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A model for the structure stabilizing effects of fluoroalcohols on peptides. A new look at an old problem

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Abstract

The stabilization of helical conformations in a synthetic 21 residue peptide in aqueous solution, by addition of hexafluoroacetone hydrate (HFA) as a cosolvent is demonstrated using CD and NMR methods. Helix stabilization in melittin at acidic pH is achieved at relatively low concentrations of the fluoroalcohols, 2,2,2-trifluoroethanol (TFE) or HFA. A model for structure stabilization based on the amphipathicity of HFA is developed. The hydrophobicity of the fluorocarbon face facilitates selective solvation of peptides, permitting folding in a sequestered, hydrophobic environment. The unique hydrogen bonding properties of fluoroalcohols, poor acceptors and good donors, precludes solvent invasion of peptide backbones, in contrast to water. Peptides accquire an effective 'teflon coat' in the proposed model.

The two major secondary structure elements in peptides and proteins, the α-helix[1] and the β-sheet[2], are stabilized by inter-peptide hydrogen bonds. In proteins, helices and sheets are generally shielded from the aqueous solvent, limiting solvation of CO and NH groups by precluding formation of solvent-solute hydrogen bonds. An extreme example of stabilization of secondary structures in hydrophobic environments is provided by the case of transmembrane segments in integral membrane proteins. In these cases the polypeptide segments spanning the phospholipid bilayer adopt either α-helical conformations as in the case of bacteriorhodopsin or form β-barrels as exemplified by the porins[3]. The driving force for chain folding in membranes is the formation of an extensive hydrogen bonding network. In contrast, isolated elements of secondary structure in oligopeptides are generally unstable in aqueous solution, largely as consequence of the extensive invasion of the backbone by water[4]. It has been known for over a quarter of a century that addition of fluoroalcohol cosolvents results in stabilization of helical structures in diverse peptide sequences[5]. 2,2,2-Trifluoroethanol (TFE) has been the most widely used additive, while hexafluoroisopropanol (HFIP) and hexafluoroacetone hydrate (HFA, hexafluoropropan-2,2-diol), Figure 1, have also found use. In most studies, addition of between 15-

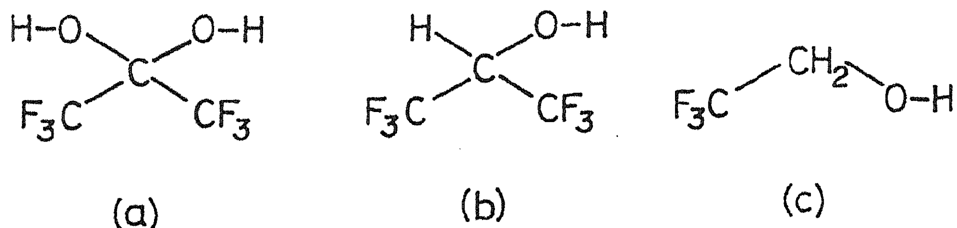

FIG. 1. Structures of fluoroalcohols (a) hexafluoroacetone hydrate (HFA) or hexafluoro-propan-2,2-diol (b) hexafluoroisopropanol (c) 2,2,2-trifluoroethanol.

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25% (v/v) of the fluoroalcohol results in significant stabilization of secondary structure, most often helices, in the presence of a large molar excess of water. Despite the extensive use of fluoroalcohol cosolvents as structure stabilizers in peptides, the mechanism by which this is achieved has not been clearly understood[5-8]. Recent studies in this laboratory have attempted to address this mechanistic issue as summarized in the following section.

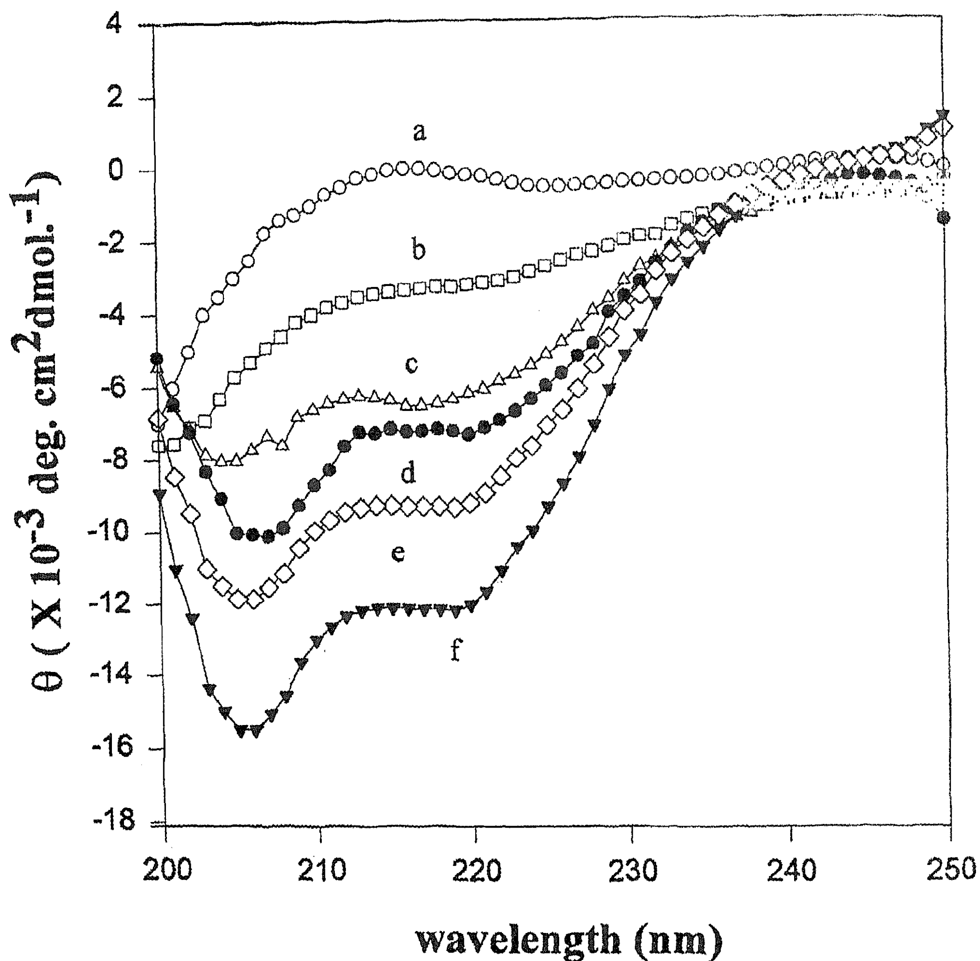

FIG. 2. CD spectra of the C-terminal antigenic peptide Y-21, residues 200-219, with an additional N-terminus Tyr, (YHACQKKLLKFEALQQEEGEE) of chicken riboflavin carrier protein, at increasing concentrations of HFA (v/v): (o) 0%, (□) 10%, (Δ) 14% (✧) 25%, (▼)50%. The CD spectrum in 50% TFE is also shown for comparison. (From ref. 10).

Helix stabilization in peptides

The helix stabilising properties of HFA and TFE may be compared by their effects on a potentially helix forming sequence in aqueous solutions. Addition of the fluoroalcohol cosolvents to

a synthetic 21 residue peptide (YHACQKKLLKFEALQQEEGEE), a C-terminal fragment of chicken riboflavin carrier protein[9], results in a significant intensity enhancement of the CD bands at 206 nm and 222 nm, diagnostic of helix formation (Figure 2). Appreciable helix induction is observed by a HFA concentration of 15% v/v. A comparison of the spectra obtained in HFA and TFE at similar cosolvent concentrations suggests that the former is a significantly more potent helix inducer[10]. Confirmatory evidence for stable helix formation is obtained from nuclear Overhauser effect (NOE) spectra where strong successive $N_iH \leftrightarrow N_{i+1}H$ NOEs are observed for the segment, residues 5–18 (Figure 3). A dramatic example of the helix inducing abilities of HFA is provided by the 26-residue, bee venom peptide, melittin (G-I-G-A-V-L-K-V-L-T-T-G-L-P-A-L-I-S-W-I-K-R-K-R-Q-Q-NH_2). Melittin has a very strongly basic C-terminus and is positively charged at pH values < 10. As a consequence of the C-terminal cluster of

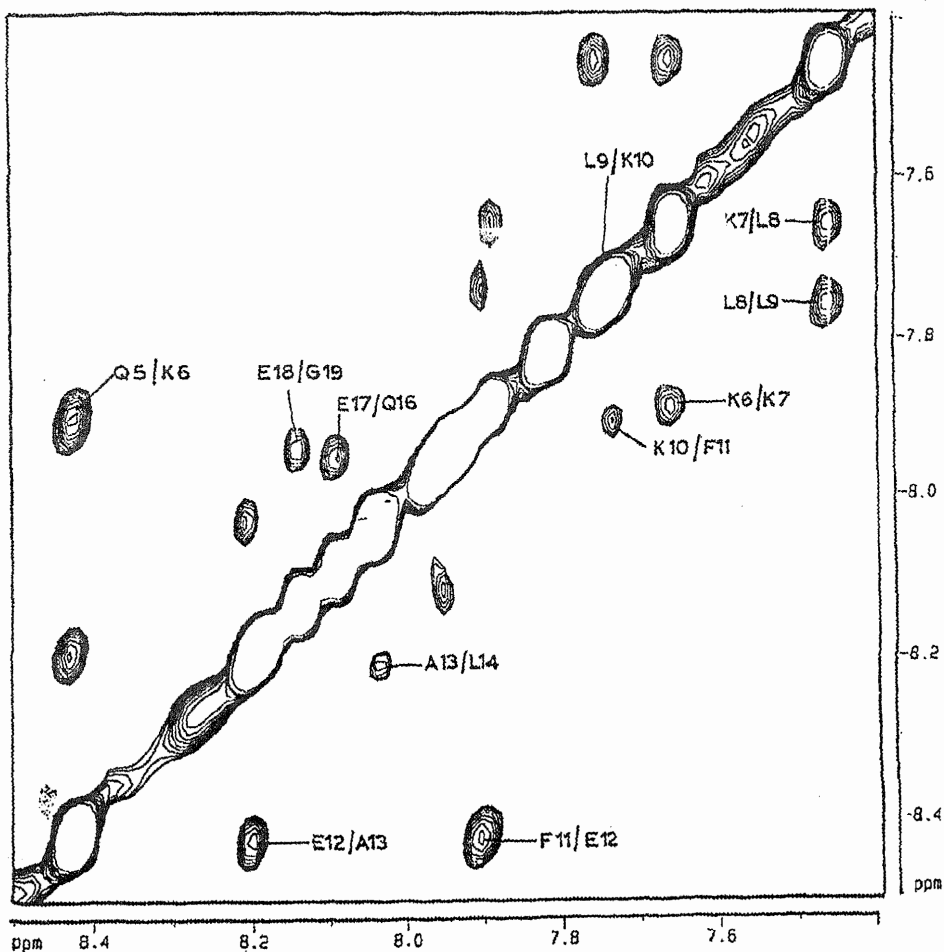

FIG. 3. 400 MHz NOESY spectra of the Y-21 peptide (see Figure 2 legend for sequence) in 50% HFA-water, pH 3.0, 315 K. Peptide concentration 5 mM. Mixing time 300 ms. (From ref. 11).

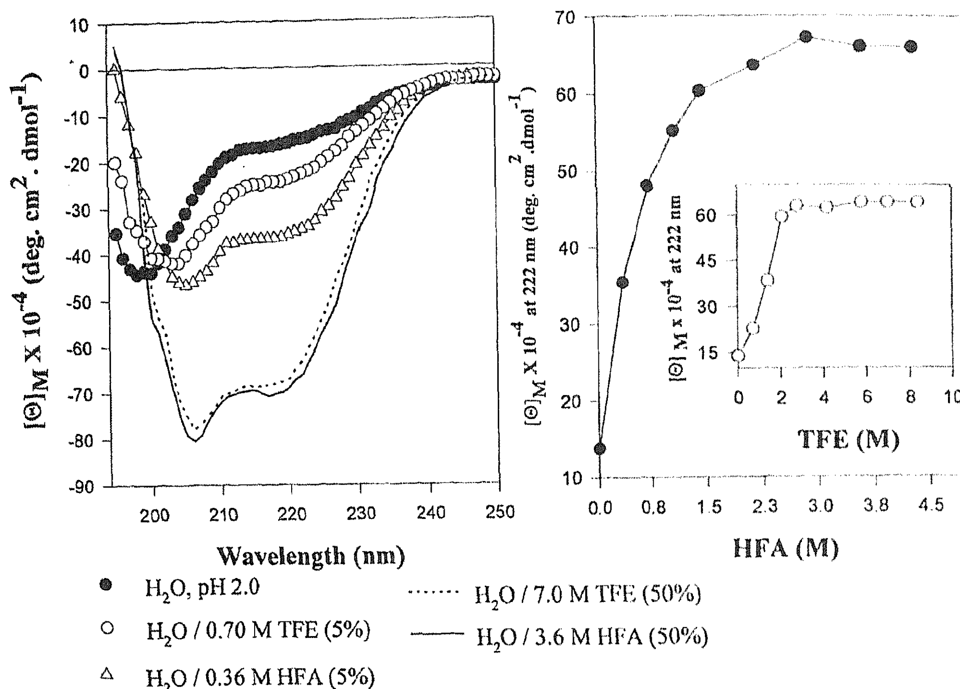

FIG. 4. (left) Far UV CD spectra of melittin in aqueous solution with different concentrations of HFA and TFE as additives. Peptide concentration 25 μM, pH 2.0. (right) Variation of molar ellipticity at 222 nm as a function of HFA concentration. (inset) Variation of molar ellipticity at 222 nm as a function of TFE concentration. Note molar fluoroalcohol concentration are represented.

positive charges melittin is largely unstructured in water, at neutral and acidic pH, in the absence of strong counterions[12–14]. Indeed a helical conformation in melittin at near neutral pH is achieved only upon addition of ~200 mM phosphate[12]. Figure 4 demonstrates the effect of TFE and HFA on melittin, in aqueous solution at acidic pH. There is a dramatic enhancement of helicity as witnessed by the strong CD bands at 208 nm and 222 nm. In the case of melittin, which has a strongly hydrophobic N-terminus segment, helix induction is complete between 10–20% TFE (v/v) (S. Bhattacharjya and P. Balaram, unpublished results).

The two examples presented above emphasise that the extent of helix induction and the fluoroalcohol concentrations required for structure stabilization are indeed sequence dependent, a feature recognized in earlier analyses of the extensive literature detailing fluoroalcohol effects on peptide conformation. No attempt is made in this report to exhaustively catalog earlier studies on fluoroalcohols (Appropriate literature citations may be found in ref. 5 and 10).

A model for conformational stabilization

A readily apparent feature of the HFA molecule is its potential amphipathicity. One face of the molecule is fluorocarbon in nature and consequently hydrophobic, while the other is distinctly hydrophilic containing the two hydroxyl groups[10]. The hydrophobicity of fluorocarbons is best

exemplified by the "non-wettability" of polytetrafluoroethylene (TEFLON). Carbon-fluorine bonds are also largely incapable of participating in hydrogen bonding despite the large electronegativity difference between the two bonded atoms. This is borne out by several analyses of the crystal structure database which reveal that C-F bonds are extremely poor hydrogen acceptors[15,16]. The ability of fluorosurfactants to micellise in aqueous solution at much lower concentrations than their hydrogenated analogs is clearly supportive of the hydrophobicity of fluorocarbon chains.

A second important feature of the fluoroalcohols is the enhanced acidity of hydroxyl group. (pKa values are HFA, 6.58; HFIP, 9.3; TFE, 12.4; H_2O, 15.3; ethanol 15.9 and isopropanol 17.1). The fluoroalcohols are therefore much better *donors* than water or the normal alcohols in hydrogen bonding interactions[10].

A most important characteristic of the fluoroalcohols is the extremely poor *hydrogen bond accepting* ability of the hydroxyl group, as compared to water or the normal alcohols. In TFE, evidence for the poor hydrogen bond accepting nature was obtained in early infrared[17] and NMR[18] spectroscopic studies. An important consequence of this property is that the fluoroalcohols do not have a strong tendency to "insert" into intramolecular hydrogen bonds in peptides. In sharp contrast water can disrupt intramolecular hydrogen bonds by invasion of the backbone, because of its ability to participate as both donor and acceptor in hydrogen bonds[19]. Fluoroalcohols can in fact, participate in a bifurcated hydrogen bond with a peptide carbonyl group, as a donor, without disrupting the intramolecular CO—HN interaction. The possibility of a cooperative solvent effect enhancing peptide hydrogen bond stabilities has been suggested from *ab initio* molecular orbital calculations on N-methylacetamide dimers and TFE[21]. The major physicochemical characteristics of the fluoroalcohols considered above may be used together in formulating a model rationalising their observed structure stabilizing effects. Figure 5 schematically illustrates a model based on selective solvation of peptides in aqueous fluoroalcohol systems[10]. The hydrophobicity of the fluoroalkyl face, best exemplified in HFA, facilitates interaction with non-polar amino acid sidechains. Indeed, experimental evidence for TFE-indole complexes in water have been obtained from fluorescence investigations (R. Rajan and P. Balaram, unpublished), where ground state complexation can be demonstrated from quenching studies. Specific interactions between fluoroalcohol cosolvents and peptides are supported by the observation of ^{19}F-^{1}H NOEs to the phenyl ring protons of Phe (8) in angiotensin II in TFE-water mixtures[22]. The fact that selective fluoroalcohol solvation of peptides is hydrophobically driven is also suggested by the recent observation of "cold denaturation" of a synthetic peptide helix in 8% HFIP-water[23]. Loss of helical structure at low temperatures is also observed for melittin in HFA-water systems (S. Bhattacharjya and P. Balaram, unpublished).

The major feature of the model in Figure is that HFA and related systems 'dessicate' the vicinity of the peptide backbone by selective solvation. The larger size of the fluoroalcohols as compared to water results in the displacement of several water molecules in the hydration shell, making the dehydration process entropically favourable[8]. The fluoroalcohol solvated peptides ("teflon coated peptides") are rendered soluble by the outer layer of hydroxyl groups. This sequestering of the peptide in a predominantly polar environment promotes secondary structure formation, involving intramolecular hydrogen bonds. The process formally resembles nucleation of secondary structure in proteins, within the confines of an initial globular state, driven by

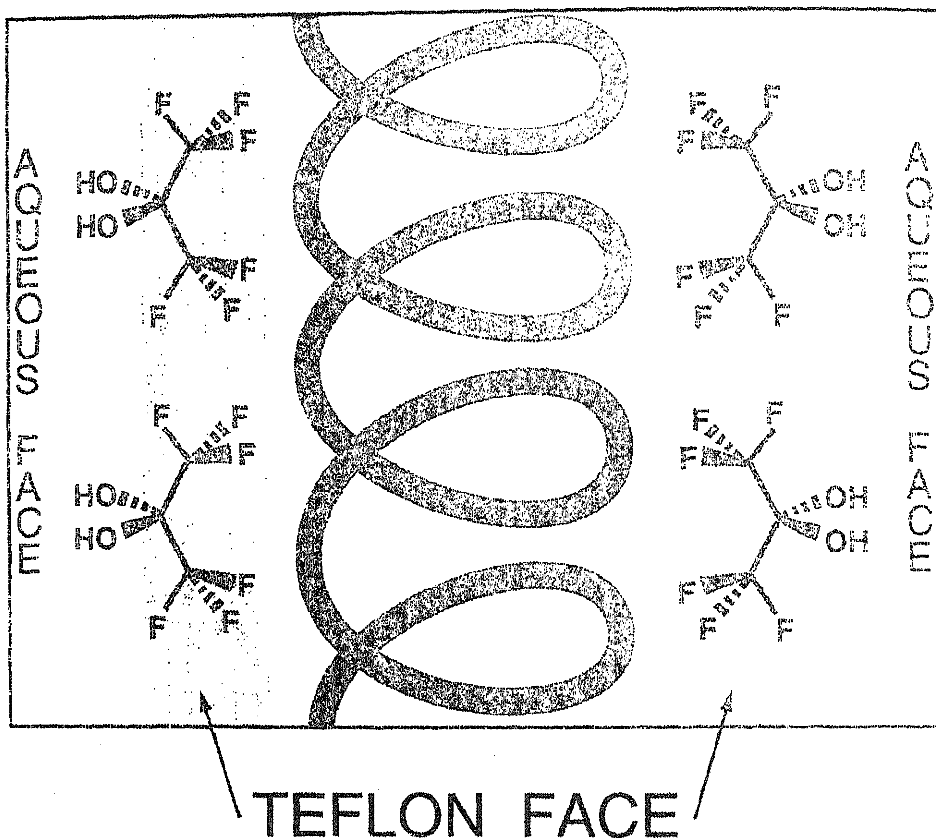

FIG 5. A schematic model for the stabilization of helical peptide concentrations in aqueous HFA, based on selective solvation driven by hydrophobic association of the fluoroalcohol. (From ref. 10).

hydrophobic collapse, in the early stages of folding[24]. The stabilization of structure by fluoroalcohols is not limited to helices and several recent reports address the formation of β-hairpins[25–27]. It is clear that fluoroalcohol cosolvents can also perturb protein structures and be effectively used to generate equilibrium, non-native structures[28,29]. Recent studies from this laboratory provide the first example of the use of HFA as a structure modulator in proteins and illustrate its use in generating a molten globule state of hen egg white lysozyme[30]. The possible use of fluoroalcohols in effecting β-sheet to α-helix transitions is illustrated by recent studies on synthetic peptides[31] and proteins[32]. The model presented in this report should aid in the design of 'super structure formers' which in turn may expand the scope of 'solvent engineering'[33] of peptide and protein structures.

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