BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 3028—3029 (1969)

The NMR Study of Poly-L-Prolines I and II

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(Received April 22, 1969)

As is well known, poly-L-proline (PLP) takes two different helical structures, the so-called I and II forms, which were established by the X-ray diffraction method; one is a right-handed helix and the peptide bonds are in a *cis* configuration,[1] whereas the other is left-handed, with the peptide bonds *trans*.[2]

The rotation about the Cα–N bond of L-proline is severely restricted because the Cα–N bond participates in a pyrrolidine ring. The rotation about the Cα–CO bond of PLP is also definitely restricted by the steric hindrance, as can be verified by using atomic models.[3] According to Steinberg *et al.*[3] the hydrogen atom attached to the alpha carbon is restricted to the *cis* or the *trans* position in relation to the C–O bond, although the configurations have some rotational freedom around the Cα–CO bond.

Both helices of the poly-L-prolines take nearly the *trans* configuration about this bond. The *cis* configuration, in regard to the bond of PLP, is expected to exist only in a concentrated salt solution of lithium bromide[3] or calcium chloride.[4]

On the other hand, the conformation and the transconformation of PLP in solutions[5] have been studied by using its optical-active properties, such as ORD[6] or CD,[7] and its hydrodynamic property.[3] The results have shown that the poly-L-proline takes the PLP-I and II-helices in solutions, unlike the homo-polypeptides of the other α, L-amino acid. Moreover, it has been also reported that poly-L-α, amino acids show different nuclear magnetic resonance spectra because of their conformations.[8] Therefore, the study of PLP by the NMR method is interesting in that the confromational characteristics of PLP should be reflected in the NMR spectrum. The NMR method may also give us additional information on the property of PLP in solutions.

This communication will describe how poly-L-prolines have different NMR spectra according to their conformations. The poly-L-proline has the I-helical form immediately after the sample was dissolved in acetic acid. However, it changed into the II-helix within two weeks after dissolution when kept at 25°C. The value of $[\alpha]_{546}^{25}$ was

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ca. +50°C for the I-helix and *ca.* −550° for the II-helix.[9] The conformation of PLP in the mixed solvent (CD_3COOD : $CDCl_3$=1 : 8) is considered to be a PLP-I-like helix, in which the value of $[\alpha]_{546}$ is +30°C and invariable. The NMR spectrum of the PLP-I-helical structure is shown in Fig. 1. A PLP solution which had a value of $[\alpha]_{546}$=−560° after the sample had been dissolved in an acetic acid and kept for two weeks was used for measuring the NMR spectrum of the PLP-II-helical structure, as is also shown in Fig. 1. The peak assignments were made from the ratios of the areas of the peaks; they are also shown in Fig. 1.

The magnetic shielding values of the hydrogen atoms a), b)-c), and d) for the poly-L-proline which has the PLP-I-helix were 5.5, 7.8—8.0, and 6.3_{ppm} respectively. The values for the PLP-II-helix were 5.2_3, 7.9_{ppm}, and 6.2_5 respectively.

The values for the peaks of the hydrogen atoms b)-c) and d) depend little on their conformations. However, the value of the hydrogen atom a) for the I-helix was in a field up by 0.3_{ppm} from that for the II-helix. The chemical shift of the αCH in the NMR spectra of the two poly-L-prolines seems to be influenced more by the configuration of the peptide group on the imino side than that on the carbonyl side; in both helices of the poly-L-prolines the hydrogen atom a) is nearly in the *trans* position relative to the C=O, where the chemical shift of αCH peaks is assumed to be decided by the relative position of the preceding or the following carbonyl group.

The difference between the magnetic shielding values of the αCH in the *cis* and *trans* configurations of the peptide groups is similar to that found for the N-CH_3 of *N*-methylformamide[10] in benzene,

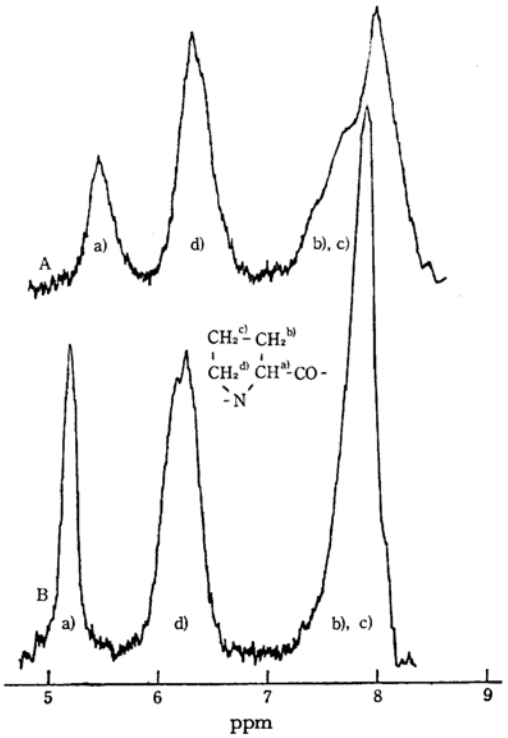

Fig. 1. The NMR spectra of poly-L-prolines I and II.
A: poly-L-proline I (solvent, $CDCl_3$: CD_3COOD =8 : 1).
B: poly-L-proline II (solvent, CD_3COOD).
Reference; TMS.
a), b), c) and d) show the assignment of the hydrogen atoms of pyrrolidine ring.

where the solvent-solute interaction is involved. The difference in the shielding values of the αCH peaks of the two poly-L-prolines can be used in the study of the *trans*-conformation of PLP.

It should also be mentioned that, during the I ⇌ II transition of the poly-L-prolines, two αCH peaks were observed at 5.2 and 5.5_{ppm}; the ratios of the areas of the two peaks changed with time.

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