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A model for the interaction of trifluoroethanol with peptides and proteins

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The structural stabilizing property of 2,2,2-trifluoroethanol (TFE) in peptides has been widely demonstrated. More recently, TFE has been shown to enhance secondary structure content in globular proteins, and to influence quaternary interactions in protein multimers. The molecular mechanisms by which TFE exerts its influence on peptide and protein structures remain poorly understood. The present analysis integrates the known physical properties of TFE with a variety of experimental observations on the interaction of TFE with peptides and proteins and on the properties of fluorocarbons. Two features of TFE, namely the hydrophobicity of the trifluoromethyl group and the hydrogen bonding character (strong donor and poor acceptor), emerge as the most important factors for rationalising the observed effects of TFE. A model is proposed for TFE interaction with peptides which involves an initial replacement of the hydration shell by fluoroalcohol molecules, a process driven by apolar interactions and favourable entropy of dehydration. Subsequent bifurcated hydrogen-bond formation with peptide carbonyl groups, which leave intramolecular interactions unaffected, promotes secondary structure formation. © Munksgaard 1996.

Key words: fluoroalcohols; peptide and protein structure; structure stabilization; trifluoroethanol

The effects of 2,2,2-trifluoroethanol (TFE) on the structures of peptides (1–32) have been studied for more than three decades. In most cases, addition of TFE (10–50 v/v%) enhances the helicity of peptides that are already helical in solution (1–7) or induces helicity in peptides that are essentially disordered to begin with (2, 4, 11, 17–30). There have also been reports of β-sheet (8, 14, 16) and β-turn (15, 18, 31) stabilization by TFE. Further, there have been examples of conformational 'switches' in peptides, triggered over a narrow range of TFE concentration, like a change from a β-structure to a helix (9–13). TFE found early use as a 'membrane mimetic' solvent (33), as it was shown to induce structures in proteins and peptides similiar to those produced by membrane environments. More recently, the structures of well characterised globular proteins in TFE/H_2O solutions have been monitored using 2D NMR and H-D exchange techniques (34–36), in addition to circular dichroism (33, 37–45). In these solutions, most proteins lose tertiary structure, while retaining significant amounts of secondary structure. Dobson and coworkers (34, 35) have shown that TFE can stabilize even non-native secondary structures. Shiraki *et al.* (37) have studied the CD spectra of 23 proteins in water and 40% TFE/H_2O solutions, establishing that, in the presence of TFE, most proteins tend to become more helical. TFE has also been known to both enhance (46) as well as disrupt (40, 47, 48) quaternary interactions. The structure-stabilizing effects of other fluoroalcohols like hexafluoroacetone hydrate (49–51) and hexafluoroisopropanol (HFIP) (52) have also been demonstrated, albeit to a lesser extent. Tables 1 and 2 provide a representative summary of the changes produced by TFE on peptides and proteins, respectively. The mechanism by which TFE is able to bring about these remarkable changes in structure, however, has not been well understood. This analysis attempts to correlate the known physical properties of TFE, results of theoretical studies, 'popular' opinions and evidence from other areas in formulating a clearer picture of the manner of interaction of TFE with peptides and proteins.

Physical properties of TFE

In order to rationalize the effects of TFE, we need to first consider the special properties that TFE is

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TABLE 1

Effect of trifluoroethanol on peptides

Ser. no.	Ref. no.	Length[a]	Structural change[b]	Hydrophobic/aromatic residues[c]
1	1	18 (11)	+α	4A, 1P, 1M, 3L, **2F**
2	1	23 (11)	+α	5A, 1V, 3L, **2F**
3	1	21 (7)	+α	2I, 1P, **2F**, **2Y**
4	1	22 (11)	+α	2A, 2P, 2I, 1V, iL, **1Y**, **2F**
5	1	25 (11)	+α	2A, 2P, 4L, 1I, **2Y**
6	1	18 (11)	+α	6L, 2I, 1P, **1W**, **1F**
7	1	38 (14)	+α	3V, 6L, 1I, 1A, 1P, **2F**
8	1	21 (7)	+α	1P, 1A, 1I, 1L, 1M, **1Y**, **1F**
9	1	18 (8)	+α	1M, 1A, 4L, **1Y**, **1F**
10	1	28 (12)	+α	1A, 6L, 1V, 1M, **2Y**, **1F**
11	2	14 (4)	+α	2A, 1M, **1F**
12	3	5 (4)	+α	3A, **1F**
13	3	5 (4)	+α	3A, **1Y**
14	4	42 (12)	+α	5L, 2I, 2P, 1M, **3Y**
15	5	38 (14)	+α	3V, 2P, 5L, 1I, 1M, 1A, **1Y**
16	6	26 (8)	+α	5A, 1P, 1M, **1F**
17	4	27 (8)	+α	4L, 1I, 1A, **1Y**, **1F**
18	7	20 (6)	+α	4A, 1M, **1F**
19	8	8 (8)	+β	8I
20	8	7 (7)	+β	7I
21	9	10 (10)	−β	8A, 2V
22	9	10 (10)	−β, +α	8V, 2A
23	10	15 (6)	β→α	1V, 1L, 1A, **1W**, **2Y**
24	11	43 (21)	β→α+r	6V, 4A, 2L, 3I, 1M, **1Y**, **3F**
25	12	15 (7)	β→α	2I, 1M, 1L, 1P, **1W**, **1F**
26	13	20	r→α (pH 7)+β (10%)→r (20%)→α (50%)	1A, 2I, 1L, **1Y**
27	11	14 (10)	β→r	2A, 3I, 1L, 3V, 1M
28	14	20 (7)	r→α (4–13); r→β (13–16)	2L, 2V, 3A
29	15	18 (5)	r→βh	3L, 1I, **1Y**
30	16	9 (5)	r+γ→β	3P, **2F**
31	17	13 (5)	r→α	4L, **1F**
32	17	11 (4)	r→α	3L, **1F**
33	2	14 (8)	r→α	3A, 2I, **2Y**, **1F**
34	2	16 (5)	r→α	iL, 1V, 1A, **1Y**, **1F**
35	11	29 (10)	r→α	2A, 3V, 1L, **1Y**, **3F**
36	18	21 (7)	r→α	3A, 2L, 1I, **1Y**
37	19	32 (19)	r→α	5M, 9A, 2V, 2P, **1F**
38	19	32 (19)	r→α	5M, 9A, 2V, 2P, **1F**
39	20	21 (7)	r→α (small)	2A, 3L, **1Y**, **1F**
40	21	40 (16)	r→α	5A, 1V, 3L, 1M, **2Y**, **1W**, **3F**
41	4	9 (3)	r→α	2L, 1I
42	4	10 (4)	r→α	2L, 1I, 1A, **1Y**
43	4	13 (6)	r→α	3L, 1I, 1A, **1Y**
44	21	46 (17)	r→α	5A, 2L, 3I, 3V, 1M, **3W**
45	22	24 (13)	r→α	3V, 2L, 6A, **1W**, **1Y**
46	23	12 (7)	r→α	6A, 1P
47	24	14 (6)	r→α	2L, 1A, 1V, 1M, **1W**
48	25	25 (19)	r→α	7A, 3V, 1I, 3L, 3M, 1P
49	26	17 (8)	r→α	1A, 1M, 1L, 1P, **2W**, **1Y**, **1F**
50	27	13 (7)	r→α	4A, 1M, 1L, **1F**
51	28	20 (12)	r→α	1M, 1V, 2L, 1P, 5A, **1F**, **1Y**
52	29	18 (8)	r→α	2A, 3L, 2V, **1F**
53	30	19 (8)	r→α	2V, 1P, 2L, 1I, 1M, **1F**
54	18	13 (5)	r→βt	3A, 2L
55	18	5 (3)	r→βt	2L, 1A
56	31	4 (3)	r→βt	1P, 1I, **1F**
57	31	5 (4)	r→βt	1I, 1P, **1Y**, **1F**

TABLE 1 (*continued*)

Ser. no.	Ref. no.	Length[a]	Structural change[b]	Hydrophobic/aromatic residues[c]
58	31	7 (5)	r→βt	1V, 1I, 1P, **1Y**, **1F**
59	31	7 (4)	r→βt	1I, 1V, 1P, **1Y**
60	32	10 (3)	r→r	1V, 1P, **1F**
61	4	7 (2)	r→r	1A, **1Y**
62	21	20 (5)	r→r	1A, 2I, 1L, **1Y**
63	21	22 (7)	r→r	1L, 1I, 1A, 2P, **2W**
64	1	13 (5)	r→r	1M, 2A, **2F**
65	2	15 (7)	r→r	4P, 1I, **1Y**, **1F**
66	14	11 (5)	r→r	3A, 1P, **1F**

[a] The number in parenthesis for each entry refers to the total number of hydrophobic and aromatic residues present in the peptide.
[b] r, random; β, β-sheet structure; βt, β-turn; βh, β-hairpin structure; +, enhancement; −, decrease.
[c] The hydrophobic and aromatic residues along with the frequency of occurrence. The aromatic residues have been highlighted.

endowed with Table 3 presents the physical properties of TFE, compared with those of water and ethanol. Properties of acetic acid and trifluoroacetic acid (TFA) are also shown to illustrate the changes brought about by the introduction of the CF_3 group.

Hydrogen bonding. The idea that introduction of a fluorine would increase the acidity of alcohols was first proposed by Swarts (53) and later elaborated by Henne *et al.* (54, 55), who showed that the dissociation constant of fluoroalcohols containing one perfluoroalkyl group was 3–4 times greater than the corresponding values for ordinary alcohols. For tertiary fluoroalcohols, the introduction of the CF_3 groups appreciably increases the acidity of the alcohol, comparable to that of carboxylic acids [the pK_a of $(CF_3)_3COH$ is 5.4, while the pK_as of carboxylic acids lie in the range 3–5] (56). When Murto (57) confirmed the pK_a values of the aliphatic alcohols, it became clear that the fluorinated alcohols were much stronger acids than the corresponding aliphatic alcohols. (For instance, pK of HFIP is 9.3 while that of isopropanol is 17.1; Table 3). Rao *et al.* (58) showed that the n–π* transition in carbonyl groups showed a greater blue shift in TFE than in ethanol (for example, the n–π* band of acetone, which is at 277 nm in heptane, is shifted to 270 nm in ethanol and 265 nm in TFE), indicating that TFE is a better proton donor than ethanol. From these spectral shifts, the strength of the C=O···H–O–R hydrogen bond strength can be estimated as 2.68 kcal/mol in ethanol and 4.86 kcal/mol in TFE. The enhanced hydrogen bond donating ability of TFE is clearly evident. Circular dichroism and IR studies on poly-L-proline in different alcohols showed that the all-*trans* form (poly-Pro II) was more stable in TFE than ethanol. TFE was shown to bind to the carbonyl oxygen of poly-L-proline (59). Results of other workers (60–65) overwhelmingly confirm the better proton-donating ability of TFE over ethanol and water.

Aside from being a good proton donor, TFE is a poorer proton acceptor when compared to water and ethanol (66–70). The lower boiling point of TFE (Table 3) (66) clearly points to the lower association in TFE, a feature recognized by Zana (67). The IR spectrum of TFE shows a 'free' O–H peak at 3628 cm^{-1} (68), revealing the presence of monomers even in pure TFE. PMR spectra of solutions of anhydrous ethanol and TFE in benzene reveal a more upfield resonance of the hydroxyl proton, at infinite dilution, for TFE (6.015 ppm) than for ethanol (7.03 ppm), suggesting weaker association in TFE as compared to ethanol (69). An early study of gramicidin S in methanol/TFE reveals an upfield shift of the peptide N–H resonances with increasing concentrations of TFE. This observation clearly suggests that when methanol molecules are displaced from the vicinity of the peptide, by the fluoroalcohol, the latter fails to make a hydrogen bond with the peptide N–H group (70).

A point to be noted is that the fluorine in fluorocarbons does not seem to engage in hydrogen bonding, although intuition tells us otherwise, as the electronegativity difference between C and F is quite high (1.7 on the Pauling scale). This fact has been shown by the crystal structures of fluorinated organic compounds over the years (71–73). 'An analysis of relevant crystal structures in the Cambridge Structural Database shows that F atoms in C–F bonds approach hydroxyl groups with minimum F···H distances of 2.3 Å, much longer than the O···H distances of 1.4–1.7 Å in strong O···H–O hydrogen bonds'. (73). According to Glusker (73), the F atom in a C–F group does not, apparently, develop much negative charge, and in addition is not readily polarized. The higher electron affinity of fluorine probably limits the ability of fluorine to share its electrons, a fact corroborated by the observation that the basicity of fluorine is significantly diminished when bonded to carbon (74).

Dielectric constant and dipole moment. The dielectric constants of TFE and ethanol are not appreciably

TABLE 2

Effects of TFE on proteins

Serial	Protein (ref.)	$[\Theta]_{222}$ (rel) in 40% TFE[a]
1	Beef heart mitochondria (33)	1.30[e]
2	Red blood cell ghost (33)	3.16[e]
3	Carbonic anhydrase B (37)	4.08
4	α-Chymotrypsin (37)	2.97
5	α-Chymotrypsiongen A (37)	3.67
6	Cytochrome *c* (37)	1.39
7	Immunoglobin C_L fragment (37)	1.69
8	β-Lactoglobin A (37)	3.41
9	β-Lactoglobin B (37)	3.35
10	Lysozyme (37)	1.88
11	Myoglobin (37)	1.05
12	Apomyoglobin (37)	1.33
13	Pancreatic trypsin inhibitor (37)	1.02
14	BPTI (37)	5.04
15	Papain (37)	1.30
16	Ribonuclease A (37)	1.13
17	Serum albumin (bovine) (37)	1.04
18	Staphylococcal nuclease (37)	1.70
19	Subtilisin BPN (37)	1.01
20	Thermolysin (37)	1.13
21	Triosephosphate isomerase (37)	1.40
22	Tryptophan synthase α subunit (37)	1.36
23	Ubiquitin (bovine) (37)	3.89
24	Pulmonary protein SP-B (38)	1.13
25	Pulmonary protein SP-C (38)	1.27
26	CRABP (39)	2.25
27	Troponin C (40)	1.06[d]
28	Hen egg white lysozyme (34)	2.10[b]
29	Bovine myelin basic protein (41)	2.25
30	TMAP lysozyme (42)	3.0
31	Monellin (43)	1.62[b]
32	Ferrodoxin (45)	4.87[c]
33	Thioredoxin (44)	16.0

[a] $[\Theta]_{222}$ (rel) is the relative ellipticity at 222 nm, as seen in the circular dichroism of the protein dissolved in the indicated v/v% of TFE/H_2O, compared to the ellipticity at 222 nm, when the protein was dissolved in water buffer.
[b] In 50% TFE.
[c] In 80% TFE.
[d] In 15% TFE.
[e] In 100% TFE.

TABLE 3

Physical properties of TFE and relevant solvents

Solvent	p*K*	Dielectric constant	Dipole moment (D)	Boiling point[d] (°C)	Density[a]
Water	15.3[a]	78.54[a]	1.84[a]	100	1
TFE	12.4[a]	26.67[a]	2.52[c]	74	1.387
Ethanol	15.93[b]	24.53[b]	1.69[a]	78	0.78
Acetic acid	~3[b]	6.15[b]	1.74[a]	118	1.04
TFA	0.2[a]	8.55[a]	2.28[a]	72.4	1.53

[a] Ref. 75.
[b] Ref. 76.
[c] Ref. 77.
[d] Ref. 66.

different, both being roughly a third of that of water (Table 3) (75). The dipole moment of TFE (2.52 D) (76) is higher than that of ethanol (1.69 D) (77).

Hydrophobicity. The presence of the CF_3 group in TFE serves to enhance the hydrophobicity of the molecule. The 'water repellant' properties of fluorocarbons are well known (78). Studies of micelles with terminal CF_3 groups (79–81), such as 8,8,8-trifluorooctyl methyl sulfoxide and 8,8,8-trifluorooctyl hexaoxyethylene glycol monoether (80), reveal that the CF_3 group can be easily accomodated in micelle interiors (with an increase of only 0.4 kcal/mol in the free energy of micellization). It was inferred that the presence of fluorine in hybrid surfactants (containing a hydrophilic head group and separate fluorocarbon and hydrocarbon chains) favoured the micellization of the surfactant (82). The introduction of a CH_2 group to the hydrocarbon chain reduced the CMC value by about 35%, while a CF_2 group introduced in the fluorocarbon chain lowered the CMC value by 75%. Shinoda and Nomura (83) found that fluorocarbon chains of 8 carbon atoms or longer were necessary to cause phase separation between fluorocarbons and hydrocarbons: while $C_7F_{15}COONa$ mixed with $C_{10}H_{21}SO_4Na$ in all proportions, $C_8F_{14}COONH_4$ mixed only partly with $C_{12}H_{25}SO_4NH_4$. The fluorescence spectra of naphthalene (Np)-labelled hydrocarbons ($NpCOOC_nH_{2n-1}$ or NpHCn) mixed with Np-labelled fluorocarbons ($NpCOOCH_2C_nF_{2n-1}$ or NpFCn) showed a reduction in excimer emission and an enhancement of monomer emission for fluorocarbons having seven methylenes, while addition of fluorocarbons with 12 perfluoromethylenes showed no change in the fluorescence spectrum (84). These findings were interpreted as the formation of coaggregates in the NpFC7 case, while in the case of NpFC12, the mixing was non-ideal. Tung and Ji (84) have also shown that in aqueous organic solvents, fluorocarbons tend to form aggregates at lower concentrations than their hydrocarbon analogs. These facts conclusively demonstrate that introduction of the difluoromethylene unit enhances hydrophobicity in polymethylene chains. The hydrophobic nature of fluoroalkyl groups must be an important feature of fluoroalcohols.

Size. Lastly, steric properties must be considered. The TFE molecule is about nine times the size of the water molecule (85). Beg and Clark found that phenybistrifluoromethylphosphine (in basic medium) could not be converted into bistrifluoromethyldi-

iodophosphorane, while replacing one trifluoromethyl group by a phenyl group made the conversion facile, arguing for the large steric effect of the CF_3 group (86).

Model for TFE-peptide interaction

(a) *Drying out the peptide backbone.* In the light of the facts presented above, we ask the question: how does TFE act in the presence of water to bring about structural changes in peptides? We suggest that the hydrophobicity of the TFE molecule (Fig. 1) plays a crucial role in permitting the cosolvent to home in on the peptide and drive out water molecules attached to the peptide backbone, thus 'drying' it. Otherwise, it becomes difficult to perceive how such drastic changes in structure can be brought about (e.g. refs. 1, 3, 12, 20, 40 and 46) within 10–40% TFE (the typical concentration range in many cases). After all, in a 30% solution of TFE/H_2O the concentration of water molecules is about 10 times that of TFE (assuming the volume changes are additive). The fact that TFE is nine times the size of the water molecules is also important here, as a single molecule of TFE can replace many water molecules within the first hydration shell of the peptide, making a large favourable entropic contribution to the overall free energy of interaction. Further, recent molecular dynamics simulations of the tripeptide Ac-Ala-Ala-Ala-NHMe in TFE/H_2O indicate that it is the CF_3 end of TFE that is closest to the peptide (87).

(b) *Direct interaction of the TFE molecule with the peptide backbone.* Two models can be proposed, regarding the manner of interaction of TFE with the peptide: a 'medium' or thermodynamic effect, where TFE does not directly interact with the peptide and direct interaction of TFE with the peptide. The former was proposed by Conio *et al.* (88) and later reiterated by Storrs *et al.* (6). Dyson (18) has suggested that TFE interactions are due to a lowering of the activity of water in presence of TFE. We, however, propose that TFE mediates its effects by a direct interaction with the peptide backbone, and not by indirectly affecting the structure of the bulk solvent (87, 88). Water is known to disrupt the hydrogen bond between the C=O and N–H groups, and thus destabilize helices (90–93) (Fig. 2). Molecular dynamics simulations of the peptide galanin in water (94) show that in presence of water, two processes occur: First, hydrogen bonding to the carbonyl oxygen takes place. This structure remains stable for a relatively extended period of time. The oxygen of the water molecule then becomes involved in hydrogen bonding with the N–H group. Simultaneously, unfolding of the peptide takes place.

Once TFE displaces the water molecules, the unique H-bonding characteristics of TFE (Fig. 1) permit it to form a H-bond with the carbonyl oxygen without disrupting the intramolecular hydrogen bond (Fig. 2). This is made possible due to the ability of the carbonyl group to engage in bifurcated hydrogen bonding (95). The MD simulations of galanin also indicate the stability of helices in TFE due to the inability of TFE to make a hydrogen bond with the N–H group, thus keeping the intramolecular hydrogen bond intact. The lower dielectric constant of TFE suggests that salt bridges would be more stable in pure TFE than pure water. In the solvent mixture, the preferential solvation of the peptides by TFE can easily result in a lower effective 'local dielectric' in the vicinity of the peptide backbone, leading to stabilization of helices containing potential $i,i+3$ and $i,i+4$ salt bridges.

A wealth of experimental evidence suggests the direct interaction of TFE with amino acids. The quenching of fluorescence in the model tryptophan compound, *N*-acetyltryptophanamide (NATA), by TFE, has been reported (37, 38, 96, 97). Recent studies have shown that the interaction of TFE with

FIGURE 1

The trifluoroethyl alcohol molecule with the three characteristic different regions indicated.

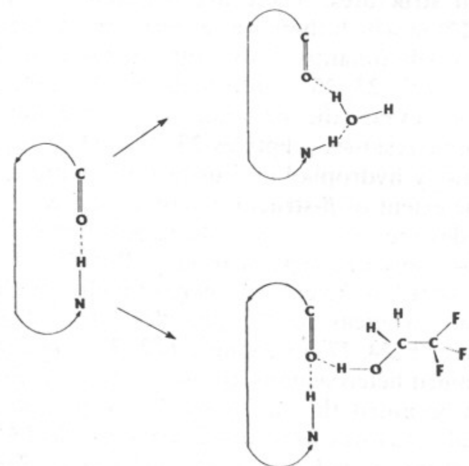

FIGURE 2

Representation of the mode of interaction of TFE with the intramolecular hydrogen bond in the peptide backbone, as compared to that of water. Note that hydrogen bonding by H_2O (top) leads to disruption of the intrapeptide H-bond, while TFE can bind to the C=O (bottom) without disrupting the C=O···H–N interaction.

tryptophan (more specifically, the indole ring of tryptophan) involves complexation in the ground state (97), followed by proton transfer in the excited state (96). The fluorescence of Tyr in presence of TFE is also quenched to a small extent, in contrast to ethanol, which enhances the intensity (97). Interaction of TFE with the aromatic ring of tryptophan (12), hydrogen bonding of TFE with the carbonyl group in poly L-proline (59) and the differential extent of structural stabilization by TFE for peptides with different hydrophobicity (98) provide strong support for TFE directly interacting with the peptides.

Observations of structure stabilization in peptides by TFE

From Table 1 it can be seen that in 19 of the 23 peptides, where a helical structure was induced from a random conformation, aromatic residues are present. Among the others (peptides **28**, **41**, **46** and **48**), two of the peptides (namely, **46** and **48**) have an appreciable (>60%) hydrophobicity. In almost all the peptides which were already helical in solution before the addition of TFE, the hydrophobicity is less than 50%. Hydrophobicity here is not as crucial as in the case of the peptides that were random in solution. In peptides **60–66**, addition of TFE did not produce any secondary structure. In many of these peptides, the number of hydrophobic residues is small and/or there are prolines present, which are known to disrupt helical structures. There are a number of peptides (**22–27**) in which there was a switch in conformation, from predominantly β-structure to random (**24**, **27**) and helical (**22–25**) conformations. A careful examination reveals the presence of a large number of aromatic residues (peptides **23–25**), while peptide **22** is entirely hydrophobic. Further, in peptides **19** and **20** the extent of β-structure actually increased. These peptides are not only wholly hydrophobic, but also contain only one type of residue. Both factors probably contribute to enhancement of hydrophobic interactions, whereas in the peptides where they were weakened (**24**, **27**) or changed (**22–25**), the sequences contained heterogenous aromatic residues. Finally, it must be noted that in many of the peptides where helical structures were stabilized (*e.g.*, **16**, **18**, **39** and **46**), there existed oppositely charged residues at a distance of 3–4 residues from each other. This supports the opinion often expressed in the current literature (1, 2, 23, 32 and 44) that TFE stabilizes helices in those peptides where there already exists helical propensity.

In short peptides, unsurprisingly, β-turns were stabilized (**54–59**). There have been very few cases where β-structures have been enhanced, the only examples being homopeptides with β-branched residues (*e.g.*, Val or Ile) (**19**, **20**) which have high propensities to form β-structures. The overwhelming wealth of examples in Table 1 are ones where there has been either an enhancement of already existing helicity or an induction of helicity (in peptides that were random in water), on addition of TFE. While sequence effects undoubtedly play a role in determining the secondary structure content, addition of TFE tilts the balance in favour of helices, in the cases where strong structure disrupting residues or side-chain interactions are not present.

Dramatic examples, however, exist for sequence specific effects of TFE. Filippi *et al.* (98) studied a series of *S*-peptide analogs, with different residues (F, Y, I, A, cyclopentylglycine (cpG), G) at position 8. They found that the extent of helicity varied as $G^8 < Y^8 < F^8 < cpG^8 < A^8 < I^8$, in direct correspondence with the hydrophobicity of the peptide. Lehrman *et al.* (1) attempted to correlate predicted and observed helicity in a 10% TFE/H_2O solution, among a series of 11 peptides spanning the primary sequence of the bovine growth hormone. For 8 of the 11 peptides, this correlation was found to be satisfactory (90%), while it was off the mark (60%) when three of the peptides were included. An examination revealed that these peptides were significantly more hydrophobic than the other members of the class. The experiments of von Stosch *et al.* revealed that the presence of the tryptophan residue, at position 12 of a 15 residue peptide fragment from the principal CD4-binding domain of gp20, was crucial in determining whether the cooperative transition from β-sheet to helix in the presence of 60% TFE took place or not (12).

Observations of structure stabilization in proteins by TFE

Urry *et al.* (33) were among the first to study the effect of TFE on the conformation of proteins. They found the helical content of proteins from beef heart mitochondria and red blood cell ghosts (as measured by the ellipticity at 222 nm in the CD experiment), to increase in the presence of 80% TFE. The increase in Θ_{222} was greater when the proteins were sonicated. This observation, along with the loss of the near-UV circular dichroism (indicating loss of tertiary structure), has been a general observation of experimentalists who measured the circular dichroism of proteins in water and in water/TFE mixtures. In Table 2 the Θ_{222} values of several proteins have been compiled, relative to the ellipticity in water. In each of the 34 proteins, there is an increase in the helical content; for some, such as bovine serum albumin and subtilisin BPN′, the helicity induced is very small, but in many cases the helicity increases considerably. There have also been examples where β-sheet proteins changed to predominantly helical conformations in TFE/H_2O mixtures. The concentrations of TFE required, however, was different for different β-sheet proteins. Whereas the cellular retinoic acid binding protein (CRABP) became helical between 15–30%

TFE, monellin required a 50% TFE/water mixture to become helical, while for concanavalin A, pure TFE, or greater than 90% TFE in H_2O, was needed for helix induction.

Dobson and coworkers reported (34), for the first time, NMR characterisation of a protein, namely lysozyme, in TFE/H_2O mixtures. The NMR data indicated a loss in the tertiary structure of lysozyme, with six regions of the polypeptide chain adopting helical structures. Five of these were native helices, while the sixth was a non-native helix (which formed a β-structure in the native state) with remarkably lower H-D protection factors as compared to the other five helices. Their data were consistent with the formation of a 'molten globule'. The TFE state of lysozyme was indeed unique, for molten globules could not be detected for lysozyme in presence of other denaturants like urea and guanidinium chloride.

Lastly, TFE has been shown to modulate quaternary interactions in proteins. Slupsky *et al.* (40) studied the effect of TFE on the dimerization of the muscle regulatory protein, troponin C, whose dissociation constant for the dimerization in water is 0.4 mM at 20 °C. On addition of just 10% of TFE, the dissociation was seen to increase 10-fold (to 4 mM). Remarkably, the protein did not lose its structure; there was only a small increase in the helicity of the protein. Similar observations of quaternary denaturation were recorded by Lau *et al.* (47, 48). The case of the human platelet factor subunit (PF4) is quite different (46). This protein exists as a tetramer, with an association rate of $18\ s^{-1}$ at room temperature, neutral pH. Addition of TFE caused a dramatic increase in the association rate, to $1000\ s^{-1}$, which is the Stokes–Einstein diffusion limit.

CONCLUSIONS

The structure stabilizing property of TFE in aqueous solutions arise from two important features of the fluoroalcohol.

(a) Hydrophobicity, conferred by the trifluoromethyl group (78–84), which permits TFE molecules to interact with hydrophobic side chains in unfolded peptides. The replacement of the hydration shell facilitates intramolecular hydrogen bond formation between peptide amide groups. The significantly larger size of TFE as compared to H_2O (85) provides an entropic bonus when water molecules are replaced by fluoroalcohols.

(2) The unique hydrogen bonding properties of TFE are particularly suited for its role as a structure stabilizer. The ability of TFE to serve as a much better H-bond donor than water or ethanol (53–56, 58–65), and its much lower H-bond accepting capabilities (66–70), play a crucial role. If both hydrogen-bond donor and acceptor tendencies are approximately equal, as in the case of water and ethanol, the solvent tends to interact with both the C=O and the N–H groups, resulting in a disruption of intramolecular hydrogen bonding (90–93). In contrast, TFE interacts largely with carbonyl groups, which have the ability of forming bifurcated hydrogen bonds without the necessity of breaking the C=O···H–N intramolecular interactions (95).

In globular proteins, the effects of TFE are likely to be a consequence of the incorporation of fluoroalcohol molecules in protein interiors. The ability of TFE to interact specifically with aromatic residues, like Trp (12, 96, 97) and Tyr (97), may be of special importance in rationalizing TFE effects on proteins like lysozyme. Entry of fluoroalcohols into interiors may result in altered packing of internal sidechains with enhancement of mobility, resulting in apparent loss of tertiary interactions as monitored by near-UV circular dichroism. The amphipathic properties of TFE suggest that effects on proteins are likely to depend critically on the nature of the system studied. The apparently contradictory effects of TFE on quaternary structures as manifested by dissociation in the case of troponin C (40) and enhanced association rates in the case of PF4 (46) may be rationalized in terms of the differences in the nature of molecular interactions at the protein–protein interfaces. These can vary widely, from interfaces which are stabilized extensively by hydrophobic contacts to cases where the interacting residues are largely polar in nature (99). The effects observed with TFE suggest that detailed investigations of other fluorinated solvents may prove fruitful in developing solvent based approaches to modulating peptide and protein structures. A detailed understanding of solvent effects should provide a convenient approach towards rational stabilization of specific structures in peptides and proteins. Promising results have also been obtained in controlling proteolysis in studies of lysozyme (100) and cytochrome *c* (101) in TFE-containing solutions.

Note added in proof: A discussion of the mechanism for helix stabilization by TFE has appeared while this manuscript was in press (102).

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