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Sequence-Specific Binding of *m*-Phenylene Ethynylene Foldamers to a Piperazinium Dihydrochloride Salt

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ABSTRACT

Binding properties of a series of isomeric *m*-phenylene ethynylene oligomers containing short amide sequences to a piperazinium dihydrochloride salt were investigated by using circular dichroism (CD) measurements. Although these isomeric oligomers exhibited similar helical conformations, high affinity was observed only for one oligomer. This behavior is presumably controlled by the orientation of amino groups of the amide sequence and the folded conformation of the oligomer.

While the "binding pocket" concept is well understood for globular proteins, it has yet to be widely implemented with synthetic macromolecules. In principle, compact polymer chains of all types can generate three-dimensional cavities and crevices. Lining these surfaces with specific functional groups whose spatial position is determined by chain sequence and conformation offers a potentially powerful and general platform for high-affinity and high-specificity molecular recognition.[1] A major challenge, however, is the need to control the conformation of chain molecules in solution, such that only a small ensemble of cavity shapes and sizes is generated in the collapsed state. Recent advances in the field of foldamers[2] have provided oligomers with a high degree of conformational order. For example, we have previously shown that *m*-phenylene ethynylene oligomers in polar solvents exhibit a unique helical conformation stabilized by solvophobic interactions.[3] This helical conformation produces an internal cavity with nonpolar surfaces capable of binding hydrophobic molecules of appropriate size in polar solvents.[4] Here we report a series of hybrid backbones derived from *m*-phenylene ethynylene oligomers and short, isomeric amide sequences. Their association with a piperazinium dihydrochloride salt demonstrates that functional groups in the helical cavity control binding selectivity.

(1) Szwajkajzer, D.; Carey, J. *Biopolymers* **1997**, *44*, 181−198.

(2) (a) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4012. (b) Zhu, J.; Parra, R. D.; Zeng, H.; Skrypczak-Jankun, E.; Zeng, X. C.; Gong, B. *J. Am. Chem. Soc.* **2000**, *122*, 4219−4220. (c) Berl, V.; Huc, I.; Khoury, R. G.; Krische, M. J.; Lehn, J.-M. *Nature* **2000**, *407*, 720−723. (d) Hirschberg, J. H. K. K.; Brunsveld, L.; Ramzi, A.; Vekemans, J. A. J. M.; Sijbesma, R. P.; Meijer, E. W. *Nature* **2000**, *407*, 167−170.

(3) (a) Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. *Science* **1997**, *277*, 1793−1796. (b) Prince, R. B.; Saven, J. G.; Wolynes, P. G.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 3114−3121. (c) Prince, R. B.; Moore, J. S.; Brunsverd, L.; Meijer, E. W. *Chem. Eur. J.* **2001**, *7*, 4150−4154.

(4) (a) Prince, R. B.; Barnes, S. A.; Moore, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 2758−2762. (b) Tanatani, A.; Mio, M. J.; Moore, J. S. *J. Am. Chem. Soc.* **2001**, *123*, 1792−1793. (c) Inouye, M.; Waki, M.; Abe, H. *J. Am. Chem. Soc.* **2004**, *126*, 2022−2027.

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A short amide sequence inserted into the middle of a *m*-phenylene ethynylene oligomer introduces hydrogen bonding and ion recognition capabilities into the backbone. Assuming that the solvophobically driven helical conformation is preserved in these hybrid backbones, the cavity will display anion and/or cation interaction receptors that are buried in the otherwise nonpolar cavity environment. The specific arrangement of these receptors in the cavity interior will depend on the amide sequence (Figure 1). For example,

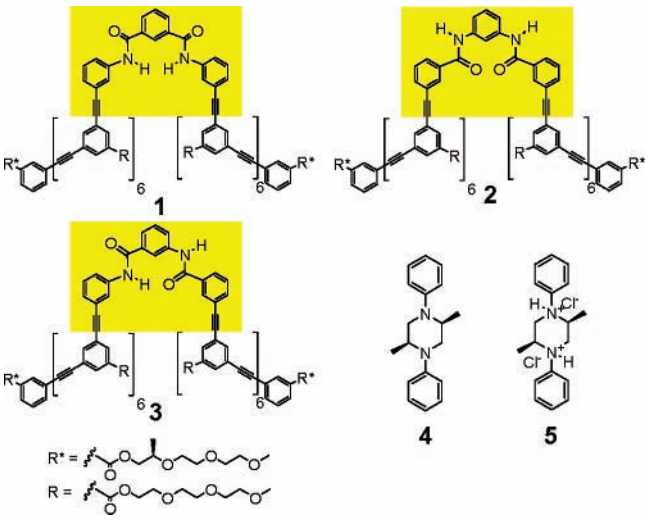

Figure 1. Structures of isomeric oligomers **1**−**3**, rodlike ligand **4**, and piperazinium dihydrochloride salt **5**.

the conformations of sequence **1** are commensurate with the phenylene ethynylene helix orienting amino groups as H-bond donors into the cavity.[5] In contrast, the helical conformations of sequence **2** position carbonyl groups as H-bond acceptors into the cavity. Sequence **3**, derived from aminobenzoic acid, is expected to create a helical cavity that has one H-bond donor and one H-bond acceptor.

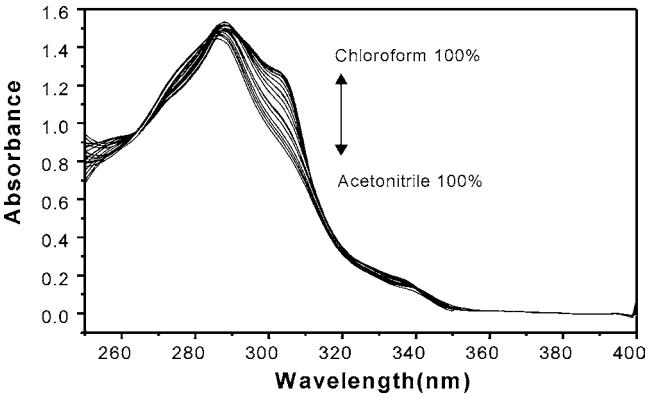

Figure 2. UV absorption spectra of oligomer **1** in mixed solvents. [**1**] = 4.3 μM. Volume % of chloroform/acetonitrile was from 100 to 0.

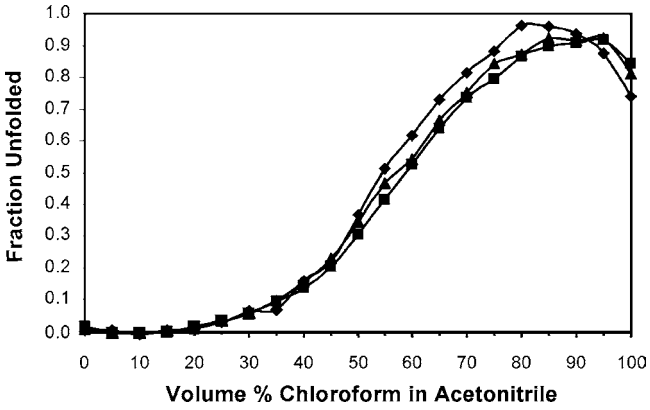

Figure 3. UV titration curves of oligomer **1** (◆), **2** (■), and **3** (▲).

Isomeric oligomers **1**−**3** were synthesized by coupling short amide sequences capped with terminal acetylene end groups to a *m*-phenylene ethynylene heptamer.[6,7] UV−vis spectra of these three oligomers in a series of chloroform/acetonitrile binary solvent compositions are similar to those of the *m*-phenylene ethynylene oligomers previously reported, suggesting a random conformation in chloroform and a helical conformation in acetonitrile (Figure 2).[3]

In addition, solvent denaturation studies indicate that these hybrid oligomers undergo a folding transition at a composition similar to the parent phenylene ethynylene backbone (Figure 3 and Table 1).[8,9]

Table 1. Comparison of the Free Energy of Folding of Denaturation for Oligomers

oligomer	$-\Delta G(CH_3CN)$ (kcal/mol)	$[CHCl_3]_{1/2}$
1	3.9 ± 0.1	52
2	3.5 ± 0.1	56
3	3.5 ± 0.1	54

On the basis of earlier studies,[4b] ligand **4** was expected to have a shape and size complementary to the helical cavity generated from oligomers **1**−**3**. However, in acetonitrile, no induced CD signal was observed when ligand **4** was added to solutions of **1**, **2**, or **3** (Figure 4 A curves labeled i). When HCl was added to the solution, there was an induced signal observed for oligomer **1** (Figure 4A curves labeled ii).[10] In contrast, without ligand **4**, oligomers **1**−**3** do not exhibit any

(5) Coles, S. J.; Frey, J. G.; Gale P. A.; Hursthouse, M. B.; Light, M. E.; Navakhun K.; Thomas, G. L. *Chem. Commun.* **2003**, 568−569.

(6) See Supporting Information.

(7) On the basis of a previous study,[3b] chiral side chains were introduced to both ends to gain information about the conformation of these oligomers. The CD signals induced by these side chains are much weaker than complexation-induced CD seen in Figure 5A.

(8) For detailed description of experimental procedure and data analysis, see Supporting Information.

(9) Apparent reduction in folding at 90−100% chloroform is likely to be caused by amide H-bonding in these nonpolar solvent conditions.

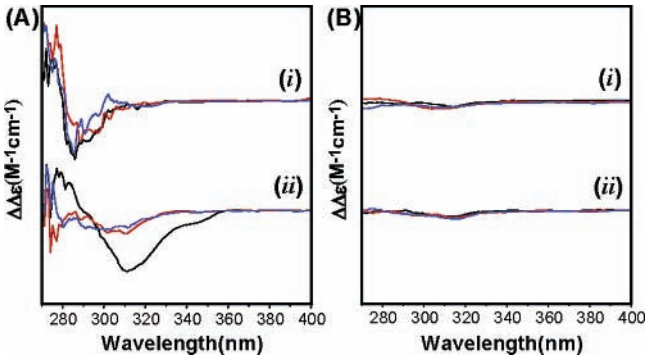

Figure 4. CD spectra of oligomers **1** (black), **2** (red), and **3** (blue) in acetonitrile with ligand **4** (A) and without ligand **4** as a control (B): (i) no HCl, (ii) [HCl] = 840 μM, [oligomer] = 4.2 μM; [ligand **4**] = 840 μM in A.

significant CD signals (Figure 4B) regardless of whether HCl is added or not. These results suggested that oligomer **1** specifically interacts with the protonated form of ligand **4**.

Given these observations, we decided to investigate the binding of oligomers **1**−**3** to the dication ligand **5**. The binding isotherm for each oligomer to ligand **5** was measured in acetonitrile by circular dichroism (CD) spectroscopy.[4b,11] Figure 5 shows a series of CD spectra obtained from the addition of different concentrations of enantiomerically pure ligand **5** to oligomers **1**−**3** in acetonitrile. For sequence **1**, ligand **5** induces a significant Cotton effect at ca. 314 nm corresponding to the backbone's diphenylacetylene chromophore. An isodichroic point is observed at ca. 292 nm. The spectra have band shapes similar to those observed for the 1:1 complex formed between *m*-phenylene ethynylene oligomers and ligand **4** in 40% aqueous acetonitrile.[4b]

As seen in Figure 5A (inset), the CD signal at 314 nm recorded over a range of ligand concentrations showed saturation behavior expected for complex-induced CD signal. The intensity of the CD signal at 314 nm plotted against the concentration of **5** can be fitted to a 1:1 binding isotherm by nonlinear least-squares analysis,[12] yielding an association constant, K_{11}, of 1.0×10^3 M^{-1}. In contrast to sequence **1**, when oligomer **2** or **3** was exposed to ligand **5**, the induced CD signal was much smaller over the entire range of ligand concentrations. No induced Cotton effect or saturation behavior was evident (Figures 5B and 5C).

Although we have not been able to rule out the possibility that this behavior stems from an insignificant helical twist sense bias in the diastereomeric complex between **5** and **2** or **3**, the most plausible explanation is a significantly lower affinity for **5** by these two sequences.

To further support the mechanism of the specific interaction between oligomer **1** and the ligand **5**, clear, short amide

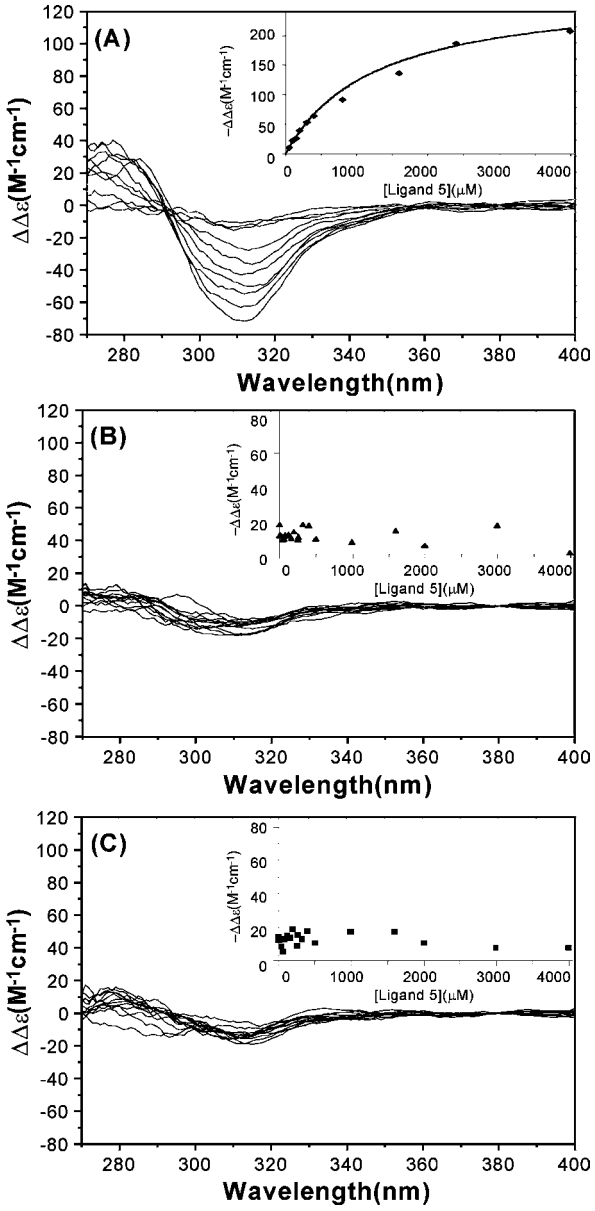

Figure 5. CD spectra of oligomer **1** (A), **2** (B), and **3** (C) as a function of concentration of **5** (range, 4.0−400 μM) in acetonitrile. [Oligomer] = 4.2 μM. The insets show plots of the $\Delta\Delta\epsilon$ values at 314 nm against the concentration of ligand **5**. The solid curve is the best fit to the data using a 1:1 binding model.

sequences capped with terminal trimethylsilylacetylene end groups (**6**−**8**) were also investigated. When ligand **5** was added to acetonitrile solutions of these short amide sequences, only **6**, which has the same amide arrangement as oligomer **1**, showed chemical shift changes of NH protons and aromatic proton.[13] For further investigation, a Job's plot analysis was performed. Keeping the total concentration of **6** and ligand **5** at 1 mM, the concentrations of **6** were varied. When products of chemical shift change by the mole fraction of **6** were plotted against the mole fraction of **6**, there was

(10) CD spectra of each oligomer in the absence of **5** do not have any significant signals. Induced CD spectra were obtained by subtracting the CD spectrum of ligand **5** from that of the complex.

(11) *m*-Phenylene ethynylene oligomers without an amide sequence do not bind **5** in pure acetonitrile.

(12) Program "Dynafit" was used for nonlinear least-squares fitting: Kuzmic, P. *Anal. Biochem.* **1996**, *237*, 260−273.

(13) For the binding study between oligomers **1**−**3** and ligand **5**, ^{1}H NMR was unsuccessful because of line broadening due to aggregation.

a peak observed at 0.67 of the mole fraction of the short amide sequence. This result suggested that the stoichiometry of the complex between **6** and ligand **5** is 2:1, rather than 1:1.[14] In addition, ^{1}H NMR titration data were also obtained by progressive addition of ligand **5** to **6** in acetonitrile. The chemical shift changes of NH protons against the concentration of ligand **5** can be fitted to a 2:1 binding, yielding association constants K_{a1} of 1.0×10^3 M^{-1} and K_{a2} of 1.0×10^5 M^{-1}.[14]

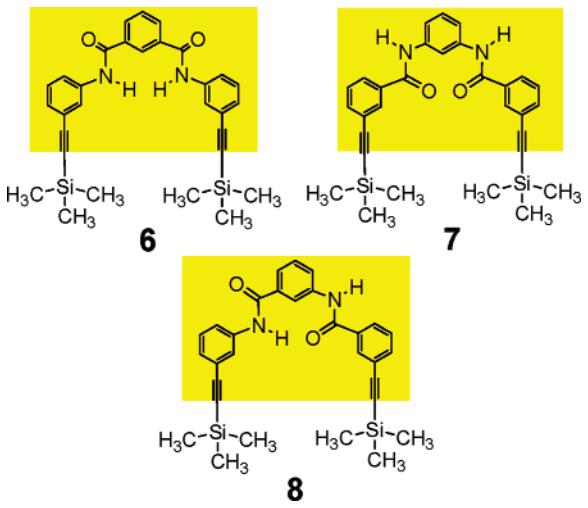

Figure 6. Structures of short amide sequences **6**−**8**.

These experiments support the notion that oligomer **1** specifically interacts with the protonated form of ligand **4**. These results are consistent with our assumption that oligomer **1** adopts a helical conformation in which the amino groups of the amide sequence are oriented toward the interior of the cavity. The presence of H-bond donors in the helical cavity and chloride anion of piperazinium salt **5** provides favorable ion interactions between **1** and **5**. We investigated the role of counterion through the study of dications of **4** with H−F, H−Br, and H−I. No clear evidence of induced CD was observed in these cases. We thus conclude that the chloride anion plays an important role in the sequence-selective binding between **1** and **5**.

In conclusion, we have demonstrated that hybrid backbones derived from *m*-phenylene ethynylene oligomers and short, isomeric amide sequences can be used to introduce ion-selective interactions into the helical cavity. The specific binding properties observed between these oligomers and piperazinium salt **5** are consistent with H-bond donor groups being oriented according to the amide sequence and controlled by the folded conformation of the *m*-phenylene ethynylene oligomer. Further studies to gain more details about the specific interactions involved in binding and the role of chlorine ion are currently underway.

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Supporting Information Available: Binding study of short amide sequences and the ligand **5**, experimental procedures, and synthesis of **1**−**3**, **5**, and **6**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) See Supporting Information.