Stability of a Minimalist, Aromatic Cluster in Aqueous Mixtures of Fluoro Alcohol

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Received September 16, 1999
Revised Manuscript Received December 14, 1999

The striking effects that fluoro alcohol cosolvents exert on aqueous peptide conformation have intrigued chemists for three decades.[1,2] Studies of simplified systems have demonstrated heuristic benefits of escaping the complex context of the peptide.[3−6] Due to the ubiquity of the solvent effect, most studies have focused on the peptide backbone. Recently, cold denaturation of hydrophobic helical peptides in ∼1.5 mol % 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)[7,8] and 1,1,1,3,3,3-hexafluoroacetone hydrate (HFA)[9] led to the hypothesis that hydrophobicity also mediates fluoro alcohol cosolvent effects. Investigators struggle to correlate physical data from solvent studies because microscopic effects of media on complex solutes are poorly understood. In particular, the influence of solvation on aromatic interactions in water remains controversial.[10−12] The solvent effect on the conformation of **1** (Chart 1) indicated that water/hydrocarbon interactions are more favorable in pure water than in modest concentrations of fluoro alcohol cosolvent. Counter to intuition, water/hydrocarbon interactions competed with intramolecular hydrocarbon interactions and destabilized this hydrocarbon cluster.

This paper presents evidence that α,α′-*m*-xylylene-*N*,*N*′-bis-2-phenylpyridinium bromide[13] (**1a**) maintained a significant fraction of its conformational distribution in a solvent-dependent intramolecular hydrocarbon cluster. Molecules **1b**,**c** and **2a**,**b** provided the controls necessary to determine the fraction of hydrophobic cluster by ^{1}H NMR spectrometry.

Solution-State Conformation. Conformational searching with the MM2* force field in water continuum dielectric[14] found the cluster conformation (Figure 1) as the global minimum for **1a**, but dynamically symmetric face-to-face, center-to-edge (FFCE) states were found within 0.3 kJ/mol of cluster. The third least energetic conformer had one ring splayed.

Chemical shifts due to anisotropy ($\Delta\delta_{\mathbf{1b-1a}}$)[15] can give much insight into the conformations of molecules;[16] magnetic anisotropy was calculated[17] using the B3LYP/6311++G(2d,2p) level of theory[18] on frozen canonical conformations of FFCE (X-ray), cluster, and splayed. To model ^{1}H $\delta_{\mathbf{1a}}$, atoms were stripped from these conformations and H atoms added to leave xylene and toluene with preserved spatial relationship. To model ^{1}H $\delta_{\mathbf{1b}}$, anisotropies in fragments of xylene and ethane were calculated. Tensors of dynamically symmetrical protons were averaged. The chemical shift tensors for Ha due to anisotropy were calculated by subtracting the calculated chemical shift of Ha in the xylene/toluene pair from that of Ha in the xylene/ethane pair. An identical analysis was performed on Hb. Conformational analysis at this point proceeded with three approximations. (1) The disposition of the phenyl substituents was either FFCE, cluster, or splayed, and this accounted for the entire conformational distribution of **1a**. Monte Carlo searching with MM2* encountered only these three conformations in the 50 lowest-energy structures. (2) $\delta_{\mathbf{1b}}$ corrected only for the bulk solvent effect on $\delta_{\mathbf{1a}}$. Either a solvent-invariant conformation for **1b** or conformationally invariant $\delta_{\mathbf{1b}}$ would allow this. Measurements indicated that a combination of both might have been operative since the solvent sensitivity of $\delta_{\mathrm{Ha}\mathbf{1a}}$ was greater than 3 times the solvent sensitivity of $\delta_{\mathrm{Ha}\mathbf{1b}}$. (3) $\Delta\delta_{\mathbf{1b-1a}}$ arose from the difference in magnetic anisotropy between phenyl and methyl. Modest inductive differences between methyl and phenyl on δ_{Ha} or δ_{Hb} would have to operate over six bonds.

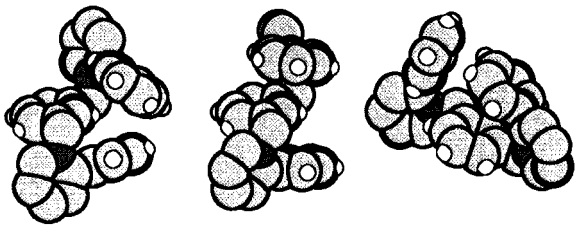
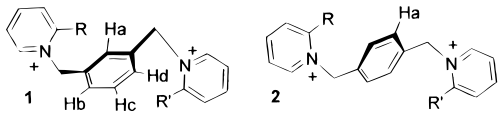


Figure 1. Cluster (left, MM2* global minimum), FFCE (middle, X-ray), and splayed (right, calculated) states of **1a** were all found by conformational searching. For clarity, methylene and pyridinium H atoms were removed. Conformationally diagnostic, Ha and Hb are visible at center and at left in cluster and FFCE. In the mathematical model, FFCE held the phenyl centroids time-average equidistant from Ha and Hb.

Chart 1[a]



[a] **1a** and **2a**, R,R′ = Ph; **1b** and **2b**, R,R′ = Me; **1c**, R = Ph, R′ = Me; **1d**, R = Ph, R′ = CH_2Br.

In D_2O, $\Delta\delta_{\mathrm{Ha}\mathbf{1b-1a}}$ was 0.93 ppm and $\Delta\delta_{\mathrm{Hb}\mathbf{1b-1a}}$ was 0.38 ppm. The difference between these two values increased when measurements were done in 2,2,2-trifluoroethanol-d_3 (TFE_{d3}) and increased further when measurements were done in $HFIP_{d2}$. A similar albeit diminished effect was observed for **1c**. $\delta_{\mathrm{Ha}\mathbf{2b-2a}}$ was nearly the mean of $\delta_{\mathrm{Ha}\mathbf{1b-1a}}$ and $\delta_{\mathrm{Hb}\mathbf{1b-1a}}$. These observations indicated cluster formation because diamagnetic anisotropy on Ha and Hb from the phenyl substituents became less symmetrical and because the phenyl substituents in FFCE cannot interact. Anisotropic $\Delta\delta$ was correlated with the distribution of conformers of **1a** with eqs 1−3:

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10.1021/ja993363b CCC: $19.00
Published on Web 01/12/2000

Table 1. Solvent-Dependent Distribution of Cluster:FFCE[a]

	solvent					
	*i*PrOD	MeOD	DMSO	D_2O	TFE	HFIP
C:F	07:93	29:71	33:67	43:57	58:42	81:19

[a] Errors are approximately 1% in precision and qualitative in accuracy.

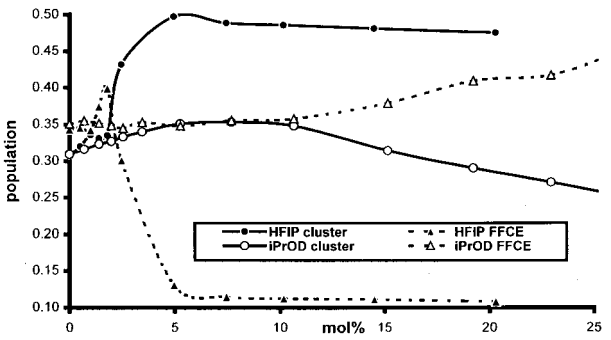

Figure 2. Conformation of **1a** as a function mol % cosolvent. The graph shows divergent, conformational behavior of **1a** with increasing mol % $HFIP_{d2}$ and *i*$PrOD_{d8}$. Mass was exchanged between cluster and FFCE, while splayed (omitted for clarity) varied less with cosolvent. With both cosolvents, splayed and cluster conformations behaved analogously. These are structurally similar; the cluster and the splayed/FFCE hybrid (shown in Figure 1 as splayed) both hide C−H surface from solvent.

$$\Delta\delta_{\mathrm{Ha}\mathbf{1b}-\mathbf{1a}} = 1.96\mathrm{C} + 0.88\mathrm{F} + (-0.04)\mathrm{S} \quad (1)$$

$$\Delta\delta_{\mathrm{Hb}\mathbf{1b}-\mathbf{1a}} = 0.14\mathrm{C} + 0.88\mathrm{F} + (-0.04)\mathrm{S} \quad (2)$$

$$\mathrm{C} + \mathrm{F} + \mathrm{S} = 1 \quad (3)$$

C, F, and S are two-ring populations of cluster, FFCE, and splayed conformation, respectively. In eq 1, the constants 1.96, (1.76/2), and −0.04 were the differences in calculated shift tensors for Ha in **1b** versus **1a** in cluster, FFCE, and splayed conformations, respectively. Treating Hb identically generated eq 2. Mass balance from eq 3 gave three equations with three unknowns. As shown in Table 1, the pure solvents $MeOD_{d4}$, *i*$PrOD_{d8}$, and dimethyl sulfoxide-d_6 ($DMSO_{d6}$) biased **1a** toward FFCE conformations, and TFE_{d3} and $HFIP_{d2}$ favored cluster. This analysis considered the effect of one phenyl on the 1,3-phenylene moiety. Thus, cluster included conformations in which one phenyl ring was in "cluster" and another phenyl was in "cluster", "FFCE" or "splayed". Mixed conformations of FFCE and splayed would also exist. Unfortunately, a better dynamic description of **1a** could not be made from this method because there was not enough information in the ^{1}H NMR spectra. Changes in the tensor predicted that $\Delta\delta_{\mathrm{Hc}\mathbf{1b}-\mathbf{1a}}$ should increase moderately as the fraction of FFCE increased; this was observed in the series in Table 1.

Cosolvent-Dependent Conformation. Figure 2 graphs the tripartite conformational distribution of **1a** with respect to mol % *i*$PrOD_{d8}$ and $HFIP_{d2}$ in water. The graph can be explained by invoking strong solvophobic effects. In this paradigm, Figure 2 shows that both cosolvents decreased the solvent shell/solute interaction early in the titration and stabilized the cluster conformation by default. Hydrocarbon interactions between *i*PrOD and solute stabilized the relatively solvent-exposed C−H groups of FFCE at the expense of the cluster later in the titration. HFIP did not have this mechanism to recover lost interaction between water and **1a** because fluorinated organic molecules interact very weakly with hydrocarbons[19] compared to the interaction between water and hydrocarbon.[20] The HFIP curve in Figure 2 also could have resulted from the disruption of an electrostatically ordered solvent shell. Field effects from dissolved ions on reactivity and conformations of organic solutes have been studied in organic solvent,[21,22] but analogous effects from electric fields on reactivity and conformation of organic material dissolved in water have received less attention. An electrostatic interpretation of the conformational dependence of **1a** was less plausible than the hypothesis offered above because molecular interactions that are electrostatic in nature behave similarly with increasing HFIP and *i*PrOH.[4] Furthermore ion pairing does not seem to affect conformational dependence greatly since the solvent dependence was the same for assays with and without sodium 3-(trimethylsilyl)-propionate (TSP).

Conclusions. Interactions between aromatic rings are weak. However, solvent-sensitive folding motifs that stack aromatic rings have been discovered in various polyphenylene derivatives in mixed organic solvent.[23−26] Here we have unveiled the dependence on aqueous cosolvent manifest in small, conformationally mobile systems that can associate three aromatic rings.

These studies were carried out to test the hypothesis that helix−coil equilibria of hydrophobic peptides shift with fluoro alcohol cosolvent in a fundamentally different manner than hydrophilic peptides. This study, coupled with other studies, suggests that fluoro alcohols synergistically operate on both sides of the hydrophobic helix−coil equilibrium by destabilizing the exposed hydrophobic side chains in the random coil[27] and stabilizing the α-helix entropically.[4] Helical states render solvophobic destabilization less effective because the hydrophobic side chains are less exposed to solvent. Nonfluorinated alcohols disrupt water/solute interactions less effectively than fluoro alcohols; furthermore, the solvent effect is nullified by favorable interactions between exposed hydrocarbon in random conformations and the hydrocarbon portion of the cosolvent.

Acknowledgment. This work was supported by NSF CHE-9702287. Thanks to Dr. Leo Bonilla for LC/MS[2] of **1a**−**2b**. Thanks to Dr. James R. Cheeseman for suggesting the optimum level of theory for chemical shift calculations.

Supporting Information Available: Details of the X-ray structures of **1a** and **2a** and details on the characterization and synthesis of **1a**−**2b** and **1c** (PDF). X-ray crystallographic data for **1a** and **2a**, in CIF format, are also available. This material is available free of charge via the Internet at http://pubs.acs.org.

JA993363B

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