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Temperature coefficients of amide proton NMR resonance frequencies in trifluoroethanol: A monitor of intramolecular hydrogen bonds in helical peptides?*

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Summary

2D ^{1}H NMR spectroscopy of two α-helical peptides which differ in their amphipathicity has been used to investigate the relationships between amide-proton chemical shifts, amide-proton exchange rates, temperature, and trifluoroethanol (TFE) concentration. In 50% TFE, in which the peptides are maximally helical, the amide-proton chemical shift and temperature coefficient patterns are very similar to each other in each peptide. Temperature coefficients from −10 to −6 ppb/K, usually indicative of the lack of intramolecular hydrogen bonds, were observed even for hydrophobic amino acids in the center of the α-helices. However, slow hydrogen isotope exchange for residues from 4 to 16 in both 18-mer helices indicates intact intramolecular hydrogen bonds over most of the length of these peptides. Based on these anomalous observations, we suggest that the pattern of amide-proton shifts in α-helices in H_2O/TFE solvents is dominated by bifurcated intermolecular hydrogen-bond formation between the backbone carbonyl groups and TFE. The amide-proton chemical shift changes with increasing temperature may be interpreted by a disruption of intermolecular hydrogen bonds between carbonyl groups and the TFE in TFE/water rather than by the length of intramolecular hydrogen bonds in α-helices.

NMR chemical shifts have been shown to provide abundant conformational and structural information in peptides and proteins (Bundi and Wüthrich, 1979a,b; Wishart, 1991,1992). In particular, ^{13}C C^{α}, C^{β}, C' and ^{1}H α-CH NMR chemical shifts are readily interpreted, correlated with structural parameters and can be fairly accurately predicted in various calculations (Ösapay and Case, 1991; De Dios et al., 1993). In contrast, our understanding of amide-proton NMR chemical shifts (δ_{HN}) is limited. However, a relationship between δ_{HN} and the intramolecular hydrogen-bond length has been demonstrated (Pardi et al., 1983; Wagner et al., 1983; Wishart et al., 1992). Also, the temperature dependence of amide-proton chemical shifts ($\Delta\delta_{HN}/\Delta T$) has been shown to correlate with the presence of intramolecular hydrogen bonds (Dyson et al., 1988). The latter method has been widely applied for peptides where the amide protons exchange too rapidly to allow determination of the rates via deuterium exchange.

Another useful tool for characterization of the peptide conformation is their behavior in mixed or organic solvent systems. Despite some limitations, the use of organic solvents or mixed solvent systems is very popular, often owing to the solvent capacity to induce a peptide structure and allows identification of structural propensities (Goodman et al., 1971; Nelson and Kallenbach, 1989;

**Supplementary Material* is available upon request, comprising seven pages with listings of experimental details and the NMR shift data for the two peptides.

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Jackson and Mantsch, 1992; Sönnichsen et al., 1992). Usually, structural NMR criteria applied to peptides in water are directly transferred to mixed solvent systems. Chemical shifts, in particular of α-CH protons, are not affected by solvent changes, and are, therefore, readily interpreted (Jimenez et al., 1986; Nelson and Kallenbach, 1989). Recently, however, Merutka et al. (1995) analyzed the effect of TFE on δ_{HN} of peptides in a random-coil configuration and found nonlinear solvent-induced resonance shifts. This effect complicates the interpretation of δ_{HN} under these conditions. However, they demonstrated that $\Delta\delta_{HN}/\Delta T$ for random-coil peptides in TFE/water mixtures are identical to those in water within experimental error. This observation would suggest that $\Delta\delta_{HN}/\Delta T$ can be correlated with the hydrogen bonding of the amide group, irrespective of the water/TFE system used. To investigate this hypothesis, we have examined $\Delta\delta_{HN}/\Delta T$ of helical peptides of known structure, and compared these shifts with the respective amide exchange rates. We observed that $\Delta\delta_{HN}/\Delta T$ coefficients can be strongly influenced by the solvent, which questions their use for hydrogen-bond identification of peptides in mixed solvents.

The amphipathic α-helix LA-18 (Ac-KLLKLAAKALLKLLKLAA-NH_2) and the non-amphipathic α-helix LN-18 (Ac-LLKTTELLKTTELLKTTE-NH_2) were investigated in this paper. In LA-18, the distribution of hydrophobic leucine and alanine residues on one side and hydrophilic lysine residues on the opposite site of the α-helix is characteristic for an amphipathic α-helix (hydrophobic moment = 0.37; Eisenberg, 1984). By comparison, in LN-18 the hydrophobic leucine and the hydrophilic lysine, threonine and glutamic acid residues are well distributed around the helix to form a non-amphipathic α-helix (hydrophobic moment = 0; Fig. 1). Circular dichroism (CD) measurements indicated that the peptides are unstructured in water (<10% helicity), and that they exhibit increasing helical content upon TFE addition (Rothemund et al., 1995,1996). Maximum helicity (90% for LA-18, 77% for LN-18) is reached at 30%-TFE/70%-H_2O (v/v). Evaluation of the molar ellipticities at 90% TFE gave identical curves, suggesting no additional change in conformation at high TFE concentrations.

^{1}H NMR resonance assignments of the two peptides at 25 °C in 50% TFE were obtained using standard 2D homonuclear spectra (DQF-COSY, TOCSY and NOESY). Significantly, the α-CH protons of residues 2 to 17 resonate upfield relative to random-coil values in both peptides (Wishart et al., 1995). This indicates stable α-helical conformations in these regions in agreement with the CD results. A plot of the amide-proton NMR chemical shifts of LA-18 and LN-18 versus the sequence position are shown in Figs. 2A and 3A, respectively. A periodic change in δ_{HN} is observed in LA-18, except for the first three residues due to the helix end effects (Blanco et al., 1992). This periodicity correlates well with the location of amide protons in the hydrophobic residues or hydrophilic face. Most amide protons of the amphipathic LA-18 helix exhibit higher δ_{HN} values in the hydrophobic face and lower δ_{HN} ones in the hydrophilic face. While δ_{HN} values of the non-amphipathic helix LN-18 lack the overall periodicity, the amide protons of hydrophobic and their neighboring amino acids similarly tend to have larger frequency shifts. Figures 2B and 3B present δ_{HN} differences relative to random-coil values ($\Delta\delta_{HN} = \delta_{HN} - \delta_{rc}$), which basically exhibit the same pattern since the chemical shift variations are significantly larger than the difference between the intrinsic shifts. The random-coil reference shifts used, however, are not completely appropriate since they are derived from aqueous solution, and TFE has been shown to induce a $\Delta\delta_{HN}$ (between 0.2 and 0.4 ppm at 90% TFE) in random-coil peptides (Merutka et al., 1995). Unfortunately, complete sets of amide-proton random-coil shifts for various TFE concentrations are currently not available.

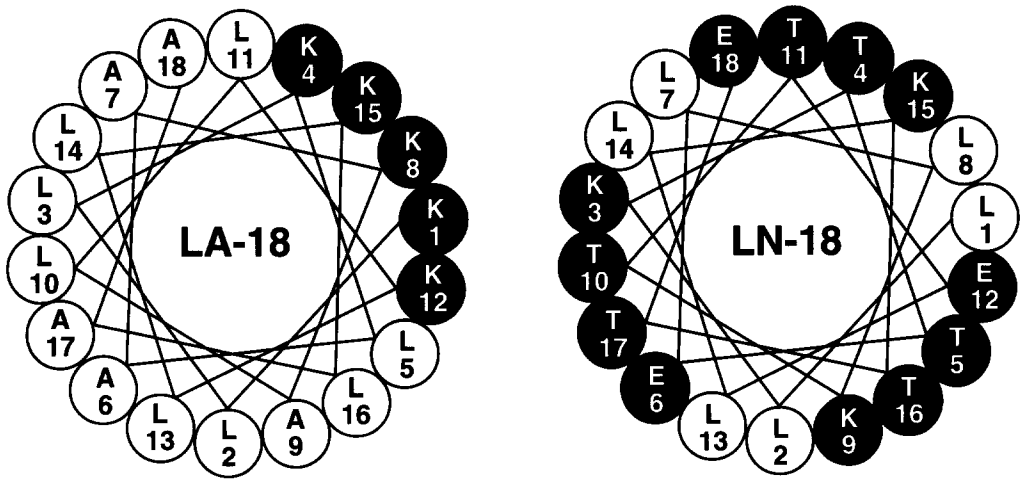

Fig. 1. Amino acid sequences of the amphipathic α-helix LA-18 and the non-amphipathic α-helix LN-18 represented by helical wheel projections. The distribution of polar residues is indicated by black circles.

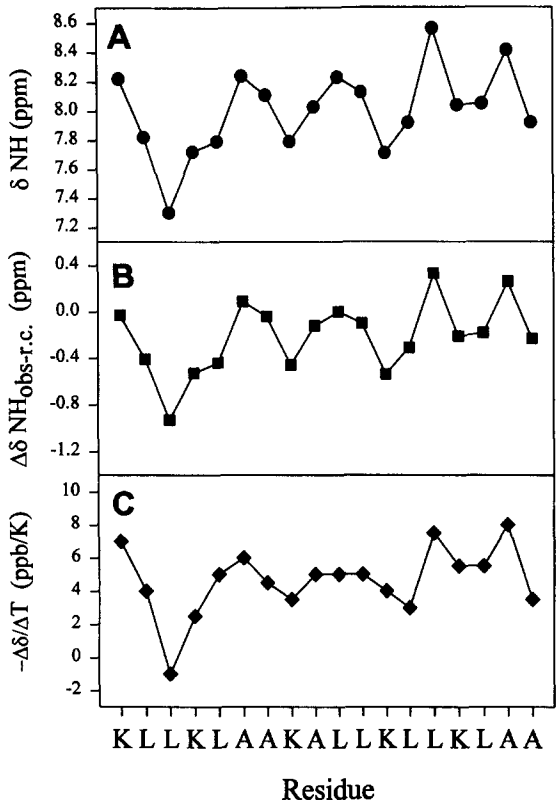

Fig. 2. Amphipathic peptide LA-18. (A) Observed amide-proton chemical shifts; (B) difference between the observed NH proton chemical shifts and random-coil chemical shifts (Wishart et al., 1995); and (C) temperature coefficients versus the amino acid residue. The shifts were recorded in 50%-TFE/50%-water (v/v) at pH 2.5.

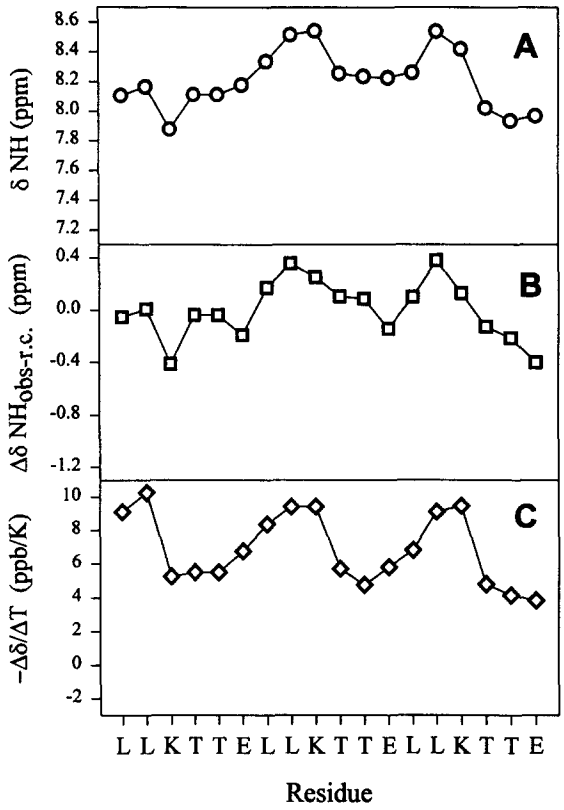

Fig. 3. Non-amphipathic peptide LN-18. (A) Observed amide-proton chemical shifts; (B) difference between the observed NH proton chemical shifts and random-coil chemical shifts (Wishart et al., 1995); and (C) temperature coefficients versus the amino acid residue. The shifts were recorded in 50%-TFE/50%-water (v/v) at pH 2.5.

In order to assess the effect of TFE on δ_{HN} for these structured peptides, NMR spectra were recorded in aqueous solutions with 50% and 90% TFE, respectively. In both peptides the observed changes in δ_{HN} are very small (<0.1 ppm) (Fig. 4). The only exceptions are the N-terminus and one proton of the C-terminal amide group, i.e. residues with increased flexibility and solvent accessibility that lack intramolecular hydrogen-bonding partners. The formation of intramolecular hydrogen bonds and concomitant α-helical structures significantly attenuates solvent-induced chemical shift changes. This result confirms (at least for helical peptides) previous suggestions (Urry and Long, 1976; Merutka et al., 1995) that the dependence of δ_{HN} on the concentration of TFE is a good monitor for structured areas or intramolecular hydrogen bonds.

The temperature dependence of δ_{HN} was determined from 2D DQF-COSY ^{1}H NMR experiments over the range 25 °C to 45 °C in 50% TFE. The $\Delta\delta_{HN}/\Delta T$ coefficients are plotted in Figs. 2C and 3C for LA-18 and LN-18, respectively. With one exception at residue Leu^3 in LA-18, the chemical shifts were found to move upfield linearly with increasing temperature. In the amphipathic helix LA-18 large $|\Delta\delta_{HN}/\Delta T|$ values (> 6 ppb/K), usually taken as an indication of the absence of intramolecular hydrogen bonds, were observed for residues Ala^6, Leu^{14} and Leu^{17}, all residues at the hydrophobic face. Overall, the $\Delta\delta_{HN}/\Delta T$ coefficients exhibited a very similar periodicity as observed for δ_{HN} (Fig. 2A).

We have also analyzed amide-hydrogen exchange rates of these helical peptides, at pH 2.5 and 25 °C, which have been shown to provide information about the formation of hydrogen bonds, backbone flexibility and accessibility. Short exchange times (<1 h) were observed for Lys^1 and Leu^2 in LA-18, and Leu^1 and Leu^2 in LN-18, due to increased flexibility at the N-termini. Protons at the C-terminus were found to exchange considerably slower than protons at the N-terminus, which can be explained by the presence of intramolecular hydrogen bonds for C-terminal amide protons. All of the remaining residues in both peptides were exchanging slowly, exhibiting exchange times of longer than 46 h; the only exceptions are lysine residues in LA-18, which exchange somewhat faster (15–20 h). No strong periodic pattern was observed for either peptide. These results strongly support the presence of stable intramolecular hydrogen bonds in both peptides. They also suggest, that apart from end effects, both helices are very stable and have little variation in hydrogen-bond lengths along the helix.

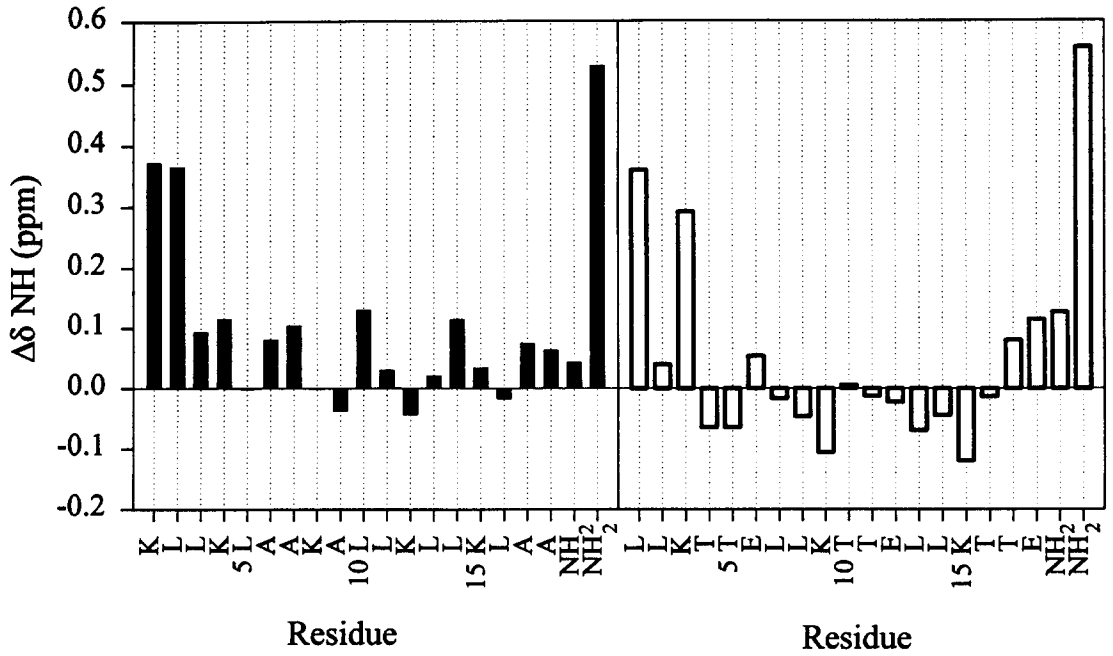

Fig. 4. Dependence of amide-proton chemical shifts on the concentration of trifluoroethanol of the amphipathic α-helix LA-18 (black bars, left) and the non-amphipathic α-helix LN-18 (white bars, right). The differences were calculated by the following equation: $\Delta\delta = \delta_{NH}$ (50% TFE) $- \delta_{NH}$ (90% TFE).

Periodic changes in amide chemical shift (δ_{HN}) have been observed previously in amphipathic helical peptides (Kuntz et al., 1991; Zhou et al., 1992). The periodicity has been explained by a curvature of the α-helix which causes a periodic change in hydrogen-bond lengths. Based on this explanation, a periodicity in $\Delta\delta_{HN}/\Delta T$ coefficients would also be expected. Matching δ_{HN} and $\Delta\delta_{HN}/\Delta T$ patterns are obtained for LA-18 and for LN-18. However, in LA-18 small $\Delta\delta_{HN}/\Delta T$ coefficients are found for hydrophilic residues, and higher values for hydrophobic ones, which is the opposite of expectations for a curved helix with shorter hydrogen bonds at the hydrophobic face. Furthermore, similar results were obtained for the non-amphipathic and presumably straight peptide LN-18. In LN-18 all leucines generally have larger $\Delta\delta_{HN}/\Delta T$ coefficients, and most residues with primarily hydrophilic character exhibit smaller $\Delta\delta_{HN}/\Delta T$ coefficients. The two exceptions are Lys^9 and Lys^{15} ($\Delta\delta_{HN}/\Delta T \approx -9$ ppb/K).

The data clearly indicate a correlation between δ_{HN} and $\Delta\delta_{HN}/\Delta T$ patterns, a phenomenon which was previously observed in endothelin peptides in TFE-containing solutions (Andersen et al., 1992). In our case, the absence of any tertiary structure, periodic changes in secondary structure, and ring-current effects leaves preferential solvation as the most obvious explanation for our observations. Solvent effects on δ_{HN} have been described earlier (Llinás and Klein, 1975; Kessler, 1982). We suggest that intermolecular hydrogen bonds are formed between the backbone carbonyl groups and TFE, and that there is a preferential interaction of TFE with hydrophobic domains in helices, due to the inherent hydrophobicity of TFE. This interaction will affect both the δ_{HN} value and its temperature dependence ($\Delta\delta_{HN}/\Delta T$), since this intermolecular solvation will be temperature-dependent, so that nearly identical patterns are obtained. This assumes dominance of the temperature dependence of the carbonyl-based TFE solvation over the aqueous peptide-bond solvation. A more detailed explanation, or the correlation of the observed periodicities with the TFE binding to specific peptide carbonyls, however, is very complicated for several reasons. Firstly, in these helical peptides δ_{HN} or $\Delta\delta_{HN}/\Delta T$ of one amide proton will be affected by TFE binding to the previous residue (inductively via the peptide bond) and to residue i−4 (via the H-bond). Secondly, the hydrophobicity of the local environment of a carbonyl group is not only determined by its own side-chain hydrophobicity, but also by that of residues i+1, i+3, and i+4.

In conclusion, we propose that a large temperature dependence of the amide protons in our model peptides in 50% TFE can be interpreted by an influence of intermolecular hydrogen bonds between TFE and peptidic carbonyl groups. In principle, temperature coefficients in mixed solvents should reflect the average temperature dependence of all solvation processes or equilibria. Thus, it should still be possible to correlate the relative extent of temperature coefficients with peptide-bond accessibility/intramolecular hydrogen bonds. However, this will only be valid if all solvating molecules are evenly interacting with all peptide bonds, i.e. the accessibilities and local concentrations are identical for all solvent components. Given the differences in size, hydrophilic and hydrophobic character, and other properties of the solvents employed, this seems unreasonable.

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