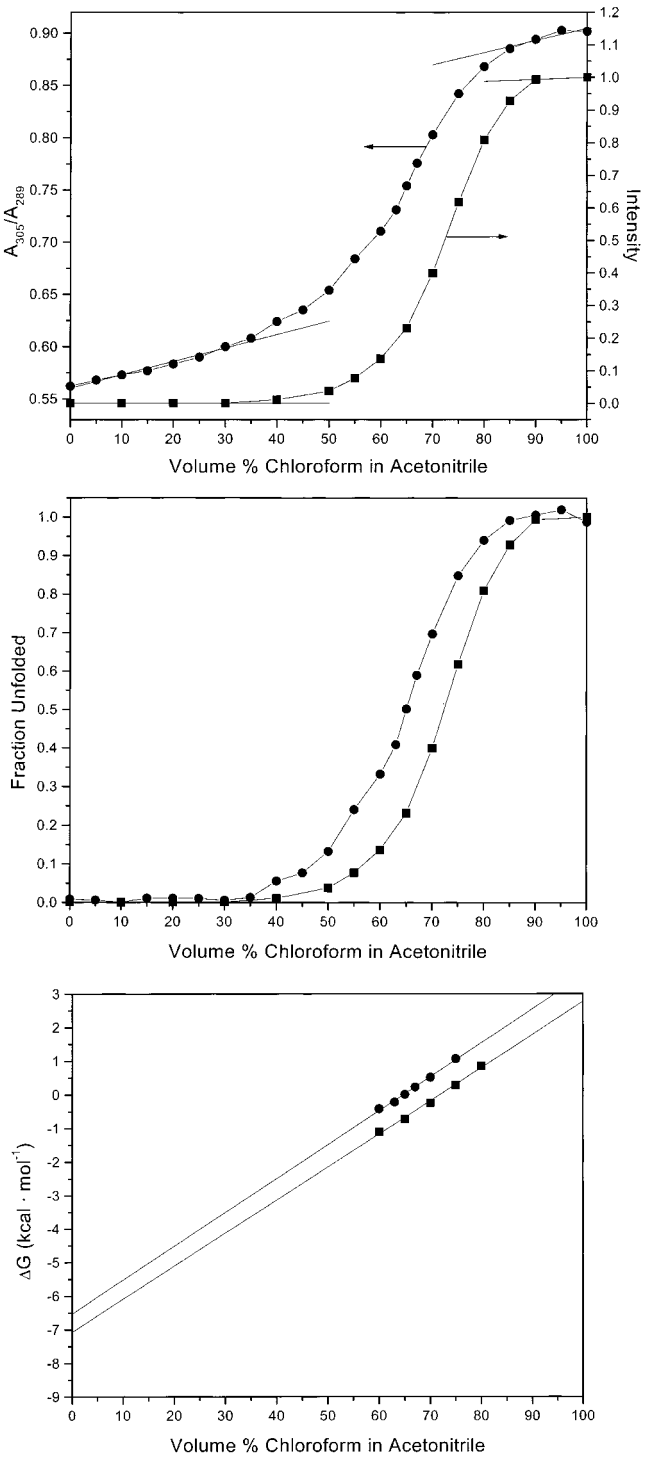

Figure 7. Comparison of titration curves generated by UV and fluorescence measurements for the octadecamer. (Top) UV absorption ratio (●) and fluorescence intensity (■) data showing the pre- and post-transition baselines. (Middle) Fraction folded vs solvent composition from UV (●) and fluorescence intensity (■) data. (Bottom) Free energy vs solvent composition data as determined from UV (●) and fluorescence intensity (■) data.

concentrations was anticipated on the basis of vapor phase osmometry (VPO) studies that we previously reported for a closely related series of oligomers.[8] Using these VPO-derived association constants we calculate that oligomer aggregation will be insignificant at concentrations below 10^{-6} M in pure acetonitrile (i.e., the mole fraction of oligomers in the unimolecular state is greater than 0.95 at 10^{-6} M in acetonitrile). In the chloroform/acetonitrile solvent mixtures used for the titration studies, aggregation is expected to be even less severe. It is worthwhile to note, however, that for other conjugated polymers, solvent induced changes in absorption spectra similar to those observed for **1** have been attributed to the formation of multichain superstructures.[44]

The fluorescence spectra from the phenylene ethynylene oligomer series are consistent with our hypothesis that chains of sufficient length undergo a solvophobically driven collapse to a helical conformation involving π-stacked aromatic residues.[8] No excimer-like emission was observed for the short oligomers ($n = 8$) in acetonitrile (i.e., a poor solvent) nor was it observed for the short or long oligomers in chloroform (i.e., a good solvent).[9] The onset of excimer-like emission in acetonitrile coincides exactly with the chain length (i.e., 10-mer) at which we previously noted upfield shifting of aromatic NMR resonances and UV hypochromism.[8] Excitation spectra from these oligomers indicated that the π-stacked arrangement is present in the ground state. The observed behavior is that expected for excimer-like emission arising from the backbone's intramolecular structure and is similar to that reported by Lehn et al. for heteroaromatic oligomers that adopted a helical conformation in solution.[45] These findings and the fact that the titration curves generated from fluorescence and UV are similar, suggest that all three spectroscopic methods (i.e., NMR, UV, and fluorescence) are sensing the same solvent-induced conformational change.

Titration curves for the oligomer series show cooperative conformational transitions whose position and sharpness increase with chain length. This behavior suggests that both the stability of the ordered state and the degree of cooperativity associated with the transition increases as the chain lengthens. Quantitative treatment of the titration data was made possible by assuming the transition to be a two-state process. The validity of the two-state approximation is best established by an experimental analysis of populations or by showing coincidence in sigmoidal curves generated by two independent methods.[1] At the midpoint of the transition for a two-state process, both the fully folded and the fully unfolded conformations will be equally populated. In contrast, a single broad population that transforms continuously from the fully folded, to intermediate, to the fully unfolded state characterizes one-state behavior. In practice, it is difficult to distinguish these situations by direct experimental observations, but evidence for a two-state process can be obtained by indirect means. For example, the spectroscopic signals from a system that undergoes a two-state transition (in the limit of slow exchange) must be able to be modeled as a linear combination of signals from the two limiting states. The fluorescence spectra of the 18-mer in the transition region could be reasonably well approximated by linear combination of the spectrum from pure acetonitrile and that from pure chloroform. However, because of the possible lack of resolution in the fluorescence spectra, no definitive conclusion could be made about the two-state character of the transition from these data alone. For this reason it was also shown that the sigmoidal titration curves measured by two independent methods (fluorescence and UV) are nearly coincident. Together these observations lend support in favor of the use of a two-state approximation for the data analysis (eq 3). More work is needed (e.g., calorimetry studies) to better establish how well the two-state model describes the transition.

We interpret the UV spectral changes as reflecting the

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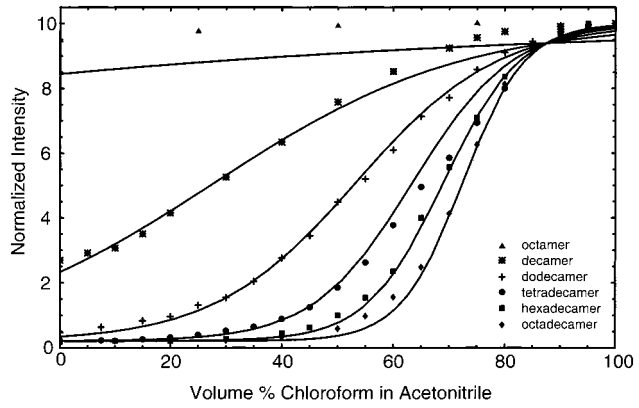
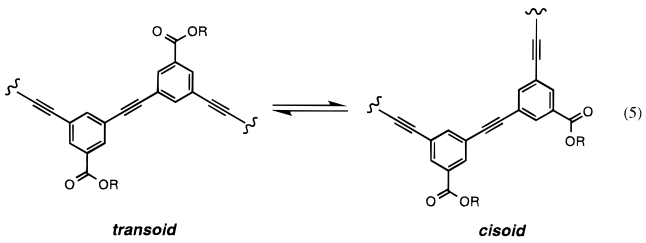


Figure 8. Fluorescence intensity at 350 nm vs the volume percent of cholorform in acetonitrile for oligomers $n = 8$ through $n = 18$. The points are the experimental data, which are normalized as described in Figure 4. The curves are the result of a fit to a two-state helix−coil equilibrium model (eq 2). The values of the parameters obtained from the fit are $a_s = -0.017 \pm 0.001$, $b_s = 1.48 \pm 0.09$, $a_{nuc} = 0.039 \pm 0.007$, and $b_{nuc} = -6.1 \pm 0.5$.

conformational equilibrium shown in eq 5. The ratio of oscillator strengths for the 305 and 289 nm bands is believe to be smaller for the *cisoid* than the *transoid* torsional state. Thus, a high population of *cisoid* units in the folded state (e.g., as in the helical conformation shown in Figure 1) would account for the observed change in the A_{305}/A_{289} absorption ratio. The excimer-like emission, on the other hand, cannot be explained purely on the basis of local torsional states (i.e., *cisoid* vs *transoid*), but rather, it is typical of π-stacked aromatic residues.[42] It is thus apparent that the fluorescence and UV methods are sensing different aspects of conformational structure, yet they generate similar titration curves. Hence, it is reasonable to use fluorescence data and the two-state approximation to analyze this conformational transition.



(5)

An important conclusion from the present study is that the stability of the conformationally ordered state is linearly dependent on chain length (Figure 6). This behavior had previously been predicted on the basis of a molecular modeling study.[8] Our experimental data suggest that each additional monomer contributes roughly 0.7 kcal/mol of stability to the folded conformation at 23 °C in acetonitrile. Linear extrapolation to the octamer yields a free energy value near zero, consistent with the absence of folding seen in Figure 4. The observation that each monomer contributes the same increment of stability to the ordered conformation is suggestive of a regularly repeating conformational structure, such as a helix. In fact, a linear free energy dependence on chain length is predicted from the helix−coil equilibrium expression given in eq 2.

To carry this analysis one step further, all of the titration curves shown in Figure 4 can be fit to the helix coil equilibrium model (eq 2). These fits are shown in Figure 8 where we have assumed that the free energy of helix propagation, ΔG_s, and the free energy of helix nucleation, ΔG_{nuc}, each depend linearly on solvent composition. The value of n_0 was set to 5 on the basis of our previous molecular modeling studies[8] (i.e., roughly five monomers per turn). As can be seen in Figure 8, the entire set of titration data for oligomers $n = 8-18$ are well-fit to eq 2. The values of m and $\Delta G(CH_3CN)$ estimated from this fit (Table 3) are in good agreement with those from Table 2. The value of σ in neat acetonitrile is 2.2×10^{-3}, indicating a fairly high degree of cooperativity that is comparable to α-helix formation of peptides in aqueous solution (Table 1). Interestingly, the value of the helix propagation constant in acetonitrile ($s = 4.4$) is similar to that measured for single-stranded polyadenylic acid in aqueous solutions.[25] In other words, phenylene ethynylene monomer pairs are driven to interact strongly in the ordered conformation, much the same as π-stacked nucleotides in helical nucleic acids. In contrast to the nucleic acids, however, oligo(phenylene ethynylene)s exhibit a highly cooperative transition.

Table 3. Values of m and $\Delta G(CH_3CN)$ As Obtained from the Two-State Helix−Coil Model

chain length	m (cal mol^{-1})[a]	$\Delta G(CH_3CN)$[b] (kcal mol^{-1})
12	46 ± 7	-2.5 ± 0.5
14	67 ± 8	-4.2 ± 0.5
16	87 ± 9	-6.0 ± 0.6
18	106 ± 10	-7.7 ± 0.7

[a] $m = -RT(a_{nuc} + (n - n_0)a_s)$. [b] $\Delta G(CH_3CN) = -RT(b_{nuc} + (n - n_0)b_s)$.

It is well established, both experimentally and theoretically, that the helix−coil transition does not strictly follow two-state behavior.[3] Use of the two-state approximation exaggerates both the stability of the helix and the sharpness of the transition.[20] Being aware of this, we fit our titration data to the one-helical sequence form of the Zimm−Bragg theory that includes partially helical conformations.[19,20] Treatment of the data in this way (see Supporting Information) did not provide any better fit than that seen in Figure 8. It is interesting to speculate that the stiff-chain nature of **1** may encourage the conformational transition to have two-state character.

Conclusions

Polar solvents drive phenylene ethynylene oligomers to undergo a cooperative conformational transition to an ordered solution structure. The transition was followed by fluorescence and UV methods. Fluorescence quenching and the growth of an intramolecular excimer-like band were observed for chains longer than 8 monomer units in polar solvents. Such spectral features are expected for π-stacked chromophores that would occur as the chain folds back on itself. Titration curves were obtained by monitoring fluorescence quenching as the solvent composition was gradually changed. The data were analyzed using a two-state model and methods analogous to the denaturation of proteins. The stability of the ordered conformation increases linearly with chain length by approximately 0.7 kcal mol^{-1} per monomer unit. This behavior is in accord with a regularly repeating structure for the ordered state, such as a helical conformation. Consistent with this finding, the data were fit to the helix−coil equilibrium model. This analysis yielded parameters indicative of a cooperative transition ($\sigma = 2 \times 10^{-3}$) that is driven by solvophobic forces and strong interactions between the monomers.

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Supporting Information Available: Synthesis and characterization data of oligomers **1**, comparison of simulated and experimental fluorescence spectra in a solvent mixture, comparison of excitation and absorption spectra, fits of the titration curves to the one-helical sequence form of the Zimm−Bragg theory. This material is available free of charge via the Internet at http://pubs.acs.org.

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