Acceleration of Short Helical Peptide Conformational Dynamics by Trifluoroethanol in an Organic Solvent

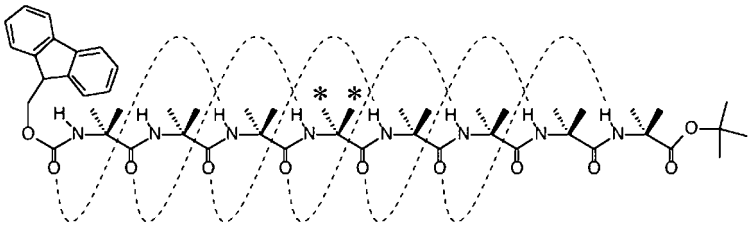
Matthew Kubasik* and Adam Blom[a]

It has been known since the 1960s that small amounts of the cosolvent 2,2,2-trifluoroethanol (TFE) can have dramatic effects on the conformational stability of peptides and proteins in solution.[1] Recent reviews conclude that no single mechanism accounts for all of the observed effects of TFE on biomolecular structures.[2,3] As a minor cosolvent, TFE is known to enhance the helical content of short peptides predisposed towards helical conformations.[4] At high concentrations, however, TFE appears to disrupt native protein structures. The primary mechanism for the helix-stabilizing effect on peptides is thought to be the displacement of water molecules,[2,5] which enhances intramolecular hydrogen bonding. (TFE is a larger molecule than water and is also known to be a better hydrogen-bond donor but a weaker hydrogen-bond accepter than water.[6]) Enhanced water/TFE solvent structure is a second suggested mechanism for TFE's enhancement of helical structure in short peptides.[7] In this mechanism, the unfavorable energetic cost of disrupting solvent structure to solvate the exposed peptide backbone leads to greater helical conformational stability. A third mechanism is grounded in the suggestion that helix-stabilizing intramolecular electrostatic interactions are enhanced by the reduction in the solvent dielectric constant afforded by TFE, which elevates the importance of helix-stabilizing intramolecular electrostatics. However, the electrostatic significance of TFE as a cosolvent has been cast into doubt due to its lack of significant effects on stability versus pH curves for a 19-mer peptide.[8]

We have used a kinetics experiment to investigate the effect of TFE on the structural stability of a highly helical peptide system in the low-dielectric organic solvent methylene chloride ($\varepsilon \sim 9$). Our peptide is an α-aminoisobutyric acid (Aib) octamer, Fmoc-Aib$_8$-O*t*Bu (Scheme 1; Fmo*c* = fluorenylmethoxycarbonyl). Aib Oligomers are known to exist in stable 3_{10} helices at the octamer and higher polymeric levels, ok.[9,10] Peptides containing Aib residues have shown remarkable thermal stability;[11] their helical conformations display resistance to "melting" at elevated temperatures.[12] 3_{10} helices make up about 10% of the helices observed in protein crystal structures and are characterized by a *i*/*i*+3 hydrogen bonding pattern in contrast to the *i*/*i*+4 hydrogen bonding pattern of the more common α-helix.[13] Scheme 1 shows the peptide structure studied in this work and its 3_{10} hydrogen-bonding pattern.

[a] *Prof. M. Kubasik, A. Blom*
Department of Chemistry, Fairfield University
1073 N. Benson Rd, Fairfield, CT 06824 (USA)
Fax: (+1) 203-254-4034
E-mail: mkubasik@mail.fairfield.edu

 DOI: 10.1002/cbic.200400198



Scheme 1. The structure of the Aib peptide octamer used in this work showing the six (*i*) C=O···H–N (*i*+3) intramolecular hydrogen bonds of the 3_{10} helical structure. The β-carbons of the fourth Aib residue are ^{13}C-enriched. The NMR resonance from the labeled carbons dominates the ^{13}C spectrum and broadens due to conformational exchange. The methyl carbons of the C-terminal *tert*-butyl group are insensitive to the peptide's conformational exchange.

Because the Aib residue is achiral, oligomers of Aib exist in a 50:50 mixture of left- and right-handed helices (Scheme 1). The racemic nature of Aib helices makes their helicity undetectable by circular dichroism spectroscopy. Furthermore, these enantiomeric helices interconvert on a millisecond timescale.[14] We have measured the rate of helix/helix interconversion by NMR spectroscopy in CH_2Cl_2 and in a TFE/CH_2Cl_2 solution (Figure 1). Although this system is composed of stable 3_{10} helices, the peptide is conformationally labile, and the rate of interconversion between helices is an indication of the stability of the helix.

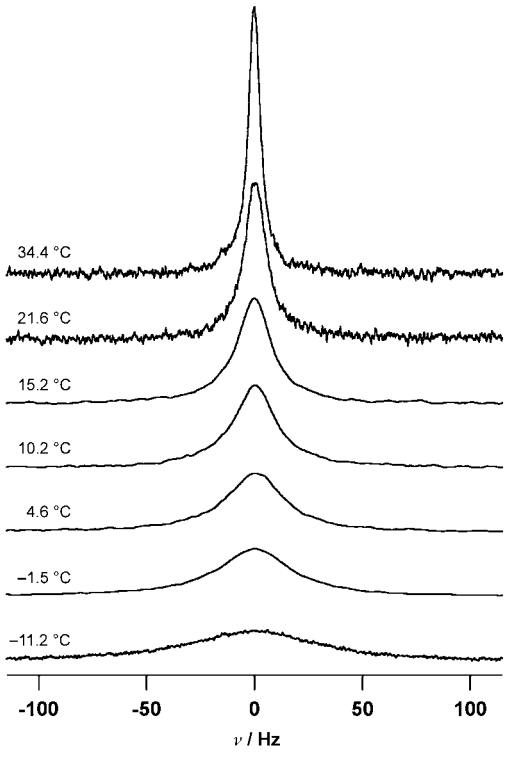


Figure 1. ^{13}C NMR data of the peptide octamer in 5% TFE in CH_2Cl_2 (*v*/*v*). These data are representative of peptide data obtained at all other TFE concentrations. The spectrum at 34.4 °C is already exchange-broadened (FWHM ~6.6 Hz) as compared to the ~0.8 Hz width of the feature belonging to the nonexchanging methyl carbons of the C-terminal O*t*Bu ester (not shown). As the temperature is lowered, the rate of exchange slows, and this diminishes the averaging of β-methyl carbon signals.

We have enriched an octameric peptide in ^{13}C at the β-carbons of the fourth Aib residue. The ^{13}C NMR spectrum of this peptide is dominated by the labeled β-carbons. Interconversion of the helix handedness changes the magnetic environment of the β-carbons, which broadens the NMR resonance (Figure 1).[14,17] Assuming a fully cooperative, two-state mechanism, the rate of chemical exchange for the two methyl groups indicates the rate of helix/helix interconversion. Note that we are not characterizing helix→random coil kinetics, rather a conformational change between two well-defined helical states. Analysis of the line width of the labeled-carbon resonance, compared to the line width of the methyl carbons of the C-terminal *tert*-butyl ester group, which is insensitive to the interconversion, indicates the rate of interconversion (see Experimental Section).

The rate of right-/left-handed helix interconversion increases monotonically with increasing amounts of TFE in solution (Figure 2). However, the catalytic effect of TFE does not show a simple concentration dependence. The acceleration of the rate by TFE appears to saturate, and this saturation displays a temperature-dependence. At high temperatures, the rate acceleration appears to be near saturation at 10% TFE, while at low temperatures only about 5% TFE nearly saturates the effect.

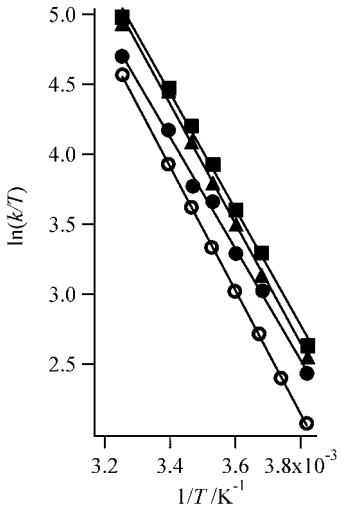


Figure 2. Eyring plots for octameric Aib peptide enantiomerization in CD_2Cl_2/TFE mixtures: ○ 0% TFE; ● 5% TFE; ▲ 10% TFE; ■ 30% TFE. Rate constants obtained through comparison of the widths of broadened β-methyl resonances undergoing conformational exchange to the line width of a methyl resonance not undergoing exchange (see Experimental Section).

Eyring plots of 0, 5, 10, and 30% (*v*/*v*) TFE in CH_2Cl_2 are shown in Figure 2. The lack of curvature in the plots indicates the absence of any enthalpically significant mechanistic change over the temperature range studied (261–307 K). The values for $\Delta H^{\ddagger}$ from the Eyring plots (Figure 2 and Table 1) show no definitive trend in TFE concentration. Although there is an initial decline in $\Delta H^{\ddagger}$ from 0% to 5% TFE, $\Delta H^{\ddagger}$ climbs

Table 1. Rate constants ($T = 30.5\,^{\circ}C$) and enthalpies of activation for helix/helix interconversion for the Aib octamer in CD_2Cl_2 and CD_2Cl_2 solution which contains TFE.

% TFE[a]	k [s^{-1}][b]	$\Delta H^{\neq}$ [$kJ^{-1}\,mol^{-1}$][c]
0	29600	36.73
5	33900	33.14
10	42600	35.56
30	44700	34.49

[a] *v/v* TFE in CD_2Cl_2. [b] $T = 30.5\,^{\circ}C$. [c] Obtained from Eyring equation: $k = k_B T/h \exp(\Delta S^{\neq}/R) \exp(-\Delta H^{\neq}/RT)$ and Figure 2. For the octamer in neat CH_2Cl_2, extending the temperature range to 200 K results in a slightly higher $\Delta H^{\neq}$ of 37.8 $kJ\,mol^{-1}$.

again in 10% and 30% TFE. A qualitative interpretation is that TFE exhibits a differential influence on the energetics of the ground conformational state and transition states in this small peptide system. This interpretation is consistent with the observations of Dobson et al., who found an initial increase and then a slowing of the folding kinetics of acylphosphotase with increasing TFE concentration (0%–18% in D_2O).[15] Preferential solvation of a peptide tetramer by TFE, but not by ethanol, in aqueous solution has been demonstrated by intermolecular NOE studies.[16]

In our system, the interconversion of helix handedness requires no amide-bond isomerization but it does require the breaking and reforming of all six intramolecular hydrogen bonds. In a previous work,[17] we presented evidence for a helix/helix-interconversion mechanism in which peptides "zipper" from one form of handedness to another, breaking and then reforming a very small number of intramolecular hydrogen bonds at a time. A plausible mechanism for the zippering between left- and right-handed helices would involve successive "flips" of the peptide-bond unit and concerted rotations of ψ_i and φ_{i+1}.[18] Such "flips" have been identified among protein conformers found in X-ray crystal structures.[19] The transition state of a "peptide-flipping" mechanism would allow a hydrogen-bonding cosolvent to reduce the enthalpic barrier for interconversion, $\Delta H^{\neq}$, through hydrogen bonding to groups exposed in the transition state. This effect could be expected to saturate at low TFE concentration. Disruption of ground-state helical conformation by TFE cosolvent in CD_2Cl_2 solution, which would also lower $\Delta H^{\neq}$, might be expected to saturate at a different TFE concentration and lead to the nonmonotonic changes observed for $\Delta H^{\neq}$.

Another mechanism for TFE to destabilize the helical-conformational ground state in our system is by increasing the solvent dielectric constant ($\varepsilon_{TFE} = 27$, $\varepsilon_{CH_2Cl_2} = 9$). This would tend to diminish the electrostatic contribution to the stabilization of the peptide helix. However, the stability of Aib-based peptides in DMSO,[20] which is a relatively high dielectric constant solvent ($\varepsilon_{DMSO} = 46$), suggests that electrostatic contributions are not sufficiently disrupted to alter the conformational preferences of the Aib octamer.

TFE-induced changes in activation entropy, $\Delta S^{\neq}$, could also have significant effects on interconversion rates. Based on the activated complex theory,

$$k = \frac{k_b T}{h} \exp\left(-\Delta G^{\neq}/RT\right)$$

where

$$\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$$

Entropy effects are often very subtle, and due to the comparatively limited temperature range used in this study, we have not attempted to determine quantitative $\Delta S^{\neq}$ values at the various TFE concentrations. Qualitatively, however, the Eyring plots suggest that the kinetics in 5% TFE solution will have the lowest, most negative $\Delta S^{\neq}$, whereas the 10 and 30% solutions will have less negative $\Delta S^{\neq}$ values than neat CH_2Cl_2. The $\Delta S^{\neq}$ for the octamer in neat CH_2Cl_2 has been previously measured as $-37\,J\,K^{-1}\,mol^{-1}$ over a temperature range of 200 K to 315 K.[17] It is possible that at low TFE concentrations, the ordering of TFE solvent around an exposed amide in the transition state leads to a *more* negative value of $\Delta S^{\neq}$. However, increasing concentrations of TFE could broaden the peptide's transition state ensemble for interconversion, eventually making $\Delta S^{\neq}$ *less* negative than in neat CH_2Cl_2.

Although elegant time-resolved ultrafast studies of helix formation and propagation in helical 21-mer peptides exist,[21] we are aware of none that investigates the effects of TFE on the dynamics of helix initiation and propagation. Mayo and coworkers investigated the effect of TFE on the equilibrium dynamics of a partially folded α-helix-forming and a β-hairpin-forming peptide.[22] Interestingly, TFE exhibits a catalytic effect for the folding/unfolding of the α-helix but slows the folding and unfolding rates in the β-hairpin peptide. Our work contrasts with these elegant experiments in that the current study does not characterize helix→random-coil kinetics. We have characterized the influence of TFE on the rate of conformational exchange between two dominantly structured, isoenergetic, enantiomeric states.

The influence of TFE on the rate of protein folding has been investigated by Dobson and co-workers, who divided a collection of proteins into either two-state or sequentially folding proteins.[23] For both types of proteins, these workers found that small amounts (< 10–15%, *v/v*) of TFE accelerate, while higher amounts inhibit the rate of folding.

Although homooligomers of Aib exhibit a dominant helical-conformational imperative, our results could also be relevant to helical peptides of more marginal helical stability in organic solvents. For example, peptides are increasingly being functionalized with side chains for metal binding or catalytic activity. Characterization of the structural and dynamic properties of peptides in organic solvents could lead to their application as catalysts in organic synthesis, nanotechnological devices, and possible data-storage systems.

Experimental Section

^{13}C-labeled Aib was prepared from $^{13}C_2$-acetone (CIL) by using the method of Oxender and Christiansen.[24] Peptides were prepared

by solution methods with Fmoc *N*-protection via 5-(4H)-oxazolone intermediates, according to the methods of Toniolo et al.[10,25] The octameric peptide was purified by flash silica gel chromatography (5% MeOH/CH_2Cl_2 *v/v*) and by recrystallization from acetonitrile. The synthesis of the peptide was confirmed by MALDI-TOF MS: $z/e = 1003$ [*M*+Na].

^{13}C NMR spectra were collected on a 300 MHz Bruker Avance spectrometer. No apodization was applied. The temperature of the NMR samples was determined by the ^1H NMR spectra of a coaxial sample of methanol.[26] Spectra were transferred to a PC for analysis by the Igor Pro software package for Lorentzian fitting.

The rate of the interconversion was determined by Equation 6.2b in Sandström's text,[27] which compares an inherent line width, W_o, with the width of the exchange-broadened peak, *W**:

$$k = \frac{\pi\delta\nu^2(W^* + W_o)\left[1 + 2\left(\frac{w^*}{\delta_\nu}\right)^2 - \left(\frac{w^*}{\delta_\nu}\right)^4\right]^{1/2}}{2(W^{*2} + W_o^2)}$$

In this equation, $\delta\nu$ = limiting separation, in Hz, of exchanging resonances. The value of $\delta\nu$ was determined from low-temperature spectra of the peptide in neat CD_2Cl_2, and the value of W_o was obtained in each spectrum from the width of the ^{13}C resonance that results from the three methyl carbons of the C-terminal tert-butyl ester group.

Acknowledgements

This work was supported by the American Chemical Society's Petroleum Research Fund, grant # 33928-GB4.

Keywords: aminoisobutyric acid · helical structures · peptides · protein structures

[1] M. Goodman, I. Listowsky, *J. Am. Chem. Soc.* **1962**, *84*, 3770 – 3771; M. Goodman, I. Listowsky, Y. Masuda, F. Boardman, *Biopolymers* **1963**, *1*, 33 – 42; M. Goodman, I. G. Rosen, *Biopolymers* **1964**, *2*, 537 – 559; M. Goodman, A. S. Verdini, C. Toniolo, W. Phillips, F. A. Bovey, *Proc. Natl. Acad. Sci. USA* **1969**, *64*, 444 – 450.
[2] R. Rajan, P. Balaram, *Int. J. Pept. Protein Res.* **1996**, *48*, 328 – 336.
[3] M. Buck, *Q. Rev. Biophys.* **1998**, *31*, 297 – 355.
[4] M. K. Luidens, J. Figge, K. Breese, S. Vajda, *Biopolymers* **1996**, *39*, 367 – 376.
[5] D. Roccatano, G. Colombo, M. Fioroni, A. E. Mark, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12179 – 12184.
[6] M. Llinas, M. P. Klien, *J. Am. Chem. Soc.* **1975**, *97*, 4731 – 4737.
[7] A. Kentsis, T. R. Sosnick, *Biochemistry* **1998**, *37*, 14613 – 14622.
[8] J. Nelson, N. Kallenbach, *Proteins Struct. Funct. Genet.* **1986**, *1*, 211 – 217.
[9] C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Biopolymers* **2001**, *60*, 396 – 419; A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, G. M. Bonora, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 1988 – 1992.
[10] C. Toniolo, G. M. Bonora, V. Barone, A. Bavoso, E. Benedetti, B. Di Blasio, P. Grimaldi, V. Pavone, C. Pedone, *Macromolecules* **1985**, *18*, 895 – 902.
[11] R. Banerjee, G. Basu, *ChemBioChem* **2002**, *3*, 1263 – 1266.
[12] J. D. Augspurger, V. A. Bindra, H. A. Scheraga, A. Kuki, *Biochemistry* **1995**, *34*, 2566 – 2576.
[13] C. Toniolo, E. Benedetti, *Contrib. Gynecol. Obstet. Trends Biochem. Sci.* **1991**, *16*, 350 – 352.
[14] R.-P. Hummel, C. Toniolo, G. Jung, *Angew. Chem.* **1987**, *99*, 1180 – 1182; *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 1150 – 1152.
[15] F. Chiti, N. Taddei, P. Webster, D. Hamada, T. Fiaschi, G. Ramponi, C. M. Dobson, *Nat. Struct. Biol.* **1999**, *6*, 380 – 387.
[16] M. Fioroni, M. Diaz, K. Burger, S. Berger, *J. Am. Chem. Soc.* **2002**, *124*, 7737 – 7744.
[17] M. A. Kubasik, J. Kotz, C. Szabo, T. Furlong, J. Stace, *Biopolymers* **2005**, *78*, 87 – 95.
[18] K. Gunasekaran, L. Gomathi, C. Ramakrishnan, J. Chandrasekhar, P. Balaram, *J. Mol. Biol.* **1998**, *284*, 1505 – 1516.
[19] S. Hayward, *Protein Sci.* **2001**, *10*, 2219 – 2227.
[20] M. Bellanda, E. Peggion, R. Burgi, W. van Gunsteren, S. Mammi, *J. Pept. Res.* **2001**, *57*, 97 – 106.
[21] P. A. Thompson, W. A. Eaton, J. Hofrichter, *Biochemistry* **1997**, *36*, 9200; S. Williams, T. P. Causgrove, R. Gilmanshin, K. S. Fang, R. H. Callender, W. H. Woodruff, R. B. Dyer, *Biochemistry* **1996**, *35*, 691 – 697; I. Lednev, A. Karnoup, M. Sparrow, S. A. Asher, *J. Am. Chem. Soc.* **1999**, *121*, 8074 – 8086.
[22] I. Nesmelova, A. Krushelnitsky, D. Idiyatullin, F. Blanco, M. Ramirez-Alvarado, V. A. Daragan, L. Serrano, K. H. Mayo, *Biochemistry* **2001**, *40*, 2844 – 2853.
[23] D. Hamada, F. Chiti, J. I. Guijarro, M. Kataoka, N. Taddei, C. M. Dobson, *Nat. Struct. Biol.* **2000**, *7*, 58 – 61.
[24] H. N. Christensen, D. L. Oxender, *Biochim. Biophys. Acta* **1963**, *74*, 386 – 391.
[25] D. S. Jones, G. W. Kenner, J. Preston, R. C. Sheppard, *J. Chem. Soc.* **1965**, 6227.
[26] C. Ammann, P. Meier, A. E. Merbach, *J. Magn. Reson. Ser.* **1982**, *46*, 319 – 321.
[27] J. Sandström, *Dynamic NMR Spectroscopy*, Academic Press, New York, **1982**.

Received: June 11, 2004
Revised: March 21, 2005
Published online on June 3, 2005

