

Temperature Dependence of Peptide NH Chemical Shifts in Benzene: Delineation of Solvent-Shielded and Exposed Amide Protons

INTRODUCTION

^1H -nmr is one of the most widely used techniques for the study of intramolecular hydrogen-bonded conformations of peptides in solution.¹ The parameters used to delineate solvent-shielded amide NH groups include rates of hydrogen-deuterium exchange,² solvent shifts,³ paramagnetic radical-induced broadening,⁴ transfer of saturation from exchangeable solvent protons,⁵ and temperature coefficients ($d\delta/dT$) of NH chemical shifts, in strongly hydrogen-bond-accepting solvents like $(\text{CD}_3)_2\text{SO}$.⁶ Of these, the use of temperature coefficients is probably the most widespread.⁷ In general, values <0.003 ppm/ $^\circ\text{C}$ in $(\text{CD}_3)_2\text{SO}$ have been taken as indicative of solvent-shielded, and presumably hydrogen-bonded, NH groups, while values >0.004 ppm/ $^\circ\text{C}$ have been assigned to exposed groups. The model compound, *N*-methylacetamide $\text{CH}_3\text{CONHCH}_3$ yields a value of 0.006 ppm/ $^\circ\text{C}$ in $(\text{CD}_3)_2\text{SO}$.⁸ The large temperature coefficients for exposed NH protons presumably arise by the breaking of solute-solvent hydrogen bonds on increasing the temperature. Peptides dissolved in CDCl_3 show low temperature coefficients for both solvent-shielded and exposed amide hydrogens, a reflection of the poor hydrogen-bonding capability of this solvent.⁹ We describe in this report the use of temperature coefficients of peptide NH groups in benzene (C_6D_6), as a sensitive parameter for the determination of the degree of solvent exposure.

MATERIALS AND METHODS

All peptides used in this study were synthesized by solution phase procedures, checked for homogeneity by thin-layer chromatography, and characterized by 270-MHz ^1H -nmr and elemental analysis. Details will be described elsewhere.

All ^1H -nmr measurements were carried out at 270 MHz on a Bruker WH-270 FT-NMR spectrometer, at the Bangalore NMR facility. Peptide concentrations used were 10 mg/ml. The octapeptide *Z*-(Aib-Pro)₄-OMe was also studied at 1 mg/ml. Sweep widths of 3012 Hz were used, with 8K real data points yielding a digital resolution of 0.367 Hz/point. All values are quoted with reference to internal tetramethylsilane. Assignments in the tetrapeptide disulfide were made unambiguously using extensive decoupling studies. In other peptides, the urethane NH was unambiguously assigned to the highest field NH resonance in CDCl_3 and C_6D_6 . $(\text{CD}_3)_2\text{SO}$ assignments were then made using solvent titrations.¹⁰

RESULTS AND DISCUSSION

The temperature dependence of NH chemical shifts for various peptides in C_6D_6 is shown in Fig. 1. The δNH and $d\delta/dT$ values for the peptides used in this study in different solvents are listed in Table I. For $\text{CH}_3\text{CONHCH}_3$, which has generally been used as a model for solvent-exposed NH groups, the $d\delta/dT$ values are 0.006 and 0.016 ppm/ $^\circ\text{C}$ in $(\text{CD}_3)_2\text{SO}$ and C_6D_6 , respectively. However, in view of the known tendency of $\text{CH}_3\text{CONHCH}_3$ to aggregate in apolar solvents,¹¹ the $d\delta/dT$ value in benzene may not be a good indicator of a fully exposed NH group. Studies down to sufficiently low concentrations are precluded by unsatisfactory signal-to-noise ratios. The $d\delta/dT$ values for the tetrapeptide *Z*-(Aib-Pro)₂-OMe in $(\text{CD}_3)_2\text{SO}$ suggest that the Aib(3) NH is solvent-shielded. Further support for this conclusion comes from the small change in values on going from CDCl_3 to $(\text{CD}_3)_2\text{SO}$. These observations are consistent with β -turn conformation, with Aib(1) and Pro(2) at the corners, stabilized by a

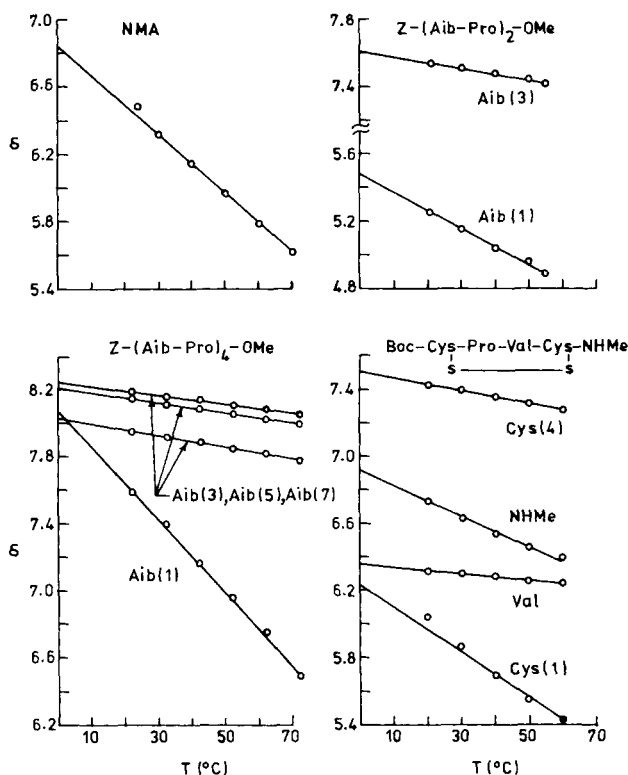


Fig. 1. Temperature dependence of NH chemical shifts of various peptides in C_6D_6 .

4 \rightarrow 1 intramolecular hydrogen bond between the urethane CO and the Aib(3) NH group. Such a β -turn conformation has been demonstrated for this tetrapeptide in the solid state by x-ray diffraction (M. Nair, M. Vijayan, Y. V. Venkatachalapathi, and P. Balaram, unpublished). Conformations of this type have been established for related -Aib-Pro- sequences in the solid state and in solution by x-ray, nmr, and ir methods.^{10,12,13} The $d\delta/dT$ values obtained for the Aib(1) and Aib(3) NH groups in C_6D_6 are dramatically different. While the former has a steep temperature dependence, the latter is much less affected by temperature changes.

The $d\delta/dT$ values for the octapeptide $Z-(Aib-Pro)_4-OMe$ in $(CD_3)_2SO$ suggest that the Aib(3), Aib(5), and Aib(7) NH groups are involved in intramolecular hydrogen bonds, while the urethane NH [Aib(1)] is free. This would correspond to a 3_{10} helical fold with every alternate 4 \rightarrow 1 hydrogen bond disrupted by the presence of Pro residues. Support for such a conformation also comes from hydrogen-deuterium exchange, solvent dependence of NH chemical shifts, and ir studies (Y. V. Venkatachalapathi and P. Balaram, unpublished). Once again the $d\delta/dT$ values in C_6D_6 clearly show that the three amide NH groups are hydrogen-bonded, whereas the urethane NH is exposed to solvent. It is relevant that the differences in $d\delta/dT$ values between an exposed and hydrogen-bonded NH are very much larger in C_6D_6 , than in $(CD_3)_2SO$. The $d\delta/dT$ values have been determined at two concentrations, 12 and 1.2 mM. In both cases, the Aib(1) NH group has a much greater $d\delta/dT$ value than the other three NH groups. This suggests that problems due to peptide aggregation are not likely to be significant.

A further example is provided by the cyclic tetrapeptide disulfide Boc-Cys-Pro-Val-Cys-

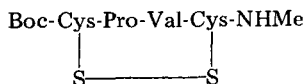
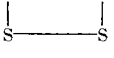


TABLE I
 NMR Parameters of Peptide NH Groups

Peptide	NH	δ NH			$d\delta/dT$ (ppm/°C)	
		CDCl ₃	(CD ₃) ₂ SO	C ₆ D ₆	(CD ₃) ₂ SO	C ₆ D ₆
<i>N</i> -Methylacetamide		—	7.75	6.41	0.0060	0.0160
<i>Z</i> -(Aib-Pro) ₂ -OMe	Aib(1)	5.51	8.08	5.27 ^a	0.0048	0.0118 ^a
	Aib(3)	7.24	7.45	7.54 ^a	0.0015	0.0033 ^a
<i>Z</i> -(Aib-Pro) ₄ -OMe ^{b,c}	Aib(1)	6.14	8.16	7.59	0.0052	0.0217 (0.0320) ^d
	Aib(3)	7.84	7.81	8.19	0.0015	0.0027 (0.0041)
	Aib(5)	7.69	7.76	8.14	0.0010	0.0028 (0.0042)
	Aib(7)	7.67	7.56	7.95	0.0016	0.0033 (0.0055)
Boc-Cys-Pro-Val-Cys-NHMe ^e 	Cys(1)	5.50	7.22	6.18	—	0.0114
	Val	6.41	7.39	6.34	—	0.0014
	Cys(4)	7.12	7.50	7.45	—	0.0036
	NHMe	6.60	7.39	6.07	—	0.0073
<i>Z</i> -Aib-Pro-Aib-Ala-OMe	Aib(1)	5.83	7.93	4.88 ^a	0.0055	0.0034 ^a
	Aib(3)	7.21	7.75	7.14 ^a	0.0049	0.0038 ^a
	Ala	7.52	7.49	7.67 ^a	0.0029	0.0032 ^a

^a These values were obtained in 1:1 CDCl₃/C₆D₆.

^b The assignments of Aib(3), Aib(5), and Aib(7) NH protons are arbitrary.

^c The $t_{1/2}$ values for H-D exchange are Aib(1), 20 min; and Aib(3), Aib(5), and Aib(7), approximately 9 hr in (CD₃)₂SO. In CDCl₃, the $t_{1/2}$ values are Aib(1) ~ 2 hr, while others are greater than 34 hr.

^d Values in parentheses are at 1.2 mM peptide (1 mg/ml).

^e $t_{1/2}$ values in CDCl₃/D₂O are Cys(1) ~ 24 hr, Val(3) > 96 hr, Cys(4) ~ 96 hr, and NHMe ~ 72 hr.

NHMe, where hydrogen-deuterium exchange rates in CDCl₃ and solvent shifts support a conformation in which the Val and Cys(4) NH groups are solvent-shielded or hydrogen-bonded (Y. V. Venkatachalapathi and P. Balaram, unpublished). The $d\delta/dT$ values in C₆D₆ are very low for these two protons, while a high value is obtained for the Cys(1) NH. The intermediate value for the methylamide NH possibly reflects a population of conformations involving this group in a moderately weak seven-membered hydrogen bond with the Val CO group. The instability of the disulfide at higher temperatures in (CD₃)₂SO precluded the determination of $d\delta/dT$ values in that solvent. In all the three cases described we have compared $d\delta/dT$ values for NH groups within the same molecule, rather than use the CH₃CONHCH₃ value as a standard. The agreement obtained between C₆D₆ and (CD₃)₂SO results strengthens our conclusion that $d\delta/dT$ values in C₆D₆ can be used to delineate exposed and shielded NH groups.

A possible limitation in the use of C₆D₆ as a solvent in peptide conformational analysis is, of course, the limited solubility of many peptides. We have therefore carried out studies in 1:1 CDCl₃/C₆D₆ mixtures to enhance peptide solubility. In the case of *Z*-(Aib-Pro)₂-OMe, this solvent mixture yielded different coefficients for the free and hydrogen-bonded NH groups. On the contrary, in *Z*-Aib-Pro-Aib-Ala-OMe, all three NH groups had similar, low $d\delta/dT$ values (Table I). This is probably due to preferential solvation of the peptide by CDCl₃, preventing a distinction between the free Aib(1) NH and the hydrogen-bonded Aib(3) and Ala(4) NH groups. This peptide has previously been shown to have two intramolecular hydrogen bonds in CDCl₃ and only one in (CD₃)₂SO.¹⁰ While solubility may impose some limitations, we have observed that a variety of peptides containing nonpolar residues are indeed soluble to the extent of 1–2 mg/ml in C₆D₆, a concentration sufficient to obtain spectra using Fourier transform spectrometers and techniques of solvent-peak suppression. A further advantage of using C₆D₆ is the generally well-resolved spectra obtained, a feature noted in earlier studies of organic molecules.¹⁴

The differentiation of free and solvent-shielded NH groups in C₆D₆ is likely to arise by the

preferential interaction of free NH groups with the π -electron cloud of benzene. Such an interaction has been proposed for $\text{CH}_3\text{CONHCH}_3$ on the basis of ir studies, which show a lowering of the NH stretching frequency in benzene while the carbonyl stretch is unaffected.¹⁵ Further evidence for the complexation of amides and benzene has also been provided by ^1H -nmr.¹⁶ Such interactions may lead to a deshielding of exposed NH groups in benzene, followed by shifts to higher field on increasing the temperature. This is indeed observed for the compounds in Table I, providing indirect support for this mechanism. The results described above suggest that benzene may prove a valuable solvent for the study of the solution conformations of apolar peptides and also emphasize the use of stereochemically constrained model peptides in developing spectroscopic methods of conformational analysis.

Financial support from the Department of Science and Technology is gratefully acknowledged.

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Received July 7, 1980
Accepted November 20, 1980

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