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Copoly(γ,DL-glutamate)s containing short and long linear alkyl side chains

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Abstract

Copoly(α-alkyl γ,DL-glutamate)s containing octadecyl and ethyl side groups were prepared from racemic poly(γ,DL-glutamic acid) of bacterial origin, and their structure in the solid state examined by DSC, polarized IR spectroscopy and X-ray diffraction methods. Copolymers with a blocky microstructure were prepared by transesterification of poly(α-ethyl γ,DL-glutamate) with octadecanol for a wide range of compositions. Random copolymers were prepared by sequential alkylation of the polyacid with ethyl bromide and octadecyl bromide in solution. All the multiblock copolymers were found to adopt the layered structure characteristic of comb-like polypeptides with the octadecyl side chains crystallized in extended conformation in a separate phase, and aligned normal to the main chains, which are arranged in helical conformation. Melting of paraffinic crystallites reversibly happened in the 50–55 °C range without significant alteration of the layered arrangement. Upon heating at 150 °C, the structure contracted but no indication on the existence of a second transition, as it is known to occur in comb-like poly(α-alkyl γ-glutamate) homopolymers, was observed by DSC. Random copolymers did show nor thermal transition neither evidence of any organized supramolecular structure.

Keywords: Poly(γ-glutamate)s; Poly(γ-glutamic acid); Poly(γ-peptide)s

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1. Introduction

It is well known that comb-like polymers bearing long linear alkyl side groups tend to be arranged in a periodical layered structure with the side chains crystallized in a separated phase [1]. These systems are of great interest, both academic and technological, for the peculiar organization that they adopt in the nanometric scale and for the practical applications that can be derived from such a type of arrangements.

The synthesis and structure of comb-like poly(α-peptide)s made of a stiff helical main chain bearing flexible side chains is a well-documented issue [2]. After the pioneer work of Watanabe describing the layered biphasic structure of poly(γ-alkyl α,L-glutamate)s [3], a good number of papers exploring this behavior in other related poly(α-peptide)s were published [4,5]. The occurrence of similar structures in comb-like poly(β-peptide)s, specifically poly(α-alkyl β,L-aspartate)s was later reported and these systems have been extensively investigated in these last years [6]. Lately, it has been revealed that this property is also shared by both racemic and enantiomerically enriched poly(α-alkyl γ-glutamate)s (PAAG) [7]. PAAGs are a distinguished class of poly(γ-peptide)s derived from poly(γ-glutamic acid) (PGGA), a biodegradable polyamide of microbial origin, which is produced by aerobic fermentation of a variety of bacteria of the genus *Bacillus* [8], and available on large scale for food industrial applications [9]. In all these systems, the polypeptide main chain is arranged in a rod-like conformation tightly coiled through an intramolecular network of hydrogen bonds with a topology close to the α-helix.

The influence of side chain heterogeneity on the structure and thermal properties of polypeptides has been examined for copoly(α-peptide)s [10,11] and copoly(β-peptide)s [12] containing short and long side chains. In both cases, it was found that the layered structure could be retained for a wide range of comonomer compositions and for large differences in the side chain lengths as well. However, crystallinity and

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the temperature at which side chains melt are negatively affected by copolymerization, this effect being much more pronounced for random copolymers than for copolymers with a block microstructure.

In this paper we wish to report on hairy copoly(γ,DL-glutamate)s containing octadecyl and ethyl side groups, abbreviated *co*PAAG-18,2($a:b$), $a:b$ being the ratio of $C_{18}H_{37}$ to C_2H_5 in the copolymer. Copolymers have been prepared by chemical modification of PGGA by two different methods, and their structure and thermal properties examined by DSC, polarized infrared spectroscopy and X-ray diffraction. Results afforded by this study are interpreted with reference to the parent homopolymers PAAG-2 and PAAG-18, and compared with those previously reported for related copoly(α,L-glutamate)s and copoly (β,L-aspartate)s.

2. Experimental

2.1. Materials and methods

All chemicals were obtained commercially from either Aldrich or Merck. They were analytical grade or higher, and used without further purification. Solvents to be used under anhydrous conditions were dried by standard methods. Poly(γ,DL-glutamic acid) with a D:L ratio of 60:40 and an average molecular weight of 390,000 was kindly supplied by Dr Kubota of Meiji Co. (Japan).

Both ^1H and ^{13}C NMR spectra were recorded at 25 °C on a Bruker AMX-300 NMR instrument operating at 300.1 and 75.5 MHz, respectively, with samples dissolved in $CDCl_3$ or a mixture of $CDCl_3$/TFA and using TMS as internal reference. Calorimetric measurements were carried out with a Perkin–Elmer Pyris I DSC instrument calibrated with indium. Sample weights of about 2–5 mg were heated or cooled at rates of $\pm 10\ ^\circ C\ min^{-1}$ under a nitrogen atmosphere. X-ray diffraction patterns were taken from oriented polymer films in a Statton-type camera using nickel-filtered Cu Kα radiation of wavelength 0.1542 nm. The patterns were recorded on flat photographic films and were calibrated with molybdenum sulfide ($d_{002} = 0.6147$ nm). X-ray thermodiffractograms were recorded from unoriented polymer films in a Siemens D-500 diffractometer using Cu Kα radiation and provided with a TPK-A Park heating stage and a scintillation counter. Dichroic infrared spectra were recorded in a Perkin–Elmer FTIR-2000 instrument equipped with an external gold polarizer. Dichroic ratios were determined from the absorbance measured for the film with orientation parallel and perpendicular to the infrared polarization vector. Polymer films used in this study were prepared by casting from chloroform and dried under vacuum. To induce orientation, rectangular pieces of these films were subjected to uniaxial mechanical stretching under controlled temperature.

2.2. Synthesis of copolymers

Two procedures, one with the polymer substrate suspended in the reaction medium (heterogeneous polymer-solvent system) and the other in solution (homogeneous polymer-solvent system), have been applied to obtain *co*PAAG-18,2 both of them using PGGA as starting material. It should be stressed that the terms ‘homogeneous’ and ‘heterogeneous’ are strictly applied to the solvent–polymer pair since $NaHCO_3$ is present as a separate phase in both cases.

Heterogeneous procedure. PAAG-2 was obtained according to Kubota et al. [13] by reaction of PGGA suspended in *N*-methylpyrrolidone (NMP) with ethyl bromide in the presence of $NaHCO_3$, as described in full detail elsewhere [7a,14]. The transesterification of PAAG-2 with octadecanol was carried out as follows: to a stirred suspensión of PAAG-2 in the alcohol (molar ratio 1:25), 0.20 mol of $Ti(OBu)_4$ were added and the mixture heated at 150 °C under a nitrogen atmosphere. To follow the advancement of transesterification, reaction mixture aliquots were intermittently taken out and analyzed by NMR. The reaction was stopped at the desired conversion by cooling to room temperature, and the excess of octanol removed by repeated washing with hot ethanol. The resulting *co*PAAG-18,2 was purified by reprecipitation from chloroform with methanol and stored under vacuum. The homopolymer PAAG-18 was prepared by the same procedure but using a reaction temperature of 190 °C for a period of time of 22 h.

Homogeneous procedure. Partially ethylated poly(γ,DL-glutamic) acid was obtained by following the procedure described for the preparation of PAAG-2. When the desired conversion was attained, and after taking out aliquots for the microstructure NMR analysis, an excess of octadecyl bromide was added and the reaction left to proceed at 55–60 °C for 6 days. The resulting *co*PAAG-18,2 was isolated by precipitation with ether, purified by reprecipitation from chloroform with ether, and stored under vacuum.

3. Results and discussion

3.1. Synthesis and microstructure

A number of copoly(γ, DL-glutamate)s containing octadecyl and ethyl side chains in a wide range of ratios and different microstructure were prepared from bacterially produced poly(γ,DL-glutamic acid) using two alternative two-steps procedures. The chemical reactions involved in such procedures are outlined in Fig. 1 and the results obtained there from are collected in Table 1.

In the heterogeneous procedure, complete esterification of PGGA with ethyl bromide to PAAG-2 was first carried out according to Kubota et al. [13]. Secondly, partial transesterification of PAAG-2 with octadecyl bromide in the presence of $Ti(OBu)_4$ led to copolymers *co*PAAG-18,2 with

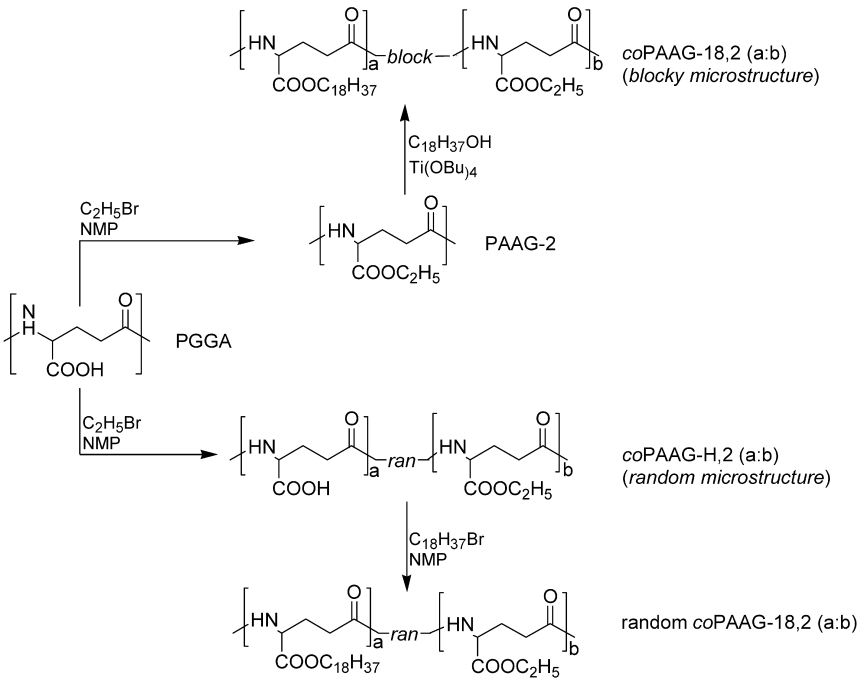

Fig. 1. Outline of the synthetic route followed for the preparation of PAAG-18,2 (*a* : *b*) copolymers.

the desired composition. We have recently reported on the general utility of this transesterification method for the preparation of poly(α-alkyl γ-glutamate)s homopolymers having long alkyl side chains [7a,14]. It was found that the octadecyl to ethyl ratio in *co*PAAG-18,2 could be closely adjusted by fixing the conversion attained in this second step. The evolution of the transesterification was followed by ^{1}H NMR. The ^{1}H NMR spectrum of the resulting *co*PAAG-18,2 (60:40) obtained by this procedure is shown in Fig. 2(a). The composition of the copolymer could be readily determined by rating the area of the signal arising from the main chain CH at ~4.7 ppm to the area of the three signals contained in the 0.7–1.8 ppm interval, which include all the side chain protons of ethyl and octadecyl groups except those on the $-OCH_2$. No signal splitting indicative of sequential microstructure effects could be detected in this spectrum. Since the transesterification reaction is carried out on PAAG-2 suspended in the alcohol, the copolymers prepared by this procedure are expected to have a blocky microstructure consisting of an undetermined number of blocks of ethyl and octadecyl glutamates of variable lengths.

The second procedure applied to prepare *co*PAAG-18,2 happened totally in homogeneous phase. It consisted of a sequential esterification of PGGA with ethyl bromide and octadecyl bromide with the polymer in solution. The synthesis in this case was restricted to *co*PAAG-18,2 (35:65) and *co*PAAG-18,2 (10:90), the ^{1}H NMR spectrum

Table 1
Results of *co*PAAG-18,2 (*a* : *b*)

Copolymer	$(a:b)^{a}$	$[\eta]^{b}$ (dL g^{-1})	Elemental analysis[c] N; C; H (%)	$T_m/\Delta H^{d}$ (°C)/(J g^{-1})	T_d^{e}
Heterogeneous phase					
PAAG-18,2 (90:10)	92:18	0.32	3.85; 70.53; 11.56 (3.85; 71.78; 11.14)	53/57	325sh, 368
PAAG-18,2 (80:20)	80:20	0.52	4.11; 70.76; 11.89 (4.16; 70.67; 10.89)	52/48	325sh, 357
PAAG-18,2 (70:30)	70:30	0.55	4.56; 69.63; 10.68 (54.46; 69.60; 10.64)	54/40	335sh, 365,
PAAG-18,2 (60:40)	61:39	0.60	5.03; 68.49; 10.39 (4.77; 60.05; 8.65)	55/43	330sh, 354,
PAAG-18,2 (20:80)	22:78	1.07	6.90; 60.05; 8.65 (6.79; 61.20; 8.34)	55/18	320, 350sh
Homogeneous phase					
PAAG-18,2 (35:65)	35:65	n.d.	n.d	−/−	291, 330sh
PAAG-18,2 (10:90)	10:90	n.d.	n.d.	−/−	293, 327sh

n.d., Not determined.

[a] Comonomer composition in the copolymer determined by ^{1}H NMR.

[b] Intrinsic viscosity measured in dichloroacetic acid.

[c] Values given in brackets are those calculated for the copolymer with the comonomer composition given in column 2.

[d] Melting temperature and enthalpy estimated by DSC at heating.

[e] Decomposition temperatures taken as the derivative curve peak of the corresponding TGA trace. (sh: shoulder).

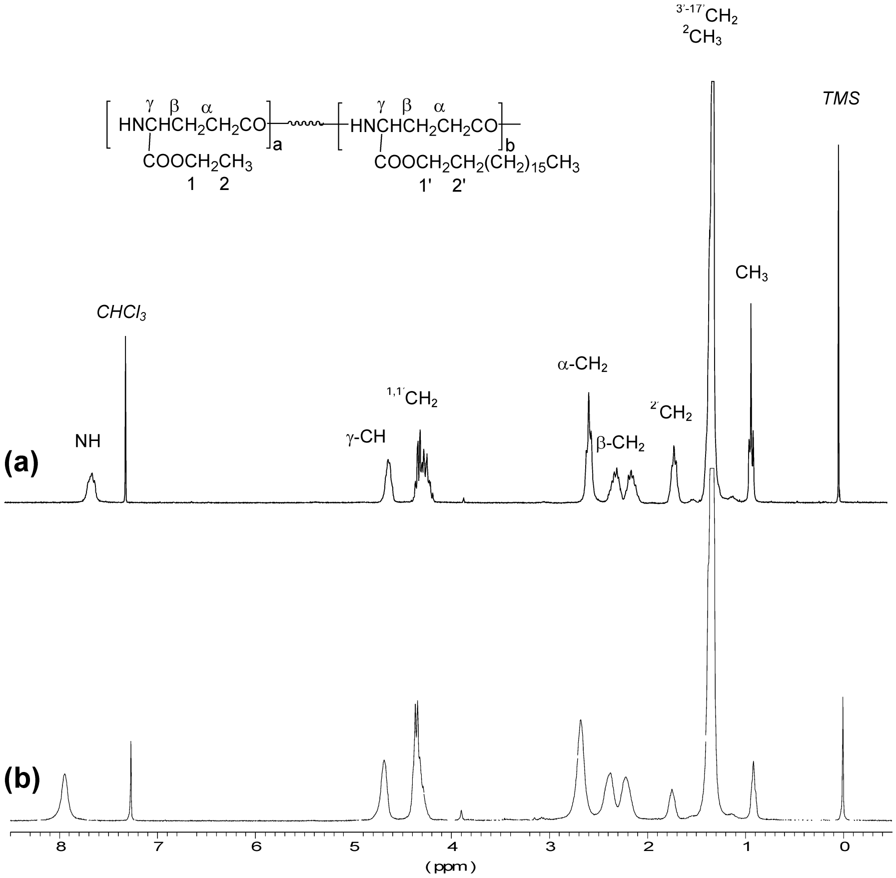

Fig. 2. ^{1}H NMR spectra of (a) *co*PAAG-18,2 (60:40) with blocky microstructure, and (b) random *co*PAAG-18,2 (35:65), with indication of peaks assignments.

of the former being shown in Fig. 2(b). As before, the comonomer composition of these copolymers could be determined from ^{1}H NMR spectra, but no information concerning microstructure was provided. On the contrary, the ^{13}C NMR spectra of the partially ethylated poly(γ,DL-glutamic acid), abbreviated *co*PAAG-H,2, proved to be sensitive to sequential effects. The complex α-CH_2 signal produced by a 45% esterified *co*PAAG-H,2 is displayed in Fig. 3. Assignment of the four peaks to the possible existing glutamic acid (H)/ethyl glutamate(E) dyads (HH, EE, EH and HE) was made on the basis of chemical shifts of dyads EE and HH observed in the spectra of the PGGA and PAAG-18 and taken into account the comonomer composition. The quantitative analysis of these peaks led to conclude that *co*PAAG-H,2 copolymers are essentially at random with average sequence lengths close to those theoretically calculated for the actual composition (Table 2). This result is fully consistent with what should be expected for a polymer modification reaction unaffected by microenvironmental factors, as it is the case of the esterification of PAAG taking place in solution. Since no rearrangement of the ethyl groups is expected to occur in *co*PAAG-H,2 upon esterification with octyl bromide, it can be concluded that *co*PAAG-18,2 copolymers obtained by sequential esterification must have a random microstructure. We will refer to them as random *co*PAAG-18,2.

3.2. Thermal behavior

PAAG-2 is a semicrystalline polymer that melts around 250 °C. Conversely, PAAG-18 exhibits a sharp endotherm at 55 °C corresponding to the fusion of the crystallized octadecyl side chains. The DSC traces recorded at heating from *co*PAAG-18,2 and PAAG-18 are shown in Fig. 4 and

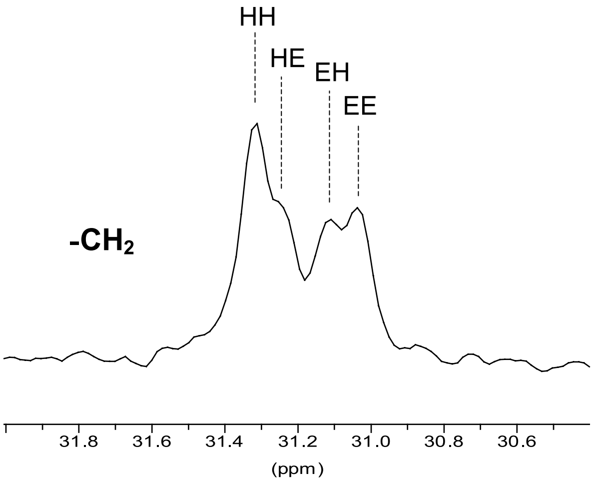

Fig. 3. α-CH_2 signal in the ^{13}C NMR spectrum of copolymer *co*PAAG-H,2 (55:45) registered in DMSO. Assignments of peaks to glutamic acid (H)/ethyl glutamate (E) dyads are indicated.

Table 2
Composition and microstructure of *co*PAAG-H,2 (55:45)

	Composition		Dyads (%)			Number average sequence length		Randomness
	x_H	x_E	HH	HE + EH	EE	n_H	n_E	
Exp.[a]	58.7	41.3	36.6	42.0	21.1	2.74	2.02	0.86
Theor.[b]	58.7	41.3	34.4	48.5	17.1	2.42	1.70	1

[a] Composition calculated from ^{1}H NMR data. Dyad contents obtained by deconvolution of the α-CH$_2$ signal in the ^{13}C NMR spectrum.

[b] Theoretical values for a random distribution of γ-glutamic and ethyl glutamate units.

thermal parameters estimated from the heating traces are listed in Table 1.

All the *co*PAAG-18,2 copolymers obtained under heterogeneous conditions show melting at the surroundings of 50 °C. As expected, the melting enthalpy steadily decreases with the content in octadecyl side chains while melting temperature appears slightly affected, the most remarkable feature being the broadening of the peak that is detected for intermediate compositions. The observed endotherm obviously arises from the fusion of the octadecyl chains and its presence reveals the ability of these chains to crystallize for any copolymer composition. On the contrary, *co*PAAG-18,2 copolymers prepared by the 'homogeneus procedure' do not show any sign of crystallinity for the two examined compositions. Such a remarkable difference in the thermal behavior supports the occurrence of the blocky microstructure that is thought to be present in *co*PAAG-18,2 copolymers obtained by transesterification. Moreover, this pattern of behavior is quite similar to that observed for block and random copoly(β,L-aspartate)s bearing octadecyl and butyl side chains, which were investigated by us a few years ago [12a].

The thermal stability of *co*PAAG-18,2 under inert atmosphere was evaluated by TGA. Degradation by heat of heterogeneous copolymers was found to start above to 250 °C and to happen in two stages with maximum decomposition rates at about 320–340 °C and 350–370 °C, respectively, with the high temperature peak increasing in intensity with the content in octadecyl units. PAAG-18 homopolymer is known to decompose in two steps at 330 and 365 °C, respectively [7a], while PAAG-2 does it in a single stage at 375 °C [14]. Random *co*PAAG prepared in solution also exhibit a two-steps decomposition but taking place near to 290 and 330 °C, the higher temperature step being of relative less importance. The thermal decomposition pattern observed for the copolymers is again in agreement with the blocky and random microstructures accepted to occur in these *co*PAAG-18,2 depending on the preparation method. Illustrative traces are shown in Fig. 5 for *co*PAAG-18,2 (20:80) and the T_d values for the whole series are compared in Table 1.

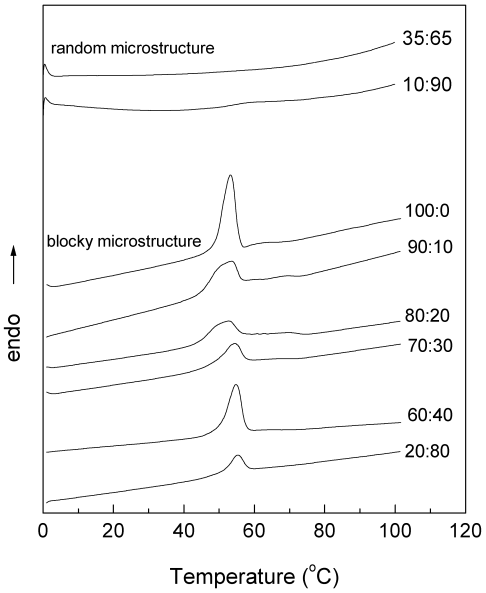

Fig. 4. DSC traces of *co*PAAG-18,2 (*a* : *b*).

3.3. Copolymer conformation and supramolecular structure

The backbone conformation of *co*PAAG-18,2 copolymers was investigated by polarized infrared spectroscopy. Fig. 6 shows the DIR spectra of an uniaxially stretched film of *co*PAAG-18,2 (80:20) with blocky microstructure registered with the polarization vector parallel and perpendicular to the orientation direction, respectively. The two spectra clearly differ in the intensity of the absorption associated to the amide group with both amide A and amide I

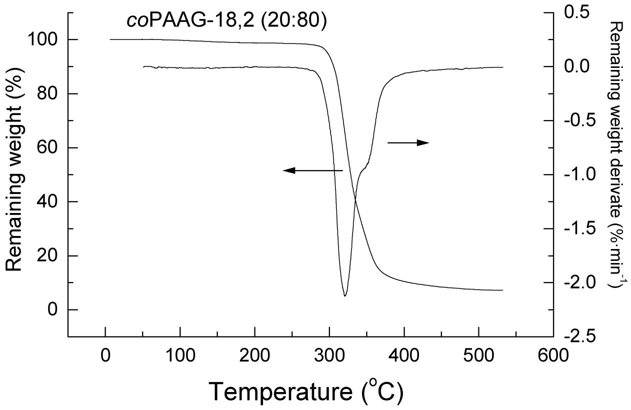

Fig. 5. TGA trace and derivative curve of the indicated copolymer.

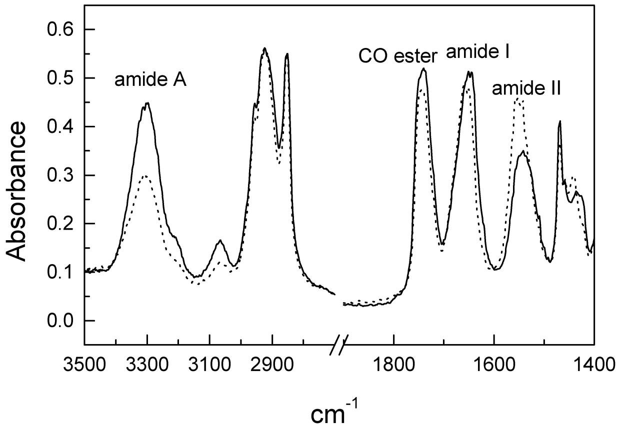

Fig. 6. Polarized infrared spectra of *co*PAAG-18,2 (80:20) recorded with the electric vector of the polarized radiation parallel (solid line) and perpendicular (dotted line) to the orientation axis of the copolymer film.

band displaying parallel dichroism and the opposite behavior being observed for amide II. Such a dichroic infrared response reveals that planar amide groups must be oriented in the sample with the C=O and N–H bonds parallel or nearly parallel to the stretching axis. It can be inferred therefore that, in analogy to what has been previously observed for bacterial poly(γ-glutamate)s bearing a wide variety of side groups [7b,15], an α-helix like conformation must be adopted by *co*PAAG-18,2. Just to note, that the occurrence of a regular coiled conformation in racemic poly(γ-glutamate)s of biosynthetic origin is perfectly feasible since a microstructure in stereoblocks is known to exist in these compounds [16].

Results obtained by X-ray diffraction of *co*PAAG-18,2 were in accordance with the DSC observations described above. *co*PAAG-18,2 with a blocky microstructure produced diffraction patterns containing discrete scattering, as those shown in Fig. 7 whichever is the composition in ethyl and octadecyl glutamate units. Spacings and visually estimated intensities of the reflections observed in these patterns are compared in Table 3. The most characteristic feature of these patterns is the set of intense reflections appearing along the equator with a basic periodicity of 3.0–3.2 nm. According to what is known for comb-like polyglutamates and polyaspartates, these equatorial reflections evidence the presence of the layered arrangement made of polypeptide helices in these copolymers. As expected the intensity of the innermost ∼3.1 reflection decreases with the *a* : *b* ratio to be almost inappreciable for *co*PAAG-18,2 (20–80). The fact that the intensity of the 1.0 nm-reflection increases with the content in ethyl groups is explained taking into account that such reflection is the most prominent one in the diffraction pattern of poly(α-ethyl γ,DL-glutamate). On the other side, the strong six 0.42 nm reflections seen in the outermost part of the pattern arise from the crystallized paraffinic phase made of octadecyl chains that are filling the interlayer space. According to spacing and relative position of the reflections, it can be concluded that the crystallized alkyl side chains are in zigzag conformation, hexagonally packed, and aligned normal or nearly normal to the main helical chains. The reflection seen on the meridian with spacing of 0.5 nm should be attributed to the pitch of some of the α-helix like conformations that have been put forward for poly(γ-glutamate)s [15,17].

The effect of temperature on the supramolecular structure of *co*PAAG-18,2 was also examined. Fig. 8(a) shows the diffractograms of *co*PAAG-18,2 (80:20) and *co*PAAG-18,2 (20:80) registered at increasing temperatures from 25 to 150 °C. The structural changes taking place upon heating are clearly displayed in the case of *co*PAAG-18,2 (80:20). At 80 °C, the sharp 0.42 nm reflection is replaced by the broad scattering centered around 0.45 nm whereas the low angle peaks do not undergo significant alterations. These are the changes that should be expected from the melting of the paraffinic phase taking place at 52 °C according to DSC. Since the long spacing does not vary significantly after upon melting, it must be interpreted that the molten octadecyl side chains essentially retain the conformation and the alignment that they had in the crystallized state. Upon heating at 150 °C, the 3.2 nm peak broadened markedly and shifted to 2.7 nm indicating that the long distance order has vanished, presumably entailing the collapse of the alkyl side chains. However, since no endotherm is detected by DSC above 55 °C, the existence of a second transition of the type reported in homopolymers

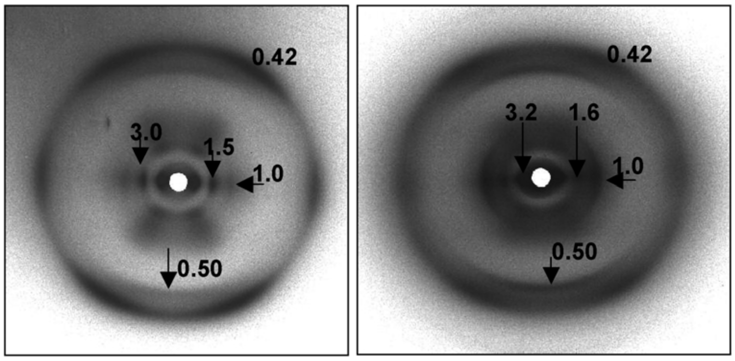

Fig. 7. X-ray diffraction patterns from oriented *co*PAAG-18,2 (80:20) (left) and 20:80 (right).

Table 3
X-ray diffraction spacings (nm) of *co*PAAG-18,2 with a blocky microstructure registered at different temperatures

T (°C)	PAAG-18	PAAG-2	PAAG-18,2 (90:10)	PAAG-18,2 (80:20)	PAAG-18,2 (70:30)	PAAG-18,2 (60:40)	PAAG-18,2 (20:80)
25 °C (Phase A)	3.2(vs)		3.0(vs)	3.1(vs)	3.2(vs)	3.1(vs)	3.1(m)
	1.5(s)		1.5(s)	1.5(s)	1.6(m)	1.6(m)	1.5(m)
	1.0(w)	1.0(vs)	1.0(w)	1.0(w)	1.0(w)	1.0(m)	1.0(s)
		0.50(m)	0.50(m)	0.50(m)	0.50(m)	0.50(m)	0.50(m)
	0.42(s)	0.46(m)	0.42(s)	0.42(s)	0.42(s)	0.42(s)	0.42(s)
80 °C (Phase B)	3.5			3.2(vs)			3.2(w)
	0.45(br)			0.45(br)			0.50(m)
							0.42(br)s
150 °C	2.7			2.7(vs)			
							0.50(m)
	0.46(br)			0.45(br)			0.42–0.49

Intensities visually estimated and denoted as: vs, very strong; s, strong; m, medium; w, weak; br, broad.

cannot be postulated in this case. The original profile was entirely recovered upon cooling back to room temperature.

The behavior observed for *co*PAAG-18,2 (20:80) was notably different. The diffraction profile obtained for this copolymer (Fig. 8(b)) can be interpreted as arising from PAAG-2 contaminated with a minor amount of PAAG-18. Changes taking place upon heating are almost unappreciable indicating that the PAAG-2 crystal structure is hardly affected by heat along the range of temperatures within it was examined. Slight changes detected in the 0.42 peak can be attributed to melting of the minor amount of layered structure present in this copolymer.

4. Conclusions

The results obtained in this study allow us to conclude that hairy copoly(γ-glutamate)s containing short (ethyl) and long (octadecyl) alkyl side chains can be prepared in a wide variety of compositions from biosynthetically produced poly(γ,DL-glutamic acid) by sequential esterification. The microstructure of the resulting copolymer is depending on the procedure applied for the modification reaction. Whereas a random copolymer is obtained when the esterification proceeded homogeneously, a copolymer with a blocky microstructure resulted when the reaction was performed in heterogeneous phase. The chemical similitude between the two side groups and the relatively large distance separating them along the polymer chain minimize the chemical environment effects hampering the discrimination of the copolymer microstructure by the NMR technique. Nevertheless, the blocky nature of the copolymers generated under heterogeneous conditions was evidenced by their capability to form the supramolecular layered structure typical of comb-like polypeptides. In this structure the polypeptide backbone is the helical conformation and the long alkyl side chains are crystallized in a paraffinic separated phase that shows sharp melting at 50–55 °C. On the contrary, random copolymers obtained under homogeneous conditions were unable to form any organized structure. The random microstructure of these copoly(γ,DL-glutamate)s could be inferred, however, from NMR results

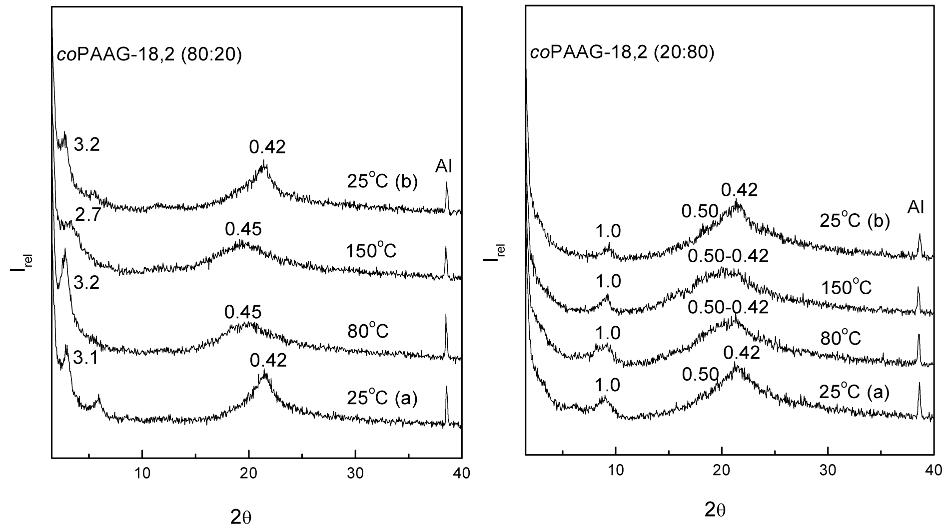

Fig. 8. Powder diffractograms of the indicated *co*PAAG-18,2 registered at the indicated temperatures. (a) Original sample; (b) sample cooled from 150 °C.

obtained for the partially ethylated poly(γ,DL-glutamic acid) precursor.

Acknowledgements

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