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"Teflon-Coated Peptides": Hexafluoroacetone Trihydrate as a Structure Stabilizer for Peptides

In the last 30 years, since the early work of Goodman and co-workers introducing trifluoroethanol as a solvent in peptide conformational analysis,[1,2] the ability of fluoroalcohols to stabilize secondary structures in peptides in aqueous solution is well documented.[3–7] However, the detailed molecular mechanisms remain to be understood.[3,4,8–12] 2,2,2-Trifluoroethanol (TFE) has been the most widely used fluoroalcohol additive to aqueous solutions of peptides and proteins.[4,13–18] We describe in this communication the superior structure-stabilization properties of hexafluoroacetone trihydrate (HFA, a covalent hydrate, of the ketone, a gem diol, hexafluoropropan-2,2-diol)[19–21] and provide a rationale for the induction of intramolecularly hydrogen-bonded conformations in peptides, in aqueous fluoroalcohol solutions.

Figure 1 shows the far-uv CD spectrum of a 21-residue peptide (YHACQKKLLKFEALQQEEGEE; **1**), a C-terminal antigenic fragment of the chicken riboflavin carrier protein,[22] in water at pH 2.8. No evidence for stable secondary structure is obtained. Upon addition of hexafluoroacetone trihydrate, intensification of the far-uv CD bands is observed, with a classical α-helical type spectrum (positive band at 192 nm, negative bands at 206 and 222 nm) appearing by a HFA concentration of 15% (v/v). The induction of helicity is completed by about 50% HFA. A comparison of the CD spectrum in 50% TFE (v/v) reveals that the extent of secondary structure induction is appreciably larger in HFA. The estimates of limiting induced helicity calculated as described[23,24] in the two solvents are 31% in TFE/water and 40% in HFA/water.

The extent of helix induction by TFE in water-soluble peptides of the length 15–22 residues ranges from 5 to 90%.[4,12] Stabilization of helical structure is also demonstrated in another 18-residue fragment of chicken RCP (YLQMNKKDMVAIKHLLSE; **2**). Figure 2 shows the variation of $[\theta_{222}]$ with increasing concentration of HFA, for both the peptides. A partial NOESY spectrum of peptide **2** in 50% HFA-H_2O is shown in Figure 3. The diagnostic d_{NN} NOEs are extremely intense and several medium-range NOEs characteristic of helices are also observed.[25] HFA is a particularly convenient cosolvent for nmr studies of peptides in water since it does not have any residual solvent protons that need to be removed by deuteration. It is therefore an attractive and inexpensive alternative to CF_3CD_2OH, with superior structure stabilizing properties. A comparison of ellipiticities in 50% HFA as against 50% TFE for **1** and **2** is in Figure 2 (inset). The $[\theta_{222}]$ value in 50% ethanol is also shown. Clearly, the extent of helix induction is sequence dependent and maximal in HFA, with the order of helix stabilization being HFA > TFE > ethanol. Pronounced stabilization of helices and hairpins has also been demonstrated in several peptides of length 17–25 residues (unpublished results).

The results establish that HFA is a far more potent structure former than TFE, for peptides in aqueous solutions. Ironically, the first report in the literature[26] on the use of HFA in protein chemistry was titled "Fluoroketone Hydrates: Helix-Breaking Solvents for Polypeptides and Proteins." In this seminal study, Longworth established[27] the utility of HFA in solubilizing silk fibroin, a predomi-

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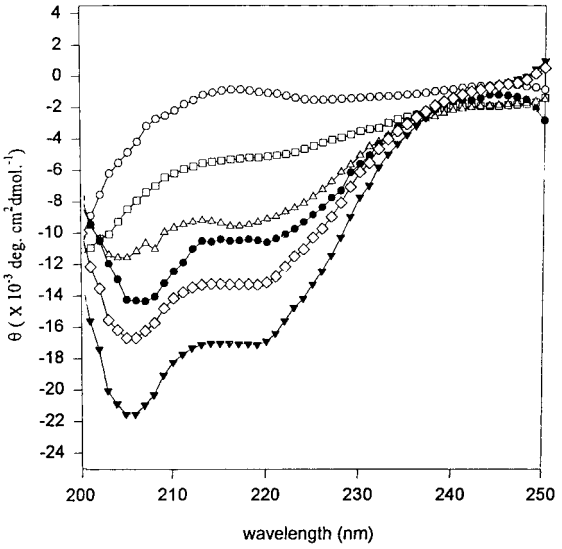

FIGURE 1 CD spectra of **1** increasing concentrations of HFA (v/v): (○) 0%, (□) 10%, (△) 14%, (◇) 25%, (▼) 50%. The CD spectrum in 50% TFE is also shown for comparison (●). Peptides **1** and **2** were synthesized by conventional Fmoc based solid phase methods, purified by reverse phase high performance liquid chromatography and characterized by matrix assisted laser desorption mass spectra [**1,** M^+ (obs) 2596.4 Mr (calc.) 2592.9, **2,** M^+ (obs) 2162.6, Mr (calc.) 2161.6] as well as by complete assignment of 400 MHz ^{1}H-nmr spectra. Peptide **1** corresponds to residues 200–219 and **2** corresponds to residues 170–186 of the chicken riboflavin carrier protein. Cysteine, at position 4 in **1,** has its side-chain protected by an acetamidomethyl group. The stabilization of helices in this peptide by TFE has been documented elsewhere.[22]

nantly antiparallel β-sheet containing fibrous protein. Almost concurrently, Goodman and Rosen[28] also reported optical rotatory dispersion studies of glutamate oligomers and concluded that HFA was a weak helix stabilizer for long polymers. Subsequently, aqueous HFA has been shown to be a very useful solvent system for dissolution of large insoluble peptide fragments.[29] Interestingly, Balasubramanian and Roche showed that while the diol form of hexafluoroacetone (abbreviated as HFPD in the original report) itself is a helix breaker, addition of water to a solution of poly γ-methylglutamate in HFPD effects a conformational transition to a α-helix, beyond a mole ratio of 1:0.6 (HFPD:H2O).[30,31] HFA has also found limited use as a solvent for CD studies of peptides, largely as a consequence of its transparency in the far uv region.[32]

The potent structure forming properties of aqueous HFA may be rationalized on the basis of the following attributes: (1) The hydrophobicity of the trifluoromethyl groups, a property that is emphasized by the water repelling properties of polytetrafluoethylene (Teflon)[33] and the dramatically lower critical micelle concentrations of fluorosurfactants, as compared to their hydrogenated counterparts.[34–36] (2) The enhanced hydrogen bond donating ability of the —OH group, as evidenced by low pKa (6.58) of HFA[19–21] [pKa TFE 12.4 (Ref. 37), H_2O 15.3] and by the ability of HFA to induce large shifts in the $n - \pi^*$ transition of the ketones.[38] In this study, we obtained $n - \pi^*$ band positions for acetone in various solvents: HFA 259 nm, H_2O 262 nm, TFE 266 nm, cyclohexane 278 nm. This corresponds to a solvent-solute hydrogen bond strength of 7.56 kcal/mole for HFA, 6.29 kcal/mole for water, and 4.65 kcal/mole for TFE relative to solvent cyclohexane. (3) The poor hydrogen bond accepting ability of the oxygen atoms in HFA, analogous to the situation in TFE,[4,39,40] underscored by recent analyses of crystal structure data bases that establish that C—F bonds hardly ever act as hydrogen acceptors.[41,42]

Figure 4 provides a schematic model that explains the ability of HFA to promote peptide helix formation in aqueous solutions. For an oligopeptide in water, hydration with formation of solvent-solute hydrogen bonds mitigates against intramolecular hydrogen bond formation, in short solvent-exposed sequences. Helix induction by fluoroalcohols may be viewed in terms of the ability of these molecules to selectively dehydrate the vicinity of the peptide backbone. Since water is present at a high concentration [for example, a 50% (v/v) solution corre-

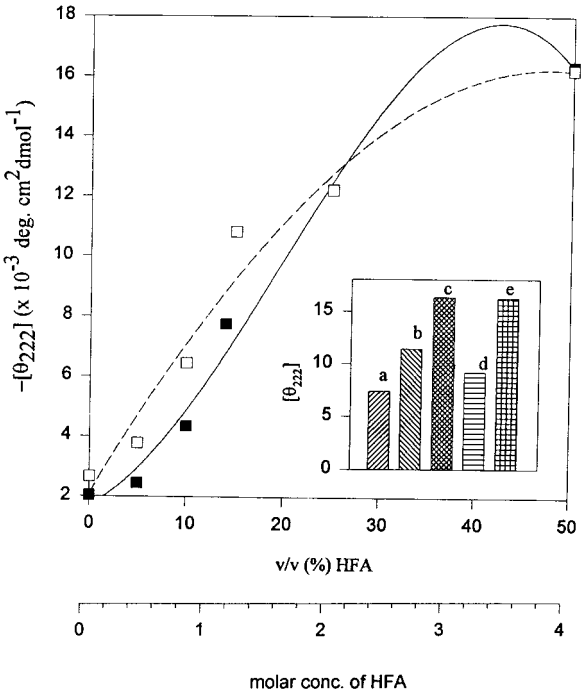

FIGURE 2 The variation of $[\theta_{222}]$ with the concentration of HFA (v/v) for the peptides **1** (■) and **2** (□) is depicted. Inset: A comparison of helical stabilization induced by different solvents in peptides **1** and **2:** (a) **1** in 50% ethanol, (b) **1** in 50% TFE, (c) **1** in 50% HFA, (d) **2** in 50% TFE, and (e) **2** in 50% HFA.

sponds to about 3.6 *M* of HFA and 35 *M* of H_2O, assuming that volumes are additive upon mixing], we suggest that the peptide fluoroalcohol association is driven by hydrophobic effects, with the trifluoromethyl substituents playing a critical role. The larger size of the fluoroalcohol should result in displacement of several water molecules, making the process of dehydration entropically favorable. In the model in Figure 4, HFA molecules effectively provide a ''teflon face,'' which secludes the peptide in a noninteracting environment, in which intramolecular hydrogen bond formation is energetically favored. These ''teflon-coated peptides'' are rendered soluble by the preponderance of O—H groups on the surface. The poor hydrogen bond acceptor property of HFA (and other fluoroalcohols) limits their ability to insert into C═O —H—N bonds in peptides, in contrast to water.[4] The extent of helix induction will undoubtedly be sequence dependent, as already noted in studies of synthetic actin fragments in aqueous TFE solution.[9] Studies in this laboratory establish structure stabilization in diverse sequences including helix induction at acid pH in the strongly basic peptide melittin (unpublished). The presence of several polar/charged residues does not preclude appreciable helix stabilization by HFA. Furthermore, other secondary structures could, in principle, be stabilized in aqueous fluoroalcohols. Indeed, evidence for β-hairpin and β-sheet stabilization in aqueous TFE have already been reported.[43] The view expressed by Kemp and co-workers that ''TFE increases helicity by selectively destabilizing amide functions that are solvent exposed''[3] is consistent with the premise that fluoroalcohols ''dessicate'' the peptide backbone. A recent report of ''cold denaturation'' in a peptide helix observed in an 8% hexafluoroisopropanol–water mixture suggests that fluoroalcohol–peptide association is indeed hydrophobically driven. Diminished fluoroalcohol interaction at low temperature would rationalize low temperature unfolding.[44] Peptide structure formation in aqueous fluoroalcohol solution resembles the nucleation of secondary structures in the early stage of protein folding within the framework of a hydrophobically collapsed globular state. The use of HFA to stabilize non-native equilibrium intermediates in protein folding has been realized in the case of lysozyme.[45]

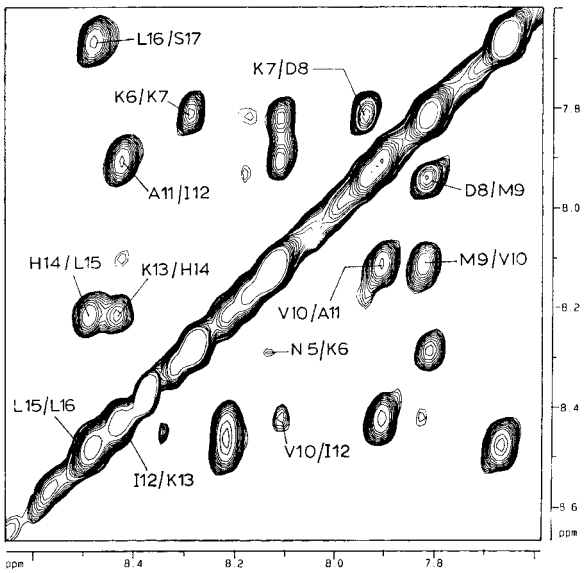

FIGURE 3 The 400 MHz partial nuclear Overhauser effect spectra spectra of the peptide **2** in 50% HFA (313 K, pH 3.0) showing NH/NH connectivities. The spectra were recorded at 200 ms mixing time. The nmr data were collected in 1 K × 512 data points and zero filled to 1 K × 1 K data matrix. The time domain data were multiplied by a $\pi/4$ sine function before Fourier transformation in both dimension. The peptide concentrations were 5–6 m*M* in all nmr experiments.

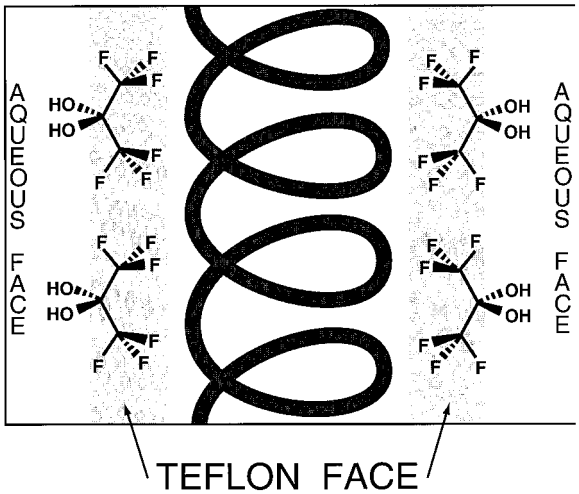

FIGURE 4 A schematic model for the stabilization of helical peptide conformations in aqueous hexafluoroacetone (see text).

This model rationalizes the enhanced structure forming ability of HFA as compared to TFE, since the latter lacks a well-defined hydrophobic face because of the absence of a second trifluoromethyl group. It further suggests that the design of superstructure formers that incorporate multiple hydrated ketone functions and fluoroalkyl substituents may be possible. Peptide helices can also be made to acquire ''fluoro coats'' by selective use of fluorinated amino acids leading to novel molecules.[46]

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