Promotion of Helix Formation in Peptides Dissolved in Alcohol and Water–Alcohol Mixtures

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The complicated influence of alcohol, trifluoroethanol (TFE) in particular, on the conformational thermodynamics of peptides and proteins is not well understood. In some instances it acts to promote the formation of local structure, as in the formation of helices.[1-9] In other cases, the role of alcohol in mixtures seems to be one which disrupts the formation of (tertiary) structure and leads to the partial or total unfolding of proteins.[10,11] In this communication, we explore the origins of helix promotion by mixed alcohol–water solvents. Conformational free energy surfaces (potentials of mean force) are computed for a blocked alanine tripeptide (Ac-(Ala)$_3$-NHMe) capable of forming a single hydrogen-bonded helical turn.[12] Our theoretical findings follow the experimentally observed trend showing that mixed solvents promote helix over pure water. In addition, neat methanol is observed to enhance the helical character of the peptide, while neat TFE shows helix content similar to that in water. Qualitative differences are predicted for helix/coil thermodynamics in solutions of neat alcohol versus water. Enhanced alcohol–peptide interactions are seen for extended conformations of the peptide in TFE mixtures, suggesting spatial and orientational ordering of the alcohol around the extended peptide.

Five series of molecular dynamics simulations were performed for the tripeptide solvated, under conditions of constant volume and temperature (300 K), in solvent composed of (i) TIP3P water,[13] (ii) a flexible version of OPLS methanol,[14] (iii) 30:70 mol % mixtures of methanol–water, (iv) a recent model of TFE,[15] or (v) a 30:70 mol % mixture of TFE–water. The potential of mean force curves (PMFs) from these calculations (Figure 1) illustrate the dependence of the Helmholtz free energy on the folding coordinate defined by the R_{O1-H5} distance. Examination of this figure reveals that the gross behavior of helix-promoting ability (HPA) for a particular solvent composition follows the order TFE–water ≈ methanol > methanol–water > water ≈ TFE. A quantitative measure of helix-promoting ability may be defined by the ratio of the equilibrium constant for the helical conformation in a given solvent relative to water, HPA = $K^{helix}_{solvent}/K^{helix}_{water}$, where $K^{helix}_{solvent}$ is the equilibrium constant for formation of helix.[12] This quantity is displayed in the last column of Table I and echoes the differences seen in the free energy functions.

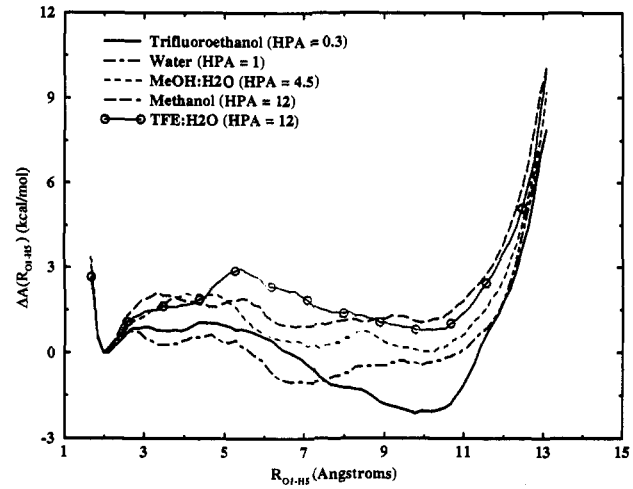

Figure 1. Potential of mean forces curves, $\Delta A(R_{O1-H5})$, for helix folding in aqueous, alcoholic, and mixed alcohol–water solutions. The free energy is plotted versus the helix folding coordinate, R_{O1-H5} in Å, for solutions of Ac-(Ala)$_3$-NHMe in water, methanol, 2,2,2-trifluoroethanol (TFE), and 30:70 (mol:mol) mixtures of methanol–water and TFE–water. All curves are adjusted to make their (arbitrary) zero of free energy correspond to the α-helical minimum. The cube edge length and number of solvent molecules for the neat liquids (before insertion of peptides and deletion of overlapping solvent) were respectively 24.8 Å and 125 molecules for TFE, 32.6 Å and 512 molecules for methanol, and 24.8 Å and 512 molecules for water. The cubic simulation volumes chosen for the mixtures assumed ideal volume mixing. Umbrella sampling calculations were performed for each system by applying a harmonic biasing potential on the O1–H5 distance (R_{O1-H5}), which was centered at distances of 2, 3, ..., 9, 10, 12, and 14 Å and employed a force constant of 2.0 kcal/mol/Å^2. Simulations were carried out for between 40 000 and 80 000 time steps for each of the 11 "windows" in R_{O1-H5}.[12] The biased histograms were processed using the constant temperature histogram method[17,18] to yield the potential of mean force. For dynamics, a time step of 2 fs and a nonbonded list generation scheme[19] which was updated every 20 time steps were used. Hydrogen–heavy atom bond lengths were fixed with SHAKE.[20] Long-range interactions were truncated at a distance of 9.75 Å using an atom–atom-based shifting function.[21] The simulations were performed with a modified version of CHARMM[22] (Version 22) using the polar hydrogen force field parameters, version 19, for the peptide.

Decomposition of the free energy difference into energetic and entropic components and the breakdown of energy into peptide–water, peptide–alcohol, and peptide–peptide interactions (Table I)[12] illustrates that the alcohol solvates the peptide in a qualitatively different manner than does water. By examining $\langle \Delta U_{uv} \rangle$, the relative solvation energy component, for water, we see that water favorably solvates the folded state, most likely due to the enhanced dipole moment of the peptide in this compact conformation.[12] Favorable solvation is partially compensated by an entropic factor favoring the extended state, presumably due to the partial "immobilization" of water during solvation of the folded state and changes in the chain entropy. In both of the neat alcohol solutions, solvation of the peptide favors the extended conformation and entropy opposes it, although the magnitude is less in methanol. The peptide–peptide energy difference favors the unfolded state for each of these three systems because of the unfavorable

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Table I. Relative Solvation Energy Components for Ac-$(Ala)_3$-NHMe in Neat and Mixed Alcohol–Water Solvent[a,b]

composition[c]	$\langle \Delta U_{uv} \rangle$ (kcal/mol) water	$\langle \Delta U_{uv} \rangle$ (kcal/mol) alcohol	$\langle \Delta U_{uv} \rangle$ (kcal/mol) combined	$\langle \Delta U_{uu} \rangle$ (kcal/mol) peptide	thermodynamic components (kcal/mol) $\langle \Delta U_{uv,uu} \rangle$	ΔA	$-T\Delta S$	HPA[d]
H_2O	6.6 ± 2.1		6.6 ± 2.1	−3.2 ± 1.1	3.4 ± 2.4	−0.4	−3.8	1.0
MeOH		−0.8 ± 2.0	−0.8 ± 2.0	−3.7 ± 0.7	−4.5 ± 0.7	1.0	5.5	12.0
MeOH:H_2O	5.3 ± 4.3	0.9 ± 3.0	6.2 ± 5.3	−4.0 ± 0.7	2.2 ± 5.3	0.0	−2.2	4.5
TFE		−10.6 ± 3.8	−10.6 ± 3.8	−2.2 ± 0.4	−12.8 ± 3.9	−2.1	10.7	0.3
TFE:H_2O	21.8 ± 5.1	−17.4 ± 2.9	4.3 ± 5.9	−3.9 ± 1.7	0.5 ± 6.1	0.9	0.4	12.0

[a] Energy and thermodynamic components for unfolding to an extended conformation (R_{O1-H5} = 10 Å window), relative to values for the helical state (R_{O1-H5} = 2 Å window), i.e., helix → coil. The Helmholtz free energy, ΔA, for this process is decomposed into the unique energetic, $\langle \Delta U_{uv,uu} \rangle$, and entropic, $-T\Delta S$, components. In addition, the energetic components are further divided between solvent–peptide, $\langle \Delta U_{uv} \rangle$, for alcohol–peptide and water–peptide pieces and peptide–peptide, $\langle \Delta U_{uu} \rangle$, contributions. [b] Reported errors for energies are from standard deviations of bin averages over 500 data points. The errors associated with the Helmholtz free energy differences are less than 0.7 kcal/mol in all cases. [c] Composition of mixtures is 30:70 (mol:mol) alcohol–water. This corresponds to 49% v/v and 64% v/v for methanol–water and TFE–water mixtures, respectively. [d] HPA denotes the helix-promoting ability, defined as the ratio of the helix equilibrium constant for a particular solvent compared to the value for pure water. For water we find K_{water}^{helix} is 0.033.

electrostatic interactions occurring in the helical conformations of the peptide.[12] When both energetic components are combined, $\langle \Delta U_{uv,uu} \rangle$, we see that our results suggest that the helix → coil energies and entropies, as measured by the temperature dependence of the helix signal in circular dichroism, should differ qualitatively in aqueous and alcoholic solvents.

In mixed solvents, the competition between solvation by water and solvation by alcohol leads to energetic and entropic factors favoring the helical state for the TFE–water mixture and offsetting results for the methanol–water mixture. This effect will vary with alcohol cosolvent and mixture composition and is most likely the origin of experimentally observed trends with changes in alcohol concentration.[3,4] In addition, the energetic contribution from TFE, which favors the extended peptide, displays a marked enhancement in the presence of water (Table I). This enhancement suggests an increased density of TFE near the extended peptide, despite the statistical dilution of TFE in the mixture. This, in turn, leads to more favorable peptide–alcohol contacts for the extended state compared to the folded state and relative to those present in neat alcohol. We also observe that the van der Waals portion of the energy is enhanced (data not shown), leading us to propose that there is an orientational preference for the less polar end of the alcohol to be closest to the peptide in its extended conformation. A more detailed hydrogen-bonding and structural analysis supporting this conclusion will be forthcoming in a future publication.[16]

The results of our calculations agree with the general experimental trends.[1-9] Additionally, for neat TFE, in contrast to methanol, we find an anomalous decrease in helix promotion. Experimental evidence suggests that mixture compositions with high-volume ratios of TFE reduce helical character below a maximum value achieved for lower volume ratios in some peptides.[9] We observe this trend, but the lowering of helical character in our case makes neat TFE comparable to water. This differs in detail from the experimental results for longer peptides of heterogeneous sequence and may be due to either the homogeneity or the length of the model peptide we studied.

Most significantly, we predict a qualitative difference in the helix → coil thermodynamics for peptides in alcoholic solvents versus aqueous solution. We see a positive energy, which should reflect a positive enthalpy, for helix → coil transitions in water and a differing sign for neat alcohols. We also predict a differing sign in the entropy, suggesting opposite behavior in the temperature dependence of helix content for alcoholic versus aqueous solutions. However, our model tripeptide system may not reflect an additive contribution to the full conformational entropy of the coil state in longer peptides. The thermodynamic differences we observe are due to inherent solvation preferences of the alcohol for the extended state of the peptide, which are further enhanced for TFE in the presence of water as a cosolvent. These observations suggest a picture for solvation of the peptide which has alcohol somewhat "ordered" both spatially and orientationally adjacent to the extended conformers of a peptide.

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