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Proton Nuclear Magnetic Resonance of Random Copoly(γ-benzyl L-glutamate, γ-methyl L-glutamate)

Toshifumi HIRAOKI, Akihiro TSUTSUMI, Kunio HIKICHI,
and Motozo KANEKO

Department of Polymer Science, Faculty of Science,
Hokkaido University, Sapporo 060, Japan.

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ABSTRACT: The conformational behavior of random copolypeptides of γ-benzyl L-glutamate and γ-methyl L-glutamate was studied by nuclear magnetic resonance in deuterated chloroform—trifluoroacetic acid under various conditions of solvent composition and temperature. The results show that the helix stability of the copolymers is intermediate between those of the two homopolymers, poly(γ-benzyl L-glutamate) and poly(γ-methyl L-glutamate). Broadening and upfield shift are observed for the CH_3 proton resonance of the γ-methyl L-glutamate residue of the copolymers. These are attributed to the ring current effect of the phenyl rings on the CH_3 protons. The triad sequence of γ-benzyl L-glutamate and γ-methyl L-glutamate in the copolymers was estimated from the CH_3 resonance.

KEY WORDS NMR / Copoly(γ-benzyl L-glutamate, γ-methyl L-glutamate) / Helix—Coil Transition / Stability / Ring Current Effect / Triad /

It is well known that synthetic polypeptides undergo the helix—coil transition with variation in temperature, the solvent composition of the mixed solvent system, or the pH of an aqueous solution. The helix—coil transition of poly(γ-benzyl L-glutamate) (PBLG) has been studied in mixtures of 1,2-dichloroethane (DCE) and dichloroacetic acid (DCA),[1-4] and chloroform and trifluoroacetic acid (TFA).[5] Poly(γ-methyl L-glutamate) (PMLG) has been also studied in the same solvent systems.[6] Comparing these two polymers in DCE—DCA, PBLG requires a higher content of DCA than PMLG to disrupt the helical form. The helix of PBLG is, therefore, thought to be more stable than that of PMLG. The same results have been obtained from nuclear magnetic resonance (NMR) studies of PBLG and PMLG in deuterated chloroform ($CDCl_3$)—TFA.[7] Further, for random copolymers composed of γ-benzyl L-glutamate and γ-methyl L-glutamate in DCE—DCA, results of optical rotatory dispersion (ORD) studies show that the helix stability of the copolymers is intermediate between those of PBLG and PMLG.[8,9]

The importance of the side chain in determining the stability of the conformation of polypeptides has been well established.[10] NMR spectroscopy has recently been applied to this problem,[11] since chemical shifts, line witdths, and relaxation times provide information about the secondary structure and mobility of the main chain and the side chain under various conditions.

In this paper, NMR investigation has been extended to the study of the conformational behavior and the side-chain interaction of random copolymers of γ-benzyl L-glutamate and γ-methyl L-glutamate in $CDCl_3$—TFA solvent systems. This copolymer will be suitable for the NMR study of side-chain interaction, since the phenyl ring of the benzyl group is expected to give rise to appreciable chemical shifts of neighboring protons due to the ring current effect.[12]

EXPERIMENTAL

Materials

The copolymers were prepared by polymerization of *N*-carboxy anhydrides (NCA) of γ-benzyl L-glutamate (BLG) and γ-methyl L-glutamate (MLG) in a mixture of dry dioxane and dichloromethane. The polymerization was initiated with triethylamine at an NCA: initiator of 200. The degree of polymerization of the copolymers was determined by the intrinsic viscosity in DCA at 25°C using an Ubbelohde type capillary viscometer. Doty's equation for PBLG[13] was used to estimate the degree of polymerization on the assumption that, in the random-coil form, the discrepancy would not be large. The composition in the copolymers was determined by NMR and IR spectra. It was found that the composition in the copolymers was almost the same as the initial mixing composition of both NCAs. The compositions and the degrees of polymerization of the copolymers are summarized in Table I.

Solvents

Deuterated chloroform ($CDCl_3$) of isotopic purity 99.8% was obtained from Merck and Stohler Isotope Chemicals, and used without further purification. Trifluoroacetic acid (TFA) was distilled before use to remove impurities and stored over molecular sieves. No trace of an impurity peak was detected in the NMR spectrum of TFA. The TFA concentration in mixed solvents is expressed in vol%.

Table I. Composition and degree of polymerization

BLG, mol %[a]	Degree of polymerization
0	640
12	2500
25	2100
40	810
57	800
77	1010
100	660

[a] Molar percent of BLG residue in the copolymers.

NMR measurements

NMR spectra were recorded on a HITACHI R-20B (60 MHz) and a JEOL PS-100 (100 MHz) spectrometer, equipped with the variable temperature accessory unit. Chemical shifts were measured relative to tetramethylsilane as an internal reference. Polymer concentrations were approximately 0.02 g/m*l*. A series of spectra was obtained at various TFA concentrations and temperatures.

RESULTS AND DISCUSSION

Figure 1 shows 100 MHz spectra of PBLG, PMLG, and a copolymer containing 57-% BLG residue at 25% TFA concentration. Spectra were recorded at a temperature of 25°C, except for PMLG, for which the spectrum was recorded at 50°C. Spectra of PMLG could not be obtained at room temperature, because PMLG is not sufficiently soluble in the range of TFA concentrations of interest at room temperature. For the

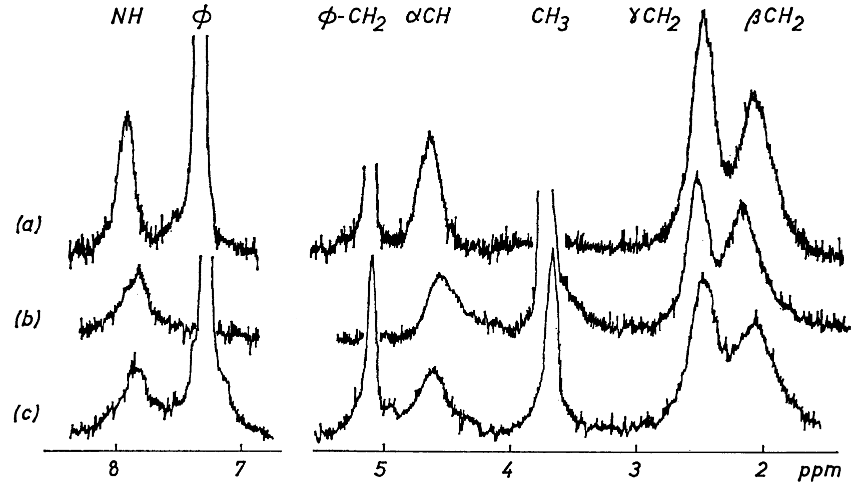

Figure 1. 100 MHz spectra of PBLG, PMLG, and 57% BLG copolymer at 25% TFA in $CDCl_3$—TFA: (a) PBLG at 25°C; (b) PMLG at 50°C; (c) copolymer (57% BLG) at 25°C.

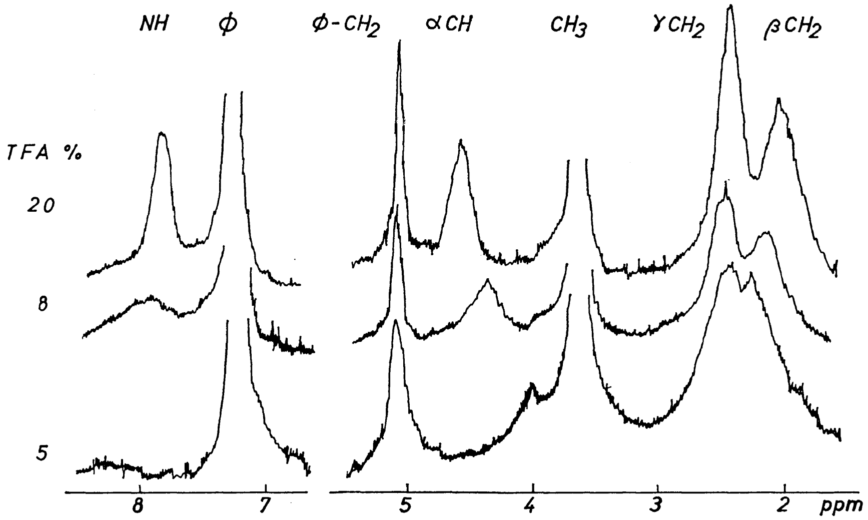

Figure 2. 100 MHz spectra of a copolymer (40% BLG) in $CDCl_3$—TFA at 25°C.

copolymers seven peaks were observed. On the basis of the spectra of two homopolymers of PBLG and PMLG, these are assigned to NH, phenyl, benzyl CH_2, αCH, CH_3, γCH_2 and βCH_2 protons in the order of increasing field of their appearance.

NH, αCH, γCH_2, and βCH_2 resonances of copolymers are all virtually single peaks. These findings are in contrast to the results for copoly(γ-benzyl L-glutamate, β-benzyl L-aspartate),[14] where peaks from two residues were observed separately. This indicates that the difference in chemical shift between the corresponding peaks of BLG and MLG residues is very small.

Figure 2 shows the spectra of a copolymer containing 40-% BLG residue at a temperature of 25°C at three TFA concentrations. The αCH peak is rather largely shifted downfield with increasing the TFA concentration. A downfield shift of the αCH has also been observed for PBLG and PMLG in the same solvent system,[7,15] and interpreted in terms of the transition from the helical form to the random-coil form. The shift of the αCH due to the conformational change is well established for organic solvent systems, in which such a strong acid as TFA is a helix-disrupting reagent. Thus the large downfield shift of the αCH of this copolymer is ascribed to the transition from the helical form to the random-coil form. The αCH peak of the random-coil form is approximately 0.5 ppm downfield from that of the helical form. This is in agreement with the results of PBLG and PMLG.[7,15]

The chemical shift of the αCH at a temperature of 35°C is plotted against the TFA concentration for six copolymers in Figure 3. This clearly shows that the transition region shifts continuously to higher TFA concentration with increasing BLG content. The sharp variation of the chemical shift of the αCH with the TFA concentration is consistent with a cooperative

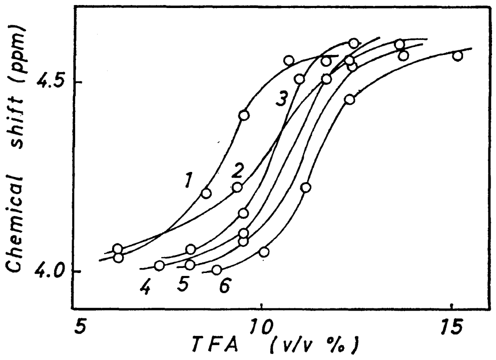

Figure 3. The αCH chemical shift of the copolymers in $CDCl_3$—TFA at 35°C: (1) 12% BLG; (2) 25% BLG; (3) 40% BLG; (4) 57% BLG; (5) 77% BLG; (6) 100% BLG (PBLG).

behavior of the helix—coil transition. This indicates that BLG and MLG residues in the copolymers participate in the helix—coil transition cooperatively, as in the respective homopolymers, although each residue is thought to be distributed randomly along the chain.

We used the TFA concentration required to disrupt the helical form as a criterion of the stability of the helix. In order to correlate the stability of copolymers with the copolymer composition, the TFA concentration at the transition midpoint at a temperature of 35°C is plotted against the BLG content in Figure 4. It is clear that the transition midpoint shifts continuously to higher TFA concentrations with increasing BLG content. Silverman and Scherage[7] concluded that PBLG undergoes the helix—coil transition at higher TFA concentrations than PMLG at a given temperature, and that the helix of PBLG is more stable than that of PMLG. The results obtained in the present work suggest that the stability of the helix of the copolymers is less than that of PBLG, but more than that of PMLG, and approaches that of PBLG with increasing BLG content.

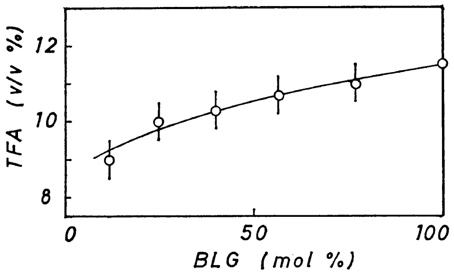

Figure 4. TFA concentration at midpoint of the helix—coil transition against the BLG content at 35°C.

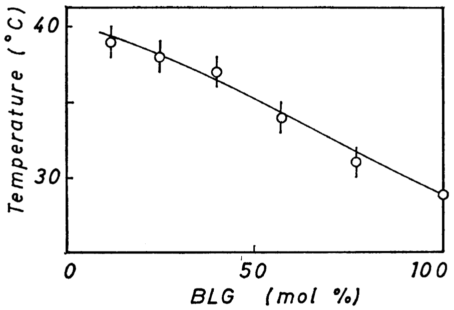

Figure 5. Transition temperature against the BLG content at 15-% TFA.

We studied the conformational transition induced by temperature. In order to observe the transition in the temperature range studied, measurements were done at a TFA concentration of 15%. In this solvent composition all the polymers are in the random-coil form at 25°C. With increasing temperature the peak of the αCH moves upfield approximately 0.5 ppm, indicating that these copolymers undergo the thermally induced coil-to-helix transition.

The transition temperature is plotted against the BLG content in Figure 5. It is found that with increasing the BLG content the transition temperature decreases. These NMR results in TFA—$CDCl_3$ are quite similar to the ORD results which were obtained in the DCA—DCE system.[8,9]

It has been suggested[7,16] that the high stability of the PBLG helix comes from the efficient shielding from acid solvents by the bulky phenyl group of the side chain and the nonbonded interaction between phenyl rings. The decrease in stability of the copolymers with increasing MLG content is considered to be a consequence of the substitution of a small methyl group, which reduces the shielding from the acid and the interaction between phenyl rings.

The NH peak in the helical form is too broad to be observed. Thus we will not discuss the NH peak.

The so-called "double peak" behavior of the αCH resonance in the helix—coil transition region has been the subject of much debate for PBLG of a relatively low-molecular weight.[17–22] One of the interpretations of the double peak is in terms of the polydispersity in molecular weight, especially in a low-molecular weight range.[17–19] The molecular weight of the polymer used here is reasonably high, as seen in Table I. Although the polymers were not fractionated, low-molecular weight parts are thought to have been cut off by the several repetitions of precipitation in the course of the sample preparation after the polymerization. Thus, the distribution of molecular weight for the polymers used here is not considered to cause the double peak. As seen in Figure 2, the αCH resonance did not show the double peak in the transition

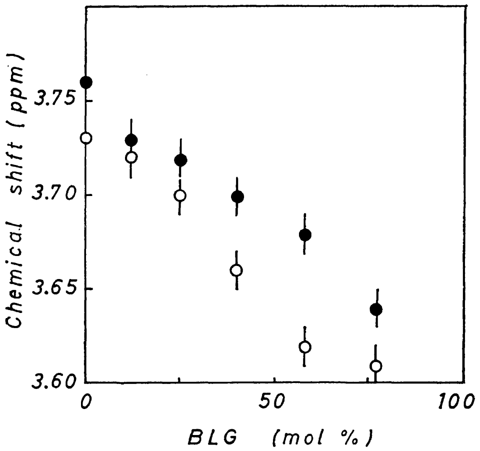

Figure 6. The peak position of the CH_3 resonance of the copolymers *vs.* the BLG content at 25°C: ○, the random-coil form at 20-% TFA; ●, the helical form at 5-% TFA.

region, but was accompanied by a broadening.

The shift of the γCH_2 peak of the copolymers remains virtually unchanged over the helix—coil transition region. However, the βCH_2 peak moves upfield about 0.2 ppm, indicating that the βCH_2 peak is dependent on the main chain conformation. Therefore, the difference between the chemical shifts of the βCH_2 and γCH_2 resonances is conformationally sensitive and could also be used to follow the helix—coil transition.[15]

The chemical shift of the CH_3 protons of the MLG residue is plotted against BLG content in the random-coil and helical forms in Figure 6, with the peak position taken as the chemical shift of the CH_3 protons. The chemical shift of the CH_3 resonance is shifted upfield with increasing BLG content, since it is dependent on the presence of the BLG residue.

The spectra of the CH_3 resonance in the random-coil form is shown in Figure 7. The CH_3 spectrum of PMLG is a single symmetric sharp peak, as shown in the figure. On the other hand, the CH_3 spectra of the copolymers are much broader and more asymmetric. The line shape changes in a rather complicated manner with the BLG content. Upon increasing the BLG content, a shoulder appears at the high field side of the spectrum; its intensity increases at the expense of the low field portion of the spectrum.

The half width of the CH_3 resonance of the copolymers is plotted against the BLG content in Figure 8. It is of interest to note that the half width changes with variation of the BLG content, the half width being a maximum at an intermediate BLG content.

These behaviors of the CH_3 resonance in the copolymers are probably due to the ring current effect of the neighboring phenyl group of the BLG residue. In general, the large upfield shift is observed when a resonating proton is located on the phenyl ring.[12] In copolymers, the magnetic environment of the methyl group will differ slightly from one group to another because of the random distribution of the benzyl group.

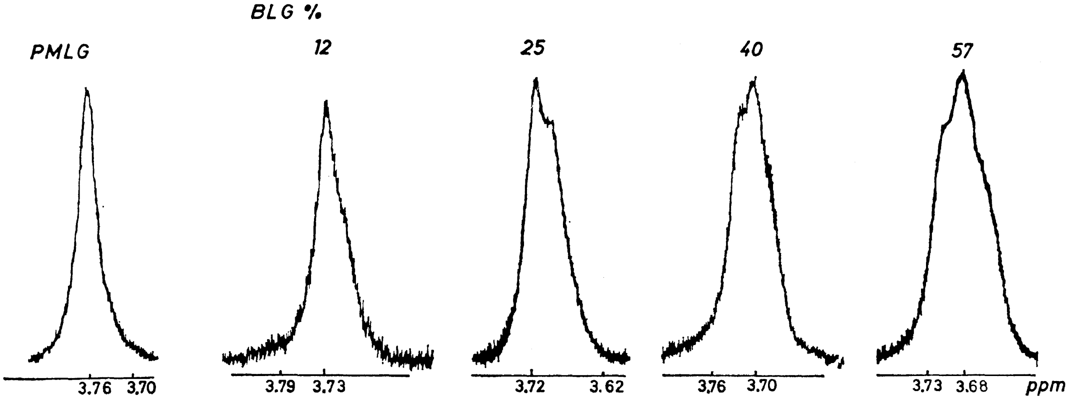

Figure 7. The CH_3 region of the 100 MHz spectra in random-coil form at 20-% TFA of the copolymers at 25°C and of PMLG at 50°C.

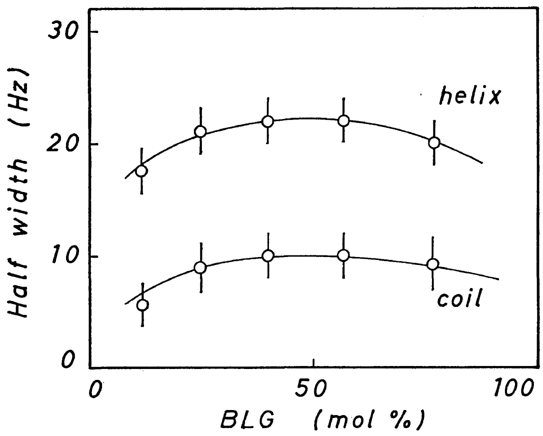

Figure 8. The half width of the copolymers at 25°C: the upper curve is for the random-coil form in 20-% TFA and the lower curve is for the helical form at 5-% TFA.

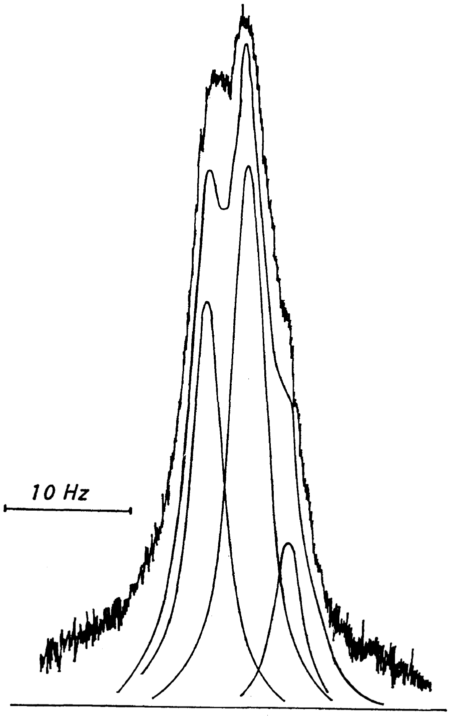

Figure 9. Spectrum (100 MHz) of the CH_3 resonance of a copolymer (40-% BLG) in random-coil form at 20-% TFA at 25°C (upper) and a simulated spectrum (lower).

A great diversity of the magnetic environment will occur at intermediate BLG contents, suggesting that the half width of the CH_3 peak of the copolymers varies with the BLG content, as shown in Figure 8.

The facts mentioned above and the careful inspetion of the spectra of the CH_3 resonance of copolymers indicate that the CH_3 resonance consists of three more or less overlapped peaks. We attempted to separate the CH_3 resonance into three peaks. Because only the nearest neighbor BLG residues are thought to have a marked effect on the CH_3 protons in the copolymers, the triad sequence is taken into account. We assume that the peak of the center CH_3 protons in the MMM triad occurs at the lowest field, as in PMLG, the peak from the BMB triad is at the highest field, and the peak from the BMM and/or the MMB occurs between the two: B and M stand for BLG and MLG residues, respectively. We simulated spectra assuming that the line shape is Lorentzian and the half widths of the three peaks are the same as the CH_3 peaks of PMLG; the resulting spectra were compared with the observed ones. An example of a simulated NMR spectra is shown in Figure 9 for a copolymer (BLG 40%), together with an observed one. By adjusting the triad fraction

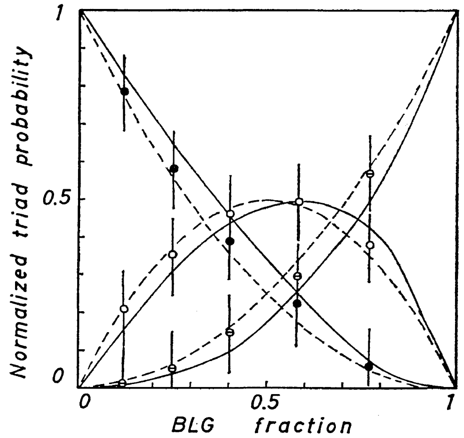

Figure 10. The normalized probability of a monomer triad in which the MLG residue is centered as a function of molar fraction of BLG residue: ●, MMM; ○, BMM and/or MMB; ⊖, BMB. The solid line is for $r_1=1.4$ and $r_2=0.7$ in ref 23, and the dotted line for $r_1=r_2=1.0$.

we can obtain the best fit of the spectra. Although there is a rather great ambiguity in separating the CH_3 resonance because of poor spectral resolution, the triad sequence thus obtained is plotted against the BLG fraction in Figure 10. In this figure, the solid line and dotted line are theoretical curves which are calculated from the monomer reactivity ratios of MLG and BLG residues, r_1 and r_2, respectively, on the assumption of the primary Markoff process of polymerization. These results show almost random distribution of MLG and BLG residues, in agreement with Kanamori's results.[23,24]

CONCLUSION

The helix—coil transition of random copolypeptides of BLG and MLG residues has been investigated by NMR in $CDCl_3$—TFA, and the results compared with homopolypeptides, PBLG, and PMLG. Judging from the chemical shift of the αCH resonance, the stability of the helical copolypeptides is found to be between those of PBLG and PMLG, and to approach that of PBLG with increasing BLG content. In the random-coil form, the chemical shift, line shape, and line width of the CH_3 resonance of copolypeptides are apparently different from those of PMLG, and depend on the content of the BLG residue. This is a consequence of the ring current effect from the phenyl ring in the neighborhood of a MLG residue. The distribution of the triad sequence was estimated by separating the CH_3 peak into three peaks corresponding to the three triads.

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