SOLVENT DEPENDENCE OF PEPTIDE CARBONYL CARBON CHEMICAL SHIFTS AND POLYPEPTIDE SECONDARY STRUCTURE: THE REPEAT TETRAPEPTIDE OF ELASTIN

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SUMMARY: It is demonstrated that carbon-13 magnetic resonance may be used to evaluate polypeptide secondary structure by noting the solvent dependence of peptide carbonyl carbon chemical shifts. Solvent pairs are utilized in which one solvent is a poor proton (or deuteron) donor and the second is a good proton (or deuteron) donor. The peptide carbonyl carbon resonance of the carbonyl shielded from the solvent by intramolecular hydrogen bonding exhibits less downfield shift on introduction of CD_3OD, D_2O or 2,2,2-trifluoroethanol-d_3 to dimethylsulfoxide-d_6 solutions than do those of peptide carbonyls that are more exposed to the solvent. The approach is illustrated with the elastin repeat tetrapeptide, Val_1-Pro_2-Gly_3-Gly_4, wherein the Val_1 $\underline{C}$-O is solvent shielded due to intramolecular hydrogen bonding to the Gly_4 N$\underline{H}$.

INTRODUCTION

Proton magnetic resonance (pmr) studies have been utilized for several years to delineate peptide N$\underline{H}$ protons in a manner that has been correlated with polypeptide secondary structure (1-7). The pmr methods provide information on relative solvent exposure and as such it should be appreciated that shielding from the solvent can occur due to reasons other than intramolecular hydrogen bonding. Once delineated other arguments must be introduced to determine if solvent shielding is due to secondary structure. The same situation holds for delineation of peptide carbonyls by carbon-13 magnetic resonance (cmr). With this caveat in mind appropriate delineation of both peptide N$\underline{H}$ moieties by pmr and peptide $\underline{C}$-O moieties by cmr would reduce the problem of determining polypeptide secondary structure in solution to the proper pairing of delineated peptide N$\underline{H}$ with delineated peptide $\underline{C}$-O groups to form the hydrogen bond.

Past efforts of pmr to yield secondary structure have been supported by x-ray diffraction studies. The studies of Kopple et. al. (2,3) on cyclohexapeptides using temperature dependence of peptide NH chemical shifts correlate well with x-ray diffraction studies of Karle and Karle (8) on cyclohexyl glycine. Similar delineation indicative of hydrogen bond formation by comparison of valinomycin with the valinomycin-potassium ion complex (4,5) was independently substantiated with x-ray diffraction studies on the complex (9). The several studies on gramicidin S using hydrogen-deuterium exchange rates (1) temperature dependence (4) and solvent dependence of peptide NH chemical shift (6) form a consistent picture which agrees with one of three possible conformations proposed by Hodgkin and Oughton on the basis of their crystal studies (10). Application of the pmr approach to non-cyclic peptides has also been substantiated. The conformation of the tetrapeptide tail of oxytocin proposed on the basis of pmr studies in this laboratory (11) was subsequently and independently proposed on the basis of x-ray diffraction studies by Rudko et. al. (12). A similar conformational feature, a β-turn with Pro as residue i+1, is the subject of the present report on the tetrapeptide, Val_1-Pro_2-Gly_3-Gly_4.

Gray and colleagues (13,14) have recently reported the presence of repeating peptide sequences in elastin. We have synthesized those sequences, and their oligomers and higher polymers and have carried out extensive pmr studies (15-18). Temperature studies on the tetrapeptide high polymer HCO-$(V_1P_2G_3G_4)_n$V OMe in Me_2SO and in MeOH demonstrated the Gly_4 NH to have a lesser temperature coefficient (slope) as shown in Table I (16). Solvent dependence of peptide NH chemical shift also showed the Gly_4 NH to be solvent shielded (16). This shielding occurs in the monomer HCO(VPGG)OMe but not in HPGGVOEt; it occurs with two Gly_4 NH protons but not

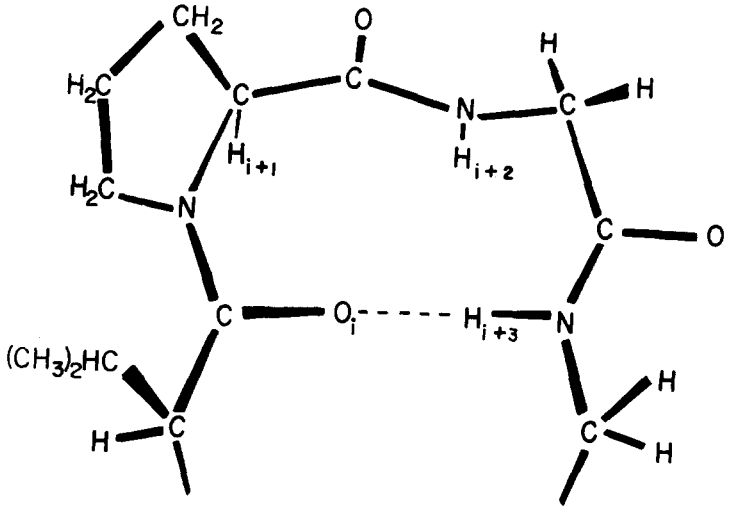


Figure 1: β-turn of the tetrapeptide VPGG

the third in $H(P_2G_3G_4V_1)_3OMe$. This implies that the Val_1 C=O is required for the shielding of the Gly_{i+3} NH. These results were interpreted in terms of the β-turn given in Fig. 1.

MATERIALS AND METHODS

Carbon-13 magnetic resonance spectra were obtained on a JEOL-PFT-100P pulse Fourier transform spectrometer at 25.15 MHz utilizing an EC-100 computer system with 20K memory. Resolution of the transformed spectrum was 0.05 ppm. Protons were decoupled at 100 MHz and an internal deuterium lock was used at 15.36 MHz. A 10 mm sample tube was fitted with a 0.45 ml insert (Wilmad Glass Co., Inc., Buena, N. J.). An internal reference of tetramethylsilane (TMS) was used for the organic solvents and dioxane at 67.4 ppm for D_2O (19). Probe temperature was 22°C.

Synthesis and characterization of the polypeptides $HCO-(VPGG)_n$ VOMe are reported elsewhere (16) as are the details of synthesis of the glycine-1-C-13 enriched peptides (20).

RESULTS

The complete cmr spectrum for $Boc-Val_1-Pro_2-Gly_3-Gly_4-OMe$ in

'ABLE I - TEMPERATURE DEPENDENCE OF PEPTIDE NH CHEMICAL SHIFT[1] FOR

$HCO-(Val_1-Pro_2-Gly_3-Gly_4)_{40}-Val-OMe$[2]

Peptide Residue	Me_2SO-d_6		MeOH	
	Slope Hz/10°C	0° Intercept	Slope Hz/10°C	0° Intercept
Val_1 NH	-15.3	1810	-17.3	1806
Gly_3 NH	-12.7	1856	-15.8	1912
Gly_4 NH	-7.9	1776	-7.3	1837

.) Slopes and intercepts given in Hz at 220 MHz.
!) Values from reference 16.

'ABLE II - SOLVENT DEPENDENCE OF PEPTIDE CARBONYL CARBON CHEMICAL SHIFTS

$HCO-(Val_1-Pro_2-Gly_3-Gly_4)_8-Val-OMe$

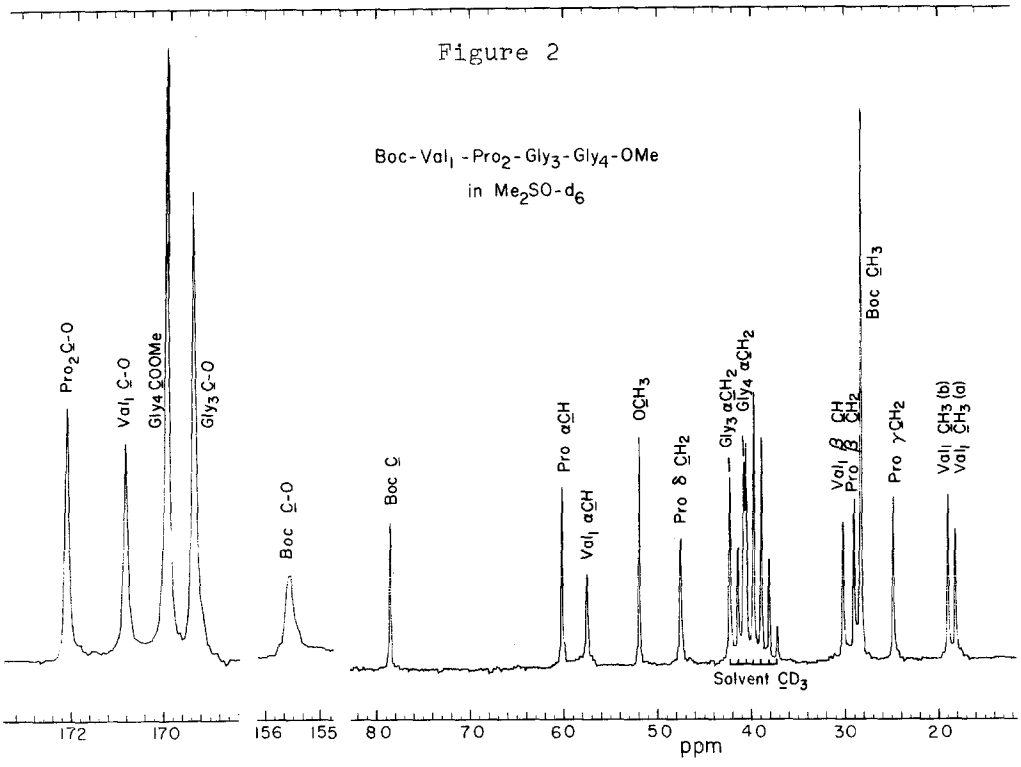
Peptide Residue	[1] Me_2SO-d_6 δ	[2] CD_3OD δ	[3] D_2O δ	[4] TFE-d_3 δ	1→2* $\Delta\delta_j$	1→3* $\Delta\delta_j$	1→4* $\Delta\delta_j$
Val_1 C-O	169.88	172.40	172.90	173.47	0	0	0
Pro_2 C-O	171.82	174.79	175.66	175.66	0.45	0.82	0.25
Gly_3 C-O	168.86	172.01	172.80	173.33	0.63	0.92	0.88
Gly_4 C-O	168.28	171.19	171.78	172.07	0.39	0.48	0.20

The change in chemical shift for the indicated solvent pair given relative to that of the Val_1 C-O i.e.
$\Delta\delta_j = \delta_j$ (C-O in proton[1]donating solvent) - δ_j(C-O in Me_2SO)-$\Delta\delta_1$
ositive values of Δδ indicate a greater downfield shift.
esolution is 0.05 ppm.

Me_2SO-d_6 is given in Fig. 2. Assignment of all resonances in the upfield region was achieved by stepwise synthesis. The carbonyl carbon resonances were assigned by glycine-1-C-13 enrichment and by chemical modification of the amino end. The Gly_3 C-O was enriched to 2% and the Gly_4 COOMe was enriched to 3%. The Val_1 C-O was assigned by its 1 ppm upfield shift on replacement of the Boc protecting group by HCO-. By elimination the lowest field resonance is the Pro_2 C-O. In the high polymer of the tetramer the highest field resonance is the Gly_4 C-O.

Table II contains the chemical shifts of the peptide carbonyl

Figure 2



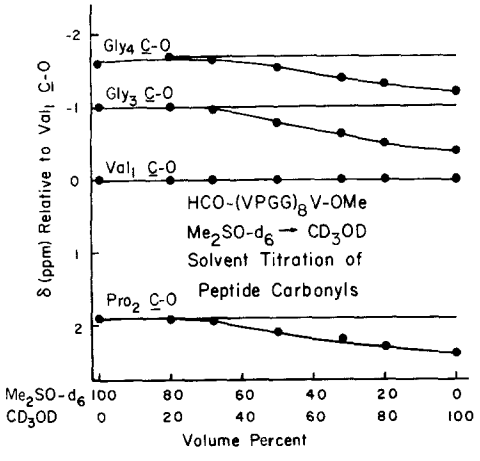


Figure 3

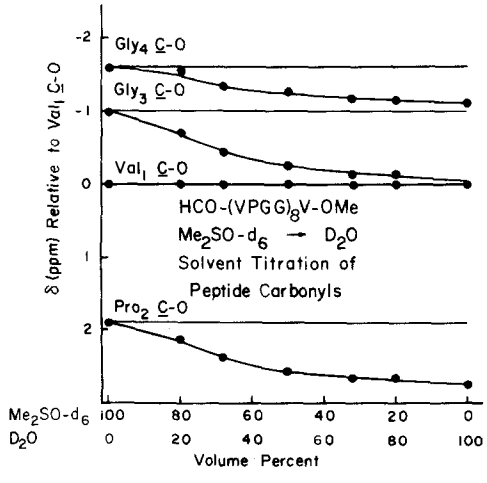


Figure 4

carbon resonances in four solvents for HCO-(Val$_1$-Pro$_2$-Gly$_3$-Gly$_4$)$_8$-Val-OMe. In each case for the transition from solvent 1, Me$_2$SO-d$_6$, to either of the other three solvents, the Val$_1$ C-O shifts the least. In the last three columns of Table II are given, for the three

solvent pairs, the solvent induced chemical shifts of each peptide carbonyl carbon resonance relative to that of the Val_1 $\underline{C}$-O. It is important to note that similar results are obtained for the monomer HCO-VPGG-OMe but not for HCO-VPG-OMe.

A plot of the solvent titration 1⟶2 i.e. Me_2SO-d_6 into CD_3OD is given in Fig. 3, where it is seen that the downfield shifts relative to the Val_1 $\underline{C}$-O occur in a regular manner. This solvent pair is of particular interest as in each solvent the temperature dependence of peptide N$\underline{H}$ proton chemical shift (See Table I) demonstrated the Gly_4 N$\underline{H}$ to be solvent shielded i.e. to exhibit the least slope whereas the other two peptide N$\underline{H}$ moieties exhibit slopes which are about twice as large. This indicates that similar conformations exist in both solvents and provides a mutual explanation for the shielding of the Val_1 $\underline{C}$-O and the Gly_4 N$\underline{H}$. A plot of the solvent titration 1⟶3, Me_2SO-d_6⟶D_2O, is given in Fig. 4. Again the titration is seen to be regular with no complicated form to the curve.

DISCUSSION

The above results demonstrate a decreased solvent sensitivity of the Val_1 $\underline{C}$-O. Since the Val_1 residue precedes proline it is necessary that the primary structural feature be considered. This may be achieved by studying the tetramer HCO-Val_i-Pro_{i+1}-Gly_{i+2}-OMe in the several solvents. In this case the Val_i $\underline{C}$-O is seen not to exhibit the shielding. For example, on going from Me_2SO-d_6 into CD_3OD the Val_i $\underline{C}$-O and the Pro_{i+1} $\underline{C}$-O exhibit the same shift within the resolution limit of 0.05 ppm. This tetramer is also of interest since it does not contain a Gly_{i+3} N$\underline{H}$, suggesting that the Gly_{i+3} N$\underline{H}$ is required for the observed shielding of the Val_i $\underline{C}$-O just as in the pmr studies the shielding of the Gly_{i+3} N$\underline{H}$ required the Val_i $\underline{C}$-O.

We have also observed selective solvent shielding in other polypeptides. In gramicidin S, a cyclodecapeptide with two-fold symmetry which was a model for the development of the pmr approaches (1,4,6), there is a preferential shielding of two peptide carbonyls whereas the other three exhibit 0.5 ppm or greater relative chemical shifts. While the assignments have not yet been made for the peptide carbonyl carbon resonances of gramicidin S, the differentiation is as expected for the secondary structure.

Correlation may also be found with oxytocin. Assignments of the peptide carbonyl carbon resonances have been made for acyclic oxytocin (non-disulfide bridged) in Me_2SO (21) and for oxytocin in D_2O (22). The three peptide carbonyl carbon resonances which exhibit the least shift are those which had been proposed to be involved in three intramolecular hydrogen bonds on the basis of pmr studies (11,23). Also the repeat pentapeptides (24) and hexapeptides of elastin show similar correlation between the cmr and pmr studies. Thus it would appear that solvent dependence of peptide carbonyl carbon chemical shift can, under favorable circumstances, be correlated with polypeptide secondary structure.

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