# The Helix-Coil Transition of Poly-(γ-benzyl L-glutamate) using $^{13}$C Resonance Spectroscopy

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*Summary* On conversion of the helical poly-(γ-benzyl L-glutamate) in chloroform–trifluoracetic acid into the random coil form, the $^{13}$C spectrum shows an upfield displacement of 3·0 p.p.m. for the α carbon and 2·7 p.p.m. for the amide carbon.

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Proton spectroscopy has been widely used to study the solution conformation of biopolymers and their synthetic analogues. The low-molecular weight cyclic polypeptides such as gramacidin[1] and valinomycin[2] have been much studied as has the helix–coil transition in synthetic poly-α-amino-acids.[3] Although the shifts of the amino-acid carbons have been determined,[4] there are no data available for poly-amino-acids. This paper outlines a study of the helix–coil transition of poly-(γ-benzyl L-glutamate) (PBLG) in chloroform–trifluoroacetic acid (TFA) by means of proton decoupled $^{13}$C spectroscopy.

The sample of PBLG (S416) having DP *ca.* 100 has already been characterised[5] and that of poly-(β-benzyl L-aspartate) (428) also had DP *ca.* 100. A polymer concentration of 15% w/v was used in 12 mm tubes at 30 °C for the complete spectra, obtained on a Varian XL-100-15 spectrometer equipped with the VFT-100 Fourier Transform attachment. For the $T_1$ measurements a polymer concentration at 30% w/v at room temperature was used in 8 mm tubes on a JEOL PS-100 spectrometer equipped with the PFT-100 Fourier Transform System. Internal $Me_4Si$ was used as reference.

$^{13}$C *Chemical shifts in p.p.m. from internal* $Me_4Si$ (*upper* 2 *rows*) *and relaxation times* (*bottom row*) *of poly-*(*γ-benzyl* L*-glutamate*) *solutions in chloroform–trifluoroacetic acid*

| Solvent | $C_\beta$ | $C_\gamma$ | $C_\alpha$ | $C_{Bz}$ | $C_{2,6-Ar}$ | $C_{1-Ar}$ | C=O (Ester) | C=O (Amide) | |
|---|---|---|---|---|---|---|---|---|---|
| 3% TFA<br>97% $CDCl_3$<br>(15% PBLG) | 25·9 | 30·7 | 56·3 | 65·9 | 126·6<br>127·1 | 133·6 | 170·5 | 173·1 | $^{13}$C Shifts |
| 29% TFA<br>71% $CDCl_3$<br>(15% PBLG) | 27·1 | 30·6 | 53·2 | 67·6 | 126·6<br>127·1 | 133·6 | 173·2 | 171·1 | |
| 3% TFA<br>97% $CDCl_3$<br>(30% PBLG) | 0·03 ±0·01 s | 0·03 ±0·01 s | 0·03 ±0·01 s | 0·10 ±0·02 s | 0·81 ±0·05 s | 3·3 ±0·1 s | 1·93 ±0·1 s | 0·9 ±0·1 s | $T_1$ Values for helix |

The Figure shows the $^{13}$C spectra at 25·2 MHz of poly-(γ-benzyl L-glutamate) through the helix–coil transition. The assignment of all peaks except those of the carbonyls follows from the amino-acid spectra. The assignment of the upfield carbonyl in the random coil spectrum (29% TFA) to the amide group is based partly on a similar assignment in the amino-acid and is supported by the fact that the upfield carbonyl resonance in the spectrum of random coil poly-L-glutamic acid can unequivocally be assigned to the amide carbonyl by comparison with the spectrum of random coil poly-L-lysine in water. The reverse assignment in the spectrum of helical poly-(γ-benzyl L-glutamate) is based partly on the greater width of the low field peak but largely on the fact that the displacement to lower fields of the upfield peak is monotonic with added TFA and exactly parallels the displacement to lower fields of the benzyl carbon. The upfield peak in the helical spectra (3% and 7% TFA) is therefore assigned to the ester carbonyl.

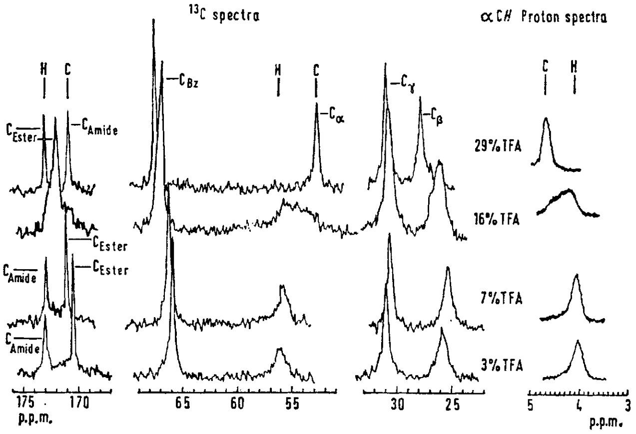

Figure. $^{13}$C *spectra at* 25·2 *MHz and* $^1$H *spectra at* 100 *MHz of poly-*(*γ-benzyl* L*-glutamate*) *in* $CDCl_3$–TFA: 3% *and* 7% TFA—*Helix,* 16% TFA—*Helix* + *Coil,* 29% TFA—*Coil.*
H—*helix,* C—*coil shifts for* $C_{Amide}$, $C_\alpha$ *and* α-C*H resonances.*

The α-carbon shift in the helix (56·2 p.p.m.) differs from that in the coil (53·2 p.p.m.). Thus in the carbon spectrum it is the coil that is upfield of the helix rather than the reverse as in the α-C*H* proton spectrum and furthermore the shift difference of 3·0 p.p.m. in the carbon spectrum can be contrasted with about 0·5 p.p.m. in the proton spectrum. It can be seen from the Figure that the α carbon spectrum and the α-C*H* proton spectrum are very similar in the partially helical state (16% TFA) and it has been argued elsewhere[5] that the shape of the signal is due largely to molecular weight polydispersity rather than to slow exchange between conformations. Since the frequency difference between the conformations (*ca.* 76 Hz) in the α carbon spectrum differs little from that in the α-C*H* proton spectrum (*ca.* 50 Hz at the same field strengths), the carbon shift cannot provide substantially new information on exchange rates between helix and coil. In the case of poly-(β-benzyl L-aspartate) the α carbon shift of the left-handed helical form in 1% TFA is 51·2 p.p.m. and of the coil in 10% TFA is 49·7 p.p.m. Thus, although the coil resonance is also upfield of the helix, the difference is only 1·50 p.p.m., *i.e.*, one half that observed for PBLG, and furthermore both shift values differ from those of the right handed PBLG. These differences between glutamate and aspartate are similar to those found in α-C*H* proton spectra.[6]

Whilst the γ carbon remains at essentially the same shift of 30·6 p.p.m. over the transition the β carbon moves downfield by about 1 p.p.m. on conversion of the helix into coil, the intermediate case (16% TFA) showing evidence of two peaks. In the proton spectrum the β-C$H_2$ resonance is also displaced, but to higher fields on formation of the coil.

As mentioned previously, the ester carbonyl peak is displaced to lower fields by 2·7 p.p.m. on changing the TFA content from 3% to 29% and thereby crosses over the amide carbonyl resonance. This displacement is probably due to hydrogen bonding of the ester carbonyl with TFA which is also the cause of the downfield displacement of the benzyl carbon peak. The amide carbonyl resonance is displaced to higher fields by 2·0 p.p.m. on conversion of the helix into coil and the spectrum in 16% TFA shows signs of a multicomponent peak in like manner to the α carbon resonance.

The line widths in the helical conformation appear greater than those of the coil as expected from the reduced mobility of the helix in a similar way to observations in the proton spectrum. The Table includes measurements of $T_1$ obtained by the 180°–*t*–90° pulse method on a 30% w/v solution of helical PBLG containing 3% TFA. The values show that accurate register of the carbonyl and aromatic resonance intensities in a Fourier transform spectrum requires a pulse interval of at least 10 s. A much faster pulsing rate suffices, however, for many other peaks, in particular the α carbon. The line widths of the helical form (30% PBLG in 3% TFA–97% $CDCl_3$) suggest $T_2$ values much lower than the quoted $T_1$'s. Moreover, although addition of TFA to the helical form results in much reduced line widths, $T_1$ values are little affected.

These results demonstrate that the amide carbonyl carbon, the α carbon, and to a lesser extent the β carbon shifts are conformationally sensitive. This could be of value in conformational studies of naturally occurring polypeptides. Furthermore, the ester carbonyl carbon and the benzyl carbon shifts are sensitive to solvent interaction.

We thank Drs. F. W. Wehrli of Varian and P. Beynon of JEOL for their assistance in obtaining the spectra.

(*Received*, 31*st December* 1971; *Com.* 2213.)

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[1] A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **61**, 734.
[2] M. Ohnishi and D. W. Urry, *Science*, 1970, **168**, 1091.
[3] E. M. Bradbury, P. Cary, C. Crane-Robinson, L. Paolillo, T. Tancredi, and P. Temussi, *J. Amer. Chem. Soc.*, 1971, **93**, 5916, and references therein.
[4] W. Horsley, H. Sternlicht, and J. Cohen, *J. Amer. Chem. Soc.*, 1970, **92**, 680.
[5] E. M. Bradbury, C. Crane-Robinson, and H. W. E. Rattle, *Polymer*, 1970, **11**, 277.
[6] E. M. Bradbury, C. Crane-Robinson, and H. Goldman, *Macromolecules*, 1971, **4**, 557.