^{17}O NMR Study of Polycrystalline l-Leucine

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INTRODUCTION

Most NMR studies of amino acids and peptides have been done in liquid phase (1-14). Many of the structural parameters obtained in these studies are basically the averaged values of parameters characterizing molecules in solid state. It is also known that there are small differences in the structure of the molecule in solid and liquid states (15). Therefore, results of NMR studies in solid and liquid state give different type of information.

Complete knowledge of the structure and dynamical properties of a given material in solid state allows one to draw many conclusions as to the properties of the same material in liquid state. Therefore studies of solids are more informative and should be conducted whenever possible.

There is experimental evidence that the conformation of proteins is determined by their amino acid sequence (16, 17). It is possible to identify the secondary structure of proteins based on sequence data alone (18). Therefore gathering information on structural and dynamical parameters of amino acids and peptides produces a good starting point for structural studies of more complicated compounds.

NMR spectroscopy is a very powerful method for structural and dynamical studies (19), and ^{1}H, ^{2}H, and ^{13}C spectroscopies have been used extensively in the studies of amino acids and peptides (1-14, 20-22). None the less, there is a very limited number of ^{17}O NMR studies in this field of research, and this is a serious gap as oxygen occupies a key position in the peptide molecules and plays a major role in their molecular conformation and in many physiological processes (23-26). The importance of a hydrogen bonding in biological systems (27-29) makes oxygen a valuable probe for NMR spectroscopy. Due to a very low natural abundance (0.037%) of ^{17}O and its low NMR sensitivity (at constant magnetic field the resonance frequency is about 7.38 times lower than that for protons) the number of papers published on the ^{17}O NMR studies of amino acids and peptides is very limlted, and they report results on liquids or solutions (6-13, 24-26, 30-35). Analogous studies in solid phase are virtually non-existent (36-38). However, studies of amino acids and peptides in solid state provide structural and dynamical informations not available from studies in liquid phase, where many of the important interactions are averaged out by Brownian motion. From the solid state ^{17}O NMR studies complete tensors of the chemical shift, electric field gradient, and dipolar interaction can be determined. The electric field gradient (at the position of ^{17}O) and chemical shift tensor are very sensitive to the electronic structure of the molecule and their knowledge reveals structural details (39) not available from X-ray or neutron diffraction experiments. The energies of irradiation in these cases are much higher than in NMR spectroscopy, and the small energy changes due to the subtle changes in electronic structure cannot be detected by these methods alone (40). This is especially evident, when main interest is in molecular dynamics and in such inter- or intramolecular interactions as hydrogen bonding.

These ^{17}O NMR studies of polycrystalline 1-leucine have been

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undertaken to gather structural and dynamical information not available from other studies.

EXPERIMENTAL METHODS.

Chemical aspects. For the ^{17}O NMR measurements, l-leucine enriched to about 20% ^{17}O at the carboxyl site by methods described previously, (41-44) was used. Synthesized material was passed through a chelex column twice, for further purification. About 1 g of so prepared l-leucine was sealed in a glass tube 10 mm in diameter and 20 mm in length.

Nuclear magnetic resonance spectroscopy. Oxygen-17 NMR spectra were obtained on FT NMR Bruker CXP 180 high power pulse spectrometer operating at 24.4 MHz. Due to very low natural abundance of ^{17}O nuclei (0.037%) and low NMR sensitivity, it was necessery to use isotopically enriched materials. For the level of enrichment used in our study (20%), it took about 4 to 5 hours to obtain the ^{17}O NMR spectrum with the S/N ratio of about 20. We developed an experimental procedure facilitating the recording of the ^{17}O NMR spectra from solids (46). Our procedure has enabled us to obtain the same spectrum with the same signal to noise ratio with only 50 to 60 minutes acquisition time.

The ^{17}O NMR spectra of the central transition ($m=1/2\leftrightarrow-1/2$) of polycrystalline l-leucine were obtain using the quadrupolar echo sequence (4, 44-46)

$$90°_x - t_1 - 90°_y - t_2 - A. \qquad [1]$$

The x and y subscripts denote the direction of the H_1 field in the rotating frame, that is the phase of the r.f. pulses t_1 and t_2 are the time delays and A stands for acquisition.

The ^{17}O solid echo signals of polycrystalline l-leucine were recorded with the delay times $t_1=60\mu s$ and $t_2=50\mu s$. Acquisition was done with $0.2\mu s$ dwell time and relaxation delay was 200 ms. Duration of the 90° pulse was $4.2\mu s$. 20,000 acquisitions were sufficient to get a spectrum with S/N ratio of about 25. The line shape of the central transition ($m=1/2\leftrightarrow-1/2$) spectrum was obtained at different temperatures in the range from 190 K to 358 K, through Fourier transform of the second half of the recorded echo signal. The temperature of the sample was monitored by a Bruker temperature control unit, with the copper-constantan thermocouple external to the sample, to within ±1 K. A minimum of 20 min. was allowed for a sample to reach temperature equilibrium. Quadrupole coupling constants and asymmetry parameters were obtained by computer simulation of the experimental spectra using the numerical procedure developed in our laboratory (47).

RESULTS AND DISCUSSION

Variable temperature study of ^{17}O NMR central transition in polycrystalline l-leucine. NMR quadrupolar echo experiments have been performed on polycrystalline l-leucine in order to gain insight into its crystalline and molecular structure and molecular dynamics. Our focus has been on N - H···O hydrogen bond which appears to be an almost universal feature of amino acid aggregation in the solid state (48).

The unit cell of leucine contains two crystallographically nonequivalent molecules, A and B (49,50). Every nitrogen atom of molecule A is hydrogen bonded to oxygen from three different molecules in nearly tetrahedral directions. In molecule B every nitrogen atom also forms three hydrogen bonds with three different molecules but one of these hydrogen bonds is the so called "bifurcated hydrogen bond" (one hydrogen atom shared between two oxygens of one molecule). Thus, we can distinguish two chemically nonequivalent oxygen sites in the lattice cell of l-leucine. One of them represents the oxygen site to which the proton is hydrogen bonded (^{17}O···H - N), whereas the other represents the site to which the proton is bonded through a bifurcated hydrogen bond. The difference in the bond length between the two sites (normal hydrogen bond - 2.8 Å) and bifurcated hydrogen bond - 3.0 Å) can cause differences in the quadrupole coupling constants and in the asymmetry parameters. There are deviations from

TABLE I.
Parameters extracted from ^{17}O NMR spectra of 1-leucine recorded at different temperatures.

Temperature [K]	Outer Component Q_{cc} [MHz]	η	Inner Component Q_{cc} [MHz]	η
190	8.0±0.2	0.05±0.02	8.0±0.2	0.8±0.05
297	7.65±0.05	0.05±0.01	7.8±0.1	0.65±0.05
353	7.6±0.1	0.05±0.02	6.0±0.2	0.5±0.05

Fig. 1

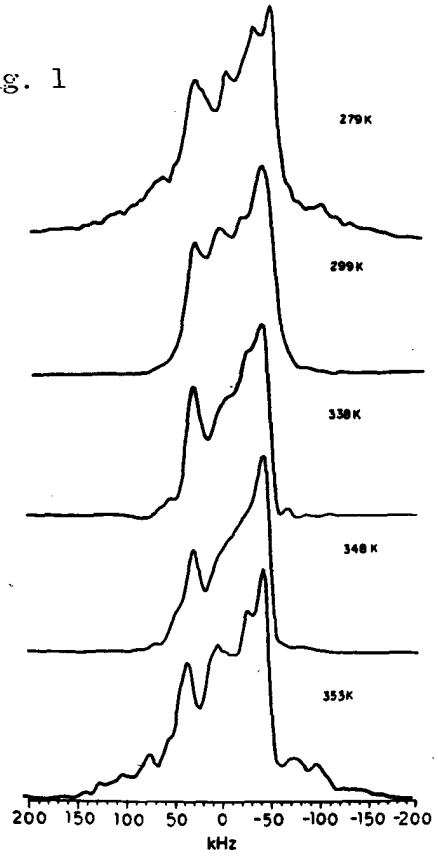

Fig. 2

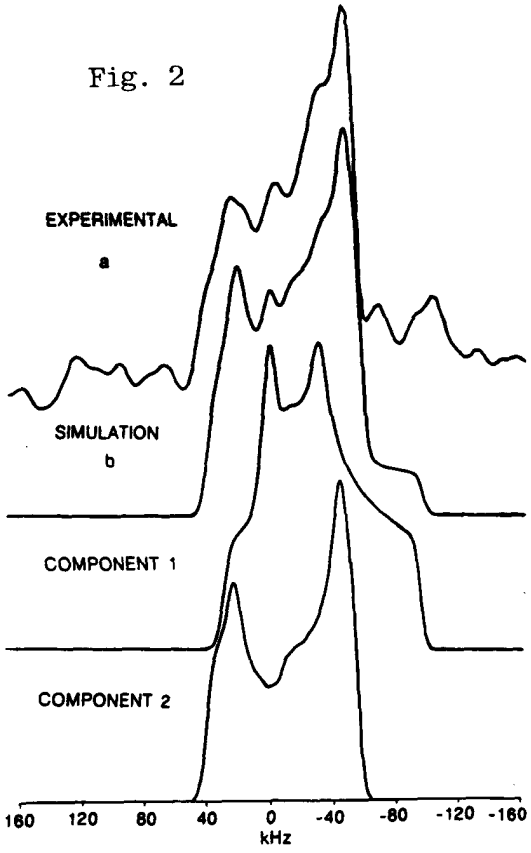

Figure 1. ^{17}O NMR spectra of polycrystalline 1-leucine recorded at different temperatures. Spectra were recorded with the following quadrupolar echo sequence:
90^{o}_{x} - t_1 - 90^{o}_{y} - t_2 - A.
Duration of the 90° pulse was 4.2, t_1 was 60μs, and t_2 was 50μs. A denotes acquisition and was done with 0.2μs dwell time. Relaxation delay was 200 ms, spectrum reflects 20,000 acquisitions.

Figure 2. Results of the numerical analysis of the ^{17}O NMR spectrum of polycrystalline 1-leucine recorded at 297 K.
a) - experimental spectrum, b) - simulated as a superposition of two components with relative integral intensities 0.4:1. Component 1 with Q_{cc}=7.8 MHz and η=0.65; component 2 with Q_{cc}=7.64 MHz and η=0.05.

linearity for N-H···O in leucine, and as would be expected, the weaker (bifurcated) bonds are more distorted than the stronger bonds. The average N - H···O angle for bifurcated and normal hydrogen bonds in leucine are 155 and 170 degrees respectively (50).

We show in Figure 1 typical spectra of 1-leucine at different temperatures, obtained by the quadrupole-echo Fourier-transform technique, at an operating field strength of 4.23 T (corresponding to a ^{17}O resonance frequency of 24.4 MHz). Quantitative analysis of ^{17}O NMR data involves fitting the theoretical line-shape to the experimental powder patterns. The best fit to the experimental spectra was obtained with the assumption that the spectrum is the superposition of two powder patterns with nearly equal quadrupolar coupling constants Q_{cc} but different asymmetry parameters η. Over the temperature range studied, very little change in the line width and line shape of the outer component of the spectrum was observed. In contrast, the inner part of the leucine spectrum showed a marked temperature dependence. An example of numerical analysis of the ^{17}O NMR spectrum of 1-leucine, recorded at 297 k, is given in Fig.2. The calculated spectrum (Fig.2b) was obtained by summing (with appropriate weights) two powder patterns, one with Q_{cc}=7.64 MHz and η=0.05 (component 2) and the other with Q_{cc}=7.80 MHz and η=0.65 (component 1). Spectra at three different temperatures were analyzed by this method. Results are listed in Table I.

We assign the more temperature sensitive component of the 1-leucine spectra to the oxygen involved in bifurcated hydrogen bonds and the other one to the oxygen involved in normal hydrogen bonds. This assignment is supported by ratio of the integral intensities of the separate spectrum components used in the fitting procedure. This ratio is 5:2, the same as the ratio of oxygen atoms involved in normal and bifurcated hydrogen bonds. One can expect more freedom for motion of the oxygen involved in bifurcated hydrogen bonds with increasing temperature, and this is what we found in our measurements. The asymmetry parameter for oxygen in bifurcated hydrogen bond in 1-leucine has higher value at all of the temperatures studied. This means larger deviation of the EFG tensors from axial symmetry for ^{17}O nuclei involved in bifurcated hydrogen bonds compared with the EFG tensor at ^{17}O nuclei in "normal" hydrogen bonds and is consistent with X-ray diffraction studies of 1-leucine.

Our ^{17}O NMR studies of 1-leucine powder pattern clearly demonstrate the sensitivity of the electric field gradient at the ^{17}O nucleus to the conformations and strengths of the intermolecular hydrogen bonds involved as well as to temperature.

CONCLUSION

Results of the ^{17}O NMR studies of polycrystalline 1-leucine demonstrate the feasibility of oxygen muclei as a probe for investigating structural and dynamical parameters of hydrogen bonds of the X - O···H type. With higher isotopical enrichment (about 50%) of ^{17}O and with a better NMR probe, which is now under construction, we expect to obtain much higher S/N ratio of the recorded spectra. This will enable us to perform more detailed quantitative analysis of the experimental results. The results presented here may be encouraging for other researchers to devote some time to ^{17}O NMR studies of solid amino acids. It is a time consuming, but very rewording methodology.

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