NMR AND X-RAY DIFFRACTION STUDY OF THE CONFORMATIONAL STRUCTURE OF POLY[N^5-(2-HYDROXYETHYL)-L-GLUTAMINE] AND POLY[N^5-(2-HYDROXYETHYL)-L-GLUTAMINE-*co*-γ-L-GLUTAMYLHYDRAZIDE]

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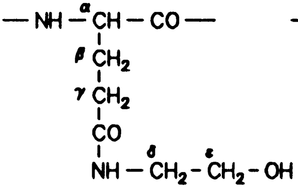
Dedicated to Professor Otto Wichterle on the occasion of his 80th birthday.

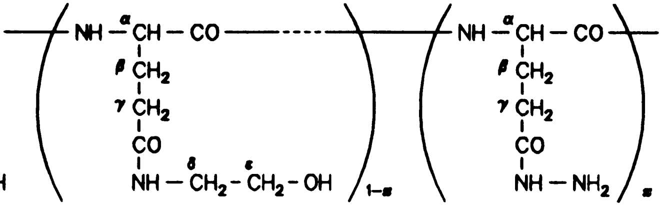
The conformation of poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) and poly[N^5-(2-hydroxyethyl)-L-glutamine-*co*-γ-L-glutamylhydrazide] (P(HEG-GH)), in solid state was studied by ^{13}C CP/MAS NMR spectrometry and X-ray diffraction. The association of the copolymers in dichloroacetic acid was investigated by following the temperature dependence of their ^{1}H NMR spectra. PHEG and P(HEG-GH)) were prepared by the reaction of poly(benzyl γ-L-glutamate) with 2-aminoethanol and 2-aminoethanol–hydrazine mixtures, respectively. The conformation of the copolymers depends on the reaction conditions. While PHEG and the copolymers prepared with the mole ratio of hydrazine to the benzyl ester $n_H/n_B = 0.25$ were almost amorphous in solid state and had a random coil conformation and/or randomly oriented helices, at increased concentration of hydrazine ($n_H/n_B = 0.5$ and 1.0) P(HEG-GH) formed more or less ordered structure characterized by assemblies of α-helices. The copolymers prepared at $n_H/n_B \geq 2.0$ showed features of β-sheets. The tendency of the copolymers to form associates in $Cl_2CHCOOH$ solution correlated well with their tendency to form ordered structures in solid state.

Polymers derived from L-glutamic acid have been studied as biomedical polymers. They conveniently combine the features of natural polypeptides, such as biodegradability, with advantages common with synthetics in the sense that their properties can be adjusted by a rational synthesis[1,2]. Poly[N^5-(2-hydroxyethyl)-L-glutamine], (PHEG), a linear, water-soluble and biodegradable polymer, has been investigated as a carrier of biologically active compounds[3]. For binding of drugs to the polymer, suitable reactive groups should be incorporated in the polymer side chains. The hydrazide groups could be particularly convenient in this respect.

Hydrazide grouping can be introduced into PHEG in one step by the reaction of poly(benzyl γ-L-glutamate) (PBLG) with mixtures of 2-aminoethanol and hydrazine[4,5].

Depending on the ratio of hydrazine to benzyl glutamate units, copolymers poly[N^5-(2-hydroxyethyl)-L-glutamine-*co*-γ-L-glutamylhydrazide] [P(HEG-GH)] with various content of hydrazide units can be prepared, which in turn have not only different capacity for chemical binding, but also exhibit strongly different physical properties derived from their conformational structure.


PHEG


P(HEG-GH)

The composition-dependent changes in the conformation of the resulting copolymers can hardly be sufficiently described by using a single method. In our studies of the conformation of poly(glutamic acid) and poly(aspartic acid), a combination of the wide-angle X-ray diffraction, infrared spectroscopy and ^{13}C cross-polarization/magic angle spinning (CP/MAS) NMR spectroscopy appeared particularly useful[6]. Although the content of hydrazide groups in P(HEG-GH) copolymers could be determined by IR spectroscopy, information from IR about the conformation of the copolymers was rather limited[4]. Therefore, for the study of the conformation of PHEG and P(HEG-GH) in solid state we used X-ray diffraction and ^{13}C CP/MAS NMR. In solution, we measured the temperature dependence of ^{1}H NMR spectra of PHEG and P(HEG-GH) in dichloroacetic acid (DCA), basing on the experience that the temperature dependences of the integrated intensities of high resolution NMR bands are particularly sensitive to the occurrence of physical associates[7,8]. The results of all three methods are correlated.

EXPERIMENTAL

Materials

Dry hydrazine was prepared from hydrazine hydrate by distillation with solid NaOH and kept under nitrogen. 2-Aminoethanol was rectified under reduced pressure and dried over a molecular sieve. *N*,*N*-dimethylacetamide (DMA) was purified by azeotropic distillation with benzene and water, rectified under reduced pressure and stored over a molecular sieve.

PBLG was prepared by polymerization of benzyl γ-L-glutamate *N*-carboxyanhydride in dioxane with triethylamine as initiator[9,10].

PHEG was prepared by aminolysis of PBLG with 2-aminoethanol in *N*,*N*-dimethylacetamide at 60 °C as described previously[10].

Poly[N^5-(2-hydroxyethyl)-L-glutamine-*co*-γ-L-glutamylhydrazide] (P(HEG-GH)) was prepared by the reaction of PBLG with the 2-aminoethanol–hydrazine mixtures[5] (Table I). Typically, 1.0 g of PBLG (4.56 mmol of monomeric units) was dissolved in 10 ml of DMA. A mixture of 2-aminoethanol and hydrazine was added and the reaction mixture was kept at 60 °C for 4 days. It was then neutralized with 5% acetic acid in ethanol and dialyzed against water until the ninhydrin test was negative. The product was isolated from the resulting solution or swollen gel by freeze-drying.

Methods

X-Ray diffractograms were taken with a HZG-4A powder diffractometer (CuK$_\alpha$ radiation, scintillation counter recording, monochromatization using a β-filter with amplitude analyzer) in the range of angles $2\Theta = 4 - 56°$.

^{13}C CP/MAS NMR spectra of solid polymers were measured at room temperature with a Bruker MSL 200 spectrometer at 50.3 MHz, in Al_2O_3 rotors at a spinning frequency of 4.5 kHz. With most samples the number of scans was 4 000, the contact time was 3 ms, pulse repetition time 2 s, spectral width 20 kHz and number of points 8 K. Where the available amount of sample was less than 200 mg, the rotor space was filled up with Teflon filling; in such cases the number of scans had to be increased up to 30 000. The spectra of these samples exhibit a band at 75.0 ppm corresponding to the Teflon filling. Chemical shifts in ^{13}C CP/MAS NMR spectra were referred to the carbonyl band of glycine at $\delta = 176.0$ ppm by sample replacement. ^{13}C NMR spectra of aqueous solutions of PHEG were measured at 50 MHz using a Varian XL-200 spectrometer. Hexamethyldisiloxane ($\delta = 2.05$ ppm) was used as internal standard.

^{1}H NMR spectra of the polymers were measured in DCA solutions ($c = 0.05$ g/ml), hexamethyldisiloxane ($\delta = 0.05$ ppm) was used as internal standard. For homogenization, the solutions in sealed tubes were kept at 100 °C for ~1 day with constant stirring. After several days at ambient temperature, high resolution ^{1}H NMR spectra were recorded at 100 MHz using a JEOL PS-100 spectrometer. The temperature was kept constant within ± 0.5 °C by means of a JES-VT-3 unit. Integrated intensities of NMR bands were determined with the multichannel data analyzer Tracor-Northern TN-4000 after previous digitization of the spectra by means of the Summagraphic Intelligent Digitizer.

TABLE I

Synthesis of poly[N^5-(2-hydroxyethyl)-L-glutamine] (PHEG) and poly[N^5-(2-hydroxyethyl)-L-glutamine-*co*-γ-L-glutamylhydrazide] (P(HEG-GH)) by simultaneous hydrazinolysis and aminolysis of poly(benzyl γ-L-glutamate)

Polymer	Reaction mixture		x^a
	n_H/n_B, mol/mol	n_{AE}/n_B, mol/mol	
PHEG	0	50.00	0.0
P(HEG-GH)1	0.25	9.75	0.1
P(HEG-GH)2	0.50	9.50	0.2
P(HEG-GH)3	1.00	9.00	0.4
P(HEG-GH)4	2.00	48.00	0.8
P(HEG-GH)5	20.00	0	1.0

Subscripts: H hydrazine, B benzyl ester, AE 2-aminoethanol. [a] Molar fraction of γ-L-glutamylhydrazide units in polymer estimated from elemental analysis and IR spectra[5].

RESULTS AND DISCUSSION

The polymer samples PHEG, P(HEG-GH)1 and P(HEG-GH)2 were soluble in water. With increasing content of hydrazide units, polymers became insoluble in water and formed a swollen gel. The formation of a gel structure was probably due to physical association, which became most apparent in samples with the highest content of hydrazide units (P(HEG-GH)4, P(HEG-GH)5). The presence of a small fraction of covalent crosslinks in the copolymer P(HEG-GH)5, due to the formation of diacylhydrazide, cannot be excluded. The effect of hydrazide content on the conformation and association of copolymers is discussed below.

X-Ray Diffraction

Diffractograms of PHEG and P(HEG-GH) are given in Fig. 1. It is evident that the samples, especially those with low amount of hydrazide units, are to a high degree amorphous. The degree of ordering of polymeric chains depends to some extent also on the way of preparation and extraction of the solid phase from the solution. Different batches of PHEG exhibited different contents of amorphous and ordered regions according to X-ray and NMR spectroscopy. When we strictly followed the preparation procedure described here, pure PHEG and copolymer P(HEG-GH)1 prepared at molar ratio n_H/n_B = 0.25 are practically amorphous but we cannot distinguish whether the amorphous phase contains polymeric chains in the form of random coil of α-helices.

The diffractogram of P(HEG-GH)2 with n_H/n_B = 0.5 is quite different. Two crystalline reflections were detected at the diffraction angle 2Θ = 7.8 and 16.5°, corresponding to the interplanar distances of 1.32 and 0.54 nm, respectively. These distances are typical of regularly arranged α-helix of polypeptides[11]. This type of structure was also found in P(HEG-GH)3 with n_H/n_B = 1.0 but the crystalline reflection at 2Θ = 16.5° is

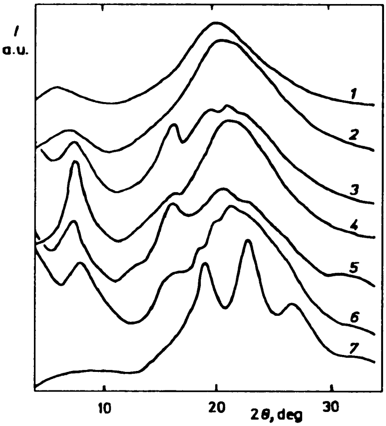


FIG. 1
X-Ray patterns. *1* PHEG; *2* P(HEG-GH)1; *3* P(HEG-GH)2; *4* P(HEG-GH)3; *5* P(HEG-GH)3 (swollen with H_2O at 4 °C for 48 h); *6* P(HEG-GH)4; *7* P(HEG-GH)5

not well developed; this means that periodicity in the direction along the helices arranged in a parallel way is rather disturbed. When this copolymer was swollen by water, kept at 4 °C for 2 days and subsequently dried, its crystalline phase was considerably improved (see additional crystalline peaks for this sample in comparison with the diffraction curve of original P(HEG-GH)3).

A further increase in the hydrazide content in P(HEG-GH)4 with $n_H/n_B = 2.0$ does not lead to an improvement of the ordered α-helix structure. On the other hand, we cannot exclude formation of β-sheet structure, which was detected in samples prepared using a large excess of hydrazine (P(HEG-GH)5, $n_H/n_B = 20.0$). The diffraction picture of P(HEG-GH)5 does not suggest the presence of α-helix. It is practically identical with the diffraction curve of homopolymer poly(γ-L-glutamylhydrazide) where the crystalline reflections at $2\Theta = 19$, 23 and 25 – 28° correspond to the interplanar distances 0.47, 0.39 and 0.32 – 0.36 nm, respectively, which are typical of the β-sheet structure of polypeptides[11].

^{13}C NMR Spectra

^{13}C CP/MAS NMR spectra of PHEG and of the copolymers are shown in Figs 2 and 3, respectively. In order to determine true line positions for poorly resolved bands, we have adjusted the shape of measured and calculated NMR spectra separately in the range of protonated and CO carbons by means of the Glinfit program. Band profiles were represented by Lorentzian and Gaussian functions and were adjusted for all parameters, i.e. amplitudes, positions and width at half-height of the respective lines. The chemical shifts obtained by separation of poorly resolved bands are shown in Table II,

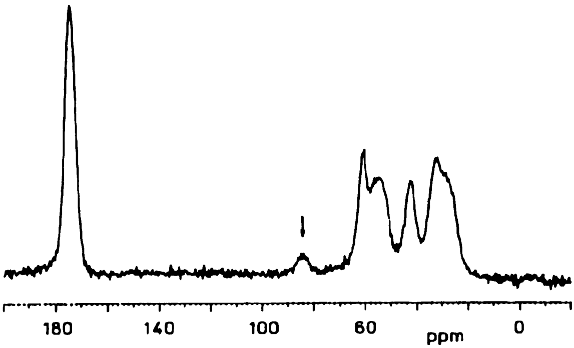


FIG. 2
^{13}C CP/MAS NMR spectrum of PHEG (the arrow denotes a spinning sideband)

together with the chemical shifts of other lines. In this table, the chemical shifts of an aqueous solution of PHEG are also shown for comparison.

Studies of the conformational structure of polypeptides by solid state NMR have been the subject of considerable attention, and the rules have been derived for the determination of conformational structure from chemical shift values of conformationally sensitive bands[12 - 14]. According to these rules, lines of C-α and CO-α carbons in the α-helical structure lie at higher δ values than for the β-sheet structure, whereas the lines of C-β carbons appear at higher δ values when the β-sheet structure predominates in the sample and at lower δ values when the presence of the α-helix is established. The lines corresponding to the random coil conformation measured in solutions often lie between the lines observed in NMR spectra of samples with α-helix or β-sheet structure. It is evident that the conformationally sensitive carbons provide an independent information on conformational structure that should be in mutual agreement.

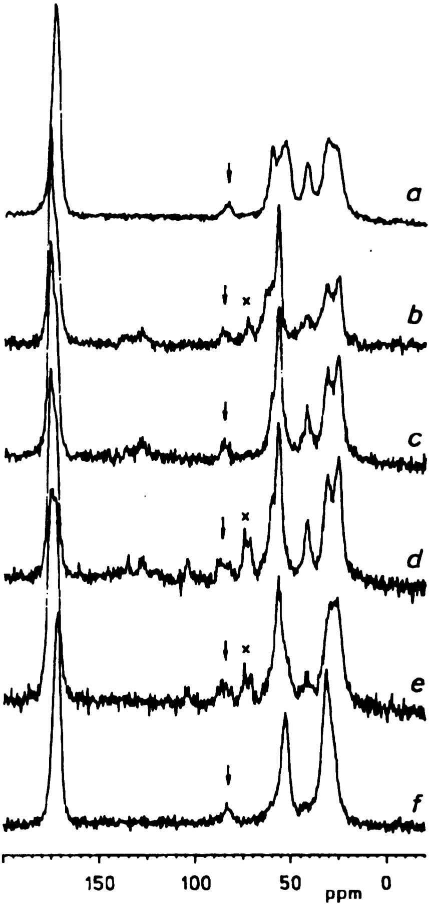


FIG. 3
^{13}C CP/MAS NMR spectra of HEG-GH copolymers. *a* P(HEG-GH)1; *b* P(HEG-GH)2; *c* P(HEG-GH)3; *d* P(HEG-GH)3 (swollen with H_2O at 4 °C for 48 h); *e* P(HEG-GH)4; *f* P(HEG-GH)5 (the arrows and crosses denote spinning side bands and Teflon signals, respectively)

By means of these rules, the α-helix and β-sheet conformations can be safely determined provided that the corresponding characteristic lines are sufficiently resolved, and the random coil conformation is absent. In order to determine the positions of the conformationally sensitive NMR bands for the random coil conformation, we prepared a sample of PHEG aimed at obtaining predominant random coil form and this was confirmed by X-ray diffraction.

It was found that in the ^{13}C NMR spectrum of this PHEG sample the lines of C-δ and C-ε carbons are best described by means of Lorentzian profiles, whereas for the other carbon lines the Gaussian profile is more suitable. The lines representing C-β and C-γ carbons almost coalescence into a single band (Fig. 2), as is usually typical of the β-sheet conformation. In this case, however, the analysis of NMR line shapes has shown that the lines overlap because of their considerable width, and that the position of the C-β line at 26.0 ppm is close to that of the random coil form in solutions (Table II), or of the helical structure in the solid state. The predominance of the α-helix structure is excluded by the positions of the C-α and CO-α bands, but the analysis of the C-α carbon band admits up to 13% of α-helix form. This content was calculated from the intensity ratio of the bands at 53.2 and 56.5 ppm, determined from NMR bandshape analysis (Table II). This analysis was made possible by previous experience in the study of the conformational structure of poly(benzyl γ-L-glutamate) showing that the dependence of C-α carbon line intensities on contact time is identical for α-helix and β-sheet forms, so that the line intensities are not affected by conformational structure[15].

TABLE II
Solid state ^{13}C NMR chemical shifts (δ, ppm) of PHEG and HEG-GH copolymers

Polymer	CO-α	C-ε	C-α	C-δ	C-γ	C-β	CO-γ
PHEG[a]	173.25	60.02	53.17	41.58	31.82	26.95	174.85
PHEG	173.9	60.1	53.2[b] 56.5[b]	41.7	31.5[b]	26.0[b]	173.9
P(HEG-GH)1	173.8	60.1	53.3	41.4[b]	31.2[b]	25.4[b]	173.7[b]
P(HEG-GH)2	176.2	60.2[b]	57.0	41.5	31.2	25.6	173.5
P(HEG-GH)3	176.0	60.2[b]	56.6	42.0	31.2	25.3	173.4[b]
P(HEG-GH)3[c]	175.6	60.1[b]	57.1	41.5	31.3	25.4	173.4[b]
P(HEG-GH)4	175.6[b]	–	52.9[b] 56.8	41.6	31.3[b]	25.5[b]	173.6[b]
P(HEG-GH)5	172.3	–	52.8	–	31.6	31.6	173.3[b]

[a] In aqueous solution. [b] Obtained by separation of the composite band. [c] Swollen with water at 4 °C for 48 h.

However, owing to the large band width in the PHEG spectrum (Fig. 2), the presence of a certain amount of the β-sheet conformation in this sample cannot be excluded.

^{13}C CP/MAS NMR spectra of the copolymers are shown in Fig. 3. Chemical shifts together with the results following from NMR bandshape analysis are summarized in Table II.

A comparison of Figs 2 and 3*a* shows that the shape of the NMR spectrum of P(HEG-GH)1 is similar to that of PHEG. Bandshape analysis has shown that Lorentzian profile is suitable only for describing the shape of C-δ and C-ε carbon lines, for the other bands of protonated carbons the Gaussian profile was used. The shape of the C-α carbon band at 53.3 ppm can be described by a single Gaussian function. In this case, similarly to PHEG, the positions and shapes of all conformationally sensitive carbon bands suggest the presence of the random coil conformation, even though a small contribution of other conformations again cannot be excluded.

An increase in the content of hydrazide units leads to a pronounced change in the shape of the NMR spectrum, as seen in Fig. 3*b* for P(HEG-GH)2, and in Figs 3*c*, 3*d* for P(HEG-GH)3 and P(HEG-GH)3 swollen with H_2O. In these samples the α-helix structure predominates, as confirmed by the positions of all conformationally sensitive carbon bands in the NMR spectrum (Table II). The bandshape analysis has shown that the lines of C-δ and C-ε carbons can be described for all samples by means of Lorentzian profile, whereas the shape of all the other lines depends on conformation, becoming Lorentzian for α-helix structure. These Lorentzian lines are always narrower than those of Gaussian shape and they correspond to predominant random coil conformation.

In the ^{13}C NMR spectrum of solid P(HEG-GH)4 the C-δ and C-ε carbon bands of pure PHEG are only weak, and the band of C-α carbon is beginning to appear at 52.9 ppm, corresponding to hydrazide units (Fig. 3*e*). The positions of conformationally sensitive bands (Table II) indicate that also in P(HEG-GH)4 the predominantly helical form persists.

An entirely different type of NMR spectrum appears in Fig. 3*f* for sample P(HEG-GH)5. The lines of C-δ and C-ε carbons of PHEG have disappeared completely, and the NMR spectrum confirms the predominance of hydrazide units. The positions of all three conformationally sensitive bands as well as the coalescence of the bands of C-β and C-γ carbons at 31.6 ppm (Table II) confirm the presence of predominating β-sheet conformation.

The results of ^{13}C CP/MAS NMR analysis of the conformational structure of all the studied samples are summarized in Table III, together with the results obtained by X-ray diffraction. This table shows that the results of both methods are consistent. They indicate the presence of either α-helix, β-sheet or random coil structure. However, the α-helix structure which is so prominently manifested in NMR spectra of P(HEG-GH)2, P(HEG-GH)3 and P(HEG-GH)3 swollen with H_2O, appears, by X-ray diffraction, as a mixture of α-helix and random coil structures. It is evident that while X-ray diffraction is very sensitive to spatial order of the helix, NMR spectra rather indicate local conformational structure. Spatially ordered helices in a three-dimensional lattice probably have an NMR spectrum identical with that of spatially completely disordered helices. This explains the higher content of α-helix structure determined by NMR, as compared to that determined by X-ray diffraction.

^{1}H NMR Spectra

Parts of ^{1}H NMR spectra (0 – 5 ppm) of PHEG and P(HEG-GH)5 in DCA solution (measured at 80 °C) as well as the assignment of bands are evident from Fig. 4. The

TABLE III
Secondary structure of PHEG and HEG-GH copolymers in the solid state by X-ray diffraction and ^{13}C NMR methods

Polymer	X-Ray diffraction	^{13}C NMR	Predominant conformation
PHEG	rc (α)	rc (α, β)	rc
P(HEG-GH)1	rc (α)	rc (β)	rc
P(HEG-GH)2	α, rc	α	α, rc
P(HEG-GH)3	α, rc	α	α, rc
P(HEG-GH)4	α, rc (β)	α (β, rc)	α, rc
P(HEG-GH)5	β, rc	(β, rc)	β, rc

α α-Helix; β β-sheet; rc random coil; in parentheses, other possible structures are given.

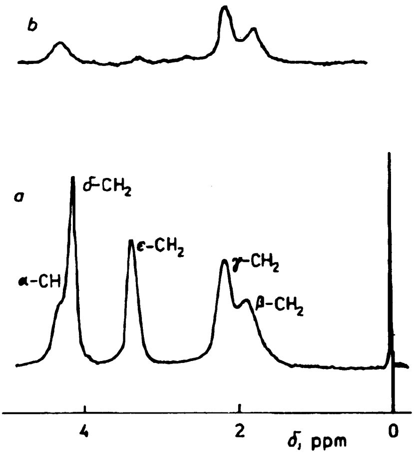


FIG. 4
100 MHz ^{1}H NMR spectra (0.05 g/ml in $Cl_2CHCOOH$, 80 °C). *a* PHEG; *b* P(HEG-GH)5

resonance peaks of NH groups lie at 6.92 and 7.54 ppm, i.e. outside the range shown in Fig. 4. The bands of CH_2-δ and CH_2-ε protons are practically absent in the spectrum of P(HEG-GH)5 (Fig. 4*b*), confirming that in this polymer more than 95% of monomeric units are of the hydrazide type.

As already mentioned, it has been proved in a number of communications[7,8,16,17] that, because of the reduced mobility, the occurrence of associated polymer structures leads to the reduction of absolute integrated band intensities in high resolution NMR spectra. A sensitive method revealing the presence of associates in polymer solutions and providing information on their thermal stability is the measurement of the temperature dependence of integrated NMR band intensities[7,8]. While in systems without associated structures the integrated intensity shows a monotonous (practically linear) decrease with increasing temperature, the decomposition of associated structures during heating is manifested by an increase in integrated intensity. The temperature variation in the integrated intensity in the spectra (1 – 5 ppm) of PHEG and P(HEG-GH) in DCA solution, measured at gradually increasing temperature, is shown in Fig. 5. Similarly as in previous communications[7,8,16,17], the variation of intensity was quite identical for all bands in the respective range. The encircled points in Fig. 5 indicate the integrated intensity values of the spectra measured 10 – 15 min after cooling from the highest temperature to 25 °C. During the whole temperature variation, all instrumental parameters were kept constant.

The anomalous temperature dependence (the increase in integrated intensity) indicating decomposition of associated structures, was observed with all studied solutions (Fig. 5). However, while with PHEG or P(HEG-GH)1 the decomposition is complete at

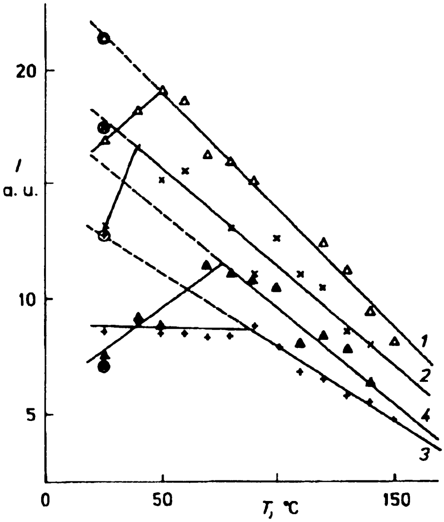


FIG. 5

Temperature dependences of integrated intensity of the 1 – 5 ppm region in ^{1}H NMR spectra (0.05 g/ml in $Cl_2CHCOOH$). *1* PHEG; *2* P(HEG-GH)1; *3* P(HEG-GH)3; *4* P(HEG-GH)5. (For the meaning of encircled points see the next)

40 – 50 °C, with P(HEG-GH)3 or P(HEG-GH)5, the temperatures of complete decomposition of associated structures are considerably higher, 80 – 90 °C.

From the integrated intensity values measured at 25 °C 10 – 15 min after cooling from the highest temperature (encircled points in Fig. 5) a very rough information on the rate of the association process can be obtained[17]. For the solutions of PHEG, P(HEG-GH)1 and P(HEG-GH)3 these values are identical with the values obtained by extrapolation to 25 °C of the integrated intensities from the linear region (above 50 or 90 °C). This indicates that the encircled points in these cases correspond to a situation without association; the association here is a relatively slow process. On the other hand, for P(HEG-GH)5 in solution, the integrated intensity measured at 25 °C after cooling is practically equal to the original value obtained before the start of the temperature variation. In this case, 10 – 15 min is sufficient for regeneration of the associated structures after their preceding disintegration by heating; hence, in P(HEG-GH)5 the association process is considerably more rapid than in the preceding cases.

From a comparison of integrated intensities, the fraction p of associated units can be determined by means of the relation[7,8]

$$p = 1 - I/I_0 , \qquad (1)$$

where I is the integrated intensity of the high resolution NMR spectrum of the system where association takes place and I_0 is the integrated intensity if no association occurs. I_0 was set equal to the integrated intensity values extrapolated to the given temperature from the region where the integrated intensity decreases linearly with temperature, and where the associates are disrupted. The obtained temperature dependences of the asso-

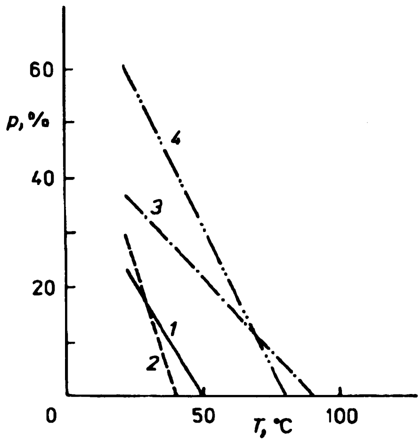


FIG. 6

Temperature dependence of percentage of associated units of polymer molecules in $Cl_2CHCOOH$ solutions (0.05 g/ml) *1* PHEG, *2* P(HEG-GH)1, *3* P(HEG-GH)3, *4* P(HEG-GH)5 ciated fraction are shown in Fig. 6. This figure shows that the values of p at 25 °C increase with the growing content of hydrazide units in the copolymer.

CONCLUSIONS

It was found that similarly as in the case of poly(aspartic acid) and poly(glutamic acid)[6], a combination of X-ray and spectroscopic methods is very useful in the studies of the conformation of PHEG and HEG-GH copolymers in the solid state. Unfortunately, in this case IR spectroscopy did not yield any structural information. On the other hand, a possibility of studying the formation and decomposition of associates in DCA solutions by means of ^{1}H NMR spectra appeared, and this contributed to our understanding of the generation of polymer conformations in the solid state as determined by X-ray and ^{13}C NMR spectroscopy.

The results of conformational analysis of PHEG and HEG-GH copolymers obtained by the latter two methods (Table III) are complementary and do not contradict each other. It appears that PHEG and P(HEG-GH)1 with $n_H/n_B = 0.25$ are practically amorphous. An increase during synthesis in the molar ratio hydrazine/benzyl ester units ($n_H/n_B = 0.5 - 2.0$) leads to copolymers with ordered α-helices; this phenomenon is most pronounced for the P(HEG-GH)3, with $n_H/n_B = 1.0$. In P(HEG-GH)4 ($n_H/n_B = 2.0$) a small part of polymer chains is probably also organized as β-sheet structure which predominates in samples obtained with a large hydrazine excess ($n_H/n_B = 20$). Besides reflections characteristic of β-sheet structure, the diffractograms of P(HEG-GH)4 also exhibit a considerable amorphous halo, which can arise by scattering from random coils or from randomly oriented α-helices, but the α-helix conformation is excluded by ^{13}C NMR spectra.

The found conformational structures of PHEG and HEG-GH copolymers in solid state correlate well with the results of the temperature variation of integrated band intensities in ^{1}H NMR spectra of these samples in DCA solution. The values of the associated fraction p at 25 °C (Fig. 6) are considerably lower for PHEG and P(HEG-GH)1 in solution (p = 21 and 25%, respectively) than for the solutions of P(HEG-GH)3 (p = 35%) or P(HEG-GH)5 (p = 57%). Likewise, the temperatures of complete decomposition of associates are considerably lower (40 – 50 °C) for polymers with coil conformation in the solid state than for polymers in which the regular α-helix or β-sheet structures predominate in the solid state (80 – 90 °C). Moreover, for the polymer with predominating β-sheet structure in the solid state (P(HEG-GH)5), the association process in solution is considerably more rapid than in the other cases (see Fig. 5). On the basis of these results we assume that there exists a direct relation between organized associated structures in solution and the regular conformation in solid PHEG and P(HEG-GH) samples.

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