JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Foldamer Structuring by Covalently Bound Macromolecules

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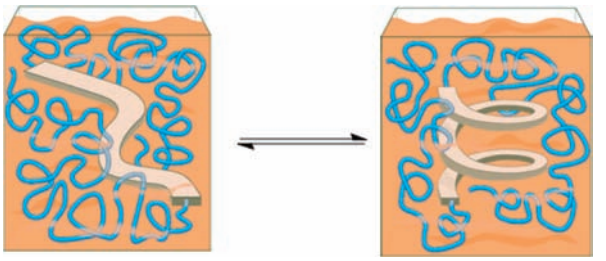
Supporting Information

ABSTRACT: We used fluorescence and electronic absorption spectroscopy to study the molecular weight dependence of macromolecule-induced folding in a chain-centered *meta*-phenylene ethynylene (*m*PE) oligomer. Analogous to the ability of intrinsically unstructured proteins (IUPs) to induce folding of globular proteins in cellular environments, we show that macromolecules attached to both ends of an *m*PE dodecamer induce the foldamer to collapse into a presumed helical conformation. The collapse is especially prominent once the macromolecule segments become larger than ca. 50 kDa. For sufficiently large macromolecules, the conformational structuring occurs even in solvents that normally denature the foldamer. Based on these findings, chain-centered foldamers might find use as models to investigate the fundamental macromolecular physics of IUPs.

Intrinsically disordered or unstructured proteins (IDPs or IUPs) lack well-defined secondary and/or tertiary structures under physiological conditions.[1] Despite their disorder, IUPs possess diverse functional capabilities.[2] While the coupling of folding and association is a common mechanism used by IUPs for recognition, regulation, signaling and assembly events,[2] IUPs can also serve as entropic chains carrying out functions that depend directly on the disordered state.[3] Assisted folding of globular proteins is one function common to IUPs whose action depends on entropic chains (e.g., some examples of intramolecular chaperone-mediated protein folding). The chaperoning mechanism is suggested to either involve (i) local loosening of kinetically trapped folded intermediates or (ii) solubilization and steric protection of the partially folded protein.[4] It remains unclear if other aspects of polymer physics, such as modulation of the local solvent environment, contribute to the mechanism of IUP chaperones.[5]

Foldamers are synthetic oligomers able to adopt ordered conformations in solution.[6] They are simple systems that can serve as models to tease out principles and thus better understand behavior found in their inherently more complex biomacromolecule counterparts. We wondered if the simple covalent attachment of entropic chains to the ends of a *meta* phenylene ethynylene (*m*PE) oligomer would enhance or inhibit structuring of the foldamer (Scheme 1). Here we show the molecular weight dependence of the entropic chain on its ability to impart structure to an *m*PE foldamer. Surprisingly, when the entropic chain is larger than ca. 50 kDa, structuring of the foldamer is enhanced, even in a solvent for which the foldamer is otherwise denatured. This observation suggests that high molecular weight entropic chains significantly alter a foldamer's local environment, facilitating the acquisition of solvophobically derived conformational order.

Scheme 1. A Foldamer, with Polymer Chains Attached at Each End, Equilibrating between Unstructured and Helical Conformations



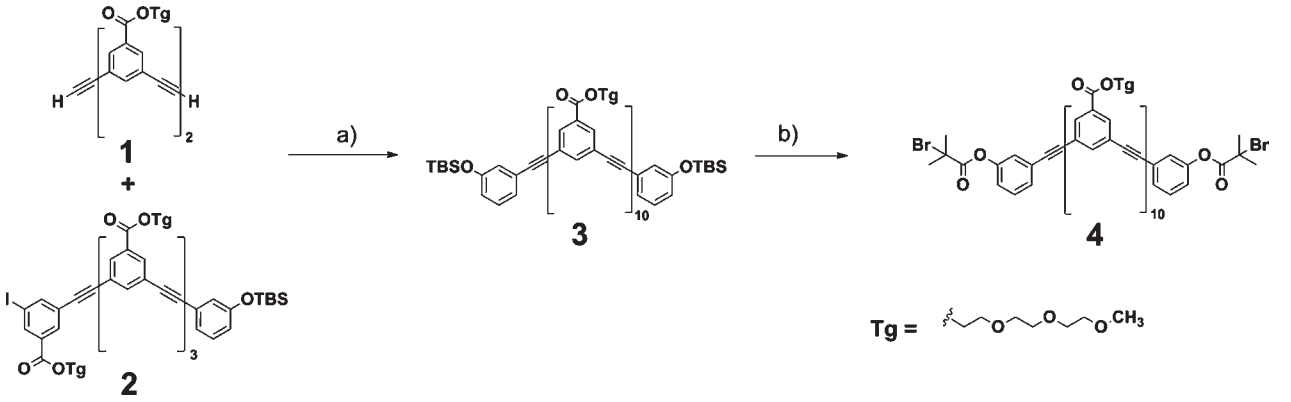
To begin, we systematically monitored the behavior of foldamer-linked polymers of varying molecular weight in different solvents. The particular foldamer used was an *m*PE dodecamer, selected because it contains the minimal oligomer length to form a stable helix in solution.[7] Dodecamer **3** was synthesized by Pd-catalyzed Sonogashira coupling of two pentamer units (**2**) with one dimer (**1**) (Scheme 2). Initiator **4** was synthesized from **3** by deprotection of the TBS groups under buffered conditions followed by esterification with α-bromo isobutyryl bromide. Single-Electron-Transfer-Living-Radical-Polymerization[8] (SET-LRP) of methylacrylate (MA) produces foldamer-linked polymers with varying molecular weights and low polydispersities (PDIs). Polymerizations were performed at room temperature in DMSO with a Cu(0) catalyst, hexamethylated tris(2-aminoethyl) (Me_6TREN) ligand, and excess MA over various reaction times. A series of foldamer-linked poly(methylacrylate) (PMA) polymers (PMA-*m*PE_{12}-PMA) were synthesized with Gel Permeation Chromatography (GPC) determined molecular weights ranging from 40 to 600 kDa. Table 1 summarizes the different reaction times and corresponding GPC data. Photodiode array detection monitoring the wavelength range 250−350 nm of the GPC eluent confirmed that a foldamer was covalently attached to the PMA polymer [see Supporting Information (SI)].

The conformational transition of *m*PE foldamers has been previously characterized by electronic absorption,[9] fluorescence,[10] and circular dichroism[11] spectroscopy. In a 'good' solvent[12] such as chloroform, both the phenylacetylene backbone and triglyme ester side chains are well-solvated so that the *m*PE foldamer forms an unstructured, random conformation. In a more polar,

Received: September 15, 2011
Published: November 15, 2011

dx.doi.org/10.1021/ja2087163 | *J. Am. Chem. Soc.* 2011, 133, 19650–19652

Scheme 2. Synthesis of *m*PE Foldamer Functionalized with an SET-LRP Initiator at Each End[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, Et_3N, THF, 36%; (b) (i) TBAF, THF, AcOH; (ii) α-bromo isobutyryl bromide, Et_3N, THF (15% over two steps).

Table 1. Molecular Weight Data for Polymers Obtained at Different SET-LRP Reaction Times

Reaction time (min)[a]	M_n (kDa)[b]	PDI
20	39	1.3
30	69	1.2
35	86	1.2
45	104	1.1
55	134	1.1
70	178	1.2
90	233	1.3
120	365	1.3
160	591	1.4

[a] Reaction conditions: 4 equiv of Cu(0), 4 equiv of Me_6TREN, 0.2−0.5 mL of DMSO, excess methyl acrylate. [b] Number average molecular weight of the PMA-*m*PE-PMA polymer in THF.

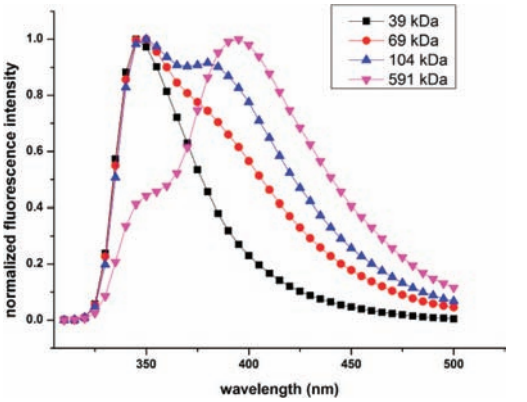


Figure 1. Fluorescence spectra of foldamer-linked polymers in chloroform. [Solution concentration is ca. 1.0 μM (concentration defined per mole of foldamer). The spectra were normalized to highest emission intensity.]

'poor' (i.e., solvophobic) solvent such as acetonitrile, the foldamer collapses. By adopting a helical conformation, the backbone reduces its solvent-accessible surface area and maximizes contacts between foldamer segments. Based on electronic absorption and fluorescence data collected during solvent titration experiments, the conformational behavior follows a cooperative, sigmoidal two-state transition.

In chloroform (a good solvent), oligomer **4** adopts an unfolded form as supported by electronic absorption and fluorescence data. The folding behavior of oligomer **4** was investigated by using typical solvent-dependent denaturation experiments by following the conformational equilibrium with electronic absorption and fluorescence spectroscopy. The sigmoidal shape of the resultant titration curve is indicative of the cooperative folding process (Figure S19). Electronic absorption and fluorescence data in acetonitrile (a poor solvent) show that the oligomer **4** and all of the polymers behave identically whereby both oligomer **4** and the chain-centered foldamers adopt a structured form. Thus, attachment of macromolecules to the ends of the foldamer causes no apparent denaturation of the foldamers (Figure S20 and S23). However, the foldamer-functionalized polymers show a conformational transition that is dependent on the molecular weight of the attached macromolecule. By monitoring emission signals at 350 and 400 nm, fluorescence spectroscopy is used to probe the conformational state of the foldamer covalently bound to polymer chains (Figure 1). A plot of fraction folded vs molecular weight shows asymptotic behavior that begins to level above ca. 130 kDa (Figure 2). This behavior remains independent of concentration in the dilute solution (i.e., μM) regime. Furthermore, the electronic absorption spectra show the typical signature of folded *m*PE oligomers. Specifically, we used the ratio of UV absorbance at 306 nm to that at 289 nm to monitor conformational ordering as a function of molecular weight of the polymers. These data are consistent with the fluorescence observations (Figures S20 and S22).

The need for covalent attachment was demonstrated by physically mixing a 104 kDa PMA homopolymer with foldamer **4** in chloroform. If noncovalent association between PMA and the foldamer is the cause of folding, we might expect to see a rise in the long wavelength fluorescence at sufficiently high PMA concentration. However, no such effect was observed (i.e., no emission was observed at 400 nm even when the concentration of PMA was as high as 100 mg/mL). We surmise that a chain-centered foldamer unit becomes structured in chloroform because the good solvent (chloroform) is displaced by the monomer segments of the entropic chain.

dx.doi.org/10.1021/ja2087163 |*J. Am. Chem. Soc.* 2011, 133, 19650–19652

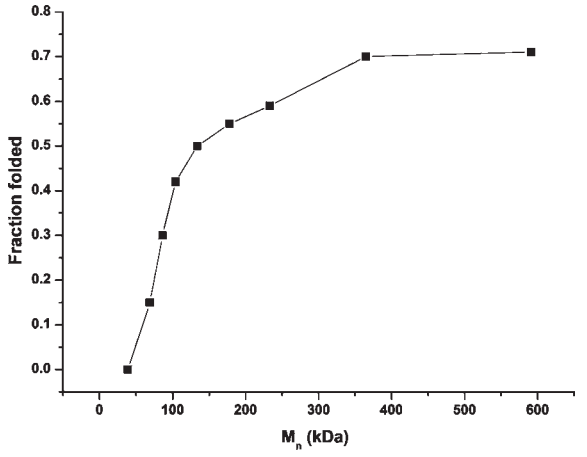


Figure 2. Plots of fraction folded verses polymer molecular weight obtained from normalized fluorescence intensity data (spectra normalized to a constant optical density OD = 0.15). The fraction folded was determined using the equation: $f_u = I_f - I/I_f - I_u$ (f_u is the mole fraction of foldamer in the unfolded state, I is the fluorescent intensity at 350 nm from a foldamer-centered polymer of intermediate molecular weight, and I_u and I_f are the intensity values characteristic of the fully unfolded in chloroform and folded states measured on oligomer **4**).

At the start of this work, it was unclear if entropic chains would enhance or disfavor folding of a chain-centered foldamer. Reasons that might disfavor the folded state are (i) steric clashing of the two entropic chains (i.e., self-avoidance) and (ii) stretching of the entropic chains by a good solvent; both reasons would exert an elongational force on the foldamer's helical structure and could drive it to uncoil. In contrast, entropic chains might promote the folded state by altering the solvent environment in the vicinity of the foldamer. Whether such a perturbation could significantly shift the equilibrium position of the folding transition was not predictable at the outset of this investigation. We have shown that when the entropic chain segment is larger than ca. 50 kDa, structuring of the *m*PE oligomer is enhanced, even in a solvent for which the foldamer is otherwise denatured. This observation supports the notion that high molecular weight entropic chains facilitate conformational ordering by altering a foldamer's local environment. On the basis of these findings, we suggest that solvophobic forces generated by the covalent attachment of an entropic chain may play an important role in intramolecular chaperone-mediated protein folding.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details, synthetic procedures, NMR, GPC, electronic absorption, fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org

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■ ACKNOWLEDGMENT

This work was supported by the Army Research Office MURI (Grant W911NF-0701-0409). The authors thank Matt Kryger, Preston May, and Windy Turchyn for helpful discussions relating to this project.

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