A Structural Study of Polyglycine II in the Solid State by ^{17}O CP MAS NMR Spectroscopy

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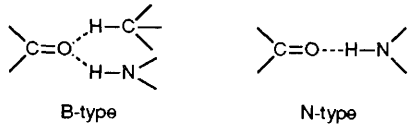
^{17}O NMR spectra of polyglycine II (3_1-helix conformation) in the solid state are measured by the ^{17}O CP MAS NMR technique; a comparison of the experimental findings with X-ray diffraction results reported previously shows that polyglycine II exists as antiparallel 3_1-helical chains.

It is known that polyglycine [$(Gly)_n$] takes two kinds of crystalline forms designated by I and II.[1] As determined from X-ray diffraction, $(Gly)_n$ I takes the β-sheet form[2] and $(Gly)_n$ II takes a 3_1-helix form.[3] The crystal structure of $(Gly)_n$ I has been conclusively determined, but for $(Gly)_n$ II two kinds of crystal structure have been proposed with some ambiguity, these are a hexagonal array of parallel chains or antiparallel chains. In the parallel chain structure, all three residues in the repeat of a single chain are equivalent (all having bifurcated hydrogen bonds) but in the antiparallel chain structure the three residues are not equivalent,[4] one participates in a B-type hydrogen bond and two are normally hydrogen-bonded to each other (N-type). Up to now however, there has been no obvious evidence whether $(Gly)_n$ II has parallel or antiparallel chains. Recently, solid-state high-resolution ^{13}C and ^{15}N NMR spectroscopies have been successfully applied to the investigation of polypeptides in the solid state due to rapid progress in the development of both the methodology and instrumentation.[5] Nevertheless, solid-state high-resolution ^{13}C and ^{15}N NMR experiments could not give information about whether $(Gly)_n$ II takes parallel or antiparallel chains as in the crystal structure. In order to overcome this problem, we have used the oxygen nucleus in polypeptides as an NMR probe because the ^{17}O chemical shift is more sensitive to structural changes than ^{13}C and ^{15}N chemical shifts. The oxygen atom in polypeptides plays a very important role in intra- and inter-molecular interactions because they are directly associated with the formation of hydrogen bonds. In this work, we attempt to prepare ^{17}O-labelled $(Gly)_n$ II and to observe the solid-state high-resolution NMR spectra of the samples obtained, in order to establish whether $(Gly)_n$ II takes parallel or antiparallel chains in the crystal structure and to provide useful perspectives in the use of ^{17}O NMR for investigating the structural analysis of polypeptides in the solid state.

6% ^{17}O-Labelled glycine (Gly) was prepared by the use of glycine methyl ester in $Na^{17}OH$–methanol solution, where $Na^{17}OH$ was prepared by reaction of 11% ^{17}O-labelled water (purchased from Cambridge Isotope Lab.) with Na metal. ^{17}O-Labelled Gly *N*-carboxyanhydride (NCA) was prepared by heterogeneous polymerization of 6%-labelled ^{17}O-glycine *N*-carboxyanhydride in acetonitrile by using n-butylamine as the initiator. The mole ratio of NCA to initiator (A : I) was chosen as 100 : 1. The conformation of this sample was converted to $(Gly)_n$ II by precipitation from aqueous lithium bromide solution. The conformational characterization was made on the basis of ^{13}C CP MAS(cross-polarization magic-angle-spinning) NMR and the complete convertion to $(Gly)_n$ II was identified. The ^{17}O MAS NMR spectra were recorded with a JEOL GSX-500 spectrometer operating at 67.8 MHz with a CP MAS accessory at room temperature. The sample was contained in a cylindrical rotor made of silicon nitride and spun as fast as 8 kHz. We used the CP MAS method with a repetition time of 5 s and a contact time of 9 ms. The ^{17}O chemical shifts were calibrated with respect to external liquid water (δ 0 ppm). Solid state ^{17}O spectra contain the quadrupole interaction, which broadens the central transition (−1/2,1/2) only to second order, and this interaction may not be reduced by MAS. Therefore, the ^{17}O CP MAS NMR signal contains three kinds of NMR parameters the nuclear quadrupole coupling constant (e^2qQ/h), the asymmetry parameter (η) of the electric field gradient tensor, and the isotropic chemical shift (δ). In order to obtain these parameters, computer-fitting was carried out by superimposing the theoretical line shape[6] to the observed spectrum. Further the ^{13}C CP MAS NMR spectra were recorded with a JEOL GSX-270 spectrometer operating at 67.7 MHz with a CP MAS accessory at room temperature.

If $(Gly)_n$ II takes a 3_1-helix parallel chain form then all the three residues in the repeated unit of a single chain are magnetically-equivalent to each other. In this case, it can be expected that the ^{17}O spectrum of $(Gly)_n$ II will show only one



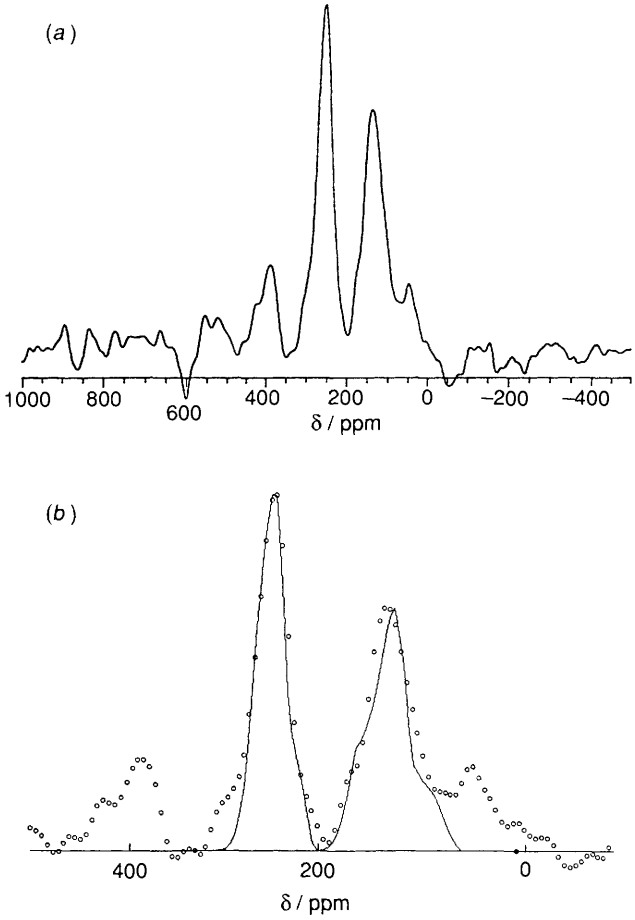


Fig. 1 67.8 MHz ^{17}O CP MAS NMR spectrum of polyglycine II in the solid state at room temperature (*a*), and theoretical simulation of polyglycine II (*b*) where the open circles and solid line indicates the observed and calculated spectra, respectively

quadrupolar spectrum pattern. On the other hand, if $(Gly)_n$ II takes antiparallel chains, these three residues are not magnetically-equivalent and only one participates in a bifurcated B-type hydrogen bond and two in N-type hydrogen bonds. In this case, the ^{17}O spectrum of $(Gly)_n$ II shows two quadrupolar spectrum patterns with two different chemical shifts corresponding to the two kinds of hydrogen bonds. Fig. 1(*a*) shows the observed 67.8 MHz ^{17}O CP MAS NMR spectra of $(Gly)_n$ II in the solid state. It is found from this spectrum that the oxygen atoms clearly are in two kinds of magnetically-inequivalent sites. The computer-fitting of the theoretical spectrum to the observed spectrum was carried out as shown in Fig. 1(*b*). From this fitting, we obtained, for one of the two kinds of sites (site A), that $e^2qQ/h = 4.0$ MHz, $\eta = 0.9$, and δ 270, and for the other site (site B) $e^2qQ/h = 5.5$ MHz, $\eta = 0.8$, and δ 170. The intensity ratio of site A : site B is about 2 : 1. From this experimental finding, it can be said that the signal for site A comes from the oxygen atoms of the two residues with N-type hydrogen bonds, and the signal for site B comes from the oxygen atom of the residue with a B-type hydrogen bond in the repeat units of a single chain. The difference in δ between the two sites is very large owing to the difference in the formation of hydrogen bonds. This never appears in the ^{13}C CP MAS NMR spectrum, this shows that solid state ^{17}O NMR is a very useful tool for studying hydrogen bonded structures. We can conclude that $(Gly)_n$ II takes the form of antiparallel chains and that there are two N-type hydrogen bonds and one B-type hydrogen bond in the repeat unit.

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