An Indirect Chaotropic Mechanism for the Stabilization of Helix Conformation of Peptides in Aqueous Trifluoroethanol and Hexafluoro-2-propanol

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Abstract: A revised indirect mechanism is proposed for the effect of 2,2,2-trifluoroethanol on peptide conformation (TFE effect) that suggests tighter solvent shells in pure water for helical states than random coil states. The alcoholic cosolvent stabilizes the helical state preferentially by disrupting the solvent shell, which causes unfavorable enthalpic and favorable entropic contributions to the free energy of helix formation. This revised mechanism was adopted because it best explained the solvent-dependent thermodynamic behavior of the coil/helix transition. To define the TFE effect, solvent-dependent physicochemical behaviors of two molecular probes for solvent character were monitored and compared with the solvent dependence of peptide helix formation. The rate of decarboxylation of 6-nitro-3-carboxybenzisoxazole was determined in aqueous mixtures as a function of concentration for DMSO, EtOH, MeOH, ^{i}PrOH, HFIP, and TFE. To relate these rate studies to the cosolvent-dependent thermodynamics of helix formation, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ as a function of concentration for EtOH and TFE were determined and interpreted. The mixed solvent dependence of the UV spectrum of a solvatochromic ketone was also monitored to correlate the behavior of the mixed solvent systems with a microscopic polarity index.

Goodman and co-workers in the early sixties discovered that 2,2,2-trifluoroethanol (TFE) coxed certain medium length peptides to adopt helical conformation (the TFE effect).[1] Although less dramatic than TFE, helix-inducing effects have been observed for other alcohols.[2] These examples of selective stabilization of polypeptide conformations by alcoholic cosolvents bore significance for scientists working in biophysical and biochemical areas. Medium length peptides tend to adopt random coil conformations in water,[3] impeding the study of polypeptide conformation outside the complex context of proteins. Low concentrations of TFE in water enable comparisons of conformational stability to be made between these peptides presumably by magnifying latent conformational biases.[4,5] Though persistent β-sheet[6] conformations have been observed in aqueous TFE, selective TFE-mediated stabilization within the protein context has been observed more often for α-helical conformations, sometimes in regions with native β-sheet preference.[7,8] These observations cast doubt on the hypothesis positing simple magnification of natural conformational tendencies in peptides by aqueous TFE.[9]

Though investigators in the peptide sciences commonly use aqueous mixtures of TFE to study structural propensities, the kinship between TFE-induced states and aqueous states is unclear. Since the TFE effect is so striking, much stands to be learned from mechanistic studies. If the mechanism of the TFE effect were known, back calculation from helix propensities in TFE/water mixtures to native helix propensity in peptide chains might be possible.

Proposed Mechanisms

Two sets of hypotheses for the conformational behavior of peptides in varying concentrations of aqueous TFE have been proposed. Direct mechanisms involve preferential binding of TFE to the helical conformer of peptides. Indirect mechanisms suggest that TFE-mediated changes in the aqueous solvent shell around polypeptides account for the solvent-induced stabilization of helical states.

In a detailed study of five peptide homologues, TFE-dependent conformational behavior was modeled as a two-state system in which TFE preferentially associated with the helical conformer.[5] Similar elements constitute another direct hypothesis for the interaction of aqueous TFE with peptides.[10] The authors reasoned that the fluorocarbon terminus of TFE should interact favorably with hydrophobic side chains, and the hydroxy terminus should preferentially interact with the amide carbonyls. A direct mechanism involving selective stabilization of intramolecular hydrogen bonds by TFE has also appeared in the recent literature.[11] The decisive assay involved monitoring ΔpK_a between salicylic acid and 4-hydroxybenzoic acid as a function

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TFE concentration. The curves describing ΔpK_a and helicity were superimposed.

An hypothesis for the mechanism of the TFE effect involving the perturbation of aqueous solvent properties alone was the first explanation for the helix-inducing effects of low aqueous concentrations of alcohols.[2] In a study of helix-coil transitions of $(L\text{-Orn})_n$ and $(L\text{-Glu})_n$ it was proposed that alcoholic cosolvents decrease the extent to which backbone amide functions are solvated, and thus, selectively destabilize the random coil state relative to closed, internally hydrogen bonded conformations. Interpretations of NMR studies have corroborated these findings. TFE-induced changes in chemical shifts of a disulfide linked peptide consisting of two helical domains failed to show evidence of binding to TFE.[12] 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP)-promoted cold denaturation of peptide helices has been explained by an indirect mechanism that focused on chaotropic, differential solvation of the nonpolar surface.[13]

In another study, strikingly similar TFE-induced phenomena in simple amidic and polyamidic molecules best supported an indirect mechanism for the TFE effect.[14] Increasing aqueous mole percent TFE (X_{TFE}) increased the amount of helical conformation in peptide conjugates covalently bound to a helix-initiating template.[15] This model effectively separated the TFE effect on helix initiation from that of helix propagation, an ambiguity present in all previous studies. The general shape and coincidence of helicity with increasing X_{TFE} for the template-bound peptide conjugates matched the acceleration of *cis*−*trans* peptide bond isomerization of *N*-acetylproline methyl ester as a function of X_{TFE}.[14] Since the proline model lacked the helical motif, selective, direct interaction between cosolvent and helical conformations was ruled out.

Relevance of the proline model as a solvent probe can be found in other studies. From studies of pure solvents and the kinetic barrier to *cis*−*trans* amide bond isomerization, ΔH was found to contribute the most to the stabilization of the cis and trans ground states.[16] Later investigations led the same authors to conclude that hydrogen bonding by the solvent also contributed much to the stability of the ground states.[17]

Inadequacies in Proposed Mechanisms

All the indirect mechanisms for the action of TFE have assumed decreasing stabilization of the random coil state with increasing X_{TFE}. Direct mechanisms have focused on favorable hydrogen bonding or hydrophobic interactions between cosolvent molecules and helix conformations. Both mechanisms predict favorable enthalpic contributions as $X_{cosolvent}$ increases. Since discrete interactions in pure water tie up the helical state in the direct mechanism and liberate the random coil state in the indirect mechanism, both models predict increasingly unfavorable entropic contributions to the free energy of helix formation as $X_{cosolvent}$ increases. Current direct and indirect mechanistic constructions of the TFE effect are inadequate because they predict $d\Delta H/d[TFE] < 0$ and $d\Delta S/d[TFE] < 0$. Opposite trends in both ΔH and ΔS have been observed for MeOH, EtOH, ^{i}PrOH, BuOH,[2] and TFE.[11] Furthermore, $d\Delta S/d[HFIP] > 0$ and $d\Delta H/d[HFIP] > 0$ of helix formation has been implicated by cold denaturation in ala-rich icosomers by low concentrations of HFIP (2−5 mol %).[13] Overwhelming evidence supports entropically controlled α-helix formation as the concentration of alcoholic cosolvents increases. Except for Andersen's explanation of HFIP-induced cold denaturation,[13] the current direct and indirect mechanisms predict exactly the opposite of what is observed for the cosolvent-dependent ΔH and ΔS of helix formation.

Revised Indirect Mechanism

Since the TFE effect has been observed with simple amides and a proline ester, we reasoned that backbone solvation is the main element in a minimalist argument, and we focused this revised indirect mechanism on the solvation of the backbone in two states. The need to include the observed solvent dependencies of ΔH and ΔS of helix formation leads to the premise that the helical state, not the random coil state, is most solvated and thus most perturbed by changes in solvent environment. In this revised mechanism, helix formation is impeded by the entropic cost of assembling the aqueous solvent shell around the helix in pure water.

The random coil was chosen as the most solvated state in previous formulations of indirect mechanisms because the solvent supposedly makes more contacts with the amide carbonyls in the random coil state than in the α-helix state. However, the α-helix state has a greater per residue dipole moment[18] and should interact electrostatically with water better than most other solvents. Random coil states are structurally similar to β-sheet conformations on average, and peptides that favor β-sheet conformation tend to precipitate from solution. Furthermore, α-helices present curved surfaces to water solvent and formation of helices reduces solvent accessible surface. Thus, the coil to helix transition should multiply and enhance water/water interactions in the solvent shells around polypeptides. Since the α-helix should promote water/water interactions, it should be more sensitive to changes in aqueous solvent environment. Focus of this mechanism on the cohesive nature of water is congruent with the growing recognition that the properties of water determine the chemical behavior of biological molecules.[19]

The diagram allows comparison of the former indirect mechanism with our revised indirect mechanism on a conceptual level. For indirect mechanisms one assumes that the enthalpic and entropic effects of solvation vastly outweigh thermodynamic contributions from conformational changes in the peptide backbone. Since fluoro alcohols at low concentration have such a striking effect on peptide conformation, this assumption seems sound. The **CS** and **HS** states are hypothetical coil and helix states that are preferentially solvated by water whereas the **C** and **H** states are comparatively unsolvated. Only three out of the four states presented in the diagram need to be considered for each mechanism. Previous indirect hypotheses involved the pure aqueous solvent system **CS** ⇌ **H** transforming to **C** ⇌ **H** upon addition of alcoholic cosolvent, which produces favorable enthalpic and unfavorable entropic contributions to helix formation as $X_{cosolvent}$ increases. The revised indirect mechanism proposed here involves **C** ⇌ **HS** progressing toward **C** ⇌ **H** as $X_{cosolvent}$ increases, which causes unfavorable enthalpic and

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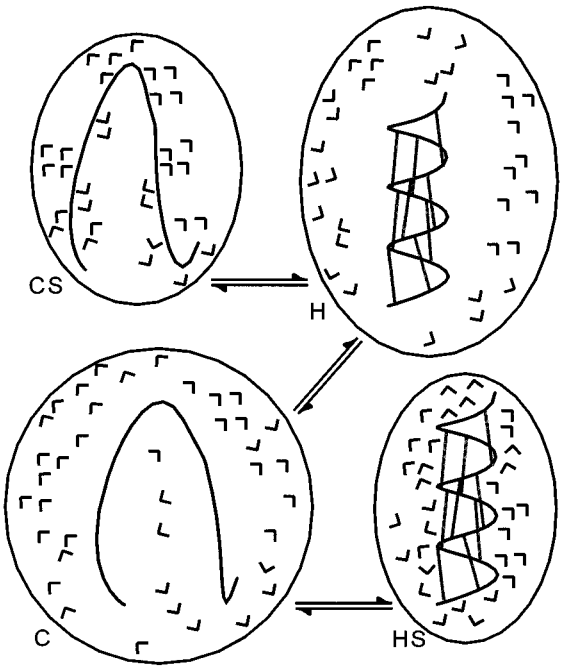
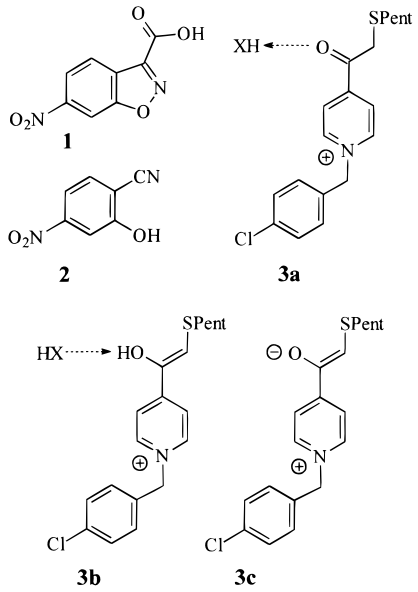
favorable entropic contributions to helix formation as the alcoholic cosolvent is added.



Herein two well-characterized molecular probes for solvent properties (**1** and **3**) were employed and the observables were titrated with TFE in water. When possible, MeOH, EtOH, DMSO, and HFIP were also included in the titrations to compare the overall magnitude of the solvent effect. These studies approached mechanistic consensus by two routes.

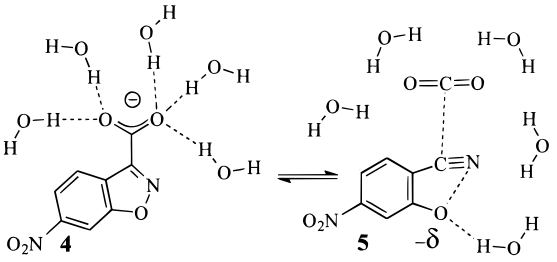


Chemical Models for the Helix/Coil Transition

One assay was designed to probe the hydrogen bonding character of the media as a function of cosolvent concentration. For this purpose the rate of decarboxylation of one of Kemp's other acids,[20−23] 6-nitro-3-carboxybenzisoxazole (**1**), to 2-hydroxy-4-nitrobenzonitrile (**2**) was used in the concentration range of 0−60 mol % cosolvent. Decarboxylation of **1** has been shown to proceed through a late transition state without intermediates[22] on the reaction pathway and to depend on hydrogen bonding with rates spanning approximately 8 orders of magnitude.[24] Recently, the solvent-dependent rate of the decarboxylation of **1** has been characterized and touted as a molecular probe for biologically relevant solvents.[25]

Two factors mainly determine the rate of decarboxylation of **1** with tetramethylguanidinium as the counterion. The reaction runs fastest in polar aprotic solvents.[24] As the polarity of the solvent system decreases, greater contact between ion pairs in solution decreases the rate of the reaction. As the hydrogen bond acidity of polar solvents increases, the rate of decarboxylation of **1** decreases. Changes in the rate of the reaction in this study should be ascribable to changes in the hydrogen bond donor ability of the solvent for a few reasons. Reaction rate deceleration due to ion contact factors less than the deceleration due to hydrogen bond acidity. Also the properties of the solvent mixtures should reflect small perturbations in water because this work focused on aqueous mixtures of polar solvents in which the major component was water. Furthermore, these results were obtained with potassium, a counterion incapable of forming hydrogen bonds as the contact ion pair. We felt that the applicability of the model outweighed gains in organic solubility conferred by the tetramethylguanidinium counterion.

Increasing $X_{\text{cosolvent}}$ perturbs the ground state **4** more than transition state **5** because **4** is more solvated; **4** is analogous to the **HS** state. Structures **C** and **HS**, when compared to **4** and **5**, show that the TFE effect on ΔH and ΔS of the coil/helix transition should be opposite to the TFE effect on the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the decarboxylation of **1**. As **4** progresses toward **5**, the extent of solvation decreases due to localized cross-conjugated negative charge in **4** diffusing to conjugated negative charge spread over the aromatic transition state, **5**. The common characteristic of both systems is differential solvation.



The helix/coil transition is a complex system consisting of many conformations with hypothetically many local minima complicated by the presence of side chains. Fortunately, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of the decarboxylation of **1** should behave predictably in the mostly aqueous systems studied here. If increasing $X_{\text{cosolvent}}$ breaks ground-state solvation, $\Delta H^{\ddagger}$ should decrease because the energy of the ground state with respect to the transition state decreases (ΔH of the ground state increases more than ΔH of the transition state). Likewise $\Delta S^{\ddagger}$ should also become less positive because the ground state will possess more entropy with respect to the transition state with increasing $X_{\text{cosolvent}}$ and decreasing solvation. For simplicity, entropic and enthalpic changes in the transition state are assumed to be small

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compared with solvent-dependent thermodynamic perturbations in the ground state. The solvent effect should produce trends in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the decarboxylation of **1** with X_{TFE} that oppose each other energetically. When interactions between water and the carboxylate are broken, lowering $\Delta H^{\ddagger}$, there should be corresponding decreases in $\Delta S^{\ddagger}$.

Aqueous solutions of another molecular probe of solvation were titrated with cosolvents. In this case, λ_{max} of 4-[(1′-pentylthio)acetyl](4′-chlorobenzyl)pyridinium chloride (**3**) reports microscopic solvent polarity, not dielectric constant. The dielectric constant as a function of X_{TFE} is known[26] and the response of λ_{max} of neither **3a** nor **3c** correlate with it. The cationic and zwitterionic forms, **3a** and **3c**, produce shifts in λ_{max} as a function of solvent that correlate with the solvent polarity indices of Snyder[27] and Reichardt,[28] respectively. λ_{max} has been shown to depend on the equilibrium **3a** ⇌ **3b**. In studies with pure solvents, λ_{max} as a function of solvent does not correlate at all with the rate for the decarboxylation of **1**. Therefore hydrogen bond donation from the solvent should be a minor factor for λ_{max}. Most striking is the difference between *k* of decarboxylation of **1** and λ_{max} of **3** in response to the pure solvents MeOH and DMSO. These two solvents are found at opposite extremes of the rate of decarboxylation of **1**, but they coincide on the polarity indices generated by both **3a** and **3c**. Both probes of solvation should respond similarly to the disruption of the aqueous solvent shell.

Results and Discussion

TFE-induced changes in the rate of decarboxylation of **1** and on the λ_{max} of **3a** and **3c** were not dramatic when compared with the behavior of the other solvents tested; see Figure 1a−c. In retrospect, a response in the opposite direction produced by the other solvents might have been expected. A negative response (i.e., *k* and λ_{max} lower than pure water) should have resulted if aqueous TFE associated to the basic sites in **1**, **3a**, or **3c** through hydrogen bonding. A few conclusions can be drawn from these data. TFE/water mixtures conserved the properties of aqueous solvent according to these probes for solvent character. Similarities in microscopic polarity between TFE and water have been observed previously.[29,30] Furthermore, TFE and HFIP showed poor hydrogen bond acceptor (electron donor) properties. Increased solvent polarity and hydrogen bond donation from the solvent stabilizes the ketone **3a**, and decreases λ_{max}, whereas decreased solvent polarity and electron donor character in the solvent stabilize the enol **3b**.[31] Similar trends were observed for both **3a** and **3c**, which laid aside all claims of direct mechanisms with TFE donating a hydrogen bond to amide carbonyl functions in peptide chains at cosolvent concentrations relevant to the TFE effect. Enolate **3c** should be a better electron donor than the amide carbonyl oxygen atom. At a glance, changes in the solvent character brought about by increasing X_{TFE} appeared inconsequential for both solvent probes. However, closer examination of the shapes of these curves reveals that the behavior of the solvent probes changed in a defined, nonlinear fashion with X_{TFE}. In Figure 2, as X_{TFE} increased in the presence of K_2CO_3 and **1** at 30 °C, an initial

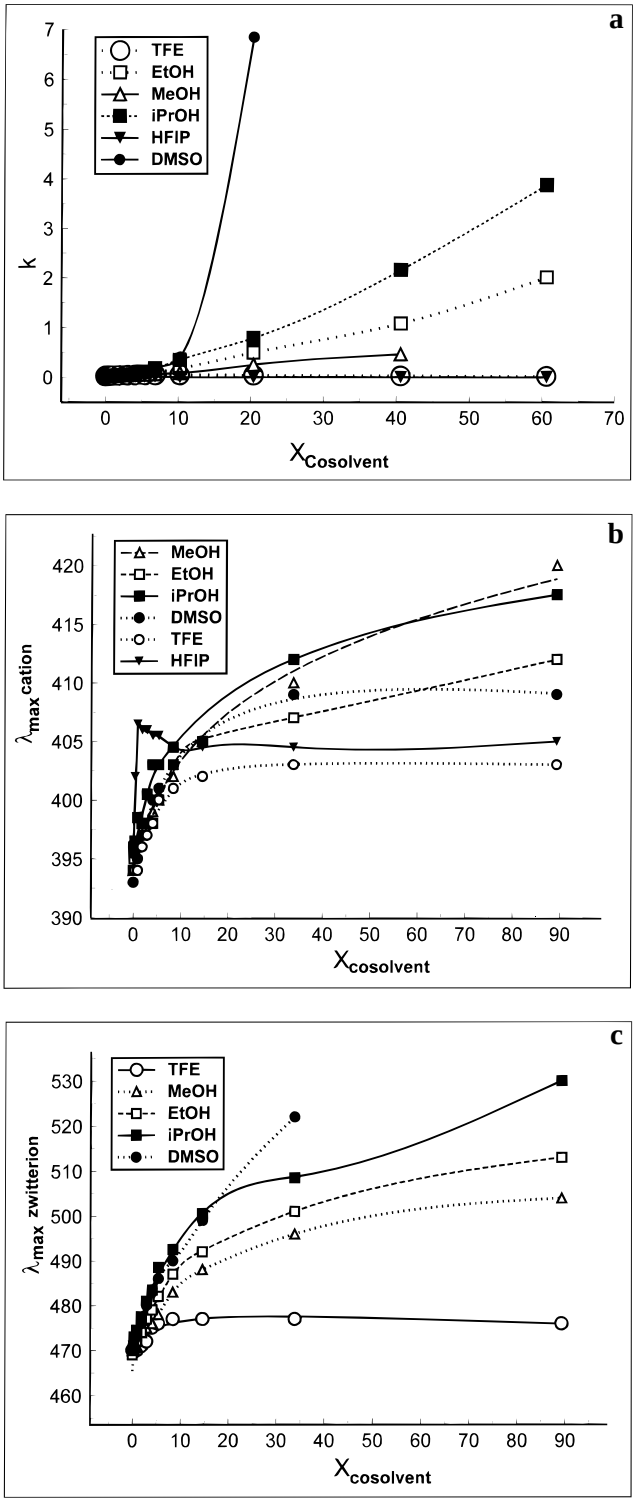

Figure 1. Nonlinear changes in *k* (s^{-1}) of decarboxylation of **1** in the low concentration region of X_{TFE} and X_{HFIP}.

nonlinear increase in the rate of decarboxylation ensued, followed by a decrease in the rate at X_{TFE} > 20 mol %. Rate depression at X_{TFE} > 20 mol % was probably a function of hydrogen bond donation to the carboxylate, which stabilized the starting material relative to the transition state. This conclusion was corroborated by rate depression at lower X_{HFIP} than X_{TFE}. Complete proton transfer is not possible from the fluoro alcohols to any of these molecular probes for solvation because the pK_a of **1**, **3a**, **3b**, TFE, and HFIP are 1.6,[22] 7.9, [31] 7.4, [31] 12.4,[10] and 9.3,[10] respectively.

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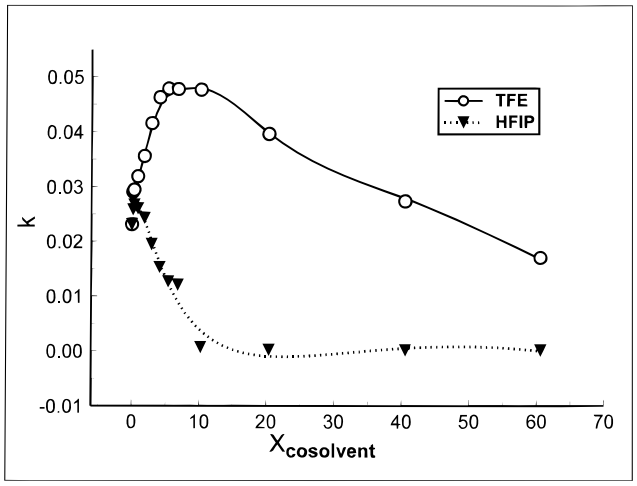

Figure 2. Nonlinear rise in λ_{max} (nm) for **3a** and **3c** in the low concentration region of X_{TFE} and X_{HFIP}. Studies with **3c** and HFIP were omitted because partial protonation of **3c** was observed in the UV spectrum.

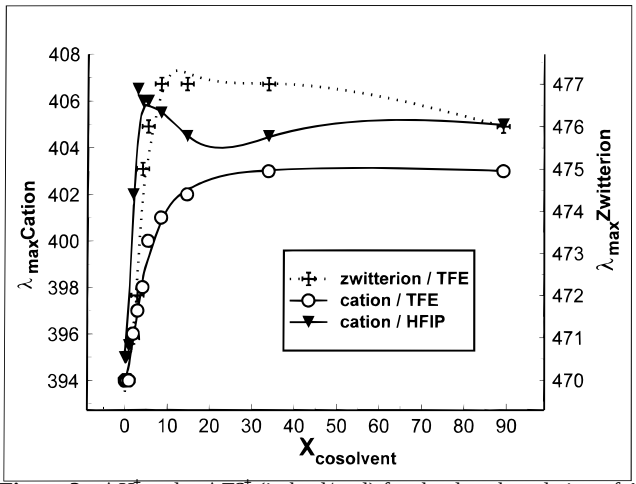

Figure 3. $\Delta H^{\ddagger}$ and $-\Delta TS^{\ddagger}$ (in kcal/mol) for the decarboxylation of **1** plotted as a function of X_{TFE}.

Analogous changes in λ_{max} in the same ranges of X_{TFE} observed for k of **1** were observed for both **3a** and **3c**, except λ_{max} did not decrease with increasing X_{TFE} for **3a**; these results are graphically displayed in Figure 3. λ_{max} holds steady with increasing X_{TFE} past 15 mol % due to the lack of electron donor character of the fluoro alcohols and the similarity between TFE and water in terms of microscopic polarity. The inability of TFE to act as a hydrogen bond acceptor has been noted previously.[32] Since the hydrogen bond acidity of both fluoro alcohol cosolvents is greater than that of water, the changes in signal observed for both probes at low concentrations of cosolvent were anomalous. Observables for both solvent probes deviated in the wrong direction for solvents with increased hydrogen bond acidity and decreased electron pair donor ability compared to water. These changes suggested cosolvent-dependent restructuring of the aqueous solvent shell at low concentration.

Analogous nonstoichiometric effects from 5 to 20 mol % TFE and 2−5 mol % HFIP have been observed for the conformational behavior of peptides as a function of $X_{cosolvent}$ as discussed above. The TFE effect on peptide helicity and the observables of these probes for solvent character coincided on the $X_{cosolvent}$ axis. In particular, the sharp nonstoichiometric rise between 5 and 15 mol % TFE has been observed for helicity in peptide chains.[14] Above 20 mol % TFE, contributions to helicity from X_{TFE} diminish. The coincidence of solvent probe response and α-helix stabilization on the X_{TFE} axis indicates that the TFE effect at X_{TFE} < 20% is a result of TFE-induced changes in water structure instead of preferential interaction between TFE and polypeptide.

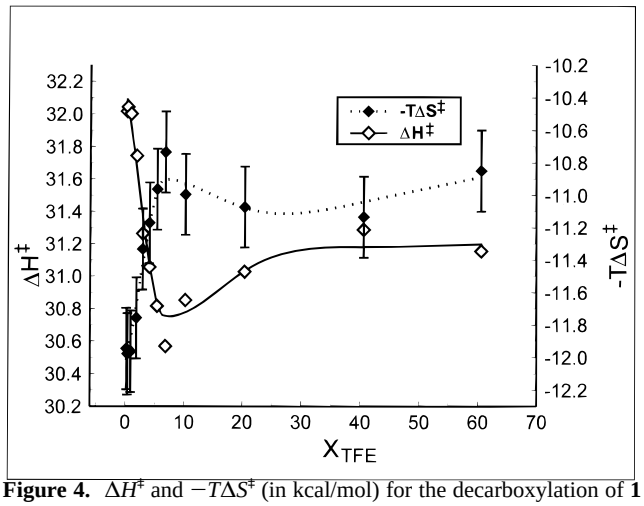

Figure 4. $\Delta H^{\ddagger}$ and $-T\Delta S^{\ddagger}$ (in kcal/mol) for the decarboxylation of **1** plotted as a function of X_{EtOH}.

The HFIP dependence of the λ_{max} of **3a** corresponded to the cosolvent concentration at which cold denaturation of peptide helices has been observed.[13] Furthermore, the TFE dependence of λ_{max} of **3c** approximated the HFIP dependence of the λ_{max} of **3a**. Increased charge on the carbonyl oxygen of **3c** versus **3a** tightens the water structure[33,34] in the solvent shell of the chromophore and thereby increases the solvent effect. These facts implicate the destruction of liquid water structure in the solvent shell as the primary factor in the TFE effect at low concentration (<20 mol %) and argue for generality in the alcoholic cosolvent effect on peptide conformation.

Applicability of the TFE-dependent rate of decarboxylation of **1** as a thermodynamic model for TFE-dependent helix/coil transitions in peptides was further questioned by studying the TFE dependence of the activation parameters for decarboxylation. Rates of decarboxylation of **1** were determined at five temperatures for X_{TFE} values between 0 and 55 mol %. Although the general trends for both $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ were similar, there were small relative changes between the two values that were apparent when the two graphs were compared; see Figure 4. Furthermore, the contributions to $\Delta G^{\ddagger}$ of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ opposed each other. Hence, the net change in $\Delta G^{\ddagger}$ was small.

Helix stabilization has been observed for TFE and other alcohols. However, stabilization by alkanol cosolvents versus TFE pales comparatively. Ethanol should enact similar changes in the activation parameters *albeit* at higher X_{EtOH}. Figure 5 shows this to be true. The activation parameters for the EtOH titration reached a minimum at ∼40 mol % EtOH instead of ∼13 mol % with TFE. Here again, entropic contributions of **4** to $\Delta G^{\ddagger}$ opposed enthalpic contributions of **4** to $\Delta G^{\ddagger}$ due to decreased solvation of **4** as a function of X_{EtOH}. Carrying this study past 55 mol % resulted in precipitation of **4**. Analogous curve shapes as a function of X_{EtOH} have been discovered by Winstein for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for S_N1 reactions.[35]

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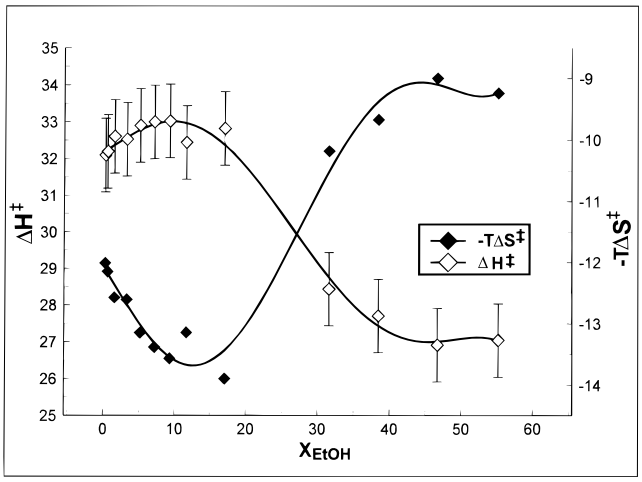

Figure 5. The graph of the observables with respect to $X_{cosolvent}$ for (a) the rate constant of decarboxylation, k (s^{-1}) of **1**. (b) λ_{max} (nm) of cation **3a**. (c) λ_{max} (nm) of the zwitterion of **3c**.

Conclusion

The solvent probes studied offer the following evidence for the revised indirect mechanism for the TFE effect on helix propensity in oligopeptides. (1) Aqueous TFE at 5−15 mol % produces similar results for a variety of physicochemical phenomena, including helicity in polypeptides.[14] This work correlated the TFE effect to two different chemical phenomena that do not involve polyamides or amidic structure; the generality of the TFE effect on various physicochemical phenomena speaks against a mechanism involving selective direct interaction between cosolvent and peptide conformers or solvent probe. (2) The rate of decarboxylation of **1** in aqueous TFE at 5−15 mol % is larger than the rate at X_{TFE} > 30 mol %. This suggests two mechanisms of interaction as X_{TFE} increases. The direct mechanism should be the one that occurs at high X_{TFE} and appears to involve hydrogen bond donation to **1**. Hydrogen bond donation to **3c** at increased X_{TFE} was also observed. (3) Trends observed for the solvatochromic probe also suggest no direct interaction by hydrogen bond donation. These results were surprising because TFE is more hydrogen bond acidic than water.[36] If aqueous TFE interacted directly with **3**, the ketone form, **3a**, should have been stabilized versus the enol **3b**. Furthermore, at X_{TFE} < 20%, there is no evidence for direct interaction with the enolate **3c**. This result was significant since the zwitterionic enolate **3c** should hydrogen bond more strongly than the peptide amide carbonyl. (4) Trends in both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the decarboxylation of **1** as a function of X_{TFE} indicate TFE-mediated changes in solvent structure. Perturbations in $\Delta H^{\ddagger}$ detected breakage of hydrogen bonds from solvent to carboxylate with increasing X_{TFE} and $\Delta S^{\ddagger}$ detected the concomitant unfettering of solute structure. (5) Attenuated effects on $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were noted for aqueous EtOH at low X_{EtOH}. Later in the titration EtOH eventually broke the aqueous solvation of the carboxylate. Again, analogy to the helix/coil transition is evident because helix induction with EtOH occurs later in the titration than with TFE. (6) The similarity between the dependence of λ_{max} of **3a** on X_{HFIP} and the dependence of λ_{max} of **3c** on X_{TFE} also points toward a general solvent shell perturbation by the cosolvent. The increased charge of the enolate **3c** tightens the water solvent shell and thereby increases the cosolvent effect of TFE.

If the helical state is identified as more water solvated than the random coil state and therefore the most perturbed by addition of cosolvent, trends in ΔH and ΔS for helix formation as a function of X_{TFE} make sense. Perhaps due to the striking nonstoichiometric establishment of helical structure with increments in X_{TFE}, a few investigators have framed the TFE effect as an associative mechanism. The behavior of molecular probes for solvation studied herein approximates the TFE-dependent conformational behavior of peptides because the observables in all these cases are a function of the structural integrity of water. Solvent dependence in λ_{max} of **3** should be a function of microscopic polarity and the decarboxylation of **1** has been shown to be dependent on hydrogen bonding parameters of the solvent. Both of these observables should vary in the same direction with perturbations in liquid water structure.

This indirect model for peptide solvation as a function of TFE offers an explanation for peptides that show β-sheet conformational preferences in water and switch to α-helical conformations upon addition of TFE. In shorter peptides, β-sheet conformations outside of the protein context that are not extensively stabilized by packing interactions are loosely ordered and random coil-like.[37] Addition of TFE entropically drives the peptide toward helicity by loosening the solvent shell around the helical conformations.

For purposes of back calculation of helix propensity in TFE to native helix propensity, solvation of the random coil states cannot be ignored even though the helix is perturbed more by the cosolvent. A few pertinent questions come to mind about the nature of solvent accessibility to the backbone donor sites in the random coil state. Are all the basic sites equally accessible for all random coils and is this independent of peptide composition? If so, peptide chemists can still discuss native helical preference as a function of TFE and correlate this preference to the purely aqueous state. If random coil states do not randomize solvent access to the hydrogen bond acceptor sites on the peptide backbone in a manner independent of peptide composition, then helix propensity initiated by TFE is a function of complex solvent contributions to the random coil state. Recent calculations indicate the composition-dependent solvation of the random coil factors to some extent because solvent access to amide carbonyls in the random coil state depends on peptide composition.[38] Regardless, native helix propensity from conformational studies in TFE could be assigned between families of peptides related by primary structure.

Our model for helix stabilization by cosolvents should be viewed as a first approximation to the TFE effect. There are complexities brought about by the complex nature of polypeptides that are beyond the scope of this model. For example, helicity in peptides bearing lysine residues has shown strong context dependence along with a tendency to melt.[39] Furthermore, our studies say nothing about changes in side chain entropy upon helix formation as a function of X_{TFE}. Side chain entropy has recently attracted the attention of investigators as a primary factor in helix stability.[40]

Experimental Section

Anhydrous solvents were prepared by refluxing reagent grade commercial material over a suitable drying agent for at least 0.5 h followed by distillation under a nitrogen atmosphere.

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^{1}H and ^{13}C NMR were recorded on a Varian 200 Gemini and a Varian VXR 300 spectrometer. UV−vis spectra were recorded with a Shimadzu UV-3101PC UV−vis−NIR scanning spectrometer with a temperature-controlled cell holder. Reaction mixtures for measurements with probe **1** where kept at a constant temperature in a Fisher Scientific 7305 thermostated circulation bath.

4-[(1′-Pentylthio)acetyl](4′-chlorobenzyl)pyridinium chloride (3). These compounds were synthesized according to the method used by Holler et al.[31] For 4-[(1′-pentylthio)acetyl](4′-chlorobenzyl)pyridinium chloride (**3**), the purification was changed to the following: After removal of the remaining thiol by distillation, the orange oil was diluted with 4 mL of anhydrous pyridine while heating at 35 °C. Ethyl acetate was added until material precipitated from solution. The mixture stood at 0 °C for 5 h. The precipitate was filtered under Schlenk conditions and washed with a 10% anhydrous pyridine solution in ethyl acetate (100 mL) and with ethyl acetate until no pyridine was present. After the mixture was dried under vacuum, compound **3** was obtained in 50% yield. The material was spectroscopically identical with the material reported in the literature.

Measurements of Solvent Character with Probe (3). For measurements with the cationic form of **3**, 50 μL of a 1.38 mg/mL 4-[(1′-pentylthio)acetyl](4′-chlorobenzyl)pyridinium chloride solution in water was added to 2.95 mL of a water/organic solvent mixture. The absorption spectrum was then measured in the 350−550 nm range. The wavelength of the absorption maximum (λ_{max}) was recorded. For observations of the zwitterionic form of **3**, solutions with 0.1 mg/mL of K_2CO_3 in both water and organic solvent were used to make up a 2.95 mL water/organic solvent mixture. The total concentration of the cationic probe was the same as for the zwitterionic probe (**3**). All spectra were taken at 30 °C.

6-Nitro-3-carboxybenzisoxazole (1). A flask was charged with 1 g (4.5 mmol) of methyl 6-nitrobenzisoxazole-3-carboxylate and 20 mL of 70% sulfuric acid; the resulting solution was warmed to 80 °C for 4 h. Slowly water was added to the solution at 25 °C until the solution was over saturated. This solution was left standing for 8 h. The resulting precipitate was washed with water and dried under high vacuum. The dried product **1** was obtained as off-white crystals in 67% yield.[41]

Measurements of the Decarboxylation Rate of 1. An aqueous solution of **1** (150 μL of 1 mg/mL) was added to 2.95 mL of water/organic solvent mixture. The absorbance was measured at an appropriate sampling rate at 396 nm with a slit width of 3 nm, until a significant amount of the total expected maximum absorbance had developed. During the measurements, the solution was kept at a constant temperature in a sealed cuvette. All measurements were taken at 30 °C unless stated otherwise. For the determination of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of the decarboxylation as a function of X_{TFE}, the rate was measured at 3, 15, 30, 40, and 50 °C. Wynne-Jones and Eyring analysis[42] was applied to the rate vs temperature data to obtain the activation parameters. Error in the plots of the Eyring analysis was estimated by assuming that the third through the eighth points in $\Delta H^{\ddagger}$ for EtOH were the same value; the standard deviation in this value was computed and used for the error for $T\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ for EtOH and TFE.

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