trifluoroacetic acid to remove the protecting group. The same treatments as above gave the monomer trifluoroacetate.

The monomer salt was dissolved in DMF or Me_2SO at the concentration listed in Table II. To the solution was added with shaking 1.2 equiv of triethylamine. The system of high concentration of the monomer salt became immediately complete gel. In this case the polymerization was allowed without shaking. The system of lower concentration of the monomer salt kept a liquid state throughout polycondensation. After the polymerization the solid or the viscous liquid was triturated with ACN and the polymer obtained was filtered, extracted repeatedly by methanol, washed with diethyl ether, and dried.

Conformational Studies of Random DL Copolypeptides in Solution Using High-Resolution Nuclear Magnetic Resonance

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ABSTRACT: The solution conformation of several DL copolypeptides is studied using the α-CH and amide NH spectra. The coexistence of helix and coil is seen from a double-peak appearance in the spectrum. It is concluded that poly(γ-benzyl DL-glutamate) of DP $\approx$ 170 is fully helical in chloroform ($CDCl_3$) and dimethylformamide but fully coiled in dimethyl sulfoxide. Reduction of molecular weight results in the formation of a coil component in $CDCl_3$ and dimethylformamide. Poly(β-benzyl DL-aspartate) of DP $\approx$ 60 is found to be over half-random coil in $CDCl_3$ while poly(β-methyl DL-aspartate) of DP $\approx$ 140 is fully coiled in chloroform.

The solution conformations of synthetic polypeptides such as poly(γ-benzyl L-glutamate) ((Bzl-L-Glu)$_n$) have been studied mainly by the use of ORD and CD, but these techniques rest ultimately on X-ray diffraction studies of the solid state together with the assumption that the conformation is the same in both states. In the case of ((Bzl-L-Glu)$_n$), however, the solution conformation has been unambiguously determined by X-ray diffraction.[2a] ORD–CD being of limited applicability to DL copolymers, their conformations have long been the subject of study and infrared spectroscopy has to date proved the most successful technique.

Inspection of α-helical models of randomly copolymerised D and L residues shows the presence of several unfavorable nonbonded atom contacts. Despite this fact, the strong preference of a number of polyamino acids for the helical conformation in the so-called helicogenic solvents led to suggestions that such DL polypeptides might have a high proportion of helix in solution. Poly(γ-benzyl DL-glutamates) ((Bzl-L-Glu)$_n$) have been much studied and it was early shown[2b] that (Bzl-L-Glu)$_n$ in chloroform can incorporate up to 30% of the D residue without disruption of the right-handed (RH) helix. In a thorough study of (Bzl-DL-Glu)$_n$ in the solid state, using in the main the amide V vibration characteristic of helix, Tsuboi *et al.*[3] came to the conclusion that a 50 D/50 L copolymer of DP $\sim$ 150 is about one-half helical, although the helix is not the regular α, but one distorted to an unknown degree. The proportion of helix was found to rise with increase of molecular weight, although the difficulty of synthesising DL copolypeptides of very high molecular weight prevented an estimation of the limiting amount of helix possible for such a polymer. To explain these findings Tsuboi *et al.*[3] postulated a polymerization scheme based on the assumption that a growing random-coil chain shows no strong preference for addition of an L or D residue. If, however, several residues of like configuration (say L) by chance polymerize in an unbroken sequence, then a helical "seed" forms and further polymerization strongly favors addition of L monomers. Since polymerization from *N*-carboxyanhydrides proceeds from the N-terminal end, the resulting polymer has an N-terminal random-coil portion and a C-terminal helical portion. Clearly, the higher the molecular weight, the greater the helicity of the polymer.

Poly(γ-methyl DL-glutamates) ((Me-DL-Glu)$_n$) have also been studied in the solid state by Masuda *et al.*[4] by using the amide V vibration and a 50 D/50 L copolymer found to be about three-quarters helical. A recent study of the same 50 D/50 L polypeptide in films by Nakajima[5] *et al.*, who also made use of the helical amide V intensity, resulted in a value of 60% helicity for the racemic polymer. On the basis of viscosity data these authors also concluded that in solution the helical and coiled sections exist as alternating sequences rather than in the form of long blocks. Both these estimates represent the sum of right- and left-handed helices and it is clear that these racemic polymers are very considerably helical in the solid state. But what are their conformations in solution? Masuda *et al.*[4] used the hyperchromicity of the 190-mμ peptide absorption to estimate the helicity of (Me-DL-Glu)$_n$ in trifluoroethanol and concluded that the polymer was 90% helical in this solvent, *i.e.*, considerably more so than that in the solid state. This method of estimating helicity in solution is limited to solvents having the transparency at 190 mμ and the same difficulties beset the use of the amide V band in solution work. A more general method of estimating solution helicity is therefore required and high-resolution nmr can provide this.

Spach[6,7] prepared two samples of (Bzl-DL-Glu)$_n$ that differed in their hydrodynamic properties. One of these, designated B and prepared in dioxane, behaved as a rigid

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Table I

Ref No.	Sample	Molar Composition	Deg of Polymerization	Mid-transition Point % CF_3COOH v/v in $CDCl_3$–CF_3COOH
375 (1)	$(Bzl\text{-DL-}Glu)_n$	D 48% L 52%	170	4.2
386 (1)	$(Bzl\text{-DL-}Glu)_n$	D 48% L 52%	159	2.2
400B	$(Bzl\text{-DL-}Glu)_n$	D 50% L 50%	68	1.5
400A	$(Bzl\text{-DL-}Glu)_n$	D 50% L 50%	21	<0.5
R10	$(Bzl\text{-L-}Glu)_n$	L 100%	92	10
425 (1)	$(Bzl\text{-L-}Asp)_n$	L 100%		1.9
425 (9)	$(Bzl\text{-DL-}Asp)_n$	D 50% L 50%	~60	
W80	$(Me\text{-DL-}Asp)_n$	D 50% L 50%	~140	

rod in dimethylformamide, suggesting an almost fully helical molecule. Form A, prepared in benzene, was much more flexible. Nmr studies[8] of both these polymers in $CDCl_3$–CF_3COOH gave very similar results and the α-CH spectrum showed the "double-peak" phenomenon characteristic of the helix–coil transition, in which separated helix and coil resonances are observed. It was thus concluded that both samples were very largely helical in chloroform. Studies have also been carried out[7-9] using nmr and ORD on "alternating" DL copolypeptides, which might be expected to possess maximal steric hindrance to helix formation. Nevertheless, it was concluded on the basis of their nmr spectra that the polymers were largely helical in chloroform.

The aim of the present work has been to use nmr to verify these conclusions as to the helicity of racemic glutamate polymers in solution and more particularly to establish their range of validity as regards the nature of the polypeptide, its molecular weight and the solvent system used. A number of DL-aspartate copolymers have also been studied to investigate whether the reduced stability of the aspartate helix, as compared to glutamate helices, results in a lowered proportion of helix in aspartate copolymers.

Experimental Section

Table I lists the samples used together with certain molecular weights estimated from viscosities in dichloroacetic acid.[10] The polymers were prepared by W. E. Hanby at the Courtaulds Laboratories, Maidenhead, and some are described in previous publications as follows: series 375, reference 2; series 425, reference 11.

ORD measurements were made on a Bellingham and Stanley Polarmatic spectropolarimeter over the wavelength range 555 to 270 mμ and b_0 values calculated using the Moffitt equation with λ_0 = 212 mμ. Nmr measurements were made at 100 MHz on a Varian HA-100-15 spectrometer at C.N.R., Arco Felice, Naples, Italy, and at 220 MHz on the S.R.C. spectrometer, then at ICI, Runcorn, England. Sample tubes (5 mm) were used with polymer concentrations of 3–4% w/v. The deuteriochloroform, deuteriodimethylformamide, and deuteriodimethyl sulfoxide were used without further purification. The trifluoroacetic acid was distilled immediately before use.

Table II
Series 375: Copoly(γ-benzyl DL-glutamates) in Chloroform

Sample No.	DP	L Composition (%)	b_0	% RH	% LH
11	1600	100	−670	100	0
7	210	80	−646	98	2
5	240	70	−503	88	12
4	200	65	−401	80	20
3	190	60	−296	73	27
1	180	52.5	−213	66	34
		(50	0	50	50)

Results and Discussion

In conformational studies of poly(γ-benzyl L-glutamate) we have found the α-CH resonance to be the most useful for conformational work and in Figure 1a–c α-CH spectra are shown for three DL copolymers having composition close to or precisely 50 L/50 D.

The highest molecular weight sample, 375 (1), Figure 1a, shows the "double peak" spectrum typical[12] for an optically pure homopolymer of the same molecular weight undergoing the helix–coil transition. The peak at 3.95 ppm is assigned to helical polymer and that at 4.50 ppm to random coil. The other samples of series 375 (having different D to L ratios and including the pure L polymer) all show very similar spectra and the same conclusion follows. The peak at 3.65 ppm is unusual. It is absent in the pure L polymer and gradually increases in intensity through to the L/L + D = 0.52 sample, in which it constitutes about 15% of the total area, in the 0.6 and 1.3% CF_3COOH spectra. Now the resonant frequency of random-coil benzyl glutamate residues in chloroform is not known with certainty, but if 375 (1) contained any random coil then it should be revealed as a peak that on CF_3COOH addition moves monotonically and asymptotically to its final position at about 4.55 ppm in 15% CF_3COOH. No such peak is apparent in 375 (1). In particular the peak at 3.65 ppm decreases in area on CF_3COOH addition without changing shift such that its area with respect to the helix peak at 3.95 ppm remains approximately constant. It cannot therefore be assigned to random coil. The spectra of Figure 1a thus indicate that 375 (1) undergoes a complete helix-to-coil change. This conclusion accords with that reached by earlier authors.[8] An advantage gained from observing the nmr spectrum of a DL copolymer is that if the total helicity is derived from the α-CH resonance, then measurement of b_0 or an equivalent parameter leads to a full conformational analysis. Table II shows this for series 375 as a simple case in which the total helicity is taken as 100%.

Sample 386 (1) of lower molecular weight (Figure 1b) differs from 375 (1) in that in pure $CDCl_3$ there is distinct resonance in the 4.2- to 4.5-ppm region. Sample 400B gave spectra very similar to those of 386 (1). Figure 1c of still lower molecular weight $(Bzl\text{-L-}Glu)_n$ (400A) shows yet more absorption centered at about 4.2 ppm in chloroform and a corresponding decrease in intensity of the helix peak at 3.95 ppm. A plot of the position of this 4.2-ppm resonance maximum against CF_3COOH content is of the form expected for a solvation shift and this suggests that random coil in chloroform absorbs at about 4.2–4.3 ppm.

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(9) P. M. Hardy, J. C. Haylock, D. I. Marlborough, N. H. Rydon, H. T. Storey, and R. C. Thompson, *Macromolecules*, 4, 435 (1971).
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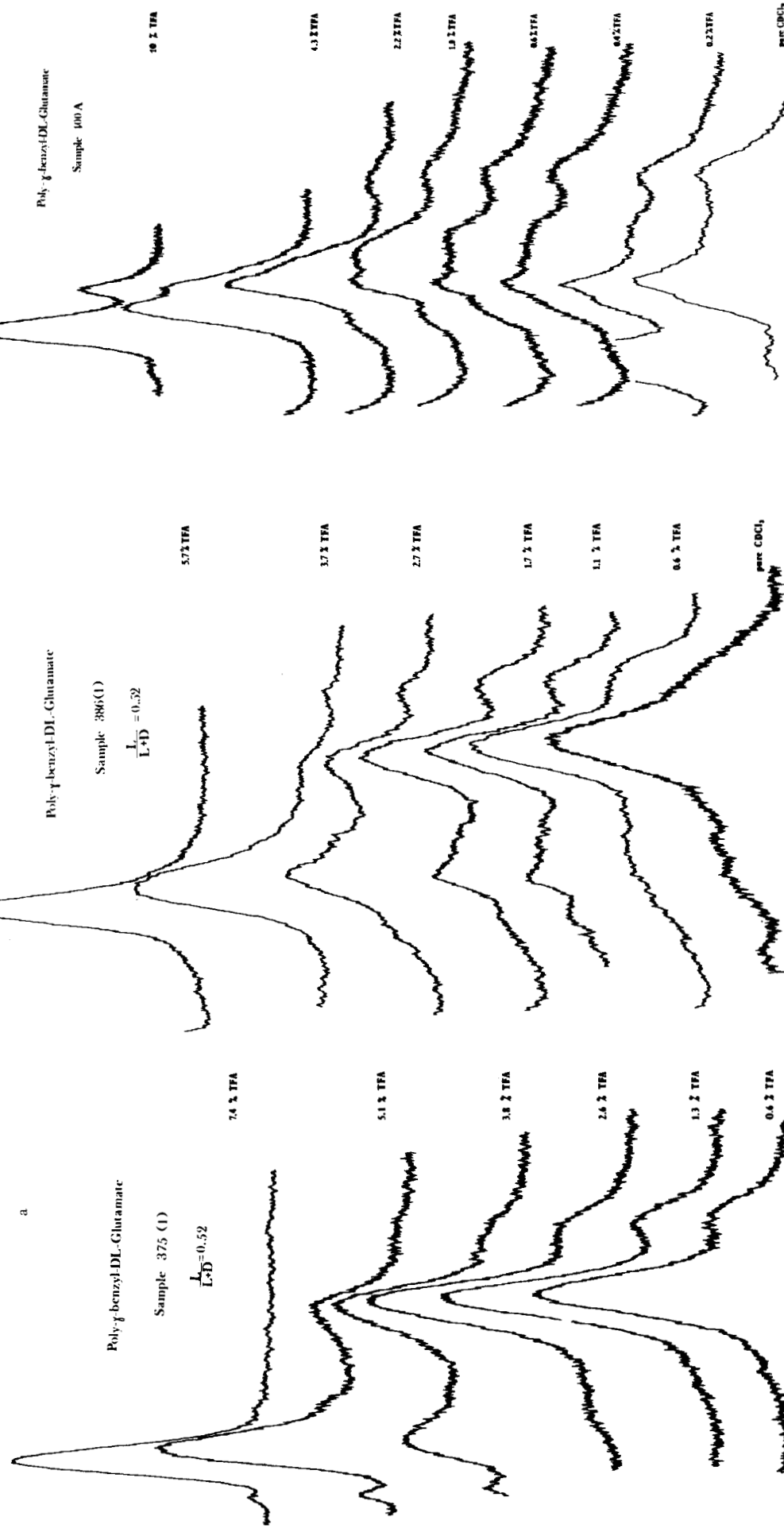

Figure 1. (a) 220-MHz α-CH spectra of poly(γ-benzyl DL-glutamate) 375 (1), 52 L/48 D, in $CDCl_3$–CF_3COOH. (b) 220-MHz α-CH spectra of poly(γ-benzyl DL-glutamate) 386 (1), 52 L/48 D, in $CDCl_3$–CF_3COOH. (c) 220-MHz α-CH spectra of poly(γ-benzyl DL-glutamate) 400 A, 50 L/50 D, in $CDCl_3$-CF_3COOH.

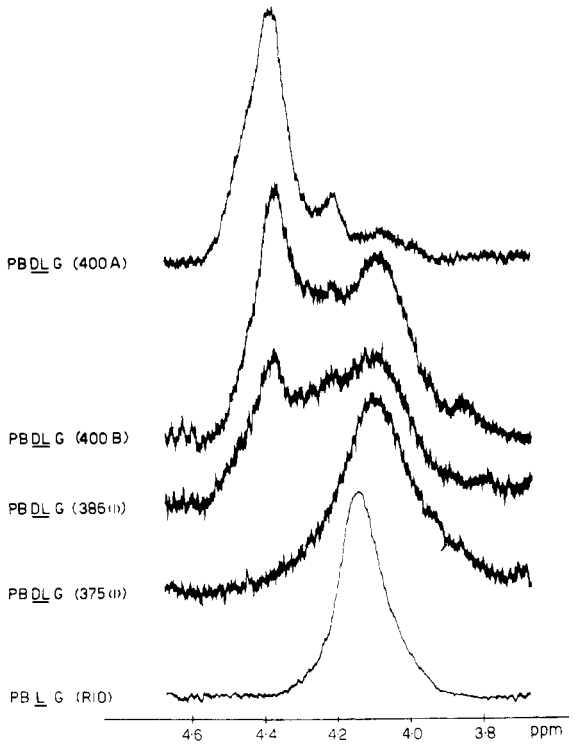

Figure 2. 220-MHz α-CH spectra of three samples of poly(γ-benzyl DL-glutamate) and one of poly(γ-benzyl L-glutamate) in dimethylformamide.

It is rather surprising that this coil peak should appear much broader than that of random-coil residues in CF_3COOH-containing solvents. However, we have noted a close similarity between the spectra of 400A at 100 and 220 MHz as regards this peak. This indicates the presence of a range of chemical shifts. This is no doubt due to the presence of a range of conformations between helix and coil resulting from a spread of molecular weights.[12]

On the basis of this assignment of coil and helix, the spectra of the (Bzl-DL-Glu)$_n$ glutamates show that 386 (1) and 400B are about one-quarter random coil in chloroform, and 400A is well over half-random coil. On CF_3COOH addition 386 (1) undergoes what is principally a helix–coil transition; the midpoint lies at 2.2% CF_3COOH, somewhat less than for the higher molecular weight 375 (1), which has a midpoint at 4.2% CF_3COOH. In both series, 375 and 386, the transition midpoints fall monotonically from about 10% CF_3COOH for the optically pure (Bzl-L-Glu)$_n$ to the above values for the racemic polymers. This no doubt reflects both the reduced stability of helical segments incorporating D and L residues and also their short length. Data on the transition midpoints is collected in Table I and the values given accord with that reported previously[8] for a racemic (Bzl-DL-Glu)$_n$. CF_3COOH addition to 400A results in both conversion of helix to coil and solvation of already existing coil; 10% CF_3COOH converts all residues into solvated random coil with identical shift of 4.55 ppm. The sharp peak at 4.37 ppm that remains unchanged in shift with CF_3COOH addition can be assigned[13] to end groups.

The resonance at 3.65 ppm remains unassigned. Its intensity in the highly helical 80 L/20 D and 70 L/30 D copolymers in chloroform–0.5% CF_3COOH is less than 20 and 30%, respectively, and it cannot therefore be due to all the D residues incorporated into RH helices. Tsuboi *et al.*[3] have presented evidence for the presence of distorted helices in random DL copolymers and the peak at 3.65 ppm could come from residues in such a conformation. The resistance to CF_3COOH denaturation of this conformation is, however, approximately that of the usual helix (3.95 ppm) and it does not therefore represent a weak point. Hardy *et al.*[9] in their study of an "alternating" poly(γ-benzyl DL-glutamate) reported the observation of an α-CH peak at 3.7 ppm in pure $CDCl_3$ that might correspond to that discussed here. (We do not, however, observe the NH peak at 8.5–8.7 ppm given by their alternating copolymers.) They attribute these unusual peaks in the alternating copolymer to a distorted helix, but to one that appears more sensitive to CF_3COOH denaturation (as judged by their spectra) than that we observe. This discrepancy might, however, be due to a molecular weight difference. A second possible assignment of the 3.65-ppm peak is to residues lying at the junctions between helical segments of opposite sense. It constitutes 15% of the total α-CH area, and this is not an unreasonable proportion for such residues under solvent conditions favouring maximum helicity. If such residues were constrained by geometric requirements of the opposing helices or by the formation of a bend or fold akin to that observed in globular proteins, then the absence of a solvation shift on CF_3COOH addition and the resistance to denaturation could be explained.

Table III

$\Delta_{\gamma\text{-}CH_2} - \Delta_{\beta\text{-}CH_2}$ (ppm) in DMF[a]	Sample Ref No. (γ-BzlGlu)$_n$
0.40	400A (DL)
0.31	400B (DL)
0.30	386 (1) (DL)
0.23	375 (1) (DL)
0.19	R10 (L)

[a] DMF = dimethylformamide.

Spectra in Dimethylformamide. Dimethylformamide is a well-known helicogenic solvent for a number of polypeptides, including (Bzl-L-Glu)$_n$, and so the spectra of the same benzyl DL-glutamates as studied above in $CDCl_3$ were observed in dimethylformamide. Figure 2 shows the α-CH spectra at 220 MHz, together with that of a fully helical (Bzl-L-Glu)$_n$ sample, R10. The upfield peak at 4.09 ppm and the low-field peak at 4.39 ppm in the copolymers can be clearly assigned to helix and coil, respectively. These shift values are more precise than those previously published.[14] The conclusion follows that the helicity of (Bzl-DL-Glu)$_n$ molecules in dimethylformamide is strongly molecular weight dependent. The sample of DP = 170 (375 (1)) is fully helical, 386 (1) is about 70% helical, while 400A of DP = 21 is almost fully coil. The small peak at 4.21 ppm is probably due to end groups and the spectra of 375 (1) show no sign of a peak at ~0.3 ppm upfield of the main helix peak that would correspond to that observed at 3.65 ppm in $CDCl_3$–CF_3COOH.

The NH resonance of the DL-glutamates in dimethylformamide has the same form as that of the α-CH in Figure 2, although as in $CDCl_3$–CF_3COOH, the coil is upfield of the helix; in particular the intensity ratios of the coil peak (at 8.28 ppm) to that of the helix peak (at 8.48 ppm) is similar to that seen in the α-CH spectra. No difference was observed between the 8.48 ppm NH helix shift of 375 (1) (Bzl-DL-Glu)$_n$ and that of R10, the homopoly(γ-benzyl

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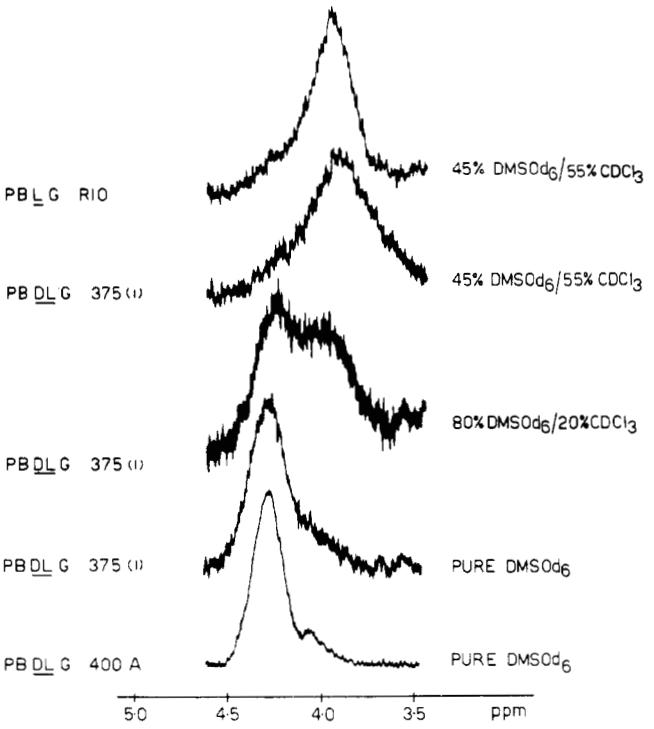

Figure 3. 100-MHz α-CH spectra of two samples of poly(γ-benzyl DL-glutamate) and one of poly(γ-benzyl L-glutamate) in dimethyl sulfoxide–chloroform.

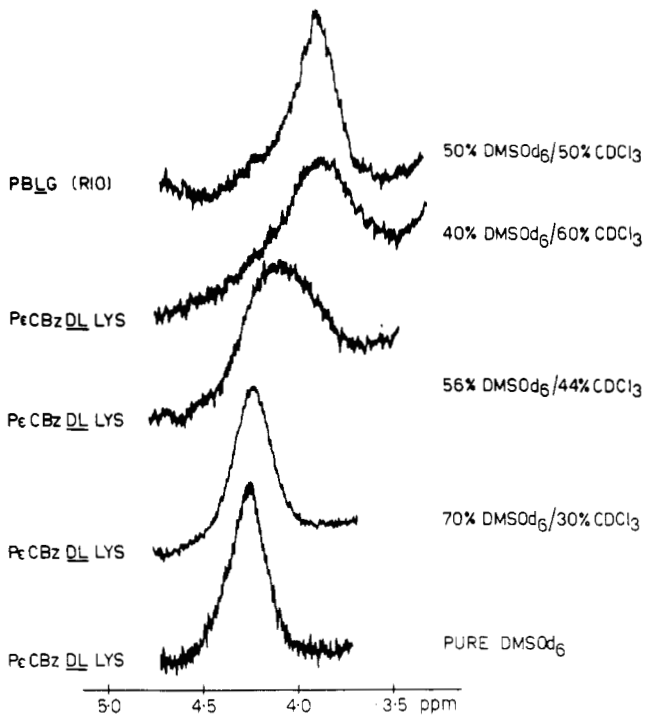

Figure 4. 100-MHz α-CH spectra of poly(ϵ-carbobenzoxy-DL-lysine) and poly(γ-benzyl L-glutamate) in dimethyl sulfoxide–chloroform.

L-glutamate). The NH peaks show the dependence on conformation less obviously than do the α-CH due both to the smaller shift difference (0.20 ppm) and to the considerable width of the helix NH peak. For this reason the NH spectra are not shown and the α-CH spectra are preferred for conformational analysis.

Another feature of the nmr spectrum that we have used[15] to follow the helix–coil transition in benzyl glutamates is the shift difference between the β- and γ-CH_2 protons. This increases as helix is converted into coil from about 0.20 to 0.40 ppm. Table III gives values for this difference measured for several glutamate samples at 220 MHz in dimethylformamide. Comparison of these data with those shown in Figure 5 of reference 15 for homopoly(γ-benzyl L-glutamate) in $CDCl_3$–CF_3COOH leads to the result that 375 (1) is almost fully helical in dimethylformamide, 386 (1) and 400B are about one-half helical, while 400A is fully random coil. This fully substantiates the conclusions based on the α-CH and NH spectra and proves that poly(γ-benzyl DL-glutamates) of intermediate and low molecular weight have a lower helicity in dimethylformamide than in $CDCl_3$.

Spectra in Dimethyl Sulfoxide. Homopoly(γ-benzyl L-glutamate) of high molecular weight is fully helical in dimethyl sulfoxide (Me_2SO)[16] while a sample of DP = 29 was found to be only about two-thirds helical at room temperature. Partly helical polymers in Me_2SO were found to exhibit the characteristic double peak α-CH spectrum, the upfield helix peak being at 3.95 ppm and the lowfield coil at 4.26 ppm. Me_2SO appears therefore to be not as strong a supporter of the helical structures as chloroform. Figure 3 shows α-CH spectra at 100 MHz of the lowest (400A) and highest (375 (1)) molecular weight (Bzl-DL-Glu)$_n$ samples in pure Me_2SO-d_6. The spectra are identical, showing a single peak at 4.27 ppm. The close correspondence with (Bzl-L-Glu)$_n$ coil shift (4.26 ppm) strongly suggests that both DL polymers are fully coil under these conditions. This is confirmed by conversion to the helix by the addition of $CDCl_3$ to 375 (1) in Me_2SO-d_6; this produces a double peak in 80% Me_2SO-d_6–20% $CDCl_3$ and a single peak at 3.95 ppm at greater than 50% $CDCl_3$. It has been demonstrated above that 375 (1) is helical in pure $CDCl_3$ and Figure 3 shows a comparison spectrum of helical (Bzl-L-Glu)$_n$ in the same solvent 55% Me_2SO–45% $CDCl_3$. In pure Me_2SO-d_6 375 (1) (Bzl-DL-Glu)$_n$ must therefore be fully coil, in contrast to the findings in $CDCl_3$ and dimethylformamide in which it is fully helical.

The α-CH spectrum was also studied of several other copolymers in the 375 series of DL-glutamates (see Table III), dissolved in Me_2SO. It was found that as the D content increased, the random-coil component grew in proportion. It follows that, unlike in chloroform, in Me_2SO no left hand helices are formed with these polymers.

Similar results have been obtained with a sample of poly(ϵ-carbobenzoxy-DL-lysine) ((Cbz-DL-Lys)$_n$) in Me_2SO-d_6–$CDCl_3$ and the α-CH spectra are shown in Figure 4. The homopolymer (Cbz-DL-Lys)$_n$ is helical in pure Me_2SO-d_6[17] (b_0 = ·−623°) and in $CDCl_3$, as in (Bzl-L-Glu)$_n$. The α-CH shift of (Cbz-DL-Lys)$_n$ is 3.90 ppm. (In pure Me_2SO-d_6 the resonance is too broad for measurement.) The close similarity of Figures 3 and 4 indicates that the same conclusions may be drawn for (Cbz-DL-Lys)$_n$ as for (Bzl-DL-Glu)$_n$, *i.e.*, a coil conformation in pure Me_2SO-d_6 which undergoes transition to a helix on the addition of $CDCl_3$.

DL-Aspartate Copolymers. A complete series (425) of poly(β-benzyl aspartates) of composition varying from pure L (425 (1)) to 50 D/50 L (425 (9)) has been studied in

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(16) E. M. Bradbury, C. Crane-Robinson, L. Paolillo, and P. Temussi, *Polymer*, 14, 303 (1973).

(17) E. M. Bradbury, C. Crane-Robinson, V. Giancotti, and R. M. Stephens, *Polymer*, 13, 33 (1972).

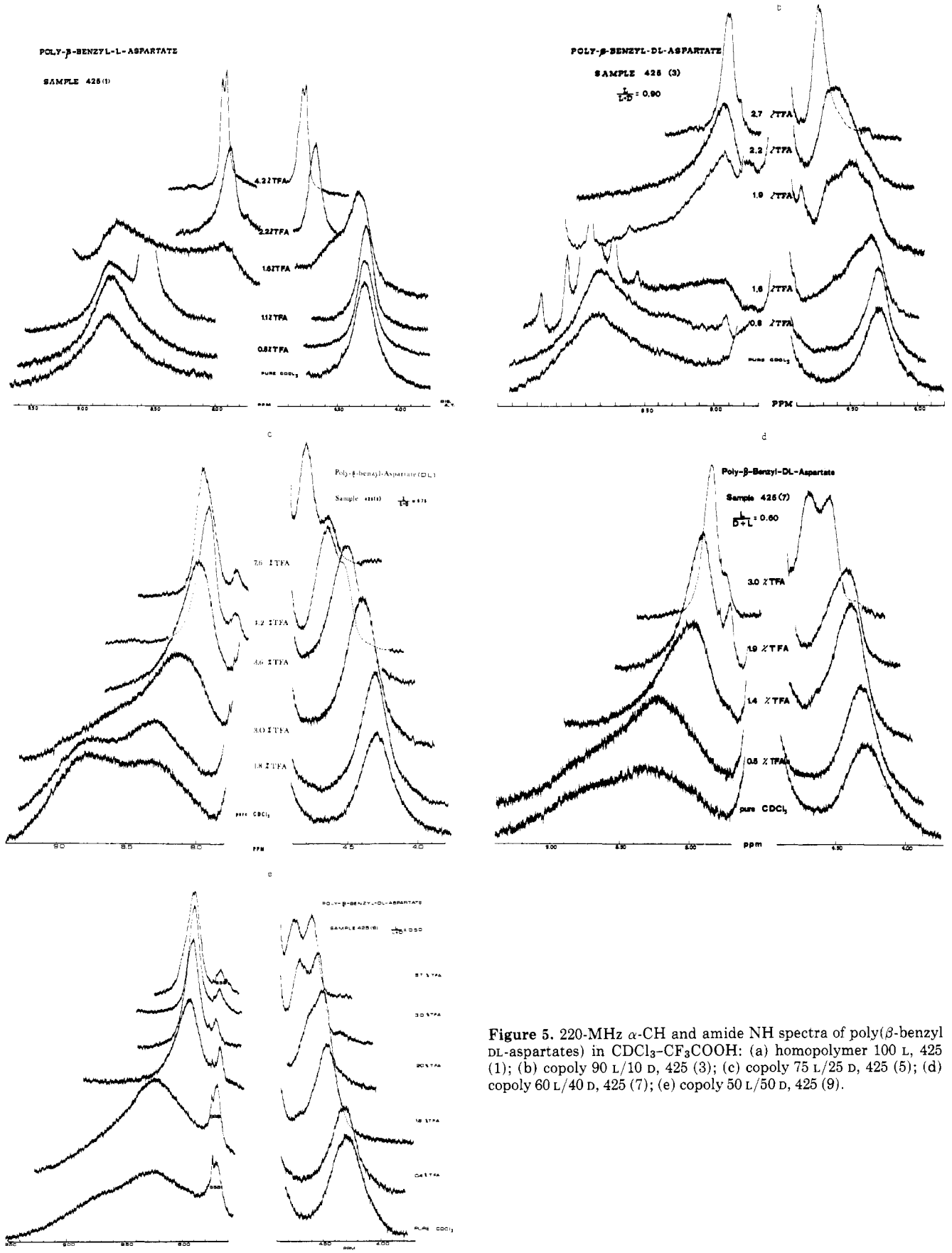

Figure 5. 220-MHz α-CH and amide NH spectra of poly(β-benzyl DL-aspartates) in $CDCl_3$–CF_3COOH: (a) homopolymer 100 L, 425 (1); (b) copoly 90 L/10 D, 425 (3); (c) copoly 75 L/25 D, 425 (5); (d) copoly 60 L/40 D, 425 (7); (e) copoly 50 L/50 D, 425 (9).

$CDCl_3$–CF_3COOH. Spectra of the α-CH and NH regions of 5 of the series are shown in Figure 5a–e. The pure L polymer shows the expected left-handed (LH) helical-to-coil transition. The LH helix shifts are: amide NH 8.75 ppm, α-CH 4.30 ppm.[18] A typical double peak appearance is seen in the transition region and the coil shifts in 3% CF_3COOH are: amide NH 7.90 ppm, α-CH 4.70 ppm.

(18) E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson, and H. Goldman, *Macromolecules*, 4, 557 (1971).

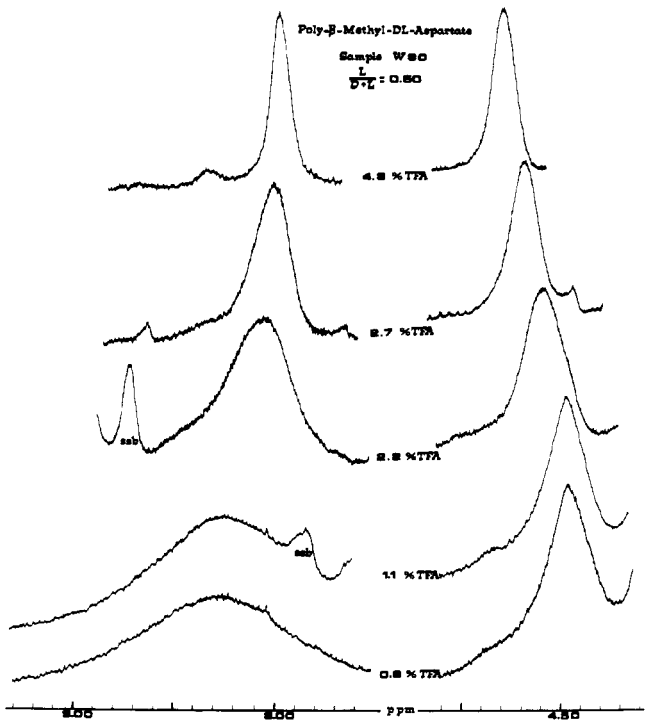

Figure 6. 220-MHz α-CH and amide spectra of poly(β-methyl 50 L/50 D-aspartate) in $CDCl_3$-CF_3COOH.

In considering the spectra of Figure 5, an important factor is the already established[18] shifts characteristic of benzyl aspartate residues incorporated into helices of the "unnatural" sense (*i.e.*, L residues on a RH helix and D residues on a LH helix); these are amide NH 8.3 ppm and α-CH 4.40 ppm. The principal question to be answered is: What is the conformation of these DL-aspartate polymers in chloroform? Comparison of the five NH spectra in pure $CDCl_3$ shows that incorporation of D residues into the L polymer results in a rise in absorption at 8.2–8.3 ppm at the expense of that at 8.75 ppm, until in the 50% L, 50% D polymer well over one-half of the intensity is in the upfield peak. This could be interpreted as due to incorporation of D residues in the existing LH L helices plus the reverse situation and thus that the polymers are fully helical in $CDCl_3$. Two observations indicate however that this cannot be the major explanation. (1) It is unlikely that the rather unstable poly(β-benzyl L-aspartate) helix could withstand the incorporation of a high proportion of D residues. Furthermore, if the 8.3-ppm peak were due solely to residues on the unnatural helix sense, then these would outnumber those on the natural helix sense (8.75 ppm), which is clearly impossible. (2) Any residues on the unnatural helix sense would give rise to α-CH resonance at 4.40 ppm;[18] however, throughout the series the α-CH peak appears symmetrical and centered at 4.3 ppm. There cannot therefore be many residues incorporated into helices of the unnatural sense. The two α-CH peaks (that must exist, to accord with the presence of two NH peaks) thus have approximately the same shift of 4.3 ppm. We therefore conclude that random-coil poly(β-benzyl L- or D-aspartate) in $CDCl_3$ has an α-CH shift of 4.3 ppm and an amide NH shift of 8.2–8.3 ppm. This implies that the sample of poly(β-benzyl 50 L/50 D-aspartate) (Figure 5e) is well over half-random coil in $CDCl_3$ whereas 425 (5) (75 L/25 D) is well over half-helical. Addition of CF_3COOH to the polymers causes an immediate drop in the helicity (a reduction in intensity at 8.75 ppm) which is accompanied by solvation of the random coil. This solvation is strikingly demonstrated by the fact that in all copolymers including 25% or more D residues, the α-CH resonance moves as a single line, with no sign of the double peak phenomenon.

An unusual feature of the random-coil spectra of the DL-aspartates is the appearance of two α-CH peaks at the higher CF_3COOH contents, separated by approximately 0.15 ppm. The lowfield peak of the pair corresponds in shift to that of homopoly(β-benzyl L-aspartates) in $CDCl_3$–3–5% CF_3COOH. Although the relative intensities correlate roughly with the L and D composition, there is no obvious reason why in the solvated coil there should be such a shift difference. None is in fact observed for the DL-glutamates (see Figures 1). That the random-coil form of (Bzl-L-Asp) is unusual is shown by the nonzero b_0 in both CF_3COOH (−250°) and Me_2SO (−150°) and this residual asymmetry might be the cause of the shift difference in the DL polymers. An alternative and more likely explanation is a primary sequence effect akin to that observed in synthetic atactic polymers. If the shift depends only on the nature of a single nearest neighbor then, assuming random copolymerization, the ratio of the peak intensities will also be the D/L ratio of the polymer. These alternatives thus cannot be distinguished on the present data. If the shift depends on a triad sequence or if the polymerization is not truly random then additional experiments such as α-deuteration, are necessary to establish a reliable assignment.

With regard to $(Bzl\text{-}DL\text{-}Asp)_n$ in the other two solvents, dimethylformamide and Me_2SO, it should be noted that the polymer is insoluble in the former, while even the homopoly(β-benzyl L-aspartate) is coil in the latter.

Figure 6 shows spectra in $CDCl_3$–CF_3COOH for poly(β-methyl DL-aspartate) ($(Me\text{-}DL\text{-}Asp)_n$). As a homopolymer, poly(β-methyl L-aspartate) ($(Me\text{-}L\text{-}Asp)_n$) is LH helical in $CDCl_3$.[19] The DL copolymer did not dissolve fully in pure $CDCl_3$ and small amounts of CF_3COOH (> 0.5%) were necessary for a clear solution. On the basis of the above discussion of $(Bzl\text{-}DL\text{-}Asp)_n$, the absence of NH resonance at 8.75 ppm and a strong peak at 8.25 ppm indicates a fully coil conformation in $CDCl_3$–0.8% CF_3COOH. Further addition of CF_3COOH produces solvation shifts of both NH and α-CH peaks to 7.9 and 4.7 ppm, respectively, in $CDCl_3$–5% CF_3COOH, values typical for coil $(Bzl\text{-}L\text{-}Asp)_n$ and $(Me\text{-}L\text{-}Asp)_n$. There is no indication of a double peak appearance that would indicate a helix–coil transition. The α-CH shift of $(Me\text{-}DL\text{-}Asp)_n$ in 0.87% CF_3COOH–$CDCl_3$ of 4.40 ppm is unexpected (for $(Bzl\text{-}DL\text{-}Asp)_n$ in Figure 5d the shift is 4.30 ppm). Together with an NH shift of 8.25 ppm this could indicate L residues on a RH helix and D residues on a LH helix; however, there seems no reason at all why such unnatural helices should form with $(Me\text{-}DL\text{-}Asp)_n$. The conclusion must remain that the polymer is random coil in $CDCl_3$–0.8% CF_3COOH.

Conclusions

High-resolution nmr is here seen to be the ideal method for conformational studies of DL copolymers in solution since helix and coil residues in a single pure solvent have been shown to have different and characteristic shifts in several cases. Furthermore, addition of a (solvating) helix-breaking solvent results in monotonic displacement of coil resonance and the double peak appearance for helical resonance. On these bases it has been shown that the helicity of racemic poly(γ-benzyl DL-glutamate) is dependent both on the molecular weight, as previously postulated, and on

(19) M. Goodman, F. Boardman, and L. Litowsky, *J. Amer. Chem. Soc.*, 84, 3771 (1962).

the solvent. Thus while all the DL-glutamates studied here are in the coil form in Me_2SO and two samples are partially coil in dimethylformamide, only the very lowest molecular weight has a coil component in chloroform. Poly(γ-benzyl DL-aspartate) of fairly low molecular weight (DP $\simeq$ 60) has been shown to be largely coil in chloroform while poly(β-methyl DL-aspartate) of somewhat greater molecular weight is entirely coil in the same solvent. The ability of the nmr spectrum to estimate the fraction of coil conformation in DL copolymers means that in combination with ORD measurements on the same solution, a precise conformational analysis is possible in terms of coil, RH helix and LH helix. This has been possible previously only by estimating the coil fraction in the solid state and the relative proportions of RH and LH helix from solutions. Such an approach is clearly subject to uncertainties as a result both of the dependence of coil content on the solvent and of the necessity to compare solid state with solution data. The nmr–ORD method is to be preferred.

Acknowledgments. E. M. Bradbury and C. Crane-Robinson are grateful to the CNR of Italy and L. Paolillo likewise to the Governors of Portsmouth Polytechnic, for Fellowships during the tenure of which part of the work was carried out. This work is also supported by the Science Research Council of Great Britain.

Further Conformational Studies of Immunoglobulin Hinge Peptides†

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Received October 5, 1972

ABSTRACT: In a previous paper, low-energy structures of human immunoglobulin G1 hinge peptide (structure I of text) were described. It was found that because of the existence of a twofold symmetry axis, a single backbone conformation could give rise to different double-strand structures when the cysteine side chains had more than one conformation compatible with the existence of such an axis. It was tentatively suggested that this fact might account for the hinge mechanism. The comparison is now made with human immunoglobulin G2 hinge peptide (see structure II of text). It is assumed that this molecule also possesses a twofold axis. Because the presence of the four disulfide bridges must lead to drastic geometrical requirements within the peptide, to begin with, a geometrical approach has been used. Two low-energy conformations of the IgG1 hinge peptide ($d^{280\ 130}$ d d $d^{290\ 270}$ and $d^{290\ 250}$ d d $d^{290\ 270}$) are taken as starting points and only structures having the assumed twofold axis are taken into account. The first results suggest that, once again, a single backbone conformation (of nine amino acids, this time) may give rise to two different double-strand structures notwithstanding the presence of the two additional disulfide bridges. Energy calculations are compatible with this model but they must be further developed, so as to confirm the hypothesis.

In a previous paper,[1] the conformational energy of the central double pentapeptide of the human immunoglobulin G1 hinge region

(Cys-Pro-Pro-Cys-(Pro))
| |
Cys-Pro-Pro-Cys-(Pro)

I

has been calculated with the aid of semiempirical potential functions. In Figure 1, the general shape of an IgG1 molecule is given so as to show the location of the peptide in the molecule. It was found that a small number of families of backbone conformations are energetically allowed and that, due to the existence of a twofold symmetry axis passing through the middle of the two S–S bridges,[2] different dimer structures can result from an identical single-chain backbone conformation when more than one cysteine side-chain conformation is compatible with the closure of the disulfide bridges. It was tentatively suggested that this fact might be related to the hinge mechanism.

Consequently, it is interesting to make the comparison with hinge peptides of other immunoglobulins. The double nonapeptide of the human IgG2 hinge region,[3]

(Cys-Cys-Val-Glx-Cys-Pro-Pro-Cys-(Pro))
| | | |
Cys-Cys-Val-Glx-Cys-Pro-Pro-Cys-(Pro)

II

which has the same C-terminal pentapeptide as the IgG1, was chosen because of the hindrances imposed by the presence of two additional disulfide bridges located only two residues away in the direction of the amino end of the heavy chain. Assuming the existence of a similar twofold symmetry axis, it can easily be tested (and without attempting a calculation of the tertiary structure of the IgG2 hinge) whether or not the model proposed for the IgG1 hinge remains valid for the IgG2, despite the additional geometrical requirements.

The following procedure is used. Two low-energy structures of the IgG1 hinge peptide are chosen. Their single chains only differ by the cysteine side-chain conformations, the backbone having an identical secondary structure. For the sake of convenience, these single chains are called nuclei. The test should establish whether or not, for both nuclei, an identical backbone conformation for the

†This paper was presented at the 10th Prague IUPAC Microsymposium on Macromolecules.

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