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J. H. Bradbury[a]; B. E. Chapman[a]
[a] Chemistry Department, Australian National University, Chnberra, A.C.T., Australia

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# Conformation of Poly-L-methionine in Solution

J. H. BRADBURY and B. E. CHAPMAN

*Chemistry Department*
*Australian National University*
*Canberra, A.C.T. Australia*



## SUMMARY

A study of poly-L-methionine by viscosity, light-scattering, and NMR spectroscopy shows 1) that the helical rodlike form of the polypeptide has approximately the same dimensions as helical poly-γ-benzyl-L-glutamate, and 2) that the random coil form in trifluoroacetic acid is charged and fits the same Mark-Houwink equation as does poly-γ-benzyl-L-glutamate in dichloroacetic acid. The relationships between NMR line widths and degree of polymerization is given for three different polypeptides.

The helix-to-coil transition has been studied for poly-L-methionine (PLM) by viscometry and other hydrodynamic methods [1] as well as by optical rotatory dispersion [1, 2]. It is found that the polypeptide exists in the helical rodlike form in various organic liquids including dichloroacetic acid (DCA), but a transition occurs to a random coil structure on the addition of trifluoroacetic acid (TFA). In this paper we are concerned with the question of the rigidity of the rod [3, 4] and the dependence on molecular weight of the line width ($\Delta\nu$) of the singlet S–$CH_3$ resonance determined by nuclear magnetic resonance (NMR) spectroscopy [5].

## EXPERIMENTAL

The preparation of samples of PLM of various molecular weights and the purification of solvents is described elsewhere [6, 7]. The viscosity measurements follow established procedures [7] and the light-scattering measurements were made with a Brice-Phoenix Photometer, series 2000 [6]. The NMR spectrometer was a Perkin-Elmer, Model R-10 operating at 60 MHz and line width measurements were made as described previously [5].

## RESULTS AND DISCUSSION

### Solvents

The polypeptide is soluble in a wide range of organic solvents some of which, e.g., benzene, nitrobenzene, m-cresol, and m-dichlorobenzene, form very viscous solutions and gels at higher concentrations. Much less aggregation is observed with ethylene dichloride (EDC) and pyridine although the intrinsic viscosity is still greater than that obtained with EDC containing about 0.5% DCA (see Fig. 1). The presence of a small amount of dichloroacetic acid (0.5-4%) removes the aggregation, since the intrinsic viscosity is the same in this solvent as it is in pyrrole (Fig. 2).

The aggregation of the helical rods in EDC, pyridine, etc., is apparently of the end-to-end variety which causes an increase of the viscosity of the solution [8]. As shown in Fig. 3 the viscosity in TFA is much less than in helicogenic solvents and the polypeptide is shown to be flexible because it expands on dilution; this confirms earlier work [1, 2] that PLM is a random coil in TFA.

### Polyelectrolyte Effect

The upward curvature of Graph 3, Fig. 2 and Graph 1, Fig. 3 shows that the polypeptide is charged in TFA. Furthermore the expansion of the polypeptide which occurs on dilution, due to the electrostatic repulsion of the fixed positive charges on the peptide groups [7], is removed by the addition of water. The latter reacts with the TFA, producing ions which screen the charges on the polypeptide and suppress the polyelectrolyte effect [7]. The occurrence of the polyelectrolyte effect confirms that charging of poly-L-amino acids occurs in strong organic acids (DCA and TFA) and further supports our contention in current controversy on the subject (summarized in Refs. 9 and 10).

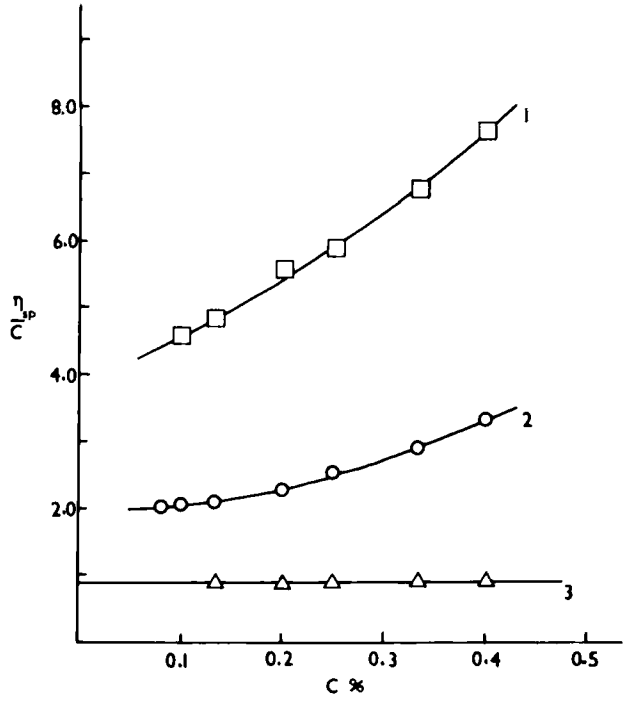


**Fig. 1.** Graph of reduced viscosity against C (g/100 ml solution) for PLM sample ($DP_W$ = 400) in (1) EDC, (2) pyridine, and (3) EDC containing 0.5% DCA.



### Viscosity Results

In Fig. 4 $[\eta]_{helix}$ is graphed against $[\eta]_{coil}$ for PLM (solvents are EDC + 2% DCA and TFA, respectively) and for poly-$\gamma$-benzyl-L-glutamate (PBLG) using the combined results of Refs. 7, 8, 11, and 12. It is seen that the PLM results fit the PBLG line within experimental error. For the linear part of the log-log graph we can write the same equation for PLM and PBLG, viz.

$$[\eta]_{helix} = \text{const } [\eta]^{a}_{coil} \tag{1}$$

By application of the Mark-Houwink equation

$$[\eta] = KM^{\alpha} \tag{2}$$

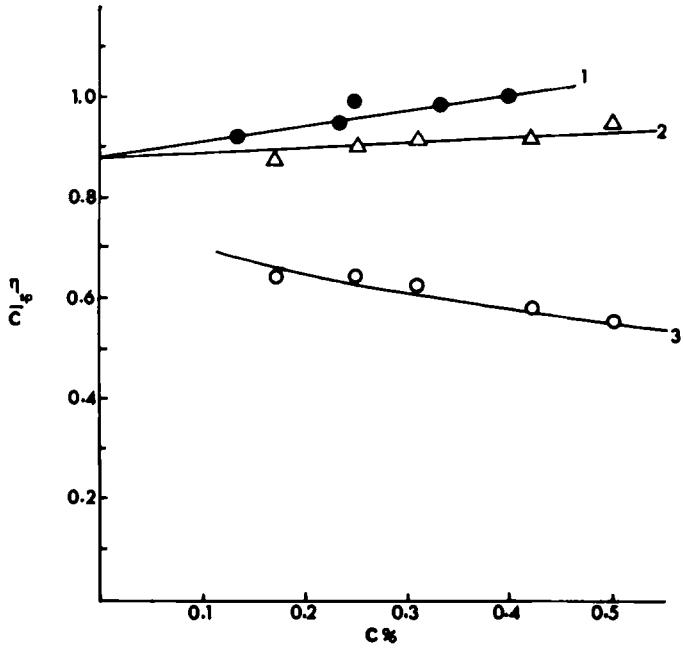


Fig. 2. Graph of reduced viscosity against C (g/100 ml solution) for PLM sample ($DP_W$ = 400) in (1) pyrrole, (2) EDC + 2% DCA, and (3) TFA.

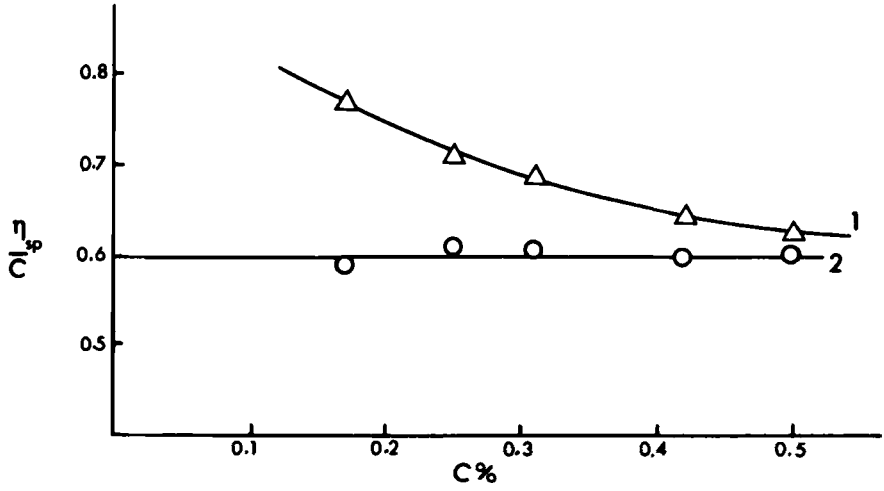


Fig. 3. Graph of reduced viscosity against C (g/100 ml solution) for PLM sample ($DP_W$ = 430) in (1) TFA and (2) TFA + 2% $H_2O$.

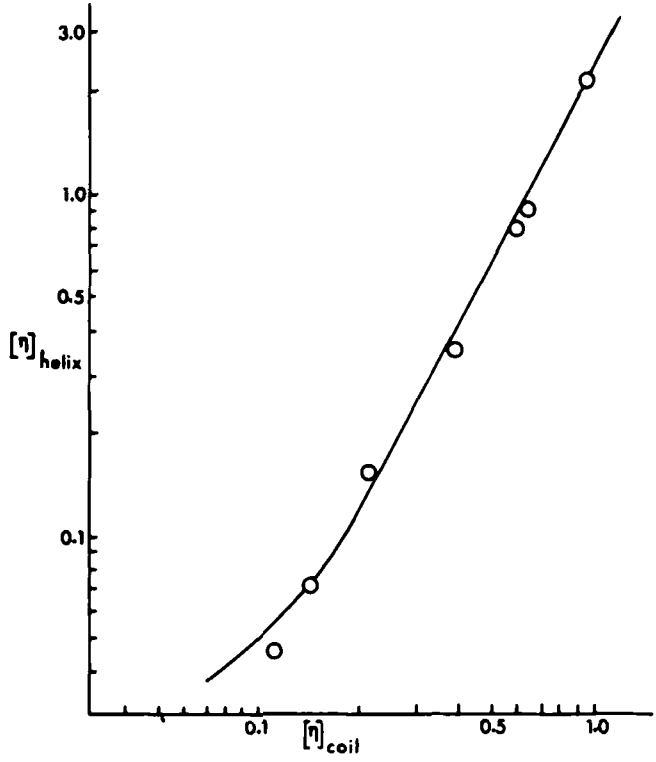


**Fig. 4.** Plot of intrinsic viscosity of helical form $[\eta]_{helix}$ against that of random coil form $[\eta]_{coil}$ of (1) PLM (○) and (2) poly-γ-benzyl-L-glutamate (full line) [7, 8, 11, 12].

where K and α are empirical constants and M is the molecular weight. For both the helical and coil forms of the polypeptide, it can be shown that the exponent a in Eq. (1) is simply the ratio $\alpha_{helix}/\alpha_{coil}$, and hence this ratio is the same for both PLM and PBLG.

### Light Scattering

The weight average degree of polymerization ($DP_w$) was determined by light scattering for three samples of PLM in TFA and the results are given in Fig. 5, together with the graph for PBLG in DCA [8]. It is seen that the agreement between the results of PLM and PBLG is within experimental

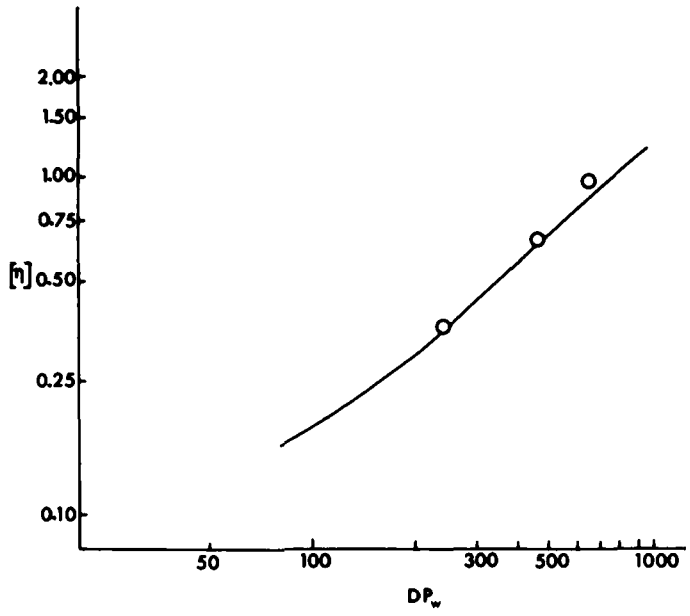


**Fig. 5.** Graph of (1) $[\eta]_{TFA}$ vs $DP_w$ of PLM (○) and (2) $[\eta]_{DCA}$ vs $DP_w$ for PBLG (full line), data of Ref. 8.



error, except perhaps for the PLM sample of highest $DP_w$ in which the point falls above the line. This shows that PLM and PBLG can be described by the same Mark-Houwink equation in TFA and DCA, respectively. Thus the exponent $\alpha_{coil}$ is 0.89 [7] for the two polypeptides in the random coil form. It is shown in the previous section that $\alpha_{helix}/\alpha_{coil}$ is also constant for PLM and PBLG, hence $\alpha_{helix}$ must be the same (1.65) [7] in both cases. There are two conclusions which can be obtained from this information as follows:

1) PLM and PBLG in TFA and DCA, respectively, are stiff random coils which are both expanded to the same degree, i.e., their expansion factor is the same [4]. In this respect they differ from other random coil polypeptides (see Table 1).

2) PLM and PBLG in the helical form have the same value of $\alpha_{helix}$ = 1.65, which indicates considerable rigidity of the helix in both cases [3, 4].

**Table 1**

| Polypeptide | Solvent composition for helix to coil transition [18] | | | Exponent in Mark-Houwink equation[a] | |
|---|---|---|---|---|---|
| | % $CHCl_3$ | % DCA | % TFA | $\alpha_{helix}$ | $\alpha_{coil}$ |
| PCLL | 63 | 37 | 0 | 1.26 [19] | 0.7 [15] |
| | | | | 1.0 [20] | |
| PELG[b] | 60 | 40 | 0 | 1.30 [21] | 0.73 [21] |
| PBLG | 25 | 75 | 0 | 1.65 [7] | 0.89 [7] |
| PLM | 50 | 0 | 50 | 1.65 | 0.89 |
| | 0 | 80 | 20 | | |

[a]References shown in brackets.
[b]PELG is poly-$\gamma$-ethyl-L-glutamate.



### NMR Line Widths

It is well known [5, 13] that the line width ($\Delta\nu$) of an NMR resonance, (e.g., $S-CH_3$ proton resonance in PLM) is independent of the molecular weight of the polymer in the random coil form, but increases with increase of molecular weight for the helical form of the polypeptide. The flexibility of a rodlike structure increases with increasing degree of polymerization and eventually at very high molecular weight the viscosity behavior of the rod approximates to that of a random coil [14]. A similar type of behavior would be anticipated with the molecular weight dependence of $\Delta\nu$ and this is shown to be the case in Fig. 6. Thus there is a linear dependence between $\Delta\nu_0$ and $DP_W$ for PLM over the whole range of samples, whereas for PBLG the linear graph flattens off at higher values of $DP_W$ and with the flexible rod poly-$\epsilon$-carbobenzoxy-L-lysine (PCLL) there is little evidence of a linear section.

An alternative explanation of these results is that the correlation time of the protons (and hence $\Delta\nu_0$) tends to a maximum value as $DP_W$ increases, due to the motion about bonds in the side chain [16]. Since the number of bonds in the side chain of PCLL > PBLG > PLM, side chain motion also

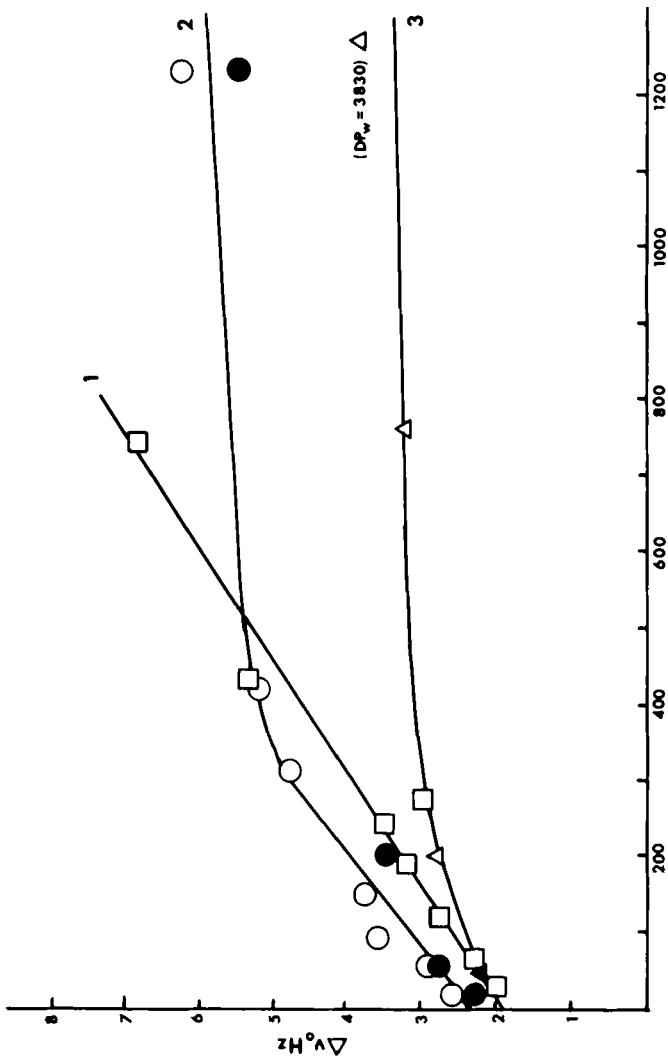


**Fig. 6.** Graph of line width extrapolated to zero concentration ($\Delta\nu_0$) against weight average degree of polymerization ($DP_w$) of (1) S–$CH_3$ protons in PLM in $CDCl_3$-dichloroacetic acid (95:5) (□), (2) phenyl protons in PBLG in dimethyl formamide (○) [5] and (●) this work, and (3) phenyl protons of PCLL in dimethylformamide (△) [15] and (▲) this work.

should follow this sequence and the maximum value of $\Delta\nu_0$ for PLM > PBLG > PCLL. Although the maximum value of $\Delta\nu_0$ has not been reached in Fig. 6 for PLM, the results obtained appear to fit this explanation. Experiments are in progress to decide between these two possibilities.

If the first explanation is the correct one, then the NMR technique may be useful to study the rigidity of rods and also the cooperativity of the helix-to-coil transition [17].

### Correlation of Stability of Helix, Rigidity of Rod, and Stiffness of Random Coil

It is seen from the results summarized in Table 1 that the order of stability of helix is PLM > PBLG > PELG > PCLL, the order of rigidity of the helix is PLM ≥ PBLG > PELG > PCLL, and the order of stiffness of the random coil in TFA or DCA is PLM ≥ PBLG > PELG > PCLL. The close correlation between helix stability and helix rigidity is to be expected, but the explanation of the equally close correlation with stiffness of the random coil is not obvious.



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