pH Dependence of the ^{15}N and ^{17}O Nuclear Magnetic Resonance Chemical Shifts of Glycylglycine

By CHARLES S. IRVING and AVIVA LAPIDOT*

(Department of Isotope Research, Weizmann Institute of Science, Rehovot, Israel)

Summary The pH chemical shift titration curves of the glycylglycine amide and ammonium nitrogens and the amide and carboxylate oxygens, measured by ^{15}N and ^{17}O Fourier transform n.m.r. spectroscopy, indicated that on going from the cationic to zwitterionic state the amide C–N double bond character increases and is accompanied by a transfer of electron density from the amide nitrogen to oxygen.

THE effect of deprotonation of the ammonium and carboxylic acid groups of di-peptides has been studied by ^{1}H and ^{13}C n.m.r.[1,2] techniques, and these chemical shift data have been used to describe changes in solvation, conformation, distribution of electron density, and linear electric field effects that accompany the transitions from cation (C) to zwitterion (Z) and zwitterion (Z) to anion (A). Even though these changes directly affect the nitrogen and oxygen atoms of dipeptides, the ^{15}N and ^{17}O n.m.r. spectra of dipeptides have not as yet been studied as a function of pH. We here report the pH titration curves of the ammonium and amide ^{15}N resonances and the amide and carboxylate ^{17}O resonances of isotopically enriched glycylglycines, which show that the distribution of electron density within the peptide bond is affected by the state of ionization of the *N*- and *C*-terminal groups in dipeptides.

As seen in the Figure, deprotonation of the carboxylate group (pK_a 3·1) of the glycylglycine cation causes the carboxylate oxygen resonance to move downfield from 238 p.p.m. (downfield from $H_2{}^{17}O$) to 257 p.p.m. The shift

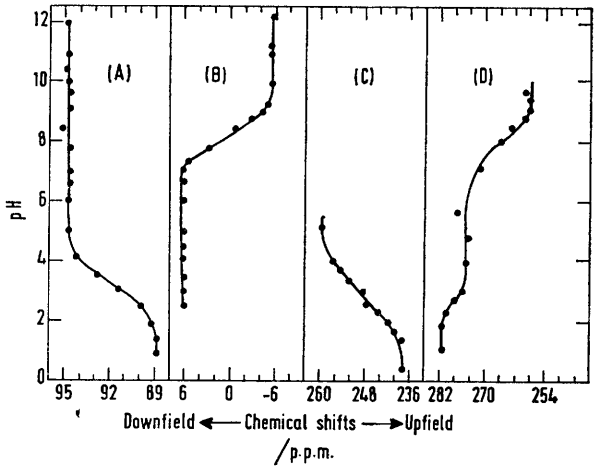

FIGURE. The pH titration curves of (A), the ^{15}N amide resonance of [^{15}N]-glycyl-[^{15}N]-glycine, (B), the ^{15}N ammonium resonance of [^{15}N]-glycyl-[^{15}N]-glycine, (C), the ^{17}O carboxylate resonance of glycyl-[^{17}O]-glycine, and (D), the [^{17}O] amide carbonyl resonance of [^{17}O]-glycylglycine. The ^{15}N chemical shifts were determined from the FT (exponential time constant = −2) of 32 90° pulses with and without ^{1}H decoupling collected in 4096 data points. A 10 s delay between pulses was used; ^{15}N chemical shifts are accurate to ±0·16 p.p.m. The ^{17}O chemical shifts were determined from the FT (t.c. = −10) of 2048 pulses collected in 2048 data points without decouplings and without a post-delay, and are accurate to ±1·3 p.p.m. ^{15}N and ^{17}O n.m.r. measurements were made on a Bruker HFX-90 spectrometer at 2·19 and 12·20 MHz, respectively.

of 19 p.p.m. compares with similar shifts of 23·6 and 21·3 p.p.m., reported for acetic[3] and formic acid,[4] respectively. Moving down the peptide chain, we find that the amide nitrogen resonance shifts downfield from 88·9 p.p.m. (downfield from 4M $^{15}NH_4NO_3$ in 2M HNO_3) to 94·7 p.p.m. The carbonyl oxygen resonance shifts upfield from 281 to 275 p.p.m. The ammonium nitrogen resonance at 6·3 p.p.m. remains unaffected. The $^1J(^{15}N-^{13}C)$ hyperfine interaction between the amide nitrogen and carbon increases in the ^{13}C Fourier transform (F.T.) natural abundance spectrum of [^{15}N]-Gly-[^{15}N]-Gly from 17·7 ± 0·15 to 18·9 ± 0·15 Hz. The $^1J(^{15}N-^1H)$ hyperfine interaction between the amide nitrogen and hydrogen remains constant at 94·1 Hz.

Deprotonation of the ammonium group (pK_a 8·3), as seen in the Figure, causes the amine nitrogen resonance to shift 11·7 p.p.m. upfield to −5·4 p.p.m. This is considerably less than the 28 p.p.m. upfield shift reported for the deprotonation of methylamine.[5] Moving down the glycylglycine chain, we find that the carbonyl oxygen resonance shifts 16 p.p.m. upfield to 259 p.p.m., while the amide nitrogen resonance does not shift at all. The ^{15}N chemical shifts of the amide and amino nitrogen atoms of glycylglycine in the (C), (Z) and (A) states were independent of concentration over the range 1—0·05M.

The downfield shift of the amide nitrogen resonance, the increase in the amide nitrogen–carbon hyperfine interaction, and the upfield shift of the amide oxygen resonance on going from (C) to (Z) are consistent with an increase in the double bond character of the peptide bond and a transfer of electron density from nitrogen to oxygen. The $^1J(^{15}N-^1H)$ amide hyperfine splitting indicates that the amide group remains *trans*[6] on going from (C) to (Z).

The upfield shift of the amide oxygen resonance, the reported downfield shift of the amide carbon resonance, and the absence of a shift of the amide nitrogen on going from (Z) to (A), indicates that deprotonation of the ammonium group enhances the polarization of the amide carbonyl.

We thank the Isotope Separation Plant of the Weizmann Institute for a supply of $H^{15}NO_3$ and $H_2{}^{17}O$, and the National Institutes of Health for a grant.

(*Received, 26th September* 1975; *Com.* 1102.)

[1] M. Sheinblatt, *J. Amer. Chem. Soc.*, 1965, **87**, 572; M. K. Kim and A. E. Marshall, *ibid.*, 1969, **91**, 872; M. von Gorkom, *Tetrahedron Letters*, 1966, **44**, 5433; A. Nakamura and O. Jardetzky, *Biochemistry*, 1968, **7**, 1226; C. A. Swenson and L. Koob, *J. Phys. Chem.*, 1970, **74**, 3376; M. Sheinblatt, *J. Magnetic Resonance*, 1972, **8**, 55.

[2] W. Horsley and H. Sternlicht, *J. Amer. Chem. Soc.*, 1968, **90**, 3738; M. Christl and J. D. Roberts, *ibid.*, 1972, **94**, 4565; C. A. Evans and D. L. Rabenstein, *ibid.*, 1974, **96**, 7312; J. G. Batchelor, *ibid.*, 1975, **97**, 3410.

[3] J. Reuben, *J. Amer. Chem. Soc.*, 1969, **91**, 5725.

[4] H. E. Weaver, B. M. Tolbert, and R. C. La Force, *J. Chem. Phys.*, 1955, **23**, 1956.

[5] W. M. Litchman, *J. Magnetic Resonance*, 1973, **12**, 182.

[6] J. A. Sogn, W. A. Gibbons, and E. W. Randall, *Biochemistry*, 1973, **12**, 2100.