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Implications of the Periodicity in NMR Chemical Shifts and Temperature Coefficients of Amide Protons in Helical Peptides

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Abstract : We obtained the chemical shifts of amide protons (NHs) in helical peptides at various temperatures and trifluoroethanol (TFE) concentrations using 2-dimensional NMR spectroscopy. These NH chemical shifts and their temperature dependence exhibited characteristic periodicity of 3-4 residues per cycle along the helix, where downfield shifted NHs showed larger temperature dependence. In an attempt to understand these observations, we focused on hydrogen bonding changes in the peptides and examined the validity of two possible explanations: (1) changes in intermolecular hydrogen bonding caused by differential solvation of backbone carbonyl groups by TFE, and (2) changes in intramolecular hydrogen bonding due to disproportionate variations in the hydrogen bonding within the peptide helix. Interestingly, the slowly exchanging NHs, which were on the hydrophobic side of the helix, showed consistently larger temperature dependences. This could not be explained by the differential solvation assumption, because the slowly exchanging NHs would become more labile if the preceding carbonyl groups were preferentially solvated by TFE. We suggest that the disproportionate changes in intramolecular hydrogen bonding better explain both the temperature dependence and the exchange behavior observed in this study.

Key words: NMR; chemical shift; temperature coefficient; amide proton; helix; TFE

INTRODUCTION

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One of most sensitive and important parameters of NMR spectroscopy is the chemical shift. Chemical shifts have been employed to predict the secondary structural elements in proteins,[1,2] and calculated theoretically to refine protein solution structures.[3,4] α-proton and carbon-13 chemical shifts have been particularly useful for characterizing the secondary structure of proteins.[5,6] However, amide proton (NH) chemical shifts have not been easily understood, as they are sensitive to intermolecular hydrogen bonding (H-bonding) between amide groups and solvent molecules, as well as to intramolecular H-bonding in the secondary or tertiary structures. The sensitivity of NH chemical shifts to experimental conditions, such as temperature, pH, and solvent composition, has further complicated their general interpretation.

An extensive study of NH chemical shift changes in various solvents has shown that an NH chemical shift is affected both by H-bonding of NH with basic solvents, and by H-bonding of the preceding carbonyl group with acidic protons in the solvent.[7] Because intermolecular H-bonding would be more easily disrupted upon temperature elevation than intramolecular H-bonding as a result of entropic consequences, the high sensitivity of NH chemical shifts to temperature has been regarded as evidence that NHs are externally oriented.[8] Analysis of the temperature coefficients of NHs in proteins has suggested that a combined use of temperature coefficients and NH exchange rates is required to reliably predict the H-bonding patterns in proteins.[9] The observed temperature dependence of chemical shifts of NHs in peptides, however, is more complicated to interpret, as the correlation with internal hydrogen bonding reported for proteins [10-12] seems, in some studies, to be lost.[13,14]

In this study, we investigated the temperature dependence of chemical shifts, at various TFE concentrations, for NHs in two peptides, one with a central proline (P14) and the other with an alanine substitution at the site of the proline (P14A). Both peptides were active analogues of an antimicrobial peptide Gaegurin isolated from frog skin.[15-17] NH chemical shifts were measured at different TFE concentrations (30%, 50%, and 70%) and a range of temperatures, and the temperature coefficients at each TFE concentration were obtained. In an attempt to explain the characteristic periodicity of NH temperature dependence, we investigated the NH exchange rates and NH temperature dependence at different solvent

compositions. As TFE has been widely used as a structure-inducing solvent for peptides, the understanding of this NMR parameter would be of general interest for conformational analysis of small peptides.

MATERIALS AND METHODS

Sample preparation

Peptides P14 (FLPLLAGLAANFLPTIISKISYKS) and P14A (FLPLLAGLAANFLAT-IISKISYKS) were synthesized and characterized as reported previously.[17]

NMR spectroscopy

For NMR spectroscopy experiments, a peptide concentration of 3 mM was used. Peptides were dissolved in a mixture of either 30 %, 50 %, or 70 % TFE-d_3/water (v/v). NMR spectra were obtained on a Bruker AMX 500 MHz spectrometer. To unambiguously determine chemical shifts, 2-dimensional (2-D) TOCSY and NOESY experiments were performed at 5- to 10-degree increments across a temperature range of 5 °C to 45 °C. TOCSY experiments were recorded with a mixing time of 75 ms using MLEV-17 composite pulses for the spin lock, and NOESY experiments were recorded with a mixing time of 250 ms. All chemical shifts were referenced to an internal sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) (0 ppm) resonance at each temperature. Data processing and sequential assignment were carried out according to previously reported procedures.[17] The temperature coefficients were determined from NH chemical shifts measured across a temperature ranges of 5 °C to 45 °C in 30%, 50 %, and 70 % TFE-d_3/water (v:v).

RESULTS AND DISCUSSION

The chemical shift differences (CSDs) for the NHs were obtained by subtracting the chemical shifts of peptide random coils from the observed chemical shifts for the P14 and

P14A peptides, where the random coil shifts were taken from Wishart et al. [18] α proton chemical shifts have been successfully employed to predict protein secondary structures.[19] P14 and P14A peptides formed α-helical structures in an aqueous TFE solution in our previous study,[17] and the negative α proton CSDs in both peptides (Figs. 1A and 2A) are typically observed in an α -helix structure.[20] Amide proton CSDs (Figs. 1B and 2B) showed characteristic periodicity of 3-4 residues per cycle along the helix except for the N-terminal part of the helix, and this periodic pattern has been reported in curved α -helical structures. [21] Both P14 and P14A peptides exhibited the same periodicity in their NH chemical shifts, and the NH chemical shifts of P14A peptide were more downfield in the central region of the helix than were those of P14 peptide. It is interesting that the temperature dependences of NH chemical shifts (Figs. 1C and 2C) showed very similar periodicity with that in NH chemical shifts (Figs. 1B and 2B). The temperature coefficients NH chemical shifts of P14 peptide were more widely varied in the central region of the helix than were those of P14A.

Amide proton CSDs have been correlated with the hydrogen bond lengths in a helix, so that NH shifts appear more downfield if they are involved in shorter hydrogen bonds.[22,23] The slowly exchanging NHs of P14 exhibited more downfield chemical shifts than did fast exchanging ones, and thus corresponded to shorter hydrogen bond lengths (Fig. 1B). It is notable, however, that the downfield shifted NHs exhibited consistently larger temperature dependences than did the upfield shifted ones (Fig. 1C). It may seem inconsistent that NHs involved in stronger hydrogen bonds exhibit larger temperature coefficients, considering that a large temperature coefficient commonly indicates the absence of intramolecular H-bonding. To understand this abnormal temperature dependence of NH chemical shifts, we considered two possible hypotheses: (1) changes in intermolecular H-bonding caused by differential solvation of backbone carbonyl groups by TFE, and (2) changes in intramolecular H-bonding due to disproportionate variations in the H-bonding within the peptide helix.

Periodicities in the temperature dependence of NH chemical shifts were previously reported in studies with model peptides, and the differential solvation of carbonyl groups by TFE was assumed to account for the observation.[24] This assumption could explain the observation, because the NH exchange rates were quite uniform in their helical peptides

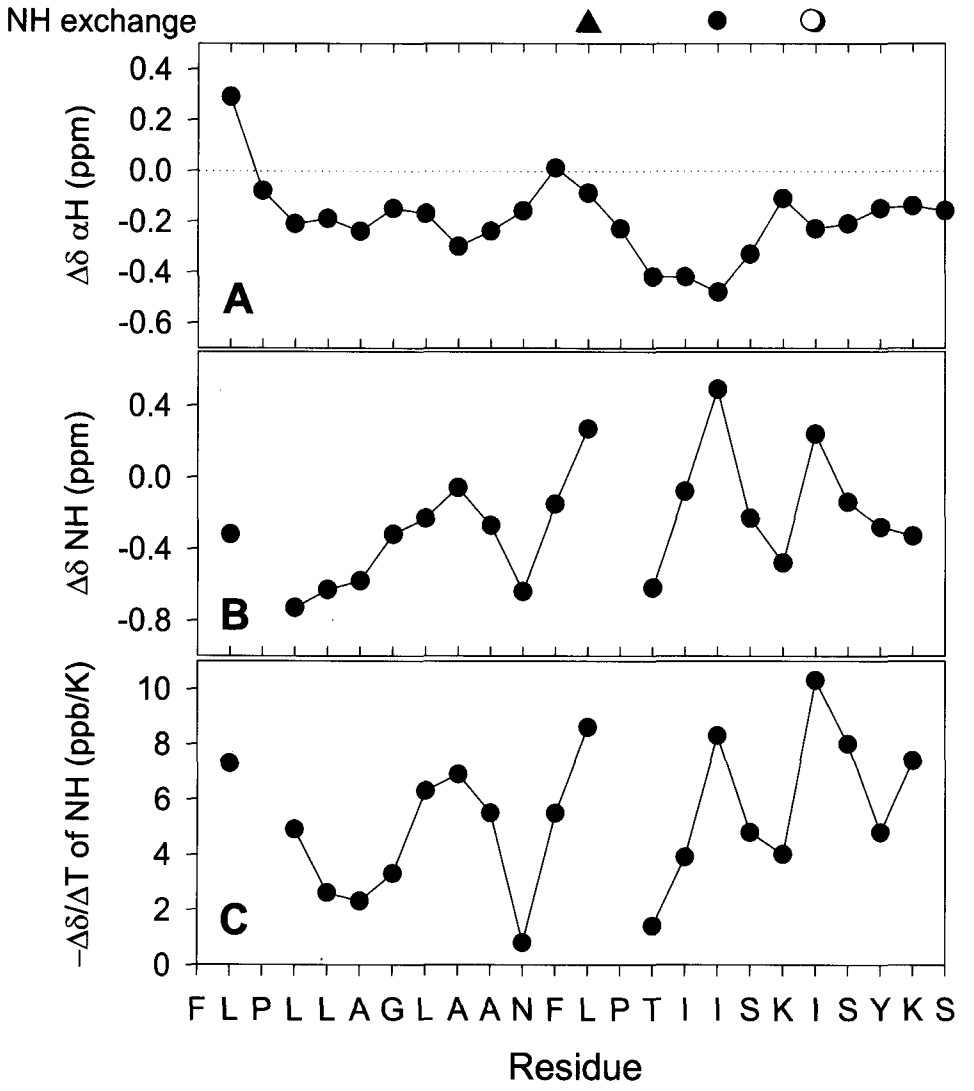

Fig. 1. Peptide P14. (**A**) Differences between observed α proton shifts and random coil shifts, $\Delta\delta_{\alpha H} = \delta_{\alpha H} - \delta_{rc}$; (**B**) differences between observed NH shifts and random coil shifts, $\Delta\delta_{NH} = \delta_{NH} - \delta_{rc}$; and (**C**) temperature coefficients of amide protons, $-\Delta\delta/\Delta T$ versus the amino acid sequence. All shifts and temperature coefficients were obtained in an aqueous TFE solution (1:1, v:v) at 25 °C, with random coil shifts supplied by Wishart et al. (18). The NH exchange profile is indicated above panel A, with designations of 48 h(●), 3 h(○), and 30 min(▲).

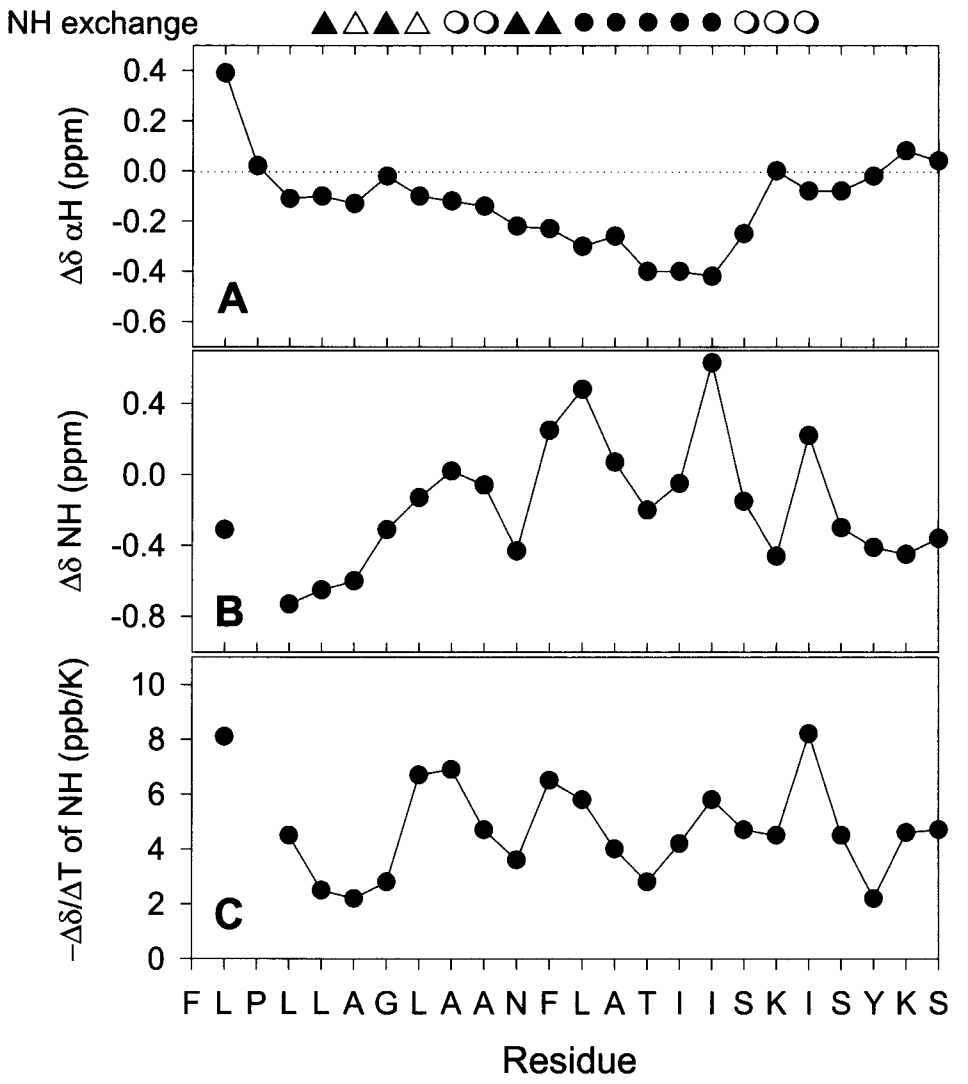

Fig. 2. Peptide P14A. (**A**) Differences between observed α proton shifts and random coil shifts, $\Delta\delta_{\alpha H} = \delta_{\alpha H} - \delta_{rc}$; (**B**) differences between observed NH shifts and random coil shifts, $\Delta\delta_{NH} = \delta_{NH} - \delta_{rc}$; and (**C**) temperature coefficients of amide protons, $-\Delta\delta/\Delta T$ versus the amino acid sequence. All shifts and temperature coefficients were obtained in an aqueous TFE solution (1:1, v:v) at 25 °C, with random coil shifts supplied by Wishart et al. (18). The NH exchange profile is indicated above panel A, with designations of 168h(●), 48h(○), 24h(▲), 4h(△).

studied. However, the exchange profile of P14 cannot be explained by the TFE solvation assumption, because the slowly exchanging NHs would become more labile if the carbonyl groups preceding the amide groups are preferentially solvated by TFE. Hence, we examined the second assumption that the intramolecular H-bonding does not change equally on the hydrophilic and hydrophobic sides of the helix upon heating. This difference results largely from the decrease in the hydrophobic interactions on the nonpolar side of the helix upon temperature elevation. The weakening of hydrophobic interactions at higher temperatures would have a greater influence on the H-bonding of residues on the hydrophobic side of the helix, and this would concomitantly affect the chemical shifts of NHs in this region of the peptide.

Changes in NH chemical shifts that occur with an increase in TFE concentration depends on the degree of exposure of the amide groups of interest and their preceding carbonyl groups. As TFE is less basic than water, it would better protonate the carbonyl oxygen and make it less available for H-bonding with peptide NHs.[7] A previous report showed that exposed NHs in a cyclic peptide experienced upfield shifts when TFE concentration was increased, and that these upfield shifts were observed whether the preceding carbonyl groups were external or internal.[25] These results imply that the decreased H-bonding of TFE to amide protons has a greater influence on NH chemical shifts than does the increased protonation of carbonyl groups by TFE. We examined the change in NH chemical shifts and their temperature dependence upon increases in TFE concentration. In our study, most NHs of residues that spanned the helical regions of the peptides exhibited very small chemical shift changes (< 0.1 ppm) (Fig 3). This indicates that TFE did not interact to a great extent with the backbone peptide bonds in the P14 and P14A helices. On the other hand, the temperature dependence of NH shifts mostly decreased with increasing TFE concentrations, and the decrease was more pronounced for residues on the hydrophobic side (Fig. 4). The observation that a reduction in the strength of hydrophobic interactions with increasing concentrations of TFE correlated with a decrease in the temperature dependence of NH chemical shifts implies that changes in hydrophobic interactions need to be considered in order to understand the temperature dependence observed in this study. We propose that the disruption of the hydrophobic interactions resulted in larger changes in the

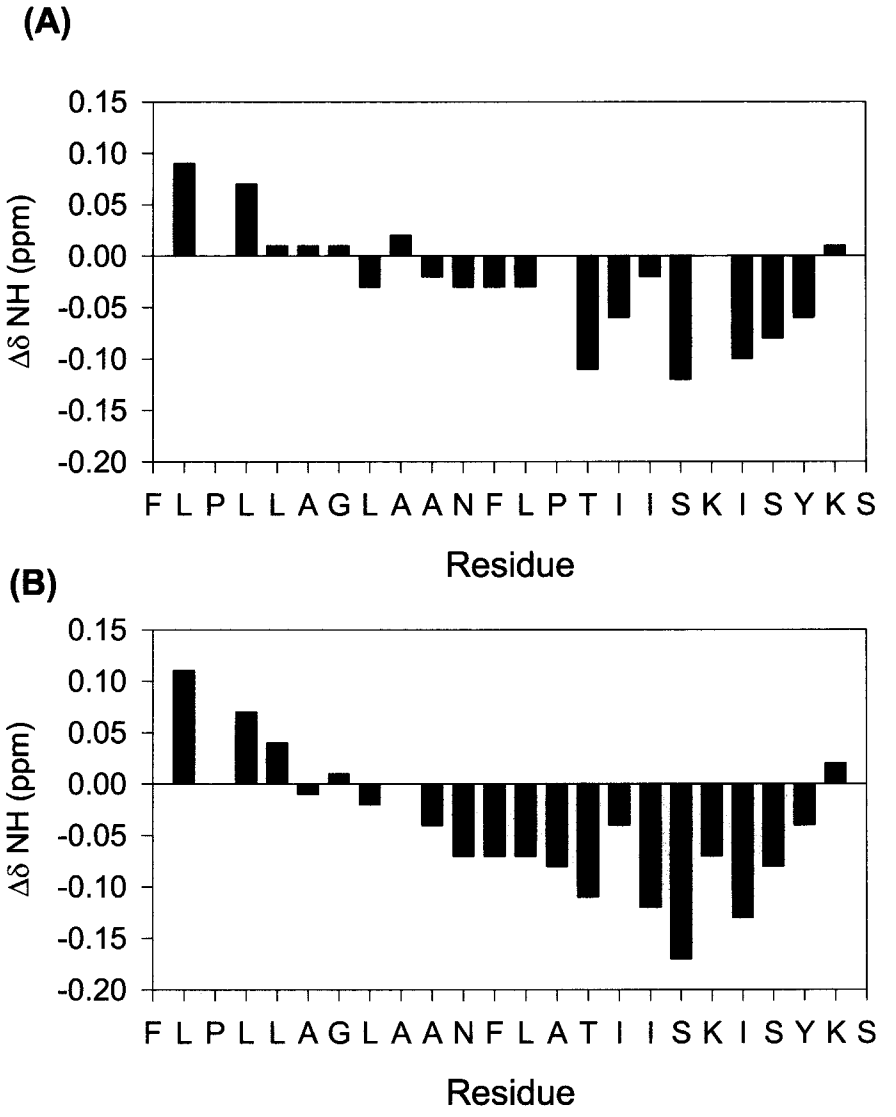

Fig. 3. Differences in amide proton chemical shifts with increasing TFE concentration at 25 °C for peptide P14 (**A**) and peptide P14A (**B**), where $\Delta\delta_{NH} = \delta_{NH}$(TFE 30%) - δ_{NH}(TFE 70%).

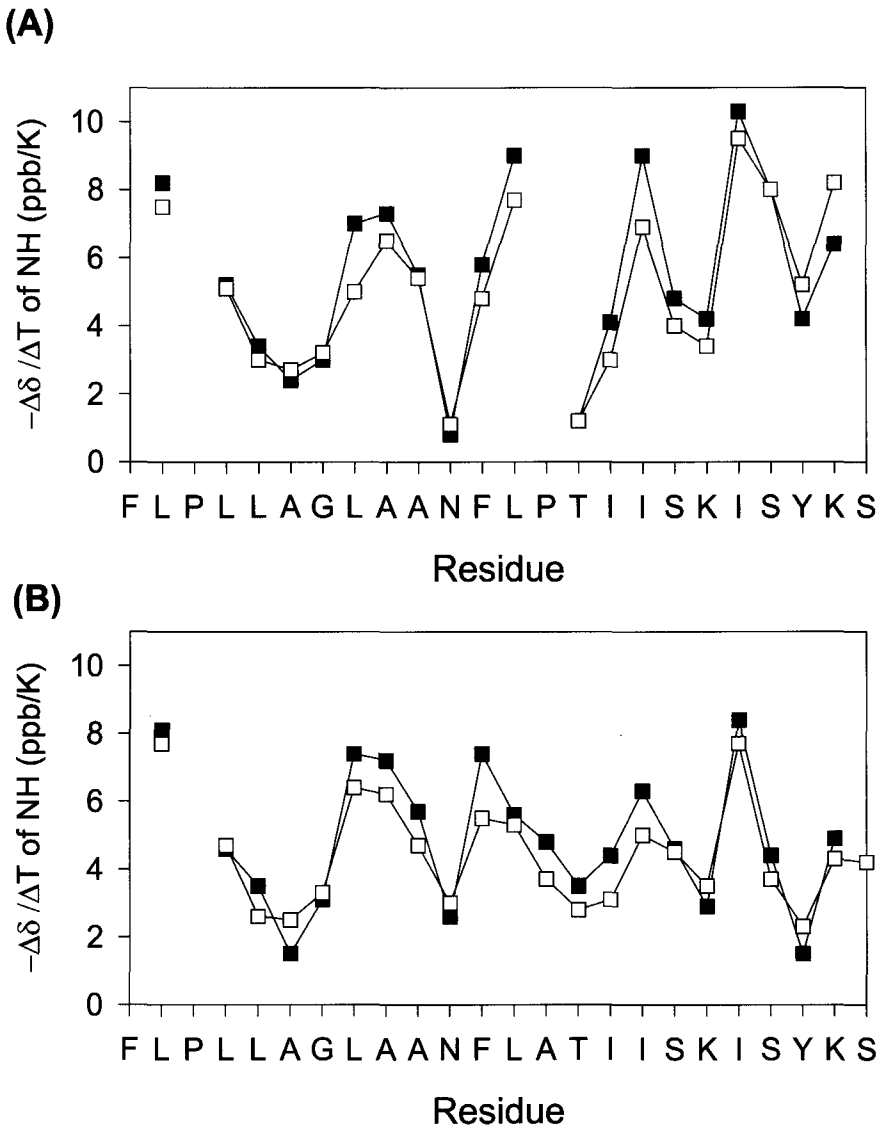

Fig. 4. Temperature coefficients of amide protons for peptide P14 (**A**) and peptide P14A (**B**) at varying TFE concentrations. Temperature coefficients were obtained over a temperature range of 5 °C to 45 °C at 30% (■) and 70 % (□) TFE concentration.

intramolecular H-bonding for residues on nonpolar side of the helix, which caused an increase in the temperature dependence of the NH shifts.

Random coil chemical shifts of NHs employed in this study were obtained from an aqueous solution, because only a limited set of random coil shifts in an aqueous TFE solution was available.[26] However, correction of our data with the available set of random coil shifts in TFE did not manifest noticeable changes in our observations in Figures 1B and 2B. This is because the inherent variations in the NH chemical shifts outweighed the differences between the random coil shifts (data not shown).

In conclusion, we propose that the NH temperature coefficients of peptide helices in an aqueous TFE solution are affected by disproportionate variations in the intramolecular H-bonding along the helix, which results from the uneven distributions of hydrophobic interactions on the two sides of the helix. Similar observations in water may also be explained by changes in the intramolecular H-bonding.[27] Future studies of helical peptides with varying degrees of hydrophobic moments would be helpful to evaluate the influence of hydrophobic interactions on the temperature dependence of NH chemical shifts. In addition, the determination of carbonyl carbon chemical shift changes at different TFE concentrations would provide valuable information on the solvation states of individual carbonyl groups. In this context, it is notable that carbonyl groups in the pentapeptide elastin exhibited similar H-bonding to both water and TFE, according to data on carbonyl carbon chemical shift changes upon solvent titration.[28] A theoretical approach using molecular dynamics is also under investigation, and a recent report demonstrated that TFE molecules stabilize peptide secondary structure by localizing around the peptide without residue-specific preference.[29,30] Because the mechanism of structural stabilization by TFE is not fully understood at this stage, when TFE is employed, the temperature coefficients of NH chemical shifts need to be interpreted carefully in order to provide conformational information.

REFERENCES

1. Pastore, A. and Saudek, V., *J. Magn. Reson.*, 90, 165-176 (1990).
2. Wishart, D.S. and Sykes, B.D., *Methods Enzymol.*, 239, 363-392 (1994).
3. Ösapay, K. and Case, D.A., *J. Am. Chem. Soc.*, 113, 9436-9444 (1991).

4. de Dios, A.C., Pearson, J.G. and Oldfield, E., *Science*, 260, 1491-1496 (1993).
5. Spera, S. and Bax, A., *J. Am. Chem. Soc.*, 113, 5490-5492 (1991).
6. Reily, M.D., Thanabal, V. and Omecinsky, D.O., *J. Am. Chem. Soc.*, 114, 6251-6252 (1992).
7. Llinas, M. and Klein, M.P., *J. Am. Chem. Soc.*, 97, 4731-4737 (1975).
8. Kessler, H., *Angew. Chem. Int. Ed. Engl.*, 21, 512-523 (1982).
9. Baxter, N.J. and Williamson, M.P., *J. Biomol. NMR*, 9, 359-369 (1997).
10. Kopple, K.D., Ohnishi, M. and Go, A., *J. Am. Chem. Soc.*, 91, 4264-4272 (1969).
11. Ohnishi, M. and Urry, D.W., *Biochem. Biophys. Res. Commun.*, 36, 194-202 (1969).
12. Zimmermann, G.R., Legault, P., Selsted, M.E. and Pardi, A., *Biochemistry*, 34, 13663-13671 (1995).
13. Stevens, E.S., Sugawara, N., Bonora, G.M. and Toniolo, C., *J. Am. Chem. Soc.*, 102, 7048-7050 (1980).
14. Williamson, M.P., Hall, M.J. and Handa, B.K., *Eur. J. Biochem.*, 158, 527-536 (1986).
15. Park, J.M., Jung, J.E. and Lee, B.J., *Biochem. Biophys. Res. Commun.*, 205, 948-954 (1994).
16. Suh, J.Y., Lee, K.H., Chi, S.W., Hong, S.Y., Choi, B.W., Moon, H.M. and Choi, B.S., *FEBS Lett.*, 392, 309-312 (1996).
17. Suh, J.Y., Lee, Y.T., Park, C.B., Lee, K.H., Kim, S.C. and Choi, B.S., *Eur. J. Biochem.*, 266, 665-674 (1999).
18. Wishart, D.S., Bigam, C.G., Holm, A., Hodges, R.S. and Sykes, B.D., *J. Biomol. NMR*, 5, 67-81 (1995).
19. Wishart, D.S., Sykes, B.D. and Richards, F.M., *J. Mol. Biol.*, 222, 311-333 (1991).
20. Wishart, D.S., Sykes, B.D. and Richards, F.M., *Biochemistry*, 31, 1647-1651 (1992).
21. Zhou, N.E., Zhu, B.Y., Sykes, B.D. and Hodges, R.S., *J. Am. Chem. Soc.*, 114, 4320-4326 (1992).
22. Pardi, A., Wagner, G. and Wuthrich, K., *Eur. J. Biochem.*, 137, 445-454 (1983).
23. Wagner, G., Pardi, A. and Wuthrich, K., *J. Am. Chem. Soc.*, 105, 5948-5949 (1983).
24. Rothemund, S., Weißhoff, H., Beyermann, M., Krause, E., Bienert, M., Mugge, C., Sykes, B.D. and Sonnichsen, F.D., *J. Biomol. NMR*, 8, 93-97 (1996).

25. Pitner, T.P. and Urry, D.W., *J. Am. Chem. Soc.*, 94, 1399-1400 (1972).
26. Merutka, G., Dyson, H.J. and Wright, P.E, *J. Biomol. NMR*, 5, 14-24 . (1995).
27. Reymond, M.T., Huo, S., Duggan, B., Wright, P.E. and Dyson, H.J., *Biochemistry*, 36, 5234-5244 (1997).
28. Urry, D.W., Mitchell, L.W. and Ohnishi, T., *Proc. Natl. Acad. Sci. USA*, 71, 3265-3269 (1974).
29. Roccatano, D., Colombo, G., Fioroni, M. and Mark, A.E., *Proc. Natl. Acad. Sci. USA*, 99, 12179-12184 (2002).
30. Diaz, M.D., Fioroni, M., Burger, K. and Berger, S., *Chem. Eur. J.*, 8, 1663-1669 (2002).