^{1}H NMR Investigation of Solvent Effects in Aromatic Stacking Interactions

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Abstract: One of the marquis challenges in modern Organic Chemistry concerns the design and synthesis of abiotic compounds that emulate the exquisite complex structures and/or functions of biological macromolecules. Oligomers possessing the propensity to adopt well-defined compact conformations, or *foldamers*, have been attained utilizing hydrogen bonding, torsional restriction, and solvophobic interactions.[1] In this laboratory, aromatic electron donor−acceptor interactions have been exploited in the design of aedamers—foldamers that adopt a novel, pleated secondary structure in aqueous solution. Herein is reported detailed ^{1}H NMR binding studies of aedamer monomers that were carried out in solvents and solvent mixtures covering a broad polarity range. Curve-fitting analysis of the binding data using a model that incorporated the formation of higher order and self-associated complexes yielded a linear free energy relationship between the free energy of complexation and the empirical solvent polarity parameter, $E_T(30)$. From these studies, the association of electron-rich and electron-deficient aedamer monomers was seen to be driven primarily by hydrophobic interactions in polar solvents. However, the magnitude of these interactions is modulated to a significant extent by the geometry of the donor−acceptor complex, which, in turn, is dictated by the electrostatic complementarity between the electron-deficient and electron-rich aromatic faces of the monomers.

Introduction

"Foldamers" are molecules designed to utilize noncovalent interactions to stabilize well-defined conformations in solution.[1] Aedamers are foldamers constructed from electron-rich 1,5-dialkoxynaphthalene and electron-deficient 1,4,5,8-naphthalenetetracarboxylic diimide units connected in an alternating array via amino acid residues (Figure 1).[2] In aqueous solutions, aedamers adopt a compact pleated structure in which the aromatic moieties stack in a face-to-face geometry. Aromatic stacking is also the basis for folding in a series of phenylacetylene oligomers reported by Moore and co-workers.[3] Herein is presented a detailed ^{1}H NMR binding analysis of aedamer monomers **1** and **2** (Figure 2) in organic solvents, methanol/water mixtures, and water. The resulting trends lend insight into the factors controlling aromatic stacking interactions in aqueous solution, the basic interaction controlling aedamer folding.

Electrostatic surface potentials calculated for 1,5-dialkoxynaphthalene and 1,4,5,8-naphthalenetetracarboxylic diimide compounds show significant polarization of the π-systems due to the electron-donating alkoxy and the electron-withdrawing carbonyl substituents attached to the aromatic units, respectively (Figure 3). The aromatic face of the 1,5-dialkoxynaphthalene unit is relatively electron rich, while that of the diimide unit is relatively electron deficient. In solution, interactions between

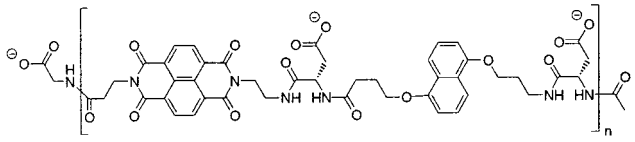

Figure 1. Molecular structure of an aedamer.

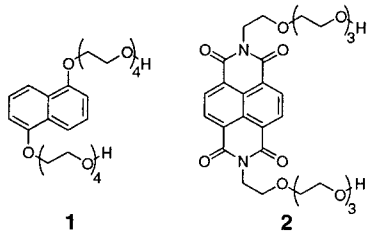

Figure 2. Electron-rich "donor" (**1**) and electron-deficient "acceptor" (**2**) aromatic species used in the ^{1}H NMR binding analyses in solvents and solvent mixtures of varying polarity.

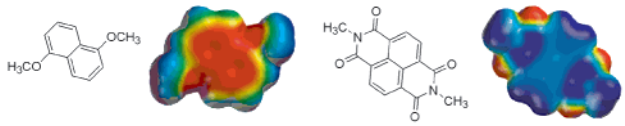

Figure 3. Electrostatic surface potentials calculated for a 1,5-dialkoxynaphthalene (left) and a 1,4,5,8-naphthalenetetracarboxylic diimide aromatic moiety (right) using the AM1 method within Spartan software. The relatively high electron density is shown in red and the shortage of electron density is shown in blue. These calculations and the color scaling used are meant for qualitative comparisons only.

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Table 1. Summary of Binding Data ($T = 298$ K)

	solvent	K_a (M^{-1}) (donor−donor)[a]	K_a (M^{-1}) (acceptor−acceptor)[a]	K_a (M^{-1}) (donor−acceptor)[b]	$-\Delta G°$ (kcal/mol) (donor−acceptor)	$E_T(30)$ (kcal/mol)[c]
1	$CDCl_3$	(1)[d]	(1)[d]	$2 \pm <0.5$[e]	0.4	39.1
2	acetone-d_6	$1 \pm <0.5$	$1 \pm <0.5$	$8 \pm <0.5$	1.2	42.2
3	DMSO-d_6	1 ± 1	$2 \pm <0.5$	$3 \pm <0.5$	0.7	45
4	CD_3CN	1 ± 1	$3 \pm <0.5$	$11 \pm <0.5$	1.4	45.6
5	CD_3OD	$1 \pm <0.5$	$8 \pm <0.5$	$30 \pm <0.5$	2.0	55.5
6	3:1 CD_3OD/D_2O	$1 \pm <0.5$	$15 \pm <0.5$	63 ± 2	2.5	57
7	1:1 CD_3OD/D_2O	$2 \pm <0.5$	28 ± 2	254 ± 41	3.3	58.9
8	1:3 CD_3OD/D_2O	10 ± 2	101 ± 28	952 ± 64	4.1	60.8
9	D_2O	20 ± 4	245 ± 101	2045 ± 63	4.5	63

[a] Self-association constants calculated using HOSTEST dimerization model (Option 2).[8] [b] Association constants calculated using HOSTEST 1:1 and 2:1 binding models (Option 3), including self-association of solutes. [c] $E_T(30)$ values are of nondeuterated solvents. These values should be a good approximation for deuterated solvents based on comparisons of deuterated vs nondeuterated solvents found in ref 10a. The $E_T(30)$ values for CD_3OD/D_2O mixtures were calculated from a linear curve-fit of $E_T(30)$ vs CH_3OHH_2O mixtures.[9] [d] Small effects of concentration of chemical shift were observed, but HOSTEST could not calculate a binding constant from dilution data. [e] The $CDCl_3$ titration data could only be fit to a binding model that excluded self-association of the solutes.

these complementary electrostatic surfaces could provide a significant driving force for face-to-face stacking and thus aedamer folding. However, the flat surfaces of aromatic molecules are traditionally considered hydrophobic.[4] As a result, aedamer conformation could also be the result of desolvation, also known as the hydrophobic effect, in which the hydrophobic surface area of the aedamer structure exposed to polar solvent is minimized upon aromatic stacking.[4,5] Note that Moore et al. have concluded that solvophobic interactions dictate the conformation of the phenylacetylene foldamers.[3]

Trends observed in the association constants between **1** and **2** in solvents of varying polarity can be used to distinguish between these two possibilities. If complexation is due primarily to electrostatic interactions, donor−acceptor interactions will *decrease* with increasing solvent polarity as more polar solvents are assumed better able to disrupt electrostatic attraction. However, if complexation is the result of a hydrophobic effect, association constants will *increase* with *increasing* solvent polarity. Note that biphasic behavior is also a possibility and would indicate that both interactions are important, but contribute differently in different solvents. Mayers et al. observed such a biphasic behavior within metal tris-bipyridine complexes,[6] indicating strong intramolecular aromatic interactions within these complexes in both nonpolar *and* polar solvents.

Results

Synthesis. Monomers **1** and **2** were synthesized utilizing a previously reported procedure (see the Supporting Information).[2c] Donor monomer **1** was found to be completely soluble in a variety of organic solvents ($CHCl_3$, acetone, DMSO, CH_3CN, and CH_3OH) and water. Acceptor monomer **2** was found to be completely soluble in all these solvents except for acetone, CH_3CN, and CH_3OH, which required the addition of up to 10% (v/v) DMSO to give homogeneous solutions.

^{1}H NMR Dilution Studies. Ideally, a 1:1 complex would be the only type of association event occurring in solution throughout the ^{1}H NMR binding studies. However, in practice, additional binding events must be considered with stacking of aromatic species. Of particular importance in this study is contributing equilibria resulting from the self-association of monomers **1** and **2** as well as the formation of donor−acceptor complexes beyond 1:1.

Prior to any binding analyses of monomers **1** and **2**, ^{1}H NMR dilution studies were carried out with both monomers in each of the nine solvents or solvent mixtures to quantify the propensity of these compounds to self-associate (see Table 1). The aromatic signals of monomers **1** and **2** were monitored as a function of concentration and quantitative analysis of the data was accomplished using the HOSTEST program developed by Wilcox and Glagovich.[7] Self-association of monomer **1** was very weak (<2 M^{-1}) in the majority of solvents, although dimerization was more significant in 1:3 CD_3OD/D_2O and D_2O (10 ± 2 and 20 ± 4 M^{-1}, respectively). The dimerization constants for acceptor monomer **2** were significantly larger and increased with increasing polarity of the solvent. In D_2O, self-association of acceptor monomer **2** was found to be 245 ± 101 M^{-1}.

^{1}H NMR Binding Studies. Since acceptor **2** has a greater tendency to self-associate than donor **1**, binding analyses were carried out in which the concentration of **2** was held constant at 0.4 mM in the presence of increasing concentrations of **1**. The Scatchard plots of the binding data were noticeably curved. Deranleau showed that a curved Scatchard plot is consistent with multiple equilibria (i.e. 1:1 + 2:1 complexes).[8] Thus, a model that accounted for multiple equilibria (1:1 and 2:1 binding) as well as self-association of the monomers was used to analyze the ^{1}H NMR binding data using the HOSTEST program. Chemical shift data obtained from aedamer and aedamer-related compounds [2c,9] are in good agreement with the chemical shifts calculated from this binding model.

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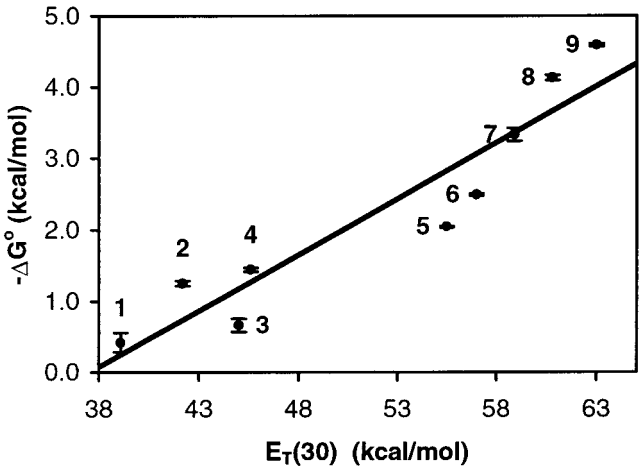

Figure 4. Plot showing the dependence of the free energy of formation, $-\Delta G°$ (kcal/mol), for a 1:1 donor/acceptor complex on the solvent polarity parameter expressed by $E_T(30)$ values (kcal/mol). The numeric labels refer to the entries of Table 1.

From the data in Table 1, it is clear that the stability of the 1:1 donor−acceptor complex increased with increasing solvent polarity. In fact, the association constant increased by over 3 orders of magnitude over the polarity range examined. Furthermore, when the free energies of formation ($-\Delta G°$) (kcal/mol) for the 1:1 donor−acceptor complex in the different solvents were plotted against the empirical solvent polarity parameter $E_T(30)$ (kcal/mol) values of the solvents, a roughly linear correlation was observed (Figure 4).[10] A comparable correlation was also observed for the 2:1 donor−acceptor complex. This linear free energy relationship is similar to that seen in a study by Diederich and co-workers in which an increase in a cyclophane−pyrene association free energy was attributed to the hydrophobic effect.[11]

Interestingly, the protic solvent systems, namely the methanol/water mixtures, display rather linear behavior with a slightly greater slope compared to all of the data taken together. This indicates, perhaps, a somewhat stronger hydrophobic effect in protic solvents. A difference between protic and nonprotic solvent effects has been seen in other systems as well.[11b]

Discussion

The observed increasing association constants with increasing solvent polarity provide compelling evidence that the hydrophobic effect, that is, desolvation of the aromatic faces, provides the dominant driving force for association between **1** and **2**. The trend seen in Figure 4 indicates that this is true over the entire range of solvent polarities examined.

However, a driving force for association based on desolvation alone does not explain all of the data. If desolvation were the only important aspect of the driving force for association in water, then self-association constants for **1** and **2** should be similar to those seen for the 1:1 donor−acceptor complexes due to roughly similar sizes of the hydrophobic aromatic surfaces. Yet the self-association constants of **2** and **1** are 1 and 2 orders of magnitude smaller, respectively, than the association constant for the donor−acceptor complex!

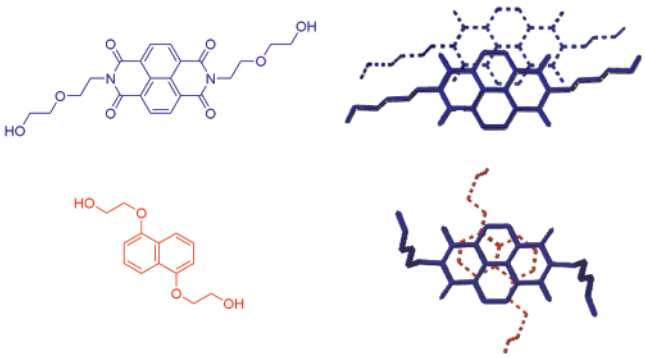

Figure 5. Left: Molecular structures of 1,4,5,8-naphthalenetetracarboxylic diimide and 1,5-dialkoxynaphthalene derivatives used for X-ray crystallographic studies. Right: (top) X-ray crystal structure of a 1,4,5,8-naphthalenetetracarboxylic diimide compound showing the offset π-stacking. (bottom) X-ray crystal structure of the donor−acceptor cocrystal. Hydrogens have been omitted for clarity.

A reasonable explanation for all of the data involves consideration of stacking geometry dictated by electrostatic complementarity in the context of a desolvation driving force. As described by Hunter and Sanders,[12] electron-rich aromatic species do not stack well because the aromatic π clouds repel each other in any stacked orientation except for edge-on geometries. Since **1** does not stack in a manner that allows for a significant reduction in solvent exposed surface area, there can be little in the way of a desolvation driving force in water, entirely consistent with the modest association constant observed. Consistent with this, solid-state structures of 1,5-dialkoxynaphthalene-based macrocycles from the Stoddart group show the aromatic moieties to be completely offset when the linker of the macrocycle is long enough to accommodate such a geometry.[13] However, a shorter linker precludes such a geometry and as a result, the chromophores adopt an edge-on geometry during the crystallization process.[14]

Electron-deficient aromatics prefer to stack in a more parallel fashion, maximizing electrostatic complementarity by placing the electron-rich heteroatoms on the periphery of one molecule directly underneath the electron-deficient aromatic core of the stacking partner.[15] Consistent with this picture, solid-state structures of derivatives of **2** show a slipped face-centered geometry as predicted by the Hunter and Sanders model (Figure 5). This stacking geometry nicely predicts the observed intermediate self-association constant in water, since this geometry produces an intermediate decrease in surface area leading to an intermediate desolvation driving force.

Electrostatic complementarity between the electron-deficient and electron-rich aromatic faces of molecules such as **1** and **2** allows face-centered stacking consistent with the stacking geometry observed for **1** and **2** in the solid state (Figure 5). Face-centered stacking maximizes the decrease in surface area

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in the complex, thereby maximizing the desolvation driving force and thus the association constant for this system.

Conclusions

In conclusion, measurement of association constants between the electron-rich and electron-deficient aromatic derivatives **1** and **2** in a variety of solvents indicates a desolvation driving force, in other words, the classical hydrophobic effect. However, the magnitude of desolvation is modulated significantly by stacking geometry, which, in turn, is dictated by electrostatic complementarity in predictable fashion. These results should contribute to the emerging understanding of stacking interactions of aromatic species.[5] Interestingly, it appears as though the electronic nature of substituents (i.e. electron donating vs electron withdrawing) on the phenyl rings may be important in the phenylacetylene oligomers of Moore and co-workers[3d] as well as the binding of some intercalating moieties to DNA.[15] We are currently pursuing variable-temperature ^{1}H NMR spectroscopy and isothermal titration calorimetry (ITC) as a means of quantifying the enthalpic and entropic components of donor−acceptor complexation in solvents of varying polarity.

Experimental Section

Determination of Self-Association Constants for Monomers 1 and 2. Stock solutions of monomer **1** or **2** were prepared in volumetric flasks (2 mL) using the appropriate solvent or binary solvent mixture containing 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt (TSP) (D_2O and 75% D_2O/CD_3OD) or tetramethylsilane (TMS). The addition of dimethyl sulfoxide-d_6 (DMSO-d_6) was necessary for the complete dissolution of diimide **2** in acetone-d_6, acetonitrile-d_3 (CD_3CN), and methanol-d_4 (CD_3OD) (10, 1, and 1% (v/v), respectively).[16] An aliquot of the stock solution (1 mL) was transferred to a dry vial using a microanalytical syringe and diluted with solvent (1 mL). This solution was then used as the stock in the preparation of a third dilution. This procedure was repeated to give a total of eight concentrations. An aliquot of each concentration (1 mL) was then transferred to a dry NMR sample tube. The NMR spectra were referenced to TSP or TMS and the aromatic signals of **1** or **2** were recorded as a function of concentration (T = 298 K) (see Acceptor and Donor Dilution Data in the Supporting Information). The dilution data were analyzed using the HOSTEST curve-fitting program developed by Dr. Neil Glagovich and Dr. Craig Wilcox at the University of Pittsburgh.[8]

Determination of Binding Constants. Stock solutions of monomer **1** or **2** were prepared in volumetric flasks using the appropriate solvent or binary solvent mixture containing TSP (D_2O and 75% D_2O/CD_3OD) or TMS. The addition of dimethyl sulfoxide-d_6 (DMSO-d_6) (10% (v/v)) was necessary for the complete dissolution of diimide **2** in acetone-d_6.[16] The diimide stock was diluted to 4 mM and a 100 μL aliquot was added to each of seven or eight dry NMR sample tubes using a microanalytical syringe. An aliquot of the donor stock (or a dilution thereof) was added to each tube (not exceeding 900 μL) and solvent added to give a total sample volume of 1 mL. The NMR spectra were referenced to TSP or TMS and the aromatic signal of diimide **2** was recorded as a function of concentration (T = 298 K) (see Donor−Acceptor Titration data in the Supporting Information).

The binding data were analyzed using the HOSTEST curve-fitting program. This program can apply a number of different models to the binding data, including 1:1 and 2:1 binding with and without the consideration of self-association of the binding partners. A constraint was applied to the chemical shifts during the analyses such that the chemical shift of the 2:1 complex was twice that of the 1:1 complex. Calculations of chemical shift carried out as previously described indicate this assumption to be reasonable (ref 2c). A sample HOSTEST output file can be found in the Supporting Information.

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Supporting Information Available: Synthesis and characterization of monomers **1** and **2**, dilution and binding titration data along with a sample HOSTEST output file, and X-ray crystallographic data for the diimide compound of Figure 5 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) According to ref 12a, the addition of DMSO (1 to 10%) as a cosolvent does not affect the complexation properties of a pure solvent like water or DMF. It was assumed that the DMSO content of these samples did not alter the complexation properties of these solvents.