Conformation of poly(glutamic acid) and of poly(aspartic acid) in the solid state. X-ray diffraction, infra-red and ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance spectroscopic study

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In order to assess the manifestation of possible conformations of poly(amino acids) in ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS n.m.r.) spectra, the conformational structures of poly(α-L-glutamic acid) (poly(Glu)), of poly(α-L-aspartic acid) (poly(Asp)) and of irregular poly(α,β,-DL-aspartic acid) in the solid state were studied by X-ray diffraction and infra-red spectroscopy and compared with the results of ^{13}C CP/MAS n.m.r. spectroscopy. None of the methods alone can fully characterize the conformation; by a combination of the results it could be determined that poly(Glu) and poly(Asp) in the free acid form exhibit both α-helical and β-structure, whereas the random-coil structure predominates in their sodium salts. Both the free acid and the sodium salt of poly(α,β-DL-Asp) exist predominantly in the random-coil form. It turns out that the sodium salts are especially suitable for determining the n.m.r. characteristics of the random-coil conformation in the solid state. The dynamic behaviour studied by the spin-lock cross-polarization experiment indicates that the studied poly(amino acids) in the free acid form are much more rigid than, for example, the poly(alkyl methacrylates).

(Keywords: poly(glutamic acid); poly(aspartic acid); conformation; infra-red spectra; X-ray diffraction; ^{13}C cross-polarization/magic angle spinning nuclear magnetic resonance spectra; solid state)

INTRODUCTION

Cross-polarization/magic angle spinning nuclear magnetic resonance (CP/MAS n.m.r.) spectroscopy, a new and promising technique, has already been applied in studies of the conformation of most poly(amino acids) in the solid state[1,2]. It was shown that the chemical shifts depend on the conformation of these polymers, and the content of α-helix and β-structure was determined from these spectra[1]. Therefore, it appears that this technique could supplement previously established methods, like X-ray diffraction and i.r. spectroscopy; moreover, it makes possible a quantitative evaluation of the contribution of various amino acids to various conformations[1]. However, the difference between the β-structure and the random coil in the spectra has not been established. Poly(α-L-glutamic acid) (poly(Glu)) and poly(α-L-aspartic acid) (poly(Asp)) may develop all these conformations depending on conditions. The study of their solid-state conformation may therefore contribute to the solution of this question.

Poly(Glu) has been studied by a number of physical techniques[3], among others also by i.r. spectroscopy and X-ray scattering[4,5], and it is known that it can assume the conformations of α-helix, β-structure and random coil in solution and in the solid state. The results of older papers on poly(Asp) are not reliable, because it was found that the studied samples probably contained a large amount of β-bonds[6]. By means of circular dichroism (c.d.), i.r. and n.m.r. spectroscopy, in our laboratory it has been shown that poly(Asp) not containing β-bonds can assume the conformations of α-helix, random coil and probably also of β-structure[7,8]. CP/MAS n.m.r. spectra of poly(Glu) and poly(Asp) have been measured by Kricheldorf *et al.*[1] and, without detailed study, based on analogy with other poly(amino acids), their shape was assigned to the β-structure. The dynamics of poly(Glu) and poly(Asp) in the solid state have not been studied so far.

The aim of this work is a confrontation of the results of conformational studies of poly(Glu), of poly(Asp), of irregular racemic poly(α,β-DL-Asp) and of their sodium salts in the solid state by the methods of X-ray scattering, i.r. and ^{13}C CP/MAS n.m.r. spectroscopy. The conditions of preparation of the samples were chosen according to the literature[4,5] so that all three conformations may develop. Particular attention is paid to the random-coil conformation and its manifestations in CP/MAS n.m.r. spectra. The n.m.r. measurements are supplemented by a study of the dynamic behaviour of these poly(amino acids) in the spin-lock cross-polarization experiment of CP/MAS n.m.r.

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EXPERIMENTAL

Materials

Poly(γ-benzyl-L-glutamate) was synthesized by polymerization of the *N*-carboxyanhydride of γ-benzyl-L-glutamate in dioxane using sodium methanolate as initiator (molar ratio, monomer/initiator = 100/1)[9] and had $\bar{M}_w = 310\,000$ (viscosimetry[10]). Poly(α-L-glutamic acid) (poly(Glu)) was obtained by the treatment of 3 g of poly(γ-benzyl-L-glutamate) dissolved in 5 ml of trifluoroacetic acid with 5 ml of acetic acid saturated with HBr, for 2 h at 60°C. The resulting polymer was washed with ethanol and ether, dissolved in 5 ml of saturated $NaHCO_3$ solution and dialysed against water for 5 days. The solution was diluted with water to 100 ml and acidified by concentrated HCl under vigorous stirring to pH 2.5. After 2 h the separated precipitate was isolated by centrifugation and vacuum dried over P_2O_5 at room temperature. It had $\bar{M}_w = 80\,000$ (viscosimetry[11]). The sodium salt of poly(α-L-glutamic acid) (poly(Glu)Na) was obtained by neutralization of 0.5 g of poly(Glu) by NaOH, dialysis against water (final volume 5 ml) and lyophilization at −20°C.

Poly(α-L-aspartic acid) (poly(Asp)), $\bar{M}_w = 52\,000$ (sedimentation equilibrium[6]), and its sodium salt (poly(Asp)Na) were prepared similarly; poly(β-benzyl-L-aspartate), $\bar{M}_w = 260\,000$ (viscosimetry[12]), was debenzylated in trifluoroacetic acid saturated with HBr for 2 h[13].

Poly(α,β-DL-aspartic acid) (poly(α,β-DL-Asp)), prepared by thermal polymerization of aspartic acid, is identical with sample I from ref. 14, $\bar{M}_w \simeq 29\,000$ (viscosimetry, sedimentation equilibrium[14]), the mole fraction of β-bonds being 0.75. Its sodium salt (poly(α,β-DL-Asp)Na) was obtained similarly as poly(Glu)Na.

The water content of all samples was 9–12%. Their structure was verified by ^1H and ^{13}C n.m.r. spectroscopy of H_2O solutions at pH 9[15]. Optical purity of L-conformers was confirmed by circular dichroism measurement as described previously[13]. Gel permeation chromatography (mixture of Sepharose 6B and Sephadex G-100 in the volume ratio 2.5/1)[16] of all polymers indicated a unimodal distribution of molecular mass with polydispersity ≈ 1.8 with no low-molecular-weight fraction (i.e. $< 10^4$).

Methods

X-ray diffractograms were obtained by means of a Hilger and Watts powder diffractometer (Cu K_α radiation, recording by scintillation counter, monochromatization by a β-filter with amplitude analyser) in the range of angles $2\theta = 4$–$70°$.

For the measurement of infra-red spectra, the powdered samples were mixed with KBr and pressed into pellets. The spectra were measured by means of a Perkin–Elmer 580 B spectrometer, with digitization and transformation into absorbance values by means of a Tracor TN 4000 spectrum analyser.

The 75.47 MHz ^{13}C CP/MAS n.m.r. spectra of the solid samples were measured on a Bruker CXP-300 *FT* n.m.r. spectrometer. The samples were measured in a deuterated PMMA rotor of inner diameter 6.3 mm, at a spinning rate of 3.5–4.0 kHz. The ^1H pulse time was 4 μs for the polypeptide free acids, and 5 μs for the sodium salts. The contact time was 3 ms and repetition time 4 s for poly(Asp); for poly(Glu) and for all the sodium salts the respective parameters were 1 ms and 1.5 s. The spectral width was 20 kHz, the number of points 4000. The setting of the magic angle was checked by means of the n.m.r. spectrum of glycine.

^{13}C chemical shifts were calibrated indirectly by means of benzene as external standard (127.66 ppm from tetramethylsilane, TMS). 4000 scans were needed for a good signal-to-noise ratio. The contact time $t_{c,1/2}$, where one-half of the total polarization is reached, was determined by means of the spin-lock cross-polarization experiment. ^{13}C n.m.r. spectra of aqueous polypeptide solutions at pH 9 were measured on a Varian XL-200 spectrometer at 50.3 MHz with hexamethyldisilane (HMDS) as external standard. Chemical shifts were related to tetramethylsilane (TMS) by means of the relation

$$\delta_{TMS_{int}} = \delta_{HMDS_{ext}} + 2.050 \text{ ppm}$$

RESULTS AND DISCUSSION

X-ray diffraction

The diffractograms of all six samples of amino acids and their sodium salts are shown in *Figure 1*. The diffractograms of poly(Glu) and poly(Asp) exhibit typical two-phase structure. Relatively weak and broad reflections, indicating a relatively low content of crystalline phase, are superimposed on an amorphous halo, which can correspond both to random coils or to randomly oriented helices. The positions of the reflections and the corresponding interplanar distances *d* are summarized in *Table 1*. The interplanar distances published by Zimmerman *et al.*[5] and by Keith *et al.*[17] for poly(Glu) with established β-structure are also shown for comparison. The diffraction patterns of both acids

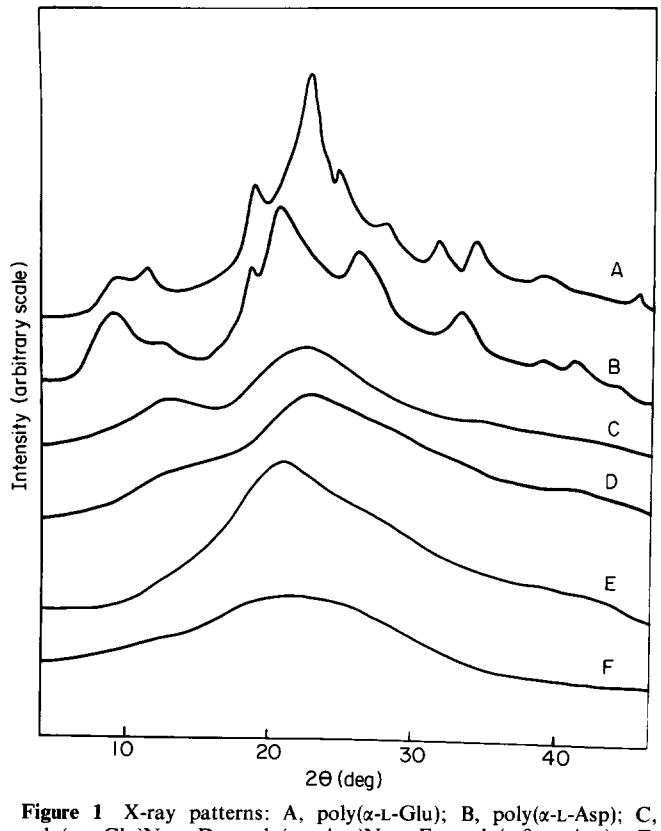


Figure 1 X-ray patterns: A, poly(α-L-Glu); B, poly(α-L-Asp); C, poly(α-L-Glu)Na; D, poly(α-L-Asp)Na; E, poly(α,β-DL-Asp); F, poly(α,β-DL-Asp)Na

Table 1 Diffraction angles 2θ and the corresponding interplanar distances d

Poly(Glu)				Poly(Asp)	
Keith *et al.*[17] d (Å)	Zimmerman *et al.*[5] d (Å)	This work 2θ (deg)	This work d (Å)	This work 2θ (deg)	This work d (Å)
7.80	7.73–7.79	9.6	9.20	9.0	9.81
4.72	4.73–4.77	11.4	7.75	12.6	7.02
3.90	3.90–3.93	19.2	4.62	18.7	4.74
3.58	3.58–3.61	22.9	3.88	20.7	4.23
3.30	3.16–3.20	27.7	3.22	26.2	3.40
2.60	2.84	31.9	2.80	33.4	2.68
	2.61	34.5	2.60	41.0	2.20
	2.30	38.9	2.30	46.3	1.96
		43.2	2.09	49.0	1.85
		45.8	1.98	51.8	1.76

exclude the presence of the α-helix in the crystalline phase; the typical α-helix reflections[3] for $d=5.4$ and 1.5 Å are missing. The reflection positions of our sample of poly(Glu) correspond well to the β-structure and this stucture most probably also corresponds to the ordering of poly(Asp) in the crystalline phase. The diffractogram of poly(Asp) contains the reflection for $d=4.74$ Å, typical for the interchain distance of polypeptides in one sheet bound by intermolecular hydrogen bonds. The reflection for $d=3.4$ Å, corresponding to a repeating peptide unit, is even more pronounced that in poly(Glu) and it probably corresponds to an antiparallel ordering, whereas the reflection for $d=3.2$ Å found for poly(Glu) indicates parallel ordering. However, it should be remembered that the shortening of the polypeptide chain with respect to the fully extended state (3.6 Å) is considerably affected by the length of the substituent[3].

The broad, overlapping reflections for $d=9.8$ and 7.0 Å of poly(Asp) and at 9.2 and 7.7 Å of poly(Glu) indicate a not very regular arrangement of the sheets into three-dimensional structure.

The diffractograms of the sodium salts of poly(Glu) and poly(Asp) (*Figure 1*) exhibit only an amorphous halo with a maximum at $2\theta \simeq 23°$. The former sample exhibits an additional weak amorphous maximum at $2\theta \simeq 13°$, which could correspond to the swelling of the sample. For poly(Asp)Na this maximum is only indicated. The position of the main maximum of these salts coincides with the position of the amorphous maximum of poly(Glu) and poly(Asp), but differs from that of poly(α,β-DL-Asp) and its sodium salt where the amorphous maximum lies at $2\theta=21°$.

Infra-red spectroscopy

Infra-red spectra of poly(amino acids) exhibit a strong band at 1730 cm^{-1} assigned to the C=O vibration of the carboxyl group. This band disappears in the sodium salts and is replaced by a band at 1580 cm^{-1} assigned to the asymmetrical vibration of the COO^- group[18]. Intermolecular order and conformational sensitivity is exhibited especially by the vibrations of the amide groups, first of all by the amide I and amide V[3,19,20] vibrations. The α-helix is characterized by the corresponding frequencies at 1655 and 625 cm^{-1}, the β-structure at 1610 and 640 cm^{-1} and the random coil at 1655 and 600 cm^{-1} respectively.

In the spectrum of poly(α-L-Glu) the amide I vibration appears as two bands of comparable intensity, at 1650 and 1608 cm^{-1}, the amide V vibration is assigned to the band at 638 cm^{-1} and possibly also to the weak band at 700 cm^{-1} (*Figure 2*, spectrum A). In the spectrum of poly(α-L-Asp) the maximum of the amide I band lies at 1660 cm^{-1}, with a shoulder at 1640 cm^{-1} (*Figure 2*, spectrum B). The amide V vibration exhibits a band at 625 cm^{-1} and evidently also a weak band at 700 cm^{-1}.

In the spectrum of poly(α,β-DL-Asp) the amide I vibration exhibits a single band at 1660 cm^{-1}; the maximum of the amide V band appears at 625 cm^{-1}, with a further broad absorption corresponding to this vibration at 600 cm^{-1} (*Figure 2*, spectrum C).

In the spectra of the sodium salts of all the studied acids the amide I vibration exhibits a single broad band at 1660 cm^{-1}, and the amide V band is broad, with a maximum at 600 cm^{-1} (*Figure 2*, spectra D–F).

Analysis of the infra-red spectra indicates that poly(α-L-Glu) contains comparable amounts of the β_2-structure (band at 1608 cm^{-1}) and of the α-helix (band at 1650 cm^{-1}). Both these forms evidently contribute to the absorption with maximum at 638 cm^{-1}. Even the β_1-structure might be present (band at 700 cm^{-1}).

In the sample of poly(α-L-Asp) the α-helix conformation predominates (bands at 1660 and 625 cm^{-1}), and a smaller amount of the β_1-structure is present (shoulder at 1635 cm^{-1}, weak band at 700 cm^{-1}). The random coil, the absorptions of which at 600 and 1650 cm^{-1} coincide with those of the α-helix (*Figure 2*, spectrum B), might also be present. The random-coil structure evidently predominates in poly(α,β-DL-Asp) (broad background at 600 cm^{-1}), but some amount of α-

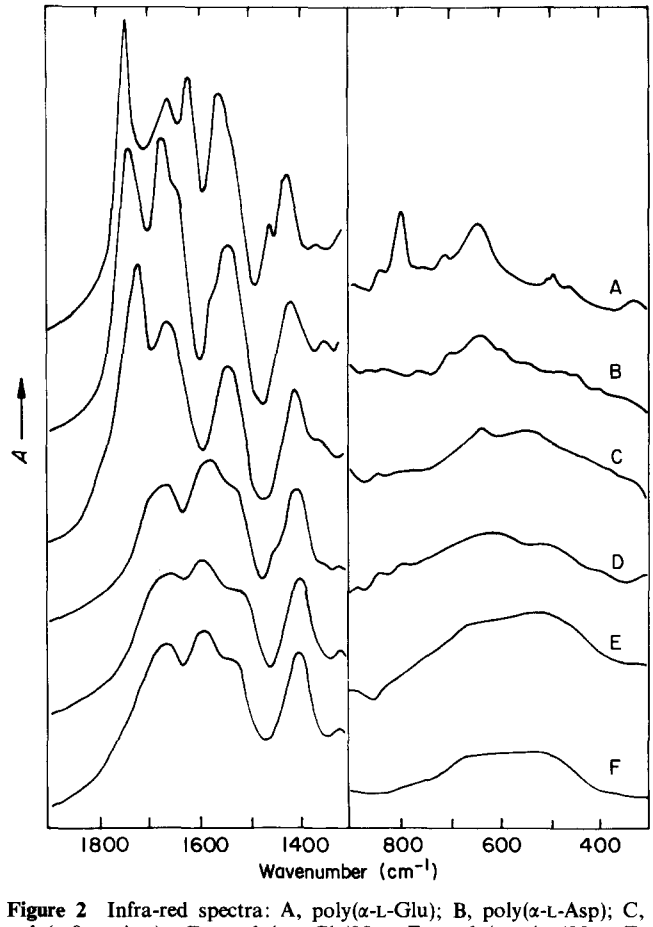


Figure 2 Infra-red spectra: A, poly(α-L-Glu); B, poly(α-L-Asp); C, poly(α,β-DL-Asp); D, poly(α-L-Glu)Na; E, poly(α-L-Asp)Na; F, poly(α,β-DL-Asp)Na

helix is also present (maximum at 625 cm^{-1}, *Figure 2*, spectrum C). The random coil is the predominant conformation in all sodium salts (bands at 600 and 1650 cm^{-1}, *Figure 2*, spectra D–F).

N.m.r. spectroscopy

^{13}C CP/MAS n.m.r. spectra. The ^{13}C CP/MAS n.m.r. spectra of all studied poly(amino acids) in the solid state, in the forms of free acids and sodium salts, are shown in *Figures 3* and *4*. The chemical shifts and widths at half-maximum height, δ, are summarized in *Table 2*. The designation of various carbon atoms is also shown in this table. From the figures it appears at first sight that the lines of the non-identical carbons in the groups –CONH– and $-COO^-$ are resolved only in the spectrum of the sodium salt of poly(α-L-Glu); in all other n.m.r. spectra these lines coincide. In all spectra, the lines of the methine

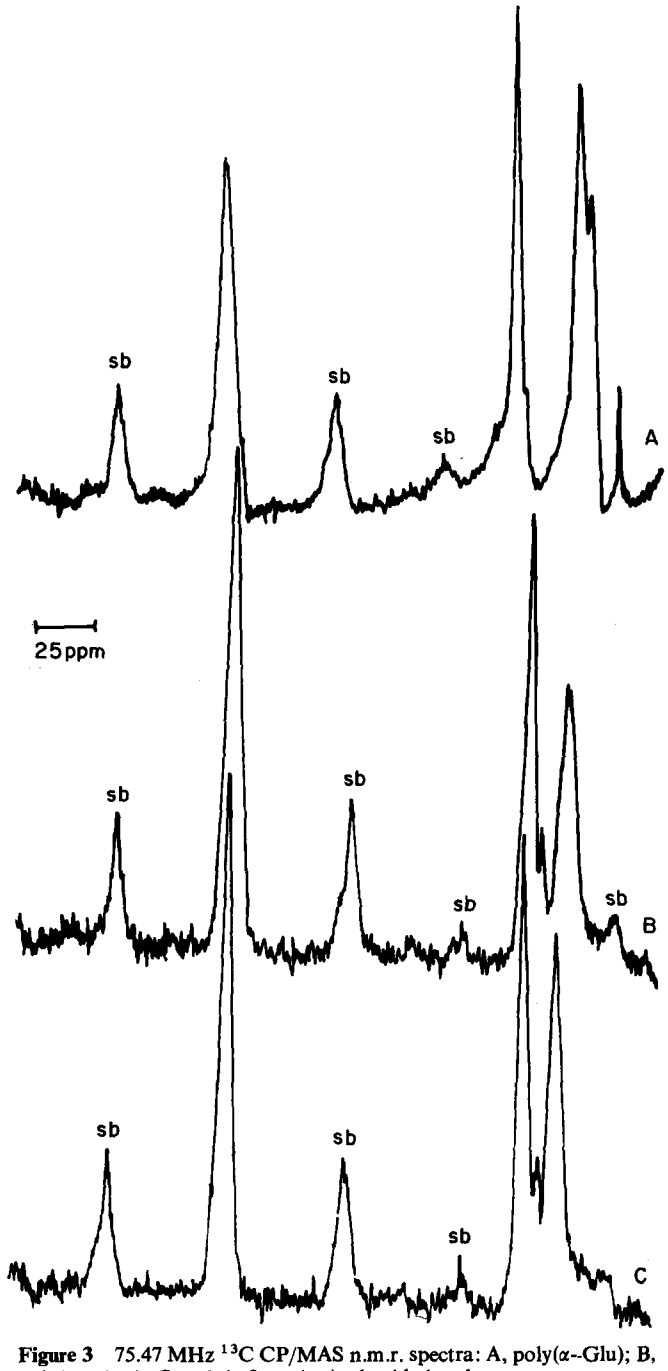


Figure 3 75.47 MHz ^{13}C CP/MAS n.m.r. spectra: A, poly(α-L-Glu); B, poly(α-L-Asp); C, poly(α,β-DL-Asp); sb, side bands

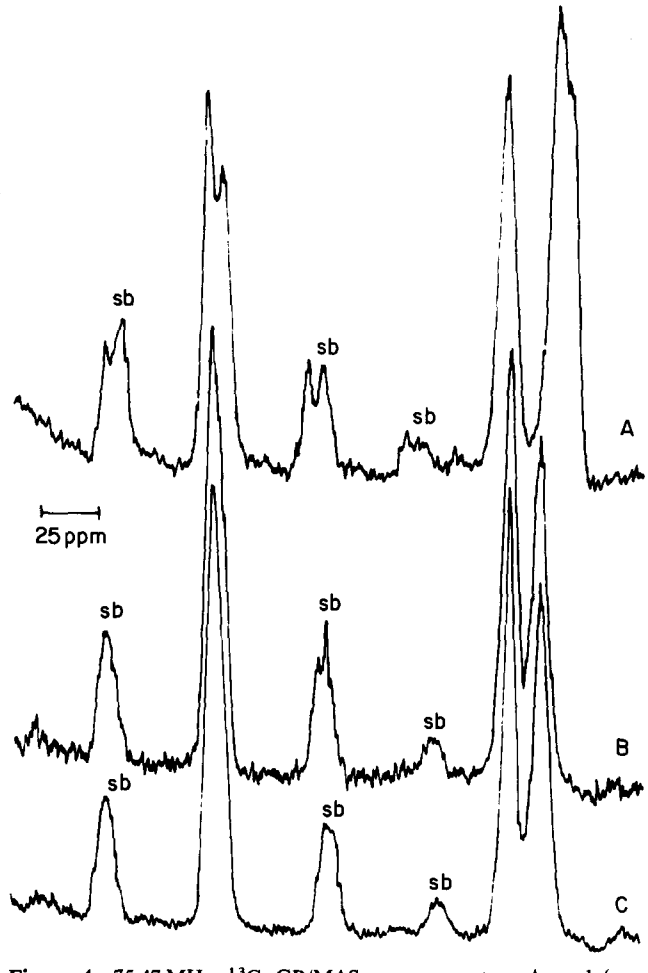


Figure 4 75.47 MHz ^{13}C CP/MAS n.m.r. spectra: A, poly(α-L-Glu)Na; B, poly(α-L-Asp)Na; C, poly(α,β,-DL-Asp)Na; sb, side bands

carbons –C(2)H– are symmetrical and do not exhibit splitting. Their widths are larger in the samples of the sodium salts than in the samples in the free acid form: for poly(α-L-Glu) by a factor of 3, and for both samples of poly(Asp) by a factor of 1.4. The spectra of the methylene carbons are the most complicated, but give most information. The spectra of both forms of poly(α-L-Glu) in the solid state, similarly as in solution, exhibit two bands, which are hardly resolved due to large linewidths. At first sight it is not clear if these lines correspond to $-C(3)H_2-$ and $-C(4)H_2-$ carbons, or to various conformational structures. The band of the methylene carbon $-C(3)H_2-$ in the spectrum of poly(α-L-Asp) in free acid form exhibits a marked asymmetry and considerable broadening ($\delta = 8.25$ ppm) as compared to the corresponding line in the spectrum of poly(α,β-DL-Asp) ($\delta = 5.77$ ppm, *Table 2*). This asymmetrical band was decomposed into two components by the method of least squares. The band profiles were represented by the Lorentz function

$$f(x) = x_1 \frac{1}{1 + x_3(x - x_2)^2}$$

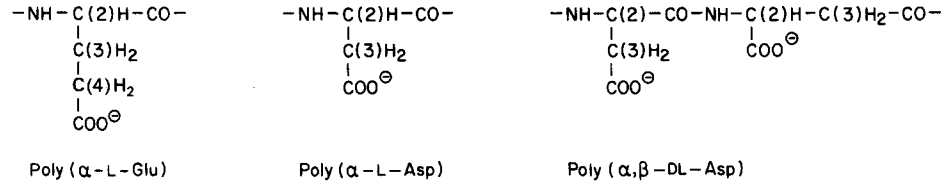
or by the product Gauss–Lorentz function

$$f(x) = x_1 \frac{\exp[-x_4(x - x_2)^2]}{1 + x_3(x - x_2)^2}$$

Table 2 ^{13}C n.m.r. chemical shifts[a] and linewidths[b] of solid poly(Glu) and poly(Asp)

Sample	–CONH–	–C(2)H–	–C(3)H_2– –C(4)H_2–	–$COO^{\ominus}$
Poly(α-L-Glu)	175.39 (3.99)	56.74 (2.75)	26.01 (8.94) 30.65	175.39 (3.99)
Poly(α-L-Asp)	172.63	49.94 (4.81)	34.78[c] (5.01)[c] 37.76[c] (4.60)[c]	172.63
Poly(α,β-DL-Asp)	175.04	52.53 (5.36)	38.78 (5.77)	175.04
Poly(α-L-Glu)Na	174.24	54.39 (8.11)	28.43 (12.37) 33.94	180.30
Poly(α-L-Asp)Na	176.32	51.32 (6.60)	38.31 (8.11)	176.32
Poly(α,β-DL-Asp)Na	174.80	51.87 (7.83)	38.71 (6.19)	176.38

[a] ppm from TMS; numbering of the carbons:



[b] Width (ppm) at half-maximum intensity, in parentheses

[c] Obtained by separation of the composite band into two components, together with the relative intensities 0.62 and 0.38 for the lines 34.78 and 37.76 ppm

Table 3 ^{13}C n.m.r. chemical shifts[a] of poly(Glu) and poly(Asp) in aqueous solutions

Sample	–CONH–	–C(2)H–	–C(3)H_2–	–C(4)H_2–	–$COO^{\ominus}$
Poly(α-L-Glu)	173.515	53.532	27.639	33.424	181.401
Poly(α-L-Asp)	173.208	51.475	38.588		177.751
Poly(α,β-DL-Asp)[b]	171.984	51.685	37.236		177.780
	172.612		38.600		177.373

[a] ppm from TMS, the numbering of the carbons is given in *Table 2*

[b] The complicated structure is due to the presence of α and β peptide bonds

with a fixed ratio $x_3/(x_3+x_4)=0.5$. All parameters, i.e. amplitude x_1, position x_2 and width at half-maximum height x_3 (or x_3 and x_4) were adjusted. The results of this separation using the product function (yielding a better fit) are given in *Table 2*.

The n.m.r. spectrum of poly(α,β-DL-Asp) and the spectra of all studied poly(amino acids) in the form of sodium salts are very similar to the spectra of these samples in solution. The chemical shifts of these compounds in aqueous solutions are given in *Table 3* for comparison. Even if the n.m.r. spectra of the liquid and solid phases of these compounds cannot be compared directly, because they were measured under different experimental conditions, the data in *Tables 2* and *3* indicate that the chemical shifts of the corresponding carbons differ only within experimental error. Because of line broadening in the spectra of solid samples, solid poly(α,β-DL-Asp) does not exhibit the fine structure of the –CONH– and –C(3)H_2– carbon lines, which is evident in solution spectra reflecting the statistical distribution of α and β bonds[14].

Conformational dependence of chemical shifts in ^{13}C n.m.r. spectra

^{13}C n.m.r. studies of solid polypeptides by means of the CP/MAS technique have shown that the ^{13}C chemical shifts of the backbone carbons –CONH– and –C(2)H and of the side-chain carbon –C(3)H_2– depend on the conformational structure of the polypeptide (α-helix, β-structure), whereas the chemical shifts of the other side-chain carbons are not appreciably affected by conformational structure[1,2,21]. The conformational dependence of ^{13}C chemical shifts can be explained by changes of electron structure dependence on the angles of the backbone peptide bonds; for some polypeptides this dependence was confirmed by calculations by means of finite perturbation theory, intermediate neglect of differential overlap (FPT INDO) methods[22].

Numerous experimental results have shown that the line of the random coil measured in solution lies between the lines observed in the spectra of the α-helix and the β-structure. For the random-coil conformation it is assumed that the chemical shifts in solution and in the solid state do not differ if solvent effects, magnetic susceptibility effects or differences in reference standard are excluded[2]. This assumption has not been experimentally verified so far. For studies of the conformational structure of polypeptides, very useful rules have been derived from ^{13}C n.m.r. spectra[1,2,21,22]: the lines of the carbonyl –CONH and of the methine –C(2)H– carbons in the β-structure appear at higher field than in the α-helix. For carbon –C(3)H_2– this is the other way round: lines of the β-structure appear at lower field than the lines of the α-helix. The chemical-shift difference between the lines of the α-helix and of the β-structure reaches a maximum value of 7 ppm, but can be so small that the lines coincide. The presence of several conformations together with relaxation effects are the reasons why in spectra of solid organic compounds the lines are 10 to 100 times broader than for the same compounds in the liquid state[23].

This has to be kept in mind in considerations of the shape of the spectra of the solid poly(Glu) and poly(Asp) in the form of free acids or sodium salts, where the width of simple symmetrical lines of protonated carbons ranges from 3 to 8 ppm (*Table 2*).

Several conformations can be resolved in ^{13}C n.m.r. spectra only in the case of regular poly(α-L-Asp). Respecting the above explained spectral rules, the two components resulting from the decomposition of the unsymmetrical band of the methylene carbon $-C(3)H_2-$ can be assigned to the α-helix (stronger line at 34.78 ppm) and to the β-structure (weaker line at 37.76 ppm) *Table 2*). In this spectrum the lines of the carbonyl and methine carbons do not yield information about conformational structure.

The assignment of lines in the spectrum of poly(α-L-Glu) is complicated by the presence of the conformationally independent line of the methylene $-C(4)H_2-$ carbon. In the spectrum of the aqueous solution, this line lies at 33.42 ppm (*Table 3*) and the chemical-shift difference of the lines of the $-C(3)H_2-$ and $-C(4)H_2-$ methylene carbons is 4.8 ppm. The spectrum of solid poly(α-L-Glu) also exhibits only two lines of methylene carbons, at 26.01 and 30.65 ppm, with a chemical-shift difference of 4.65 ppm; therefore the spectrum of solid poly(α-L-Glu) could also be interpreted as that of a single conformation (*Table 2*). However, Kricheldorf and Müller[1] published carbon chemical shifts of poly(Glu) in the conformation of the β-structure. From these data it follows that in this case the $-C(3)H_2-$ and $-C(4)H_2-$ methylene carbon lines coincide at 29.9 ppm. Therefore, it is possible that the line at 26.01 ppm in the spectrum of poly(α-L-Glu) corresponds to the $-C(3)H_2-$ carbon in the α-helix conformation (in agreement with the spectral rules), and the band at 30.65 ppm contains not only the conformationally independent line of the $-C(4)H_2-$ methylene carbon, but probably also the line of the $-C(3)H_2-$ carbon in the β-structure. The n.m.r. spectra thus indicate that the fraction of the α-helix structure in poly(α-L-Glu) is not negligible as compared to the β-structure. This conclusion, obtained from an estimate of the relative intensities of the lines at 26.01 and 30.65 ppm, is confirmed by the chemical shifts of the carbonyl and methine carbons, which lie at somewhat higher ppm values (lower field) as compared to the chemical-shift data of the pure β-structure in the sample of Kricheldorf *et al.*[1] In view of the considerable linewidth in poly(α-L-Glu) and poly(α-L-Asp), the presence of the random-coil form cannot be excluded.

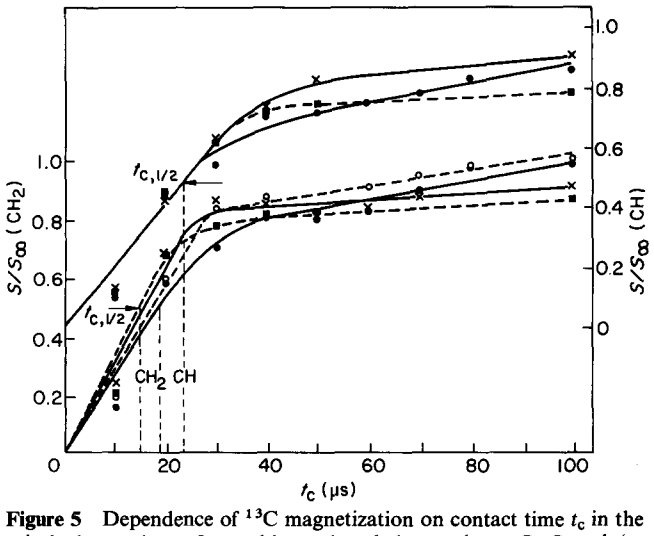


Figure 5 Dependence of ^{13}C magnetization on contact time t_c in the spin-lock experiment for methine and methylene carbons: ○, ●, poly(α-L-Glu); ■, poly(α-L-Asp); ×, poly(α,β-DL-Asp)

By their shape and chemical shifts ^{13}C n.m.r. spectra of irregular poly(Asp) and of all studied samples in the form of the sodium salts are similar to the spectra of their aqueous solutions, but they differ by linewidth, as mentioned above (*Tables 2* and *3*). From this it could be concluded that, even in the solid state, the random-coil form predominates in these compounds, even though the presence of the α-helix or β-structure cannot be completely excluded due to the large linewidth. For the irregular poly(α,β-DL-Asp) and for both poly(Asp) sodium salts, further information may be obtained from the shape and position of the $-C(3)H_2-$ methylene carbon band. The presence of the α-helix form in all these compounds can be excluded based on the absence of a sufficiently pronounced band at 34.78 ppm.

Segmental mobility

The dynamics of the solid poly(amino acids) in free acid form was followed by means of the spin-lock cross-polarization experiment. The dependence of magnetization on the contact time t_c for the protonated carbons is shown in *Figure 5*. The contact times $t_{c,1/2}$ for which one-half of total magnetization is reached were evaluated from these curves. In the case of carbons directly bound to protons, the contact times $t_{c,1/2}$ depend on the strength of the dipolar coupling $\langle M_{CH}^{(2)} \rangle$ of the carbon and the protons bound to it[24]. In *Figure 5*, the plots for the protonated carbons indicate rapid non-exponential growth of magnetization during the first 20 μs of contact. The $t_{c,1/2}$ values of methylene carbons were 15 μs for poly(α-L-Asp), 15.8 μs for poly(α,β-DL-Asp) and 17.2 μs (26.01 ppm) and 18.5 μs (30.65 ppm) for poly(α-L-Glu); $t_{c,1/2}$ of methine carbons for all these compounds is 24.0 μs.

Thus the $t_{c,1/2}$ values did not exceed the value 19 μs for methylene carbons and 24 μs for methine carbons in any of the measured samples. A comparison with the calculated $t_{c1/2}$ values of methylene and methine carbons in the rigid lattice (20 μs for CH_2 and 28 μs for CH)[24] indicates that all three measured samples of poly(amino acids) are rigid.

CONCLUSIONS

The results obtained by all three methods (*Table 4*) are complementary and not in conflict. A determination of the predominant conformation requires the application of all three methods (*Table 4*). X-ray diffraction is sensitive mainly to crystalline ordering and therefore suitable for detecting the β-structure; it is insensitive to the disordered chains both in the α-helix and in the random-coil forms. I.r. spectroscopy can in principle resolve all three conformations; it can best differentiate the α-helix or random coil from the β-structure, but it is less sensitive to the presence of the random coil next to the α-helix. On the other hand, n.m.r. spectroscopy can easily differentiate the α-helix from the β-structure or random coil but it cannot differentiate the β-structure from the random coil.

Our conformational analysis is in agreement with the previous findings stating that poly(α-L-Glu) may assume

Table 4 Results of the conformational analysis

Sample	X-ray diffraction	I.r. spectroscopy	N.m.r. spectroscopy	Most probable conformation
Poly(α-L-Glu)	β	α and β	α and β	α and β
	α and (or) rc	rc not excluded	rc not excluded	rc not excluded
Poly(α-L-Asp)	β	α and β	α and β	α and β
	α and (or) rc	rc not excluded	rc not excluded	rc not excluded
Poly(α,β-DL-Asp)	rc and (or) α	rc	rc or (and) β	rc
Poly(α-L-Glu)Na	rc and (or) α	rc	rc	rc
			α and β not excluded	α not excluded
Poly(α-L-Asp(Na	rc and (or) α	rc	rc or (and) β	rc
Poly(α,β-DL-Asp)Na	rc and (or) α	rc	rc or (and) β	rc

α, α-helix; β, β-sheet; rc, random coil

both the α-helix and the β-structure in the solid state[3]. Poly(α-L-Glu)Na mostly forms only the random coil[3], but it can also form the α-helix[25,26]. The presence of a small fraction of α-helix cannot be excluded in our sample of poly(α-L-Glu)Na. *Table 4* indicates that poly(α-L-Asp) and its sodium salt behave similarly to poly(α-L-Glu). As expected, poly(α,β-DL-Asp) and its salt do not assume any ordered conformation, as in solution[7].

Although poly(α-L-Glu) was formed under conditions of so-called α-precipitation[4] where the α-helix should be formed predominantly, our samples reproducibly always contained a considerable fraction of the β-structure. It is known that the β-structure is the main conformation of oligomeric poly(α-amino acids)[27–29], but the presence of an oligomeric fraction in our samples was excluded (g.p.c., see 'Experimental' part); therefore the β-structure found must be associated with long chains.

Kricheldorf *et al.*[1] who have assigned their n.m.r. spectra of poly(α-L-Glu), poly(α-L-Glu)Na and poly(α-L-Asp)Na only to β-structure, probably studied only low-molecular-weight samples which actually cannot contain any significant fraction of α-helices.

The sodium salts of poly(Glu) and of poly(Asp) are especially suitable for studies of the n.m.r. manifestations of the random-coil structure in the solid state. Whereas with the other poly(α-amino acids) it is possible that the transition from random coil to ordered conformation takes place after the removal of an order-disrupting solvent, with poly(Glu)Na and poly(Asp)Na it is probable that the electrostatic repulsion of the ionized side chains is at least partly preserved after lyophilization and precludes regular ordering. So far the solid-state n.m.r. characteristics of the random-coil conformation of poly(α-amino acids) has been extrapolated only from the results obtained with solutions[1,2,30]. The only paper discussing the random coil in the solid state was based on work with low-molecular-weight and insufficiently characterized samples, with a thermodynamically unstable conformation[31]. Our measurements indicate that in n.m.r. spectra the β-structure cannot be differentiated from the random coil. This follows from a comparison of the results of various methods, and from the comparison of the n.m.r. spectra of poly(α,β-DL-Asp)Na, in which an ordered structure cannot be expected to exist, and of poly(α-L-Asp)Na.

The mobilities of poly(amino acids) in the solid state have not been studied by n.m.r. so far. Our measurements indicate that poly(Glu) and poly(Asp) are rigid. This is probably caused by the presence of peptide bonds preventing free rotation, and further by the possibility of hydrogen-bond formation. This aspect will be the subject of further work.

REFERENCES

1 Kricheldorf, H. R. and Müller, D. *Macromolecules* 1983, **16**, 615
2 Saitô, H., Tabeta, R., Shoji, A., Ozaki, T. and Ando, I. *Macromolecules* 1983, **16**, 1050
3 Fasman, G. D. (Ed.) in 'Poly-α-Amino Acids', Dekker, New York, 1967
4 Zimmerman, S. S. and Mandelkern, L. *Biopolymers* 1975, **14**, 567
5 Zimmerman, S. S., Clark, J. C. and Mandelkern, L. *Biopolymers* 1975, **14**, 585
6 Saudek, V., Štokrová, Š. and Schmidt, P. *Biopolymers* 1982, **21**, 1011
7 Pivcová, H. and Saudek, V. *Polymer* 1985, **26**, 667
8 Saudek, V., Štokrová, Š. and Schmidt, P. *Biopolymers* 1982, **21**, 2195
9 Blout, E. R. and Karlson, R. H. *J. Am. Chem. Soc.* 1956, **78**, 941
10 Doty, P., Bradbury, J. H. and Holtzer, A. M. *J. Am. Chem. Soc.* 1956, **78**, 947
11 Wada, A. *Mol. Phys.* 1060, **3**, 409
12 Hayashi, Y., Teramoto, A., Kahawara, K. and Fujita, H. *Biopolymers* 1969, **8**, 403
13 Saudek, V., Pivcová, H. and Drobník, J. *Biopolymers* 1981, **20**, 1615
14 Pivcová, H., Saudek, V. and Drobník, J. *Polymer* 1982, **23**, 1237
15 Pivcová, H., Saudek, V., Drobník, J. and Vlasák, J. *Biopolymers* 1981, **20**, 1605
16 Saudek, V., Drobník, J., Havranová, M. and Čechová, D. *Makromol. Chem.* 1982, **183**, 1473
17 Keith, H. D., Padden, F. J. Jr and Giannoni, G. *J. Mol. Biol.* 1969, **43**, 423
18 Rao, C. N. R. in 'Chemical Applications of Infrared Spectroscopy', Academic Press, New York and London, 1963, p. 193
19 Matsuda, Y., Miyazawa, T. and Goodman, M. *Biopolymers* 1969, **8**, 515
20 Itoh, K., Foxman, B. M. and Fasman, G. D. *Biopolymers* 1976, **15**, 419
21 Taki, T., Yamashita, S., Satoh, M., Shibata, A., Yamashita, T., Tabeta, R. and Saitô, H. *Chem. Lett.* 1981, 1803
22 Ando, I., Saitô, H., Tabeta, R., Shoji, A. and Ozaki, T. *Macromolecules* 1984, **17**, 457
23 Hart, van der, D. L., Earl, W. L. and Garroway, A. N. *J. Magn. Reson.* 1981, **44**, 361
24 Lauprêtre, F., Monnerie, L. and Virlet, J. *Macromolecules* 1984, **17**, 1397
25 Mitsui, Y. *Biopolymers* 1973, **12**, 1781
26 Lenormant, H., Baudras, A. and Blout, E. R. *J. Am. Chem. Soc.* 1958, **80**, 6191
27 Ozaki, T., Shoji, A. and Furukawa, M. *Makromol. Chem.* 1982, **183**, 771
28 Kricheldorf, H. R., Müller, D. and Förster, H. *Polym. Bull.* 1982, **8**, 487
29 Kricheldorf, H. R., Mutter, M., Maser, F., Müller, D. and Förster, H. *Biopolymers* 1983, **22**, 1357
30 Saitô, H., Tabeta, R., Shoji, A., Ozaki, T., Ando, I. and Miyata, T. *Biopolymers* 1984, **23**, 2279
31 Kricheldorf, H. R. and Müller, D. *Polym. Bull.* 1983, **10**, 513