BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 43 359—361 (1970)

Steric Structure of L-Proline Oligopeptides. IV. Proton Magnetic Resonance Studies of L-Proline Oligopeptides*[1]

Hirofumi OKABAYASHI*[2] and Toshizo ISEMURA

Division of Physical Chemistry, Institute for Protein Research, Osaka University, Kita-ku, Osaka

(Received August 16, 1969)

The NMR spectra of *t*-amyloxycarbonyl-L-proline oligopeptides were measured and compared with those of two poly-L-prolines, I and II. The α-CH resonance peaks of the oligopeptides were observed at sheilding values of 5.2 and 5.4 ppm, values which were in accord with those of the α-CH resonance peaks of the poly-L-prolines I and II, respectively. The behavior of the α-CH peaks was related to the conformation of the L-proline oligopeptides. Moreover, the contribution of the two terminal L-proline residues to the α-CH resonance peaks was discussed.

In our preceding papers,[1,2] we have made configurational studies of L-proline oligopeptides by such methods as infrared absorption spectra, far-ultraviolet absorption spectra, optical rotatory dispersion, and circular dichroic spectra. The results have led to the conclusions presented below.

In *t*-amyloxycarbonyl-L-proline oligopeptides, the helical structures of the poly-L-proline II-type begin to appear at the tetramer, and the molecules of the pentamer, hexamer, and octamer have a helical structure similar to that of the poly-L-proline II.

The structures of the poly-L-prolines I and II have already been established by the X-ray diffraction method.[3,4] The I form has a right-handed helical structure with a residue translation of 1.85 A, in which the peptide bonds are in the *cis* configuration, while the formII is left-handed with a residue translation of 3.12 A and with the peptide bonds *trans*.

In this work, high-resolution proton magnetic resonance spectroscopy was used for the study of the steric structure of *t*-amyloxycarbonyl-L-proline oligopeptides; the configuration of the peptide groups of the oligomers will be discussed by comparing the NMR spectra of these oligomers with those of the poly-L-prolines I and II. Especially, the differences between the configurations of the two terminal L-prolyl residues and those of the other L-prolyl residues will be described.

Sakai, Okabayashi and Isemura[5-7] have already observed a marked difference in the magnetic shielding values of the α-CH peaks of the poly-L-prolines I and II.

Experimental

The L-proline oligopeptides used in the present investigation were as follows: *t*-amyloxycarbonyl-L-proline (monomer), *t*-amyloxycarbonyl-L-prolylproline (dimer), *t*-amyloxycarbonyl-L-prolylprolylproline (trimer), *t*-amyloxycarbonyl-L-prolylprolylprolylproline (tetramer), *t*-amyloxycarbonyl-L-prolylprolylprolylprolylproline (pentamer), *t*-amyloxycarbonyl-L-prolylprolylprolylprolylprolylproline (hexamer), and *t*-amyloxycarbonyl-L-prolylprolylprolylprolylprolylprolylprolylproline (octamer).

These oligopeptides were synthesized by Dr. S. Sakakibara (Peptide Center, Institute For Protein Research, Osaka University, Osaka) and one of the present authors (H. Okabayashi).

The poly-L-proline I was synthesized by the polymerization of L-proline-*N*-carboxyanhydride in acetonitrile. The molecular weight was *ca.* 6000. The poly-L-proline II was prepared by transforming the poly-L-proline I into the form II in an acetic acid solution.

The deuterochloroform (isotopic purity: 99%), tetradeuteroacetic acid (isotopic purity: 99%), tetradeuteromethanol (isotopic purity: 99%), and tetramethylsilane used for spectroscopy were manufactured

*1 Parts of this paper were presented at the 21th Annual Meeting of the Chemical Society of Japan, April, 1968, and at the 17th Symposium on High Polymer of the Chemical Society of Japan, October, 1968.

*2 Present Address: Department of Industrial Chemistry, Nagoya Institute of Technology, Gokiso, Showa-ku, Nagoya.

1) T. Isemura, H. Okabayashi and S. Sakakibara, *Biopolymers*, **6**, 307 (1968).

2) H. Okabayashi, T. Isemura and S. Sakakibara, *ibid.*, **6**, 323 (1968).

3) W. Traub and U. Shmueli, in "Aspects of Protein Structure," ed. by G. N. Ramachandran, Academic Press, New York (1963), p. 81.

4) P. M. Cowan and S. McGavin, *Nature*, **176**, 501 (1955).

5) H. Okabayashi, R. Sakai and T. Isemura, Preprint of the 21th Annual Meeting of the Chemical Society of Japan, IV (April, 1968), p. 2685.

6) H. Okabayashi, R. Sakai and T. Isemura, Abstract, at the symposium on High Polymers of the Chemical Society of Japan (October, 1968), p. 721.

7) R. Sakai, H. Okabayashi and T. Isemura, This Bulletin, **42**, 3028 (1969).

by E. Merck Aktiengesellschaft, Darmstedt, Germany. These solvents were used without further purification. Tetramethylsilane (TMS) was used as an internal reference for all the resonance peaks. All the proton magnetic resonance spectra were obtained on a Japan Electron Optics Laboratory (Jeolco) model JNM-4H-100 NMR spectrometer at a frequency of 100 MHz. The spectra of L-proline oligomers at a temperature of 26 ± 2°C were accumulated thirty-two times, using a spectrum accumulator (Jeolco model JPA).

Results and Discussion

According to the NMR studies which have been made by Sakai, Okabayashi, and Isemura,[5-7] the magnetic shielding value of the α-CH peak of the poly-L-proline I was different from that of the form II. The α-CH peaks of the form I appeared at the shielding value of 5.5_{ppm} in a mixed solvent (deuterochloroform : tetradeuteroacetic acid=8 : 1), and that of the poly-L-proline II, at 5.2 ppm in tetradeuteroacetic acid.

This difference might be due to the different configurations of the peptide groups of the two poly-L-prolines.

Figure 1 show the NMR spectra of the pyrrolidine rings of *t*-amyloxycarbonyl-L-proline and of poly-L-prolines in CD_3COOD. The peak assignments are also included in Fig. 1. The NMR spectrum of the poly-L-proline I in tetradeuteroacetic acid was obtained by measuring it rapidly, as soon as the sample had been completely dissolved. In tetradueteroacetic acid the α-CH peak of the poly-L-proline I has a shielding value of 5.4_{ppm}, and that of *t*-amyloxycarbonyl-L-proline, one of 5.7_{ppm}.

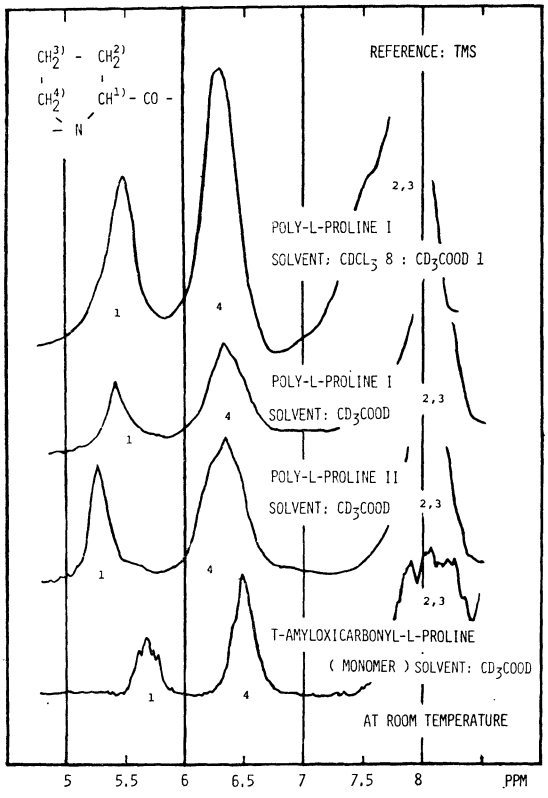

Fig. 1. NMR spectra of *t*-amyloxycarbonyl-L-proline, poly-L-prolines I and II at 26°C.

Figure 2 gives the NMR spectra of *t*-amyloxycarbonyl-L-proline oligopeptides in comparison with those of the poly-L-prolines I and II. The spectra of the oligomers were accumulated thirty-two times by means of the spectrum accumulator.

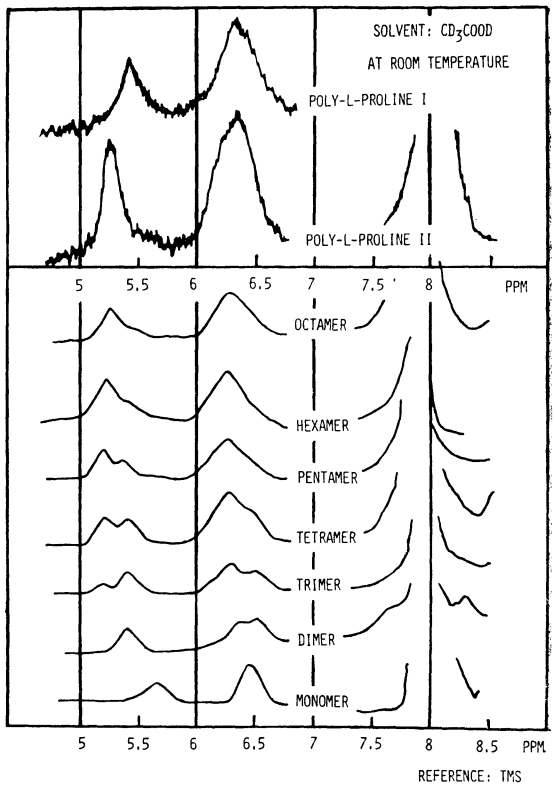

Fig. 2. NMR spectra of *t*-amyloxycarbonyl-L-proline oligopeptides, poly-L-prolines I and II at 26°C.

In the NMR spectra of the dimer, the resonance peak of 5.4_{ppm} is assigned to the two α-CH protons of the dimer. Since the shielding value of the α-CH resonance peak of the dimer is in agreement with that of the poly-L-proline I, it seems that the coincidence suggests the configuration of the dimer. However, it does not always follow that the α-CH resonance peak of the dimer corresponds to the *cis* configuration of the peptide groups of the poly-L-proline I. For the two L-proline residues of the dimer can be regarded as the terminal residues. It is unreasonable to identify the two terminal residues of the dimer with the two residues of the poly-L-proline I. Although there is a probability that the dimer has the *cis* configuration of the peptide group,

TABLE 1. THE RATIOS OF THE AREAS OF THE α-CH PEAKS AT 5.2_{ppm} TO THOSE OF THE OTHER α-CH PEAKS AT 5.4_{ppm}

Oligomers	Trimer	Tetramer	Pentamer	Hexamer	Octamer
Experimental values* (B/A)	2.01 ± 0.03	1.08 ± 0.02	0.66 ± 0.02	0.51 ± 0.02	0.35 ± 0.01
Theoretical values (B/A)	2	1	$2/3\fallingdotseq0.67$	0.5	$1/3\fallingdotseq0.33$

A = Area of the peak at 5.2_{ppm}. B = Area of the peak at 5.4_{ppm}.
* These experimental values were calculated from the NMR spectra of L-proline oligopeptides accumulated for thirty-two times by spectrum accumulator.

the NMR measurement at 100 MHz does not a resolution sufficient to make clear the configuration of the dimer. The NMR spectra at 220 MHz will give the configuration of the two L-proline residues of the dimer.

In the NMR spectra of the trimer, tetramer, pentamer, hexamer, and octamer, two α-CH peaks were observed. The magnetic shielding values of the two peaks in the five oligomers are 5.2 and 5.4_{ppm}. The resonance position of the α-CH peak at 5.2_{ppm} is the same as the α-CH resonance spectrum of the II poly-L-proline gives. Furthermore, as L-prolyl residues increase, the α-CH resonance peak of 5.2_{ppm} increases in intensity, whereas that of 5.4_{ppm} decreases in intensity, as can be well seen in Fig. 2. The behavior of the α-CH resonance peak of 5.2_{ppm} indicates that the peptide groups of the oligomers assume a *trans* configuration with an increase in the chain length.

The fact that the oligopeptides larger than the tetramer assume the conformation of the poly-L-proline II (peptide bonds: *trans*) was established by various methods, as has already been mentioned. Nevertheless, the peak attributable to the conformation of the poly-L-proline I (peptide bonds: *cis*) appears fairly clearly in the NMR spectra of the oligopeptide. If we assume that the α-CH peaks of terminal residues appear around the same position as that for the *cis* configuration, the α-CH peaks of terminal residues may be expected to decrease very regularly with the function of the length of the peptide chain. This inference was clearly suggested by the experimental findings.

In order to discuss the effect of the terminations, it is convenient to assume that, in the α-CH resonance peaks of L-proline oligopeptides, all the α-CH peaks at the magnetic shielding value of 5.4_{ppm} in tetradeuteroacetic acid are to be attributed to the α-CH protons of the two terminal L-proline residues. As has already been mentioned, one of the α-CH peaks at 5.2_{ppm} may be ascribed to the trans configuration of the peptide bonds in the oligopeptides. On the basis of the above assumptions, the ratios of the intensities of the two terminal α-resonance peaks to those of the other α-CH peaks can easily be calculated.

The experimental values of the ratios are obtained from the ratios of the areas of the α-CH resonance peaks at 5.2_{ppm} to those of the other α-CH peaks at 5.4_{ppm}. These experimental ratios are summarized in Table 1, along with the theoretical values.

The experimental values are compared with the theoretical values in Table 1. The agreement of the experimental values with the theoretical values is satisfactory. These facts show that the previous inference is plausible. That is, it may be concluded that the *L*-proline residues placed between the two terminal residues have a poly-L-proline II-like configuration.

In the NMR spectra of the oligopeptides, the change in the δ-CH_2 resonance peaks should be noteworthy. These δ-CH_2 resonance peaks might be related to the conformation of the oligomers. However, it is difficult to obtain information about the configuration of these oligopeptides from the δ-CH_2 resonance peak.

Further information about the effect of the terminal residues on the α-CH resonance peaks and the δ-CH_2 resonance peaks of L-proline oligopeptides can be obtained from the NMR spectra of L-proline oligopeptides, including the deuterized L-prolines.

The authors are grateful to Mr. O. Masuda for recording the NMR spectra.