

Neighbouring Residue Effects on the ^{15}N Chemical Shifts of Some Aliphatic Dipeptides

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Summary The ^{15}N chemical shifts of a number of simple aliphatic dipeptides have been determined in aqueous solution and while the amine nitrogen shift is independent of the nature of the neighbouring residue, the peptide nitrogen shift shows a marked dependence upon the nature of the adjacent amino-acid.

STUDIES on simple peptides using ^{13}C n.m.r. spectroscopy have shown that there is a distinct effect on the carbonyl chemical shift depending upon whether an amino-acid residue is *N*-terminal, *C*-terminal, or non-terminal.¹ However, this shift is independent of the nature of the adjoining residues² and, as such, cannot be used to obtain full sequence information in peptides containing more than three amino-acids. Very little information is available on nitrogen chemical shifts in amino-acids and peptides.³ Consequently, we investigated the ^{15}N chemical shifts in a series of simple dipeptides, to see if a neighbouring residue effect could be discerned.

In order to minimize possible complicating factors due to differential solvent⁴ and substituent⁵ effects the study was restricted to dipeptides composed of the simple aliphatic amino-acids glycine, alanine, valine, and leucine. Since in this case we are restricting ourselves to observations of the effect of varying the size of the aliphatic side chain substituent, the variations observed in this series might be expected to be amongst the smallest observed and, as such, give a simple indication of the potential usefulness of such a technique. All samples used were commercially available and were made up, unbuffered, as 0.2M aqueous solutions in the pH range 5.0—6.2. Spectra were obtained at natural abundance on a Bruker WH-180 spectrometer operating at 18.230 MHz for ^{15}N . Typical running conditions were a pulse delay of 2 s for a 30 μs (38°) pulse angle, giving an accumulation time of about 6 h. The results are given in the Table.

An important question posed by these studies is the intrinsic sensitivity of the nitrogen chemical shifts to the pH

TABLE. ^{15}N Chemical shifts^a of some aliphatic dipeptides

| | -Glycine | | | -Alanine | | | -Valine | | | -Leucine | | |
|---------------|----------|-------|---------|----------|-------|---------|---------|-------|---------|----------|-------|---------|
| | (pH) | Amine | Peptide | (pH) | Amine | Peptide | (pH) | Amine | Peptide | (pH) | Amine | Peptide |
| Glycyl- | (5.8) | 348.9 | 260.4 | (5.1) | 348.4 | 245.9 | (5.7) | 348.6 | 252.2 | (5.8) | 348.9 | 248.1 |
| Alanyl- | (5.6) | 335.0 | 260.4 | (6.2) | 335.4 | 246.1 | (5.8) | 335.1 | 252.2 | (5.3) | 335.1 | 248.7 |
| Valyl- | (5.9) | 340.2 | 255.8 | (6.1) | 340.6 | 242.6 | (5.6) | 340.6 | 247.8 | (5.1) | 340.9 | 244.9 |
| Leucyl- | (5.4) | 336.8 | 257.1 | (5.4) | 336.7 | 244.0 | (5.7) | 336.8 | 250.0 | (5.0) | 337.2 | 246.2 |

^a Positive shifts in p.p.m. upfield from external 1.0 M D^{15}NO_3 .

of the solution (a high intrinsic pH shift would necessitate obtaining all comparative measurements in accurately buffered solutions). The results in the Table show quite clearly, however, that over the pH range employed the nitrogen chemical shift of the amine nitrogen appears quite insensitive to the pH of the solution, the largest variation in a horizontal row (the same *N*-terminal residue) being the shift of 0.7 p.p.m. on going from valyl-glycine to valyl-leucine.

It is clear that, for the compounds studied, the *N*-terminal amine shift appears to be independent of the nature of the adjoining amino-acid. It can also be seen that, even in this simple series, the range of chemical shifts is quite large (13.9 p.p.m.), implying that the amine shifts could prove useful as a means of *N*-terminal residue assignment.

Having established that the amine nitrogens (and therefore presumably the peptide nitrogens) are essentially pH independent over the range studied, it is then possible to examine the results obtained for the peptide nitrogens. As can be seen, not only is the shift dependent upon the nature of the *C*-terminal residue (as would be expected), but also

shows a significant dependence on the nature of the preceding *N*-terminal residue, the overall variations being 4.6, 3.5, 4.4, and 3.8 p.p.m. for glycine, alanine, valine, and leucine. This result demonstrates that ^{15}N spectroscopy may have a significant role for the non-destructive sequence determination of small peptide.

As can be seen, valine and leucine produce a marked effect on the subsequent residue shift compared to glycine and alanine (which produce very similar effects to one another). The effect of the replacement of alanine by valine leads to low-field shifts of 4.6, 3.5, 4.4, and 3.8 p.p.m. for glycine, alanine, valine, and leucine, respectively, while corresponding replacement of alanine by leucine leads to low field shifts of 3.3, 2.1, 2.2, and 2.5 p.p.m. respectively. The similarity of these results suggests a possible additive contribution to the peptide nitrogen shifts in these systems.

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