Trifluoroethanol induces the self-association of specific amphipathic peptides

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Received 28 August 1997

Abstract We have examined the effect of trifluoroethanol (TFE) on the solution behaviour of three amphipathic peptides. One of the peptides, containing three heptad repeat units (Ac-YS-(AKEAAKE)$_3$GAR-NH$_2$), remained monomeric under conditions where TFE induced a two-state transition from a random coil to an α-helix. In contrast, the TFE-induced α-helical formation of two peptides derived from human apolipoproteins C-II and E was accompanied by the formation of discrete dimers and trimers, respectively. The apolipoprotein C-II peptide further aggregated to form β-sheet at higher concentrations of TFE (50% v/v). The results suggest a class of peptides capable of discrete self-association in the presence of cosolvents which favour intramolecular hydrogen bonding.

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Key words: Apolipoprotein; α-Helical peptide; Lipid-peptide interaction; Trifluoroethanol; Circular dichroism; Analytical ultracentrifugation

1. Introduction

The propensity of specific amino acid sequences to adopt secondary and higher order structures can be explored using model peptides and solution conditions which mimic typical environments found within proteins. Trifluoroethanol (TFE) has been widely used as a cosolvent to mimic the microsolvation effects created by long-range interactions within protein tertiary structures [1,2]. The importance of such interactions is illustrated by recent studies showing environment-dependent folding of an amino acid sequence located at different positions within the same protein sequence [3]. TFE provides a more hydrophobic and less basic environment than that existing in aqueous solution. The decreased capacity of TFE to accept hydrogen bonds is considered to weaken the interactions between peptide and solvent leading to stronger intramolecular interactions and increased formation of secondary structure [4,5]. TFE also affects tertiary and quaternary interactions in proteins and peptides. For hen egg-white lysozyme [6], TFE generates a partially denatured form resembling a molten globule. Zhou et al. [7] observed the monomerisation of a dimeric α-helical coiled-coil in solvents containing 50% TFE.

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Abbreviations: TFE, 2,2,2-trifluoroethanol; SDS, sodium dodecyl sulphate; apo C-II, apolipoprotein C-II; apo E, apolipoprotein E; TFA, trifluoroacetic acid; MRE, mean residue ellipticity; CD, circular dichroism

We considered the possibility that promotion of helix formation by TFE may facilitate self-association of amphipathic helices to form coiled-coil structures. We examined three amphipathic α-helices. One contained three heptad repeats similar to those found in a secreted polymorphic malarial antigen [8] while the other two were based on sequences found in human apolipoprotein (apo) C-II and apo E corresponding to A and G* class helices respectively [9]. TFE promoted α-helix formation in all three peptides and in the case of the apo C-II and apo E peptides generated discrete dimers and trimers respectively.

2. Materials and methods

2.1. Peptide synthesis and purification

Peptide C (apo C-II$_{19-39}$ Ac-KESLSSYWESAKTAAQDLYEK-NH$_2$; M_r = 2475 [10]), corresponding to the indicated sequence in mature human apo C-II with the addition of an acetylated N-terminus and an amide group at the C-terminus and peptide E (apo E$_{263-286}$ SWFEPLVEDMQRQWAGLVEKVQAA, M_r = 2818 [11]) corresponding to the indicated sequence in mature human apo E, were synthesised by solid-phase peptide synthesis using an Applied Biosystems model 431A peptide synthesiser. Rink resin and F-moc amino acids were purchased from AusPep (Victoria, Australia), and Wang (HMP) resin was purchased from NovaBiochem. Crude peptide C was purified on a Brownlee semi-preparative reversed phase C-18 HPLC system employing a linear gradient of 0.5% min^{-1} of 20–40% acetonitrile containing 0.1% TFA at 4 ml/min to elute the peptide. Fractions were analysed by MALDI-TOF mass spectrometry and the desired fractions pooled and lyophilised three times from water. Peptide E was purified by reversed-phase FPLC on a 3 ml Resource column (Pharmacia) equilibrated in 0.1% (v/v) TFA at a flow rate of 1 ml/min and eluted with a 50 ml gradient of 0–100% acetonitrile, 0.1% TFA. Desired fractions were identified by MALDI-TOF mass spectrometry, combined and re-purified on a 3 ml Resource column, with a 10 ml, 0–100% gradient of acetonitrile containing 0.1% TFA, lyophilised and stored at −20°C. Peptide H3 (Ac-YS(AKEAAKE)$_3$GAR-NH$_2$; M_r = 2777) with an acetylated N-terminus and an amide group at the C-terminus was kindly provided by Robin Anders (Walter and Eliza Hall Institute for Medical Research, Melbourne) and synthesised using procedures similar to those described elsewhere [8]. Purity was assessed by mass spectrometry.

2.2. Determination of the densities and viscosities of aqueous mixtures of TFE

Density measurements of mixtures of 2,2,2-trifluoroethanol (purchased from Sigma, St. Louis, MO) and water were performed at 20°C using an Anton Paar DMA02C density meter. The variation in density (ρ) with increasing TFE content followed the relation

$$\rho = 0.9982 + 5.0242x - 0.1096x^2 \quad (1)$$

where x denotes the volume fraction of TFE per total volume of added water and TFE. Viscosities of aqueous mixtures of TFE and water were measured at 20°C with a Schott capillary viscometer. All viscosities (η) were measured relative to water and methanol standards [12]. The viscosities of aqueous mixtures of TFE followed the relationship

$$\eta = 0.9355 + 2.04x + 1.355x^2 - 7.532x^3 + 4.464x^4 \quad (2)$$

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PII S0014-5793(97)01224-6

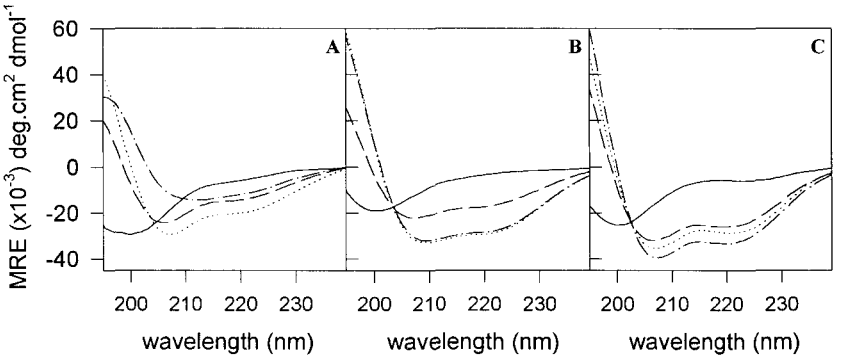

Fig. 1. Circular dichroism spectra of peptides C (16 μM, panel A), E (14 μM, panel B) and H3 (16 μM, panel C) in 0 (solid line), 20 (dashed line), 30 (dotted line) and 50 (dash-dotted line) % (v/v) trifluoroethanol and 0.1 M KF, 20 mM potassium phosphate buffer, pH 7.4.

2.3. Circular dichroism

Circular dichroism spectra were recorded at 20°C on an AVIV 62DS model spectrometer, using a 1 mm pathlength quartz cuvette. The instrument was routinely calibrated with an aqueous solution of *d*-10-camphorsulphuric acid. Ellipticities are reported as mean residue ellipticity (MRE) in deg cm^2/dmol. Peptide concentrations were determined by absorption spectroscopy at 280 nm with extinction coefficients calculated according to Gill and von Hippel [13]. The α-helicity was calculated using the equation described by Chen et al. [14], which takes into consideration the chain-length dependence of helices. The calculated maximum MREs at 222 nm for peptides H3, C-II, and E were 35 596, 34 666 and 35 270 deg cm^2/dmol, respectively. The proportion of β-structure in peptide C at high concentrations of TFE was determined by fitting the CD spectrum between 190 and 240 nm to the sum of contributions from four structural species: α-helix, β-sheet, random coil and β-turn. The sample spectra used for the fitting procedure were derived from Yang et al. [15].

2.4. Analytical ultracentrifugation

Sedimentation experiments were conducted using a Beckman XL-A analytical ultracentrifuge equipped with absorption optics, an An60-Ti rotor and either conventional or synthetic boundary double-sector charcoal-filled epon centrepieces containing quartz windows. Sedimentation coefficients (s) were calculated by direct fitting of sedimentation velocity boundary data by numerical integration of the Lamm equation (Schuck, MacPhee and Howlett, in preparation). These values were corrected for the density (ρ) and viscosity (η) of aqueous mixtures of TFE to obtain $s_{20,w}$ values. Sedimentation equilibrium experiments were conducted at 20°C and 40 000 rpm for approximately 15 h. At equilibrium, scans of the solution column were acquired at three different wavelengths. In the absence of TFE, the rotor speed was then increased to 50 000 rpm to allow simultaneous fitting of data at 40 000 and 50 000 rpm to obtain the molecular weight of the peptide and the contribution of non-sedimenting species to the baseline optical density. In all cases the baseline optical density contributed less than 5% of the total optical density. The data at three wavelengths and two speeds were analysed using the program SEDEQ1B (kindly provided by Allen Minton, Bethesda, MD). Values for the molecular weights (M) and partial specific volumes ($\bar{v}$) of the peptides were calculated from the amino acid composition [16].

3. Results

The circular dichroism spectra of peptides C, E and H3 were measured in aqueous mixtures of TFE ranging from 0 to 60% (v/v). Peptides E and H3 (Fig. 1B,C) both show a two-state transition from random coil to α-helix, with a characteristic isodichroic point at approximately 203 nm. In contrast, peptide C (Fig. 1A) shows a complex transition, suggesting a conversion from random coil to α-helix and to β-sheet at high TFE concentrations. Fig. 2 shows the variation in α-helix content with TFE concentration. Peptides E (open triangles) and H3 (open circles) show a steady increase in α-helical content with TFE concentration, approaching a maximum of 80–90% at approximately 30% TFE (v/v). The behaviour of peptide C (filled circles) parallels that of the other two peptides at low TFE concentrations while the α-helix content decreases above 40% TFE, concomitant with the formation of β structure (approximately 40% β-sheet in 50% (v/v) TFE).

Fig. 3 shows the sedimentation equilibrium distributions of peptide C, E and H3 in 0.1 M KF, 20 mM potassium phosphate buffer pH 7.4 and in the presence and absence of TFE (30%). The data are presented as plots of ln(absorbance) vs radius^2 and normalised for the density of TFE. The linearity of the data indicates molecular weight homogeneity. Molecular weight values for the peptides determined from non-linear regression of the data are summarised in Table 1. The molecular weight values for peptide H3 in the presence and absence of TFE are close to the values expected for the monomeric form of the peptide. The values obtained for peptides C and E suggest oligomerisation of the peptides in TFE to form discrete dimers and trimers respectively. Molecular weight values for peptides C and E in the presence of 30% TFE were determined over a range of starting concentrations from 0.04 to 0.5 mg/ml. Analysis of the data showed no significant dependence of molecular weight on concentration suggesting stable, high affinity complexes. The small difference between the measured molecular weights and the predicted molecular weights of the oligomers may indicate the presence of a small fraction of non-associating peptide or result from a direct effect of TFE on the partial specific volume of the peptides. This latter effect, which might arise from preferential interac-

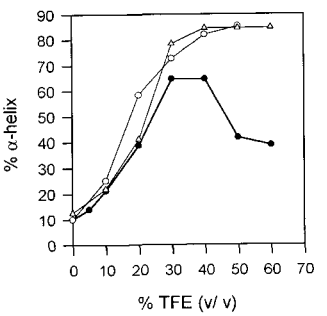

Fig. 2. The variation in the α-helix content of peptides E (open triangles), H3 (open circles) and C (filled circles) with TFE concentration. The ellipticity was derived from the TFE titrations in Fig. 1; the ellipticity at 222 nm was converted to percentage α-helix using the value calculated for the maximum ellipticity for a helical peptide of a given length (see Section 2).

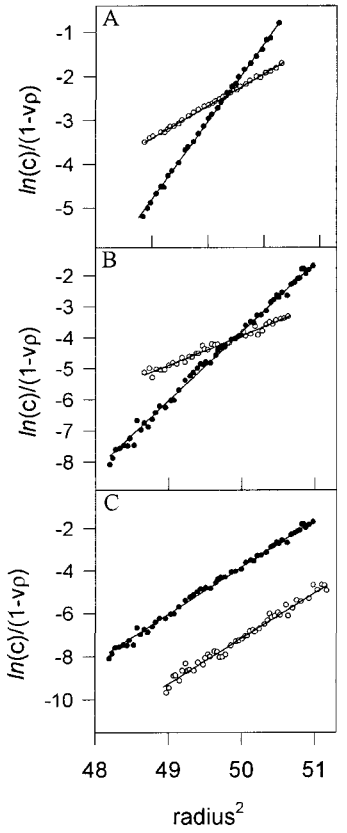

Fig. 3. Sedimentation equilibrium profiles for peptides E (14 μM, panel A), H3 (16 μM, panel B) and C (16 μM, panel C) in 0.1 M KF, 20 mM potassium phosphate buffer, pH 7.4, and in the presence (open triangles) and absence (open circles) of 30% (v/v) TFE. The data are normalised for the effect of the density (ρ) of TFE and are presented as plots of ln(c) vs radius^2 where the radial position is in cm and c represents the absorbance at 240 nm.

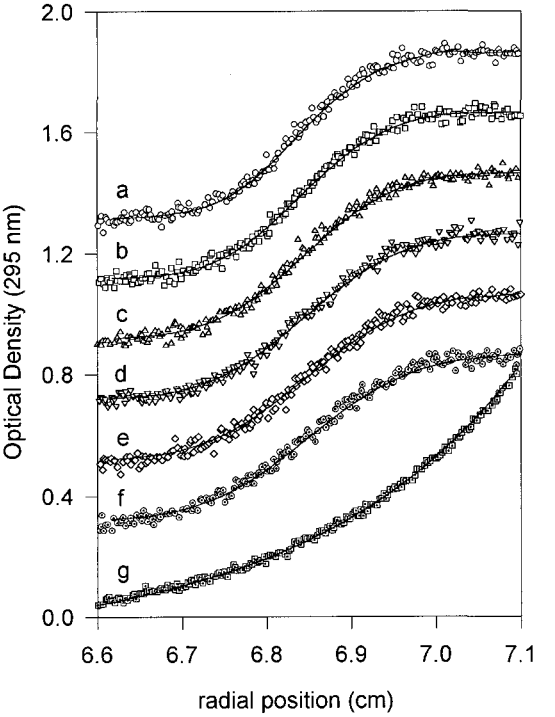

Fig. 4. Sedimentation velocity scans acquired at 15 min intervals at 40 000 rpm for 0.2 mM peptide E in 30% (v/v) TFE containing 0.1 M KF, 20 mM potassium phosphate buffer, pH 7.4. Scans (a–g) were acquired at 15, 30, 45, 60, 75, 90 and approximately 1080 min after the start of centrifugation. The first scan has been offset from zero by 1.2 optical density units. Scans b–f have been offset by 1.0, 0.8, 0.6, 0.4 and 0.2 optical density units respectively. The final scan, at 18 h, represents the peptide at equilibrium. The solid lines are simulations for the best-fit value of $s = 0.97$ S and a constrained value of the buoyant molecular mass, M(1−$\bar{\nu}\rho$), of 1197.

tions of peptide with solvent in solutions of TFE and water, would be expected to apply similarly to the three peptides studied.

The oligomers were further characterised by sedimentation velocity experiments. An important advantage of the sedimentation velocity approach is the ability to constrain the fitting procedure to values of the buoyant molecular mass values, M(1−$\bar{\nu}\rho$), obtained from sedimentation equilibrium analysis. This strategy avoids the need to assign independent values to $\bar{\nu}$, yielding sedimentation coefficients directly. The results in Fig. 4 show sedimentation velocity profiles for peptide E in the presence of 30% TFE at various times after the start of centrifugation. The fitted lines through the data assume a single sedimenting species. Sedimentation velocity data for peptide E in the absence of TFE, and peptides C and H3 in the presence and absence of TFE also fit to a single species (Table 1). The increases in the sedimentation coefficients of the peptides in the presence of TFE can partly be attributed to the formation of more compact helical structures as indicated by the results in Fig. 1. The much larger increases observed for peptides C and E compared to peptide H3 are best explained by a TFE-induced self-association of these peptides.

Table 1
The hydrodynamic properties of amphipathic peptides in the presence and absence of TFE

Peptide	Concentration of TFE in % (v/v)	MW predicted[a]	MW expected[b]	$s_{20,w}$ (S)[c]
C	0	2475	2560	0.46
C	30	(4950)	4650	1.19
E	0	2818	2840	0.47
E	30	(8450)	7940	0.97
H3	0	2777	2770	0.45
H3	30	2777	2830	0.52

[a]The predicted molecular weight of the peptide based on the amino acid sequence. The predicted molecular weights of self-associated species assuming dimer and trimer for peptides C and E, respectively, are shown in parentheses.
[b]The molecular weight of the peptide from sedimentation equilibrium data. Values of $\bar{\nu}$ used in the analysis were calculated from amino acid composition [16]. These values were 0.723, 0.738 and 0.730 ml/g for peptides C, E and H3 respectively.
[c]The sedimentation coefficients in svedberg units ($S = 10^{-13}$/s) are reported as $s_{20,w}$ values, corrected for the viscosity and density of the solvent.

4. Discussion

The addition of TFE to peptide H3 induces α-helix formation under conditions where the peptide remains monomeric. This behaviour may be compared with that of a similar amphipathic peptide E(AEKAAKE)$_2$AEK where the heptad repeat differs from peptide H3 only in the order of the lysine and glutamate residues in the b and c positions [17]. While

this peptide contained 65% α-helical structure in solution it was also monomeric in the presence and absence of TFE. Replacement of the alanine by leucine in this peptide caused self-association of the peptide in aqueous solution, an effect prevented by the addition of 25% TFE. The ability of TFE to prevent peptide association has also been observed for other amphipathic peptides. For the peptide series Ac-$(KLEALEG)_n$-K-NH_2 where n=4 or 5, the presence of dimers in 0.1% aqueous trifluoroacetic acid was prevented by the addition of TFE under conditions where the α-helical content was conserved [18]. These studies, which are similar to the results obtained with peptide H3 (Figs. 1–3), lead to the view that TFE stabilises α-helices in the monomeric state. The ability of TFE to stabilise monomeric α-helical peptides was attributed to the partially hydrophobic nature of TFE which stabilises the hydrophobic face of the helix while the weaker basicity of TFE decreases hydrogen bonding of amide protons with the solvent leading to a strengthening of intramolecular hydrogen bonds.

For peptides C and E, TFE induced α-helix structure and the formation of dimers and trimers respectively. These results differ from the results described above where TFE disrupted tertiary and quaternary interactions. Multimeric amphipathic peptide complexes in aqueous solution have been extensively studied and, in the case of GCN4 leucine zipper derivatives, rules have been developed to predict the state of oligomerisation [19,20]. The structural basis for the TFE-induced self-association of peptides C and E to form discrete complexes is currently unclear. These peptides are typical of the amphipathic repeating units within apolipoprotein sequences. Peptide C is classified as a 'class A' helix characterised by negative amino acids in the centre of the polar face and positive amino acids at the polar-non-polar interface whereas peptide E is a 'G* helix', which possesses a more random distribution of charges on the polar face, and is typical of helices found in globular proteins [9]. These amphipathic regions are considered key structural determinants of the interactions of apolipoproteins with lipid surfaces. Circular dichroism studies indicate both peptides E [11] and C (results not shown) form a high degree of α-helicity upon binding to model lipid vesicles. In addition, NMR studies show peptide E binds to SDS micelles forming a helix-bend-helix structure [11]. One explanation for the effect of TFE on peptides C and E is that these sequences have evolved to present a hydrophobic face to the lipid surface with ionic interactions mediating intrachain interactions at the lipid surface. A similar model has been proposed to describe the peptide-induced formation of phospholipid discs, in which extensive self-association involving ionic interactions between peptides stabilises the edges of a lipid bilayer structure [21,22]. In the absence of lipid, TFE may promote these ionic interactions with the formation of discrete peptide oligomers.

Peptide C is monomeric, random coil and stable in aqueous solution, and is dimeric and α-helical in aqueous solutions containing 30% (v/v) TFE. However, in high concentrations of TFE (50% v/v), the peptide acquires a high proportion of β-sheet structure (Fig. 1). CD spectroscopy and turbidometric studies show no indication of the formation of large, insoluble aggregates; however, analytical ultracentrifugation of the peptide in the β-sheet conformation indicates the formation of sufficiently high molecular weight complexes as to rapidly sediment out of solution. This is similar to a result observed by Zhang et al. [23], studying peptides derived from the prion protein PrP. PrP_{90-145} is random coil in low salt media, but at physiological concentrations of salt, or in the presence of acetonitrile, the peptide forms oligomeric β-sheet structures. In both cases the acquisition of secondary structure appears to drive the formation of aggregates. These results for peptide C suggest a relationship between the acquisition of α or β structure and the nature of the oligomers or aggregates formed.

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