BIOPHYSICAL APPLICATIONS OF ^{17}O NMR

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Oxygen plays a major role in many biophysical processes: in the determination of molecular structure and biological activity, in weak and in strong interactions, in being an important constituent of the peptide bond and nucleic acid bases, and in being a part of the carboxyl, hydroxyl and carboxamide groups of peptide side chains and end groups. It is important to emphasize the major role that weak interactions as van der Walls including hydrogen bonding have in physiological processes: in the binding of hormones (as peptide hormones to the receptor site) and in generating a stimulus that triggers a chain of intermediary steps leading to the final biological response; in determining the code of information transfer in the action of the hormone; and in the transfer of genetic information. Weak interactions play a major role in stabilizing the biologically active conformation of molecules and in the kinetics of biological processes.

The utilization of oxygen magnetic resonance as a probe in the investigation of biologically related processes encounters difficulties due to the low natural abundance of the ^{17}O isotope (.037%) that possesses a magnetic moment. Its utilization requires enrichment of biomolecules in the ^{17}O isotope. We have developed and utilized synthetic methods for ^{17}O enrichment of amino acids (1,2), peptides (2), and nucleic acid constituents (3) and studied the molecular conformation in solution, solute solvent interactions and metal ion complexation at the oxygen and nitrogen sites. We have found ^{17}O NMR to be an effective probe in studies of the intra- and intermolecular interactions and molecular dynamics of peptides and nucleic acid bases.

We have found that 1) hydrogen bonding has a marked effect on the ^{17}O chemical shift of the carbonyl group, by 2-3 orders of magnitude larger than its effect on ^{1}H (4,5) and 2) that bond character of the acyl group has a much larger effect on the ^{17}O chemical shift (6 - 8) than it has on the ^{13}C chemical shift. (See Table 1) Although the line widths of the ^{17}O resonance are broader than that of ^{1}H and ^{13}C, the much higher chemical shift variations more than compensate for the line widths, e.g., for a tripeptide the line width is a few hundred Hz at a field of 42 kG (2,7,9) and, therefore, the above variations in the chemical shift could easily be detected.

Recently, we have studied by means of ^{17}O and ^{14}N NMR the conformation, solute solvent interactions, pH effects, and determination of protonation sites of amino acids (8,10 - 12), carboxamides and methyl ester derivatives (13), amides (5,14), peptides (2,7 - 9), and nucleic acid constituents (3). We also studied metal ion binding to amino acids and peptides (15). Studies have been carried out as a function of pH, concentration, mixed solvent composition and temperature. Studies have also been carried out on model compounds of peptides in liquid crystals (16) yielding information on the ^{17}O nuclear quadrupole coupling constant of ^{17}O labeled compounds. A short description of our studies will be presented in this report.

I. pH EFFECTS ON THE ^{17}O CHEMICAL SHIFTS OF AMINO ACIDS, PEPTIDE CARBOXAMIDES AND METHYL ESTER DERIVATIVES

pH effects on the ^{17}O chemical shift of the carbonyl group in its several functional groups: ketones, aldehydes,

Table 1

^{17}O and ^{13}C Chemical Shifts of the Peptide Bond
(references for the chemical shifts: ^{17}O - H_2O; ^{13}C - TMS)

Bond	Length A	C-13 ppm	O-17 ppm
C-O	1.43	40 - 80	-30 - +30
C=O (carbonyl)	1.22	170 - 220	520 - 590
C=O (amide)	1.23	162 - 173	300 - 360
C=O (peptide)	1.24	162 - 173	300 - 360

C-13 data, ref. (26)
O-17 data, ref. (27)

Table 2

^{17}O Nuclear Magnetic Resonance Parameters in Highly Acidic Solvents (ref. 28)

Amino Acid	1 N HCl (ppm)	$HFSO_3$ (ppm)
Val	256	239
Leu	252	234
Ile	256	238
Pro	249	234
His	257	243
Gly	253	233

peptides and its model compounds as amides, hetrocyclic compounds as in nucleic acid bases and urea (Fig. 1) are most interesting in shedding light on the protonation sites and the electronic structure of these molecules.

A. Amino Acids

The pH dependence of the ^{17}O chemical shift shows a paramagnetic shift at around the pK value of amino acids of about 13 to 18 ppm for alanine, glycine, proline, leucine, histidine, valine, isoleucine, aspartic and glutamic acids. The paramagnetic shift at around the pK value for acetic acid (17), formic acid (18), glycylglycine (19), the β COOH of aspartic acid (20) and the γ COOH of glutamic acid (10) results in a downfield shift greater than 22 ppm. This shift is most likely due to overriding effects of the bond order-charge density term in the paramagnetic contribution towards nuclear shielding (21). At highly acidic solvents a diamagnetic shift takes place.

In Table 2 the chemical shifts of several amino acids in $HFSO_3$ are shown to be shifted 14 to 20 ppm relative to their chemical shift in 1 N HCl. With further increase in acidity of the medium to magic acid ($HFSO_3/SbF_5$ 1:1 molar ratio), the glycine resonance

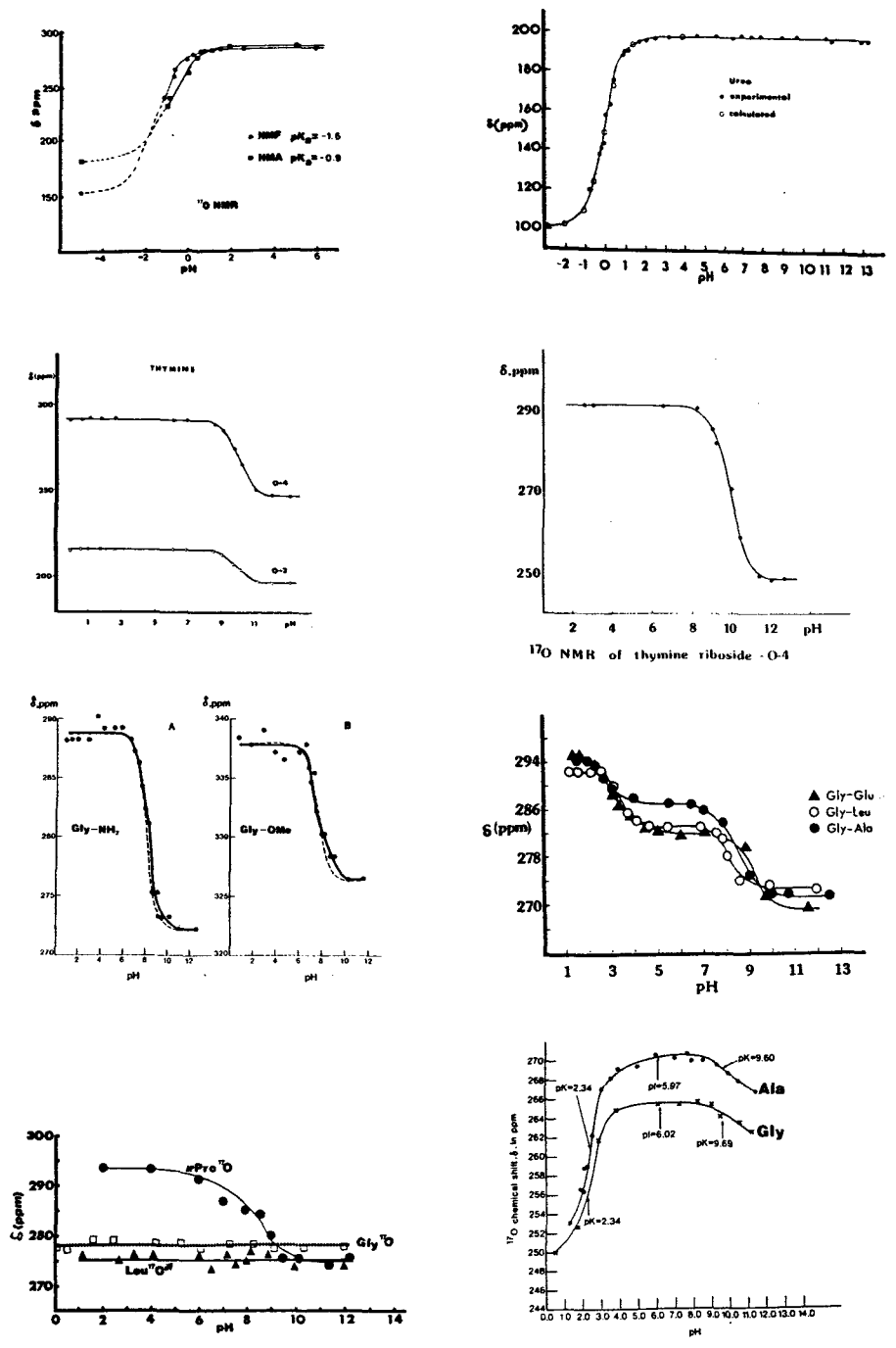

Figure 1. pH dependence of acyl oxygen-17 chemical shifts.

shifted to 209 ppm. We interpret 1) the chemical shift of 209 ppm of glycine in magic acid to represent the diprotonated form and 2) the chemical shifts in $HFSO_3$ to represent a contribution from a fraction of the molecules in the diprotonated form. Using a value of 45 ppm (the average value observed for glycine (44 ppm), acetic acid (46.5 ppm) and propionic acid (45 ppm) (22)) to represent the ^{17}O diamagnetic shift resulting from protonation of a carboxylic acid, the amino acids are estimated to be 30-40% diprotonated in $HFSO_3$ (assuming there is no significant amount of diprotonation at pH 0).

B. Peptide Carboxamides

The pH dependence of the chemical shift of Gly-NH_2 ^{17}O in aqueous solution showed a diamagnetic shift of 17 ppm at its pK value of 7.9 (23). This shift is due to deprotonation of the NH_3^+ group through a larger contribution of resonance structures in which the N donates its lone electron pair to the C-N bond.

C. Methyl Ester Derivatives

The pH dependence of [^{17}O]-Gly-OMe chemical shift showed a diamagnetic shift of 12 ppm at the pK value of 7.51 (24). This shift is due to deprotonation of the ester oxygen through an increase in the single bond character of the acyl oxygen.

The ^{17}O chemical-shift value of urea in the pH region 3 to 13 is about 195 ppm whereas that of ^{17}O in amides and peptides is between 275 and 295 ppm. The large diamagnetic shift of the ^{17}O of urea relative to that in amides and peptides is due to the increased single bond character of the carbonyl group in urea through resonance with two adjacent NH_2 groups.

^{17}O NMR studies of urea were practically pH independent in the region from pH 13 up to pH 3 and showed a large diamagnetic shift of 96 ppm at about pH 0 which we attributed to a protonation reaction at the urea oxygen having a pK of 0.1. A paramagnetic shift of 36 ppm was observed in magic acid which is attributed to diprotonation of the urea where the second protonation takes place at the site of the nitrogen atom. These findings may be rationalized in terms of the acyl group bond character. The first protonation at the oxygen atom increases the single bond character of the carbonyl bond whereas the second protonation at the nitrogen atom eliminates one of the possible resonance structures of the urea resulting in an increase in the double bond character of the carbonyl bond and consequently ^{17}O resonance shift to a lower field.

Protonation of amides at about pH -1 is followed by a diamagnetic shift of about 40 ppm due to protonation at the site of the oxygen atom. pH dependence of nucleic acid bases as thymine shows a large region of pH independence between pH 0 and 9. The ^{17}O chemical shifts at position 4 of thymine are similar to that of amides and peptides, and that of oxygen in position 2 are similar to oxygen in urea as one could expect from the similarity in structure of the relevant molecular segments.

II. PEPTIDES

Previously reported studies primarily concerned with model compounds (5, 6,8,14) have been extended to oligopeptides and their derivatives, hexapeptides and nonapeptides (2,7,9). ^{17}O chemical shifts of peptides cover a region of approximately 70 ppm depending upon the solvent, pH and the neighboring amino acid residues and protecting groups. A correlation between the solvent proton donating ability and the ^{17}O chemical shift to high field exists e.g., upon addition of water to a solution of cationic Gly-Leu in DMSO an upfield shift of 30 ppm was observed.

Solvent dependence studies are most informative in providing means of discerning between the inter- and intramolecular H bonding (of utmost importance in understanding the molecular structure and biological activities of

peptides). One example will be given here. Pro-Leu-Gly-NH_2 (MIF) is biologically active in the CNS of rodents and is thought to inhibit MSH release in amphibians. It forms the C-terminal tripeptide in oxytocin and therefore studies of its conformation in solution constitutes an essential part of the investigation of the physiological activities of peptide hormones. Despite a substantial amount of experimental evidence, the conformation of MIF in solution remains ambiguous. ^{17}O chemical shift of the tripeptide Pro-Leu-Gly-NH_2 (MIF) was measured as a function of the mole fraction of CH_3CN in a solution of mixed solvents of H_2O and CH_3CN (Fig. 2). These studies have answered an open question and have shown that intramolecular H bond is formed between the Gly NH_2 group and the proline peptide oxygen (9).

^{17}O NMR Studies of Peptides and Model Compounds in Liquid Crystals (Fig. 3)

One example will be given of ^{17}O labeled urea in lyotropic liquid crystalline phase of type II. ^{17}O, ^{14}N and ^{2}H quadrupolar splitting of the Zeeman levels of urea were observed. Also, ^{17}O and ^{2}H quadrupolar splitting of H_2O molecules in type I and type II mesophases were used to determine the order parameters. The ^{17}O nuclear quadrupole coupling constant was determined to be 10.2 mHz (16). The ternary lyotropic liquid crystalline mesophase formed by decyl ammonium chloride, sodium chloride and water was used as type II phase, and sodium decyl sulphate, sodium sulphate and water was used as type I phase in our investigation.

III. NUCLEIC ACID CONSTITUENTS

Only a short description of the pH dependencies of O-2 and O-4 for uracil and thymine will be given here. The pH dependencies are similar for both molecules; a large upfield shift for O-4 (46 and 44 ppm, respectively) and a small upfield shift for O-2 (18 and 19 ppm, respectively) have been determined. The diamagnetic shift is due to the deprotonation of N-3 at a pH of 9.6 (3) in agreement with the literature value of pK 9.5 (25). The absolute value of the ^{17}O chemical shift of O-4 is similar to the chemical shift of amides and peptides whereas the chemical shift of O-2 is similar to that of urea due to the similarity in structure.

In conclusion ^{17}O was found to be an effective tool in studies of the electronic structure (e.g., conformation, cis-trans isomerism, H bonding sites) of amino acids, peptides, nucleic acid constituents and their derivatives. Synthetic methods have been developed to label the above materials with ^{17}O. Several biologically active molecules as MIF and oxytocin have been examined. Sites of inter- and intramolecular H bonding have been determined and solute solvent interactions studied. Investigation of metal ion binding to amino acids and peptides have been found most illuminating. ^{17}O resonance of complexes of proline bound to the cobaltous ion was observed (Fig. 4). Relative concentrations of the complexes have been determined and kinetics of metal ion bindings studied. ^{17}O NMR of solutes in liquid crystalline media yielded information on the nuclear quadrupole coupling constants. In the future we hope to extend this work to biologically related studies of membranous materials.

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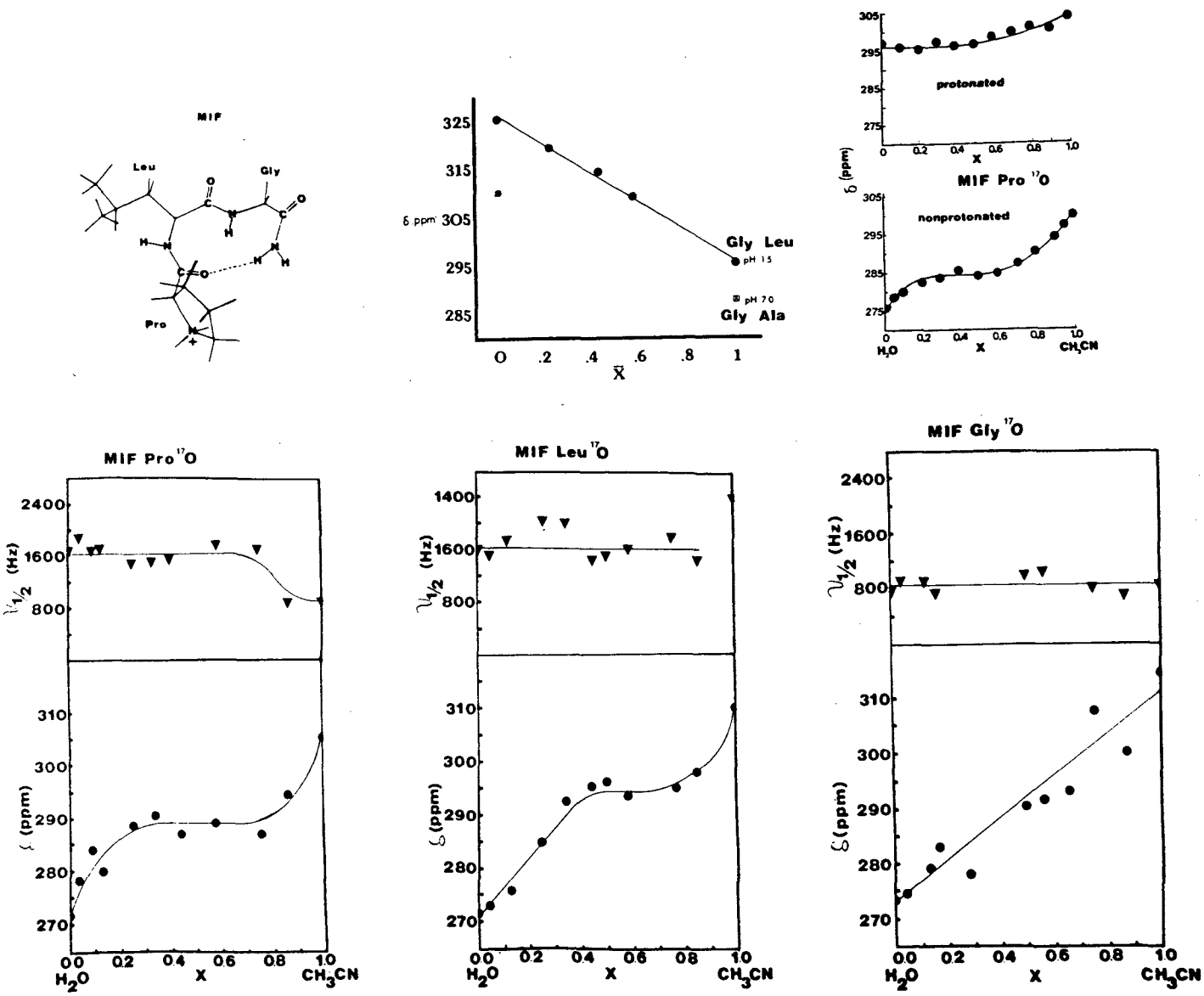

Figure 2. Solvent effects on peptide oxygen-17 chemical shifts.

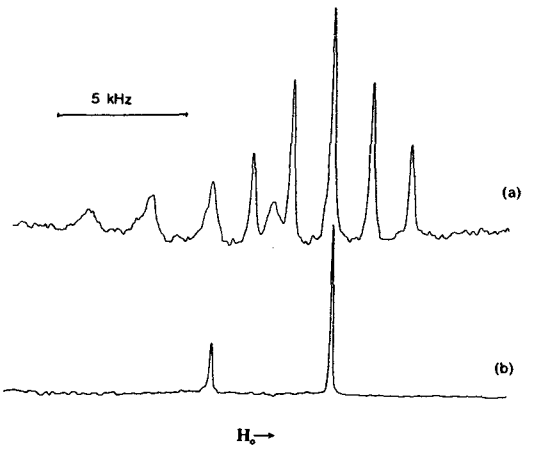

Figure 3. Oxygen-17 spectra of urea (a) in a lyotropic liquid crystalline medium of type II and (b) in the isotropic phase at 55°C.

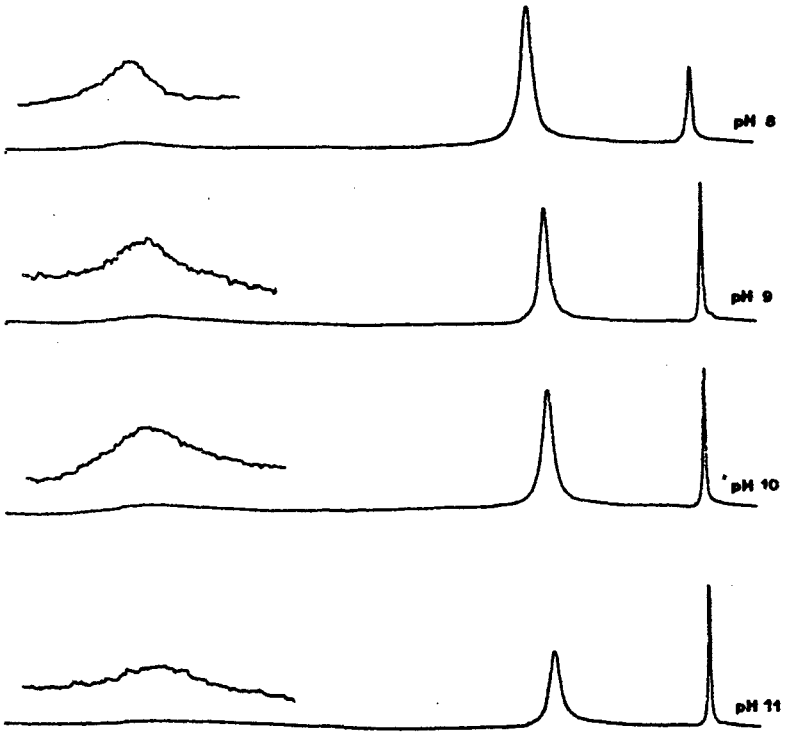

Figure 4. ^{17}O NMR spectra of 0.27 M ^{17}O labeled proline in 0.053 M cobaltous ion aqueous solution at 25°C at several pH values, (8;9;10;11).

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