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Helical Poly(β-peptides): The Helix−Coil Transition of Poly(α-alkyl-β-aspartate)s in Solution

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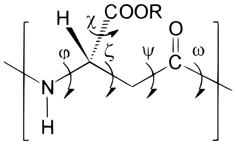
ABSTRACT: The helix−coil conformational transition taking place in a family of poly(β-peptide)s, namely, poly(α-alkyl-β-aspartate)s (M_w > 100 000), upon addition of acid or by changes in temperature has been investigated. Results obtained for a set of optically pure polymers differing in the size and shape of the alkyl side chain evidenced that the transition is a phenomenon common to all poly(β-L-aspartate)s. Experimental conditions required for transition were found to be independent of the constitution of the poly(β-peptide). Investigation of poly(β-D,L-aspartate)s with different enantiomeric composition and configurational sequence revealed the occurrence of the helix−coil transition in block stereocopolymers for any D/L ratio but not in stereocopolymers with a statistical distribution of the antipode units.

Introduction

The conformational interconversion between the α-helix and the random-coil state is a well-known transition experienced by poly(α-peptide)s in solution that was discovered by Doty[1] in the middle of the 1960s. Since then, thousands of papers dealing with this topic have appeared, and a fair number of excellent reviews covering both theoretical and experimental aspects of the phenomenon have been published.[2] The helix−coil transition is a reversible process that entails the formation−disruption of the definite intramolecular hydrogen-bonding network that holds the helical structure. The interconversion is known to happen as a function of both solvent composition and temperature, and it is usually detected by CD and NMR spectroscopy, in the former case as an abrupt change in ellipticity and in the latter as a displacement in the chemical shifts of the signals arising from the protons attached to backbone atoms. In the past few years, new studies carried out on synthetic polypeptides and polypeptides excised from proteins have prompted further developments of the classical helix−coil theories to include effects specifically arising in biopolymers, such as the influence of main chain ends and side chains on helix content.[3]

Poly(α-alkyl-β-L-aspartate)s are poly(β-peptide)s with an alkoxycarbonyl group stereoregularly attached to the β-carbon of the repeating unit.



Although these polypeptides are more flexible than their analogous poly(α-peptide)s because of the additional carbon atom present in the main chain, they are able to adopt helical structures which are topologically similar to the α-helix.[4] Right-handed helices containing between three and four residues per turn with hydrogen bonds set between every third or every fourth residue have been described for these compounds in the solid-state depending on the size and shape of the alkyl side group.[5] A complete description of the helical arrangements observed in the family of poly(β-L-aspartate)s has been afforded in our previous works. On the other hand, two independent research groups[6,7] have recently shown that small β-oligopeptides containing as few as six residues are able to form stable helices in both solid state and solution. The molecular arrangement in such helices are very close to those found in poly(α-alkyl-β-L-aspartate)s.

In this work we have carried out a detailed investigation on the helix−coil transition taking place in poly(α-alkyl-β-L-aspartate)s. With this objective in mind, a series of polymers covering a wide variety of side chain sizes and shapes was selected for the study. The effects of the solvent, temperature and enantiomeric composition on the helix−coil transition have been examined for each compound using both ^{1}H NMR and CD spectroscopies. On the other hand, the helix- and random-coil states of poly(α-alkyl-β-L-aspartate)s have been modeled in solution using force-field and quantum mechanical calculations, respectively. The simulated conformations are used to predict NMR coupling constant of the disordered state and chemical shift changes concomitant to the transition.

Methods

Polymer Samples. The poly(α-alkyl-β-L-aspartate)s used in this study were prepared by anionic ring opening polymerization of the corresponding optically pure (S)-4-alkoxycarbonyl-2-azetidinones.[8,9] The stereocopolymers poly(α-isobutyl-β-D,L-aspartate)s with a microstructure in blocks were prepared by the same method from mixtures of the two enantiomeric azetidinones with D/L compositions of 1:9, 1:3, and 1:1.[10] On the other hand, the racemic stereocopolymer with a random microstructure was obtained by polycondensation in solution of an equimolar mixture of the pentachlorophenyl α-isobutyl β-D- and β-L-aspartates.[10] A detailed account of the synthesis of all these polymers and their respective monomers has been reported elsewhere.[8−10] A selection of data of the polymers which are of relevance for the investigation carried out in the present work is given in Table 1. Although molecular

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Table 1. Selected Data for the Poly(α-alkyl-β-L-aspartate)s Studied in This Work

R	acronym	$[\eta]$ (dL g^{-1})[a]	$M_v \times 10^{-5}$ [b]	PD[c]	T_d (°C)[d]
		Homopolymers			
lineal					
-CH_2-$(CH_2)_2$-CH_3	PAALA-4	1.95	3.7	2160	286−377
-CH_2-$(CH_2)_4$-CH_3	PAALA-6	1.21	2.1	1050	291−384
-CH_2-$(CH_2)_6$-CH_3	PAALA-8	2.24	4.4	1930	326−380
-CH_2-$(CH_2)_{10}$-CH_3	PAALA-12	2.16	4.2	1480	333−380
-CH_2-$(CH_2)_{16}$-CH_3	PAALA-18				324−380
branched					
-$CH(CH_3)_2$	PAIPLA	1.82	3.4	2160	308−368
-CH_2-$CH(CH_3)_2$	PAIBLA	1.40	2.5	1460	265−355
-CH_2-CH_2-$CH(CH_3)_2$	PAIALA	1.94	3.7	2000	345−379
cyclic					
-c-C_5H_9	PACPLA	1.22	2.2	1200	297−376
-c-C_6H_{11}	PACHLA	1.53	2.8	1420	308−371
		Stereocopolymers			
-CH_2-$CH(CH_3)_2$	PAIB(D,L)A				
block		7.00	>5	>3000	
D/L (1:9)		5.85	>5	>3000	
D/L (1:3)		5.00	>5	>3000	
D/L (1:1)					
random					
D/L (1:1)					

[a] Intrinsic viscosity measured in dichloroacetic acid at 23 °C. [b] Average viscosity molecular weights estimated by using the equations reported for poly(γ-benzyl-α-L-glutamate).[11] [c] Average polymerization degrees calculated from estimated viscosity molecular weights. [d] Melting-decomposition temperatures determined by DSC-TGA.

weights estimated by viscosimetry have only an approximate value,[11] it may be ascertained that we are dealing with high-weight polymers having polymerization degrees higher than 1000.

NMR Measurements. ^{1}H NMR spectra were recorded on a Bruker AMX-300 spectrometer operated at 300.13 MHz. For each sample, 64 scans were recorded using 16 K data points, a spectral width of 4132 Hz and a recycling delay time of 2 s. All chemical shifts are expressed in parts per million relative to the methyl carbon resonance of tetramethylsilane (TMS).

The solvent-induced helix−coil transition was investigated in samples of polymer (50 mg) dissolved in 5 mL of $CDCl_3$ (ca. 1% w/v) into which 10 μL portions of trifluoroacetic acid (TFA) were successively added to cover a range of concentrations from 0 to 3% (v/v). All of the spectra were recorded at 25 °C. On the other hand, the thermally induced helix−coil transition was investigated using a solution of 10 mg of polymer in 1 mL of a mixture of TFA/$CDCl_3$ (2% v/v) which was subjected to heating and cooling to cover a range of temperatures from −5 to +40 °C. Samples were left 10 min at each temperature in order to arrive at thermal equilibrium. The temperature of the sample in the NMR probe was maintained by the Bruker variable temperature unit B-VT1000E. Standards of ethylene glycol and methanol were used for calibration at high and low temperatures, respectively.

Circular Dichroic Measurements. Circular dichroism (CD) spectra were recorded on a Jasco J-720 spectrometer using a 0.02 cm jacketed quartz cell thermostabilized at 25 °C. Samples were prepared as described above for NMR measurements but using a much lower chromophore concentration (1.97 × 10^{-3} mol L^{-1}). All the spectra were obtained in the wavelength region between 180 and 260 nm.

Molecular Modeling. The conformation of the poly(β-L-aspartate)s in the random-coil state has been characterized by computing the local conformational preferences of the α-alkyl-β-L-aspartamyl residue, i.e., without considering specific nonlocal interactions between different residues. For this purpose, the conformational preferences of N-acetyl-N'-methyl-α-methyl-β-L-aspartamide have been predicted from ab initio quantum mechanical calculations at the HF/6-31G(d)[12] level using the Gaussian 94[13] computer package. Frequency analysis of the optimized conformations was performed to verify that they are minima, as well as to determine the zero point energies (ZPE) and the thermal corrections to the energy. The final estimation of the conformational energies was attained by performing single-point calculations at the MP2/6-31G(d) level[14] on the minima previously characterized at the HF/6-31G(d) level. The free energies of solvation (ΔG_{sol}) in $CHCl_3$ solution were determined using a semiempirical AM1 adapted version[15] of the SCRF developed by Miertus et al.[16] (MST/AM1) as is implemented in *MOPAC93*, revision 2[17] computer program.

The helical conformation was modeled with *AMBER 3.0*, Revision A[18] using the all-atom *AMBER* force-field with electrostatic parameters previously developed by us. Simulated polymer chains were blocked at the ends with acetyl and N-methylamide groups. Energy minimizations were performed without any restriction using the conjugated gradient method.

Results and Discussion

Chemical Shift Measurements. Previous studies using NMR and CD spectroscopy revealed that poly(β-L-aspartate)s are able to retain the helical conformation in chloroform solution.[5] In this section, the effect of the solvent and the temperature on the helix−coil transition of poly(β-L-aspartate)s is examined. We investigate furthermore the influence exerted by the size and shape of the alkyl side group, as well as by the enantiomeric composition of the polymer.

Solvent-Induced Helix-Coil Transition. NMR measurements have been performed to relate the stability of the helical conformation in solution with the amount of TFA required to induce the helix−coil transition. ^{1}H NMR spectra in $CDCl_3$ solution show an upfield displacement for both the of NH and CH backbone protons by about 0.6 and 0.2 ppm, respectively upon addition of TFA in the 0−3% range. Figure 1 reports the ^{1}H NMR spectrum for one of these polymers, i.e., PAALA-12, in a mixture of TFA/$CDCl_3$ (3% v/v) at 25 °C. It is worth noting that all of the peaks are so broad in pure $CDCl_3$ that they are hardly detectable. Similar behavior has been observed in poly(α-amino acid), which is commonly interpreted as being due to both conformational rigidity and molecular aggregation.[19] When small amounts of TFA are added to the sample, the resolution of the signals greatly improves so that a well resolved spectra is obtained for concentrations of the acid in the vicinity of 3%.

Figure 1 also shows the changes experimented by the CH signal with the concentration of TFA varying in the 0−3% (v/v) range. It is seen how the initial broadness of the signal is greatly reduced at the same time that it moves upfield from 5.08 to 4.85 ppm when the concentration of acid increases from 2 to 2.6% (v/v). A similar behavior is observed for the NH signal (not shown) for which δ changes from 8.6 to 7.96 ppm for the same range of variations in the concentration of the acid. The positions of all other signals present in the spectra remain essentially unaltered upon the addition of TFA. It should be mentioned that the shift toward upfield observed for the CH signal in the coiled form of poly(β-L-aspartate)s is opposite to the downfield displacement commonly found for such signals on the helix−coil transition of poly(α-amino acid)s.[19] As will be shown later, such difference in behavior arises from the differences existing in the backbone dihedral angles of the respective types of helices. The decay in helix content taking place over the transition can be estimated by comparing the chemical shift exhibited by the methine proton signal at TFA concentrations intermediate between 0 and 3% (v/v) for which the helix content is assumed to be 100 and 0% respectively. Figure 2 shows

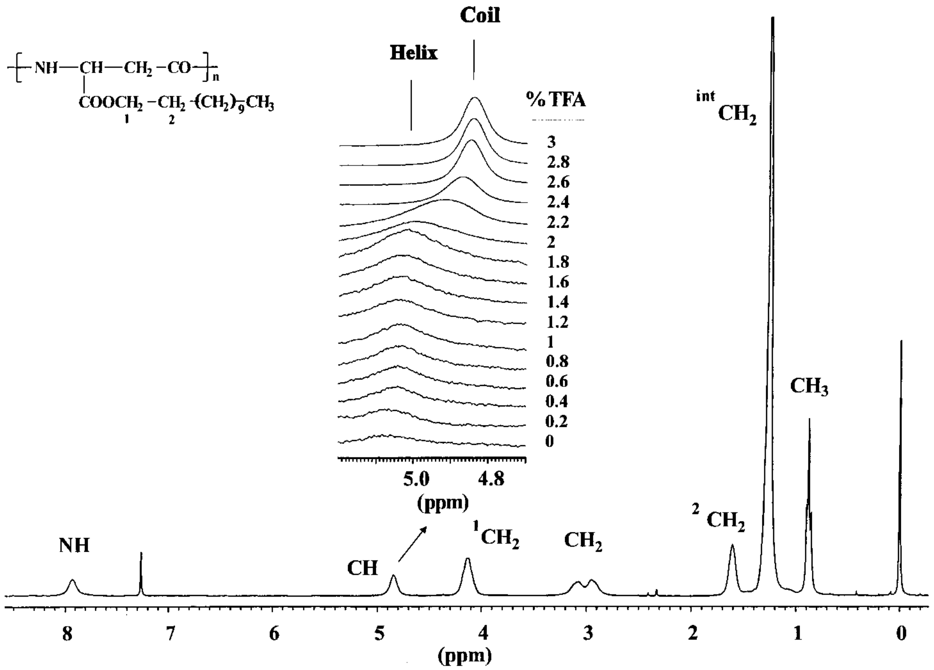

Figure 1. 300 MHz ^{1}H NMR spectrum of PAALA-12 in $CDCl_3$ containing 3% TFA (v/v) at 25 °C. Inset: Displacement of the CH signal with the concentration of acid in the 0–3% range.

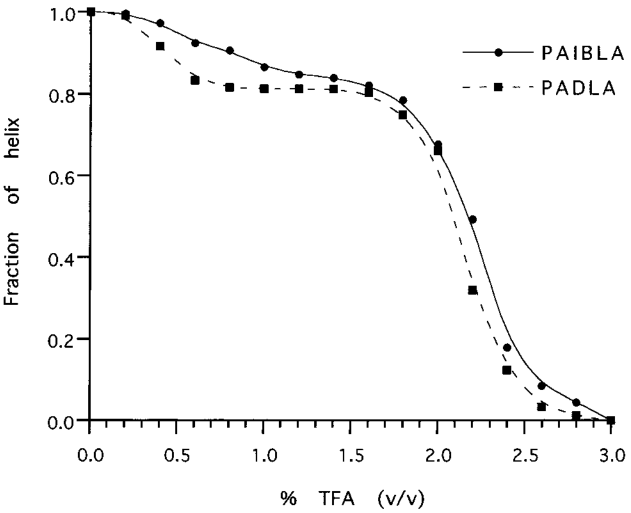

Figure 2. Variation of the fraction of helix content with the concentration of TFA for PAALA-12 and PAIBLA.

plots for two representative cases of the isobutyl and dodecyl derivatives; a sudden fall in the helix content was found to occur for a TFA concentration changing from 2.0 to 2.6%.

Similar changes have been observed for all of the other optically pure poly(β-L-aspartate)s investigated, regardless of the size of the alkyl side chain. In some cases, two peaks were observed in the region of the transition that was attributed to the polydispersity of the samples. It is known that conversion from helix to coil is a very fast process in the NMR time scale, and therefore only one peak should be expected to appear in the spectra. As it has been demonstrated in the analysis of poly(α-amino acid)s, the origin of the two peaks arises from the presence of chains with different length, with the short chains requiring less acid to undergo the helix–coil transition than the large ones.[20] CH and NH chemical shifts for the helix and coil states as well as the concentration of TFA attained in the middle point of the transition for the different polymers investigated in this work are compared in Table 2. The average midpoint is at 2.6% TFA with deviations being less than 0.4%. These results indicate that, unlike the case of poly(α-amino acid)s, the influence of the size and shape of the alkyl side chain on the helix–coil transition of poly(β-L-aspartate)s is almost negligible.

Table 2. Solvent-Induced Helix-Coil Transition Observed in Poly(β-L-aspartate)s

	δ_{CH} (ppm)		δ_{NH} (ppm)		
polymer	helix[a]	coil[b]	helix[a]	coil[b]	% TFA
PAALA-4	5.02	4.9			2.8
PAALA-6					2.8
PAALA-8					2.6
PAALA-12	5.04	4.84	8.61	7.93	2.4
PAALA-18					2.8
PAIPLA	5.00	4.82	8.56	7.96	2.6
PAIBLA					2.6
PAIALA					2.2
PACPLA	5.01	4.82	8.54	7.95	2.4
PACHLA	4.99	4.80	8.55	7.96	2.8

[a] In $CDCl_3$. [b] In TFA/$CDCl_3$ (3% v/v).

Thermally Induced Helix–Coil Transition. A second view of the helix–coil transition was provided by thermal experiments. We have used a mixture of TFA and $CDCl_3$ with a concentration close to that in which the helix–coil transition is observed (2% v/v) to study the effect of heating on the transition. The effect of the temperature in the ^{1}H NMR spectra of PAIALA is displayed in Figure 3. It is worth noting that both CH and NH signals move upfield when the sample is cooled from +40 to −5 °C, revealing that unfolding of the helix has taken place. The coil conformation predominates at low temperatures where the acid molecules may interact strongly with the amide groups. The inverse transition (coil to helix) was observed to occur upon heating within the same range of temperatures. Exactly the same behavior was found for PAALA-12 with both direct and inverse transitions taking place in the same range of temperatures. The thermally induced transition has been well-studied in poly(α-

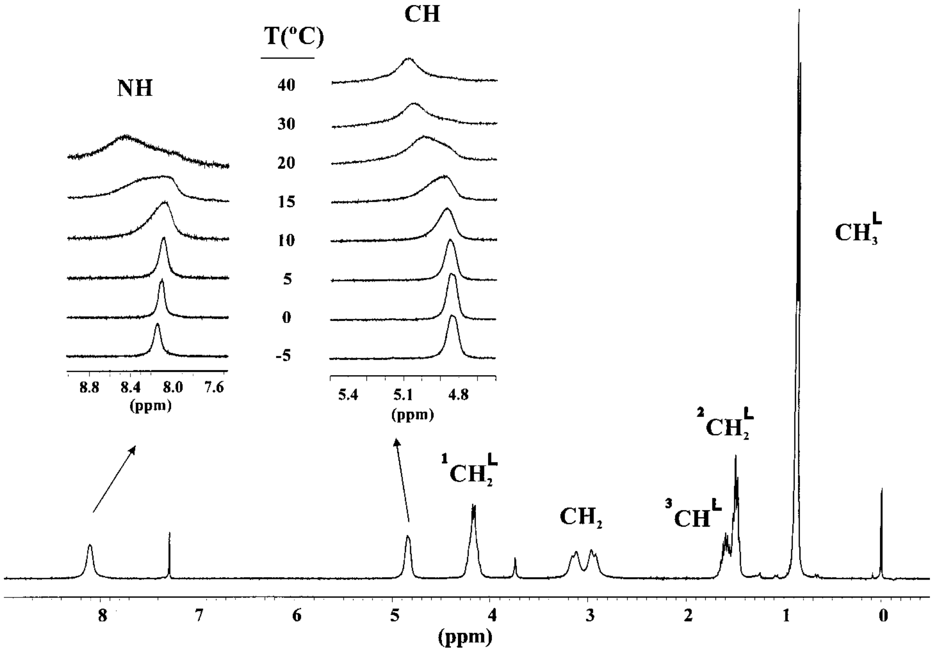

Figure 3. 300 MHz ^1H NMR spectrum of PAIALA in $CDCl_3$ containing 2% TFA (v/v) at 5 °C. Inset: displacement of the CH and NH signals with temperature. L indicates side chain protons.

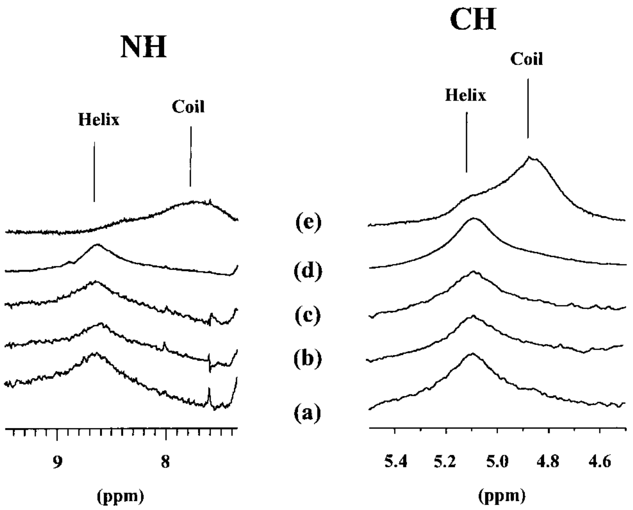

Figure 4. 300 MHz ^1H NMR spectra in $CDCl_3$ at 25 °C in the region of NH and CH of PAIBLA homopolymer (a), PAIB(D,L)A stereocopolymers in blocks with D/L ratios 1:1 (b), 1:3 (c), and 1:9 (d) and the random PAIB(D,L)A (1:1) stereocopolymer (e).

amino acid)s, and it can be understood by assuming that the gain in configurational entropy at helix disruption is offset by the loss of entropy caused by the solvation of the polymer in the coil state.[21]

Influence of the Enantiomeric Composition on the Helix−Coil Transition. The effect that the incorporation of D-units has on the conformational stability of a poly(β-L-aspartate) chain was also examined. For this purpose, we investigated the helix−coil transition in a series of poly[α-isobutyl-β-(D,L)-aspartate] stereocopolymers, abbreviated PAIB(D,L)A, with either a random or blocked microstructure and for different enantiomeric compositions. The same methodology used in the investigation of the optically pure compounds was applied in this case. In Figure 4, the ^1H NMR spectra of the homopolymer and the stereocopolymers recorded in pure $CDCl_3$ are compared. For the racemic PAB(D,L)A with a statistical distribution of D- and L-units, the chemical shifts of the CH and NH signals appear at 4.86 and 7.74 ppm, indicating the presence of the random-coil conformation. This result is in agreement with the expectation that the polymer is unable to adopt a helical conformation because the steric clashes between the side chains preclude the continuous formation of intramolecular hydrogen bonds. This also demonstrates that chemical shift differences observed upon the addition of acid or heating and cooling are due to changes in conformation and not to solvation effects. On the contrary, stereocopolymers with a microstructure in stereoblocks appear to be able to form whichever helical conformation is the enantiomeric composition. In this case, the amount of TFA required for the helix−coil transition was found to decrease steadily with the content in D-units to a limiting value of 2% for the racemic stereocopolymer. The chemical shifts and solvent compositions determined for the midpoints of the helix−coil transition for all the investigated stereocopolymers are compared in Table 3.

Table 3. Solvent-Induced Helix-Coil Transition Observed in the Stereocopolymers Poly(α-isobutyl-β-D,L-aspartate)s

D/L	δ_{CH} (ppm)		δ_{NH} (ppm)		
	helix[a]	coil[b]	helix[a]	coil [b]	% TFA
block					
0:1	5.09	4.88	8.63	7.95	2.6
1:9	5.08	4.88	8.60	7.95	2.6
1:3	5.09	4.87	8.63	7.94	2.4
1:1	5.09	4.87	8.64	7.95	2.0
random					
1:1		4.86[c]		7.90,7.80[c]	

[a] In $CDCl_3$. [b] In TFA/$CDCl_3$ (3% v/v). [c] Shift values in pure $CDCl_3$ independent of TFA concentration.

It is worthwhile to mention that two peaks appear for the CH in the region of TFA concentrations at which the transition takes place in PAIB(D,L)A block stereocopolymers. On the analogy of homopolymers, this fact should be attributed to the polydispersity of the stereoblock lengths in the copolymer chains. In this case however, a second reason may be given for the origin of the two peaks. It is fully reasonable to assume that

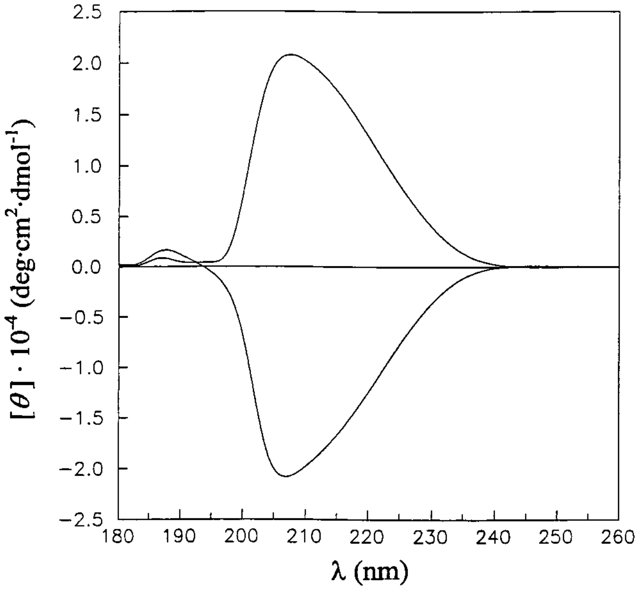

Figure 5. Circular dichroism traces for the D- and L-enantiomorphs of PAIBLA in trifluoroethanol solution.

unfolding of the helix must occur in the proximity of the points at which stereoblocks of opposite configuration are connected. With this picture in mind, two peaks should be expected for the polymer at any TFA concentrations below the coil transition point.

Circular Dichroism Spectra. The CD spectra of poly(β-L-aspartate)s in trifluoroethanol solution show a positive peak ($[\theta] \approx 2 \times 10^4$ deg cm^2 mol^{-1}) at 220 nm (Figure 5) consistent with the calculated spectrum for a right-handed $^{13}/_{4}$ helix,[22] which is the conformation most frequently observed in the solid state. As expected, mirror CD signatures consisting of a negative peak were obtained for the respective poly(β-D-aspartate)s enantiomorphs. Seebach and co-workers have reported similar CD patterns for β-hexa-[6] and β-heptapeptides[23] with an almost identical helix conformation in solution.

Figure 6a shows the variation of the ellipticity at 222 nm with the addition of TFA in the 0−15% range for PAIPLA. The curve exhibits a sudden fall in the vicinity of 3% (v/v), characteristic of helix−coil transition and coincident with the results obtained by NMR. A similar behavior was observed for the other poly(β-L-aspartate)s investigated. On the other hand, the variation of the CD spectra with temperature at a TFA concentration close to that required for the transition was studied parallel to the NMR work. Figure 6b shows the molar ellipticity of PAIALA at 220 nm as a function of the temperature with the polymer dissolved in $CHCl_3$ containing 2% (v/v) of TFA. A sharp decrease of the ellipticity is observed to take place at about 10−20 °C. This range of temperatures is in full agreement with that determined by ^{1}H NMR measurements on the same type of experiment.

Modeling Calculations. The Random-Coil State. Specific nonlocal interactions between residues may be presumed to be absent in the disordered state. Therefore, the random-coil state of the polymer can be modeled by considering only the local conformational preferences of its constituting residues, which in the simplest case will be represented by the minimum energy conformations of their potential energy surfaces. A model for the coil state of poly(β-L-aspartate)s has been built by predicting the conformational preferences of the α-methyl-β-L-aspartamyl dipeptide using quantum-mechanical calculations at the ab initio HF/6-31G(d) level. A total of 13 minima were characterized by frequency analysis. The conformational energies in bulk chloroform solution (ΔG_{conf}) were estimated by adding the free energy of solvation (ΔG_{sol}) to the gas-phase energies estimated by single-point calculations at the MP2/6-31G(d) level (ΔE).[14] Table 4 displays the conformational angles for those minima with a ΔG_{conf} lower than kcal/mol.

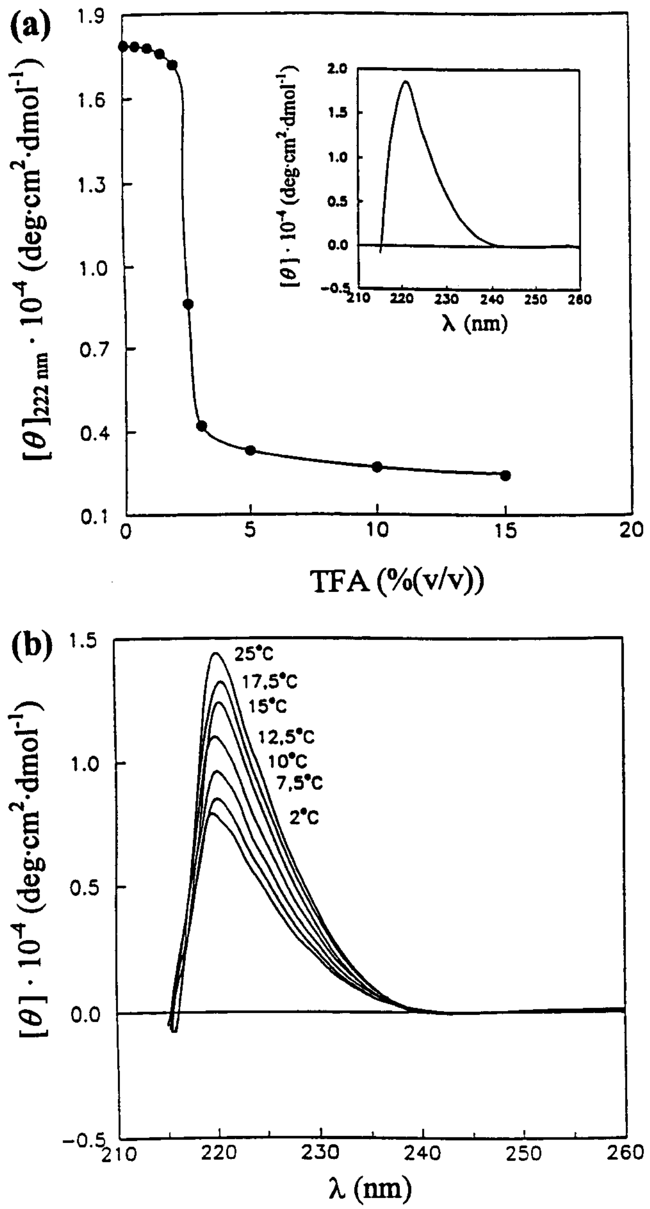

Figure 6. Ellipticity decay of PAIBLA upon addition of acid at 25 °C (a) and upon cooling a solution of the polymer in $CHCl_3$ with 2% of TFA (b).

The minimum energy conformations listed in Table 4 have been used to predict the $^3J_{\text{NH−CH}}$ coupling constant for poly(β-L-aspartate)s in the random-coil conformation according to the equation developed by Vuister and Bax for proteins.[24] This equation was explicitly developed for the dihedral angle φ of α-amino acids defined by the sequence C(=O)-N−C$^{\alpha}$−C(=O). We have defined a similar dihedral angle τ for the sequence C(=O)−N−C−COO of poly(β-L-aspartate)s. The values of τ for the characterized minima are included in Table 4. By considering a population analysis of the minima,

Table 4. Conformational Angles (deg) and Energies (kcal/mol) for the Eight Lower Minima Calculated for the α-Methyl-β-L-Aspartamyl Unit

#	φ	ξ	ι	χ_1	$\Delta G_{conf}{}^a$	τ^b
1	77.6	63.2	−149.6	−174.4	0.0	−156.2
2	161.4	62.7	121.5	169.8	1.5	−73.9
3	74.2	−136.1	58.9	171.4	1.9	−163.2
4	177.0	−56.9	−105.0	149.5	3.0	−63.8
5	−59.5	−52.0	114.2	−173.4	3.5	68.7
6	91.1	−52.5	−88.2	162.3	3.7	−148.3
7	−70.3	−156.4	−149.2	−150.9	3.9	58.8

[a] Conformational free energies in chloroform solution. [b] Dihedral angle τ defined by the sequence C(=O)−N−C−C(OO).

a $^3J_{NH-CH}$ coupling constant of 7.93 Hz was predicted. NMR experiments provide a $^3J_{NH-CH}$ coupling constant of 8.5 Hz for all of the poly(β-aspartate)s in the random-coil conformation, in good agreement with the predicted values.

The Helix Conformation. In an early work, we hypothesized on the basis of CD measurements that the helix adopted by poly(β-L-aspartate)s in solution should be close to the right-handed $^{13}/_4$ helix usually found in the solid state.[22] More recently, several small oligopeptides constituted by β-amino acids have been synthesized, and their structure has been studied by NMR spectroscopy and X-ray crystallography.[6,7,23] It is remarkable that in the three small β-oligopeptides reported in the literature the helical conformation in solution appears to be stabilized by i and $i + 3$ hydrogen bonds, which is the scheme known to happen in the $^{13}/_4$ helix of poly(β-L-aspartate)s.

Energy minimizations performed on polymer chains constituted by 30 residues led to a stable conformation retaining the initial hydrogen-bonding scheme of the $^{13}/_4$ helix. Figure 7 shows the axial and equatorial projection of the resulting helix for the case of PAIBLA. Multiple side-chain conformations capable of fitting into the same helical backbone are not expected to introduce significant differences in energy. Such a conformational search step, therefore, was not undertaken, because we need only an approximate description of the helix in solution. The resulting model is able to explain the upfield displacement experimentally observed for the CH backbone signal along the helix−coil transition. It was striking that the sign of such a shift is opposite to the downfield displacement observed for the same signal on the helix−coil transition in poly(α-peptide)s. Comparison between the respective helices reveals that such discrepancy arises from the differences in the backbone dihedral angles existing between the two types of helices. Thus, Wishart's equation[25] renders a $\Delta\delta$ of −0.25 ppm for the minimized helix of poly(β-L-aspartate)s in which $\varphi = 156°$. This value is in excellent agreement with the experimental one ($\Delta\delta = -0.2$ ppm). However, positive values close to 0.2 ppm were predicted when the same equation was applied to the right-handed α-helix in which φ takes values close to −60°.

Concluding Remarks

In this paper, the helix−coil transition typical of poly(α-peptide)s and proteins is investigated in poly(β-peptide)s in relation to their chemical constitution and configuration. Main conclusions drawn from this study are the following:

The helix−coil transition taking place in poly(α-alkyl-β-L-aspartate)s has been fully characterized and experimental results have been provided to evidence that the

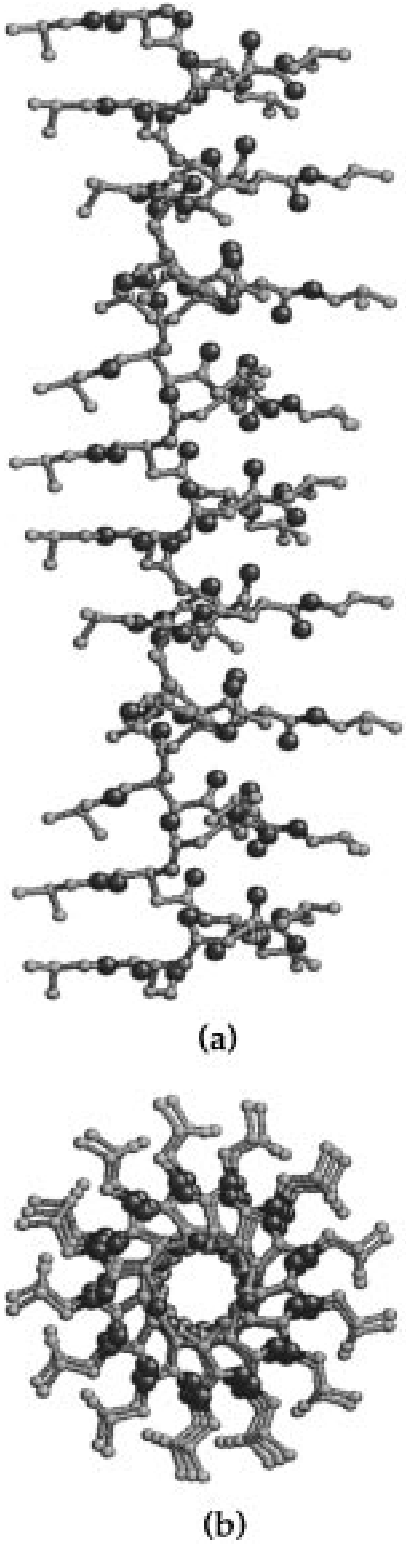

Figure 7. Axial (a) and equatorial (b) projections for the minimized helix of PAIBLA in solution.

phenomenon is common to the whole family of these poly(β-peptide)s. The overall response of the system to external stimuli, such as solvent effect or temperature, fits well in the pattern characteristically observed for poly(α-peptide)s. Differences found in the sign of the displacement of NMR signals are duly accounted for by differences in the conformational parameters of the respective helices.

The influence of the constitution of the alkyl side chain on the stability of the $^{13}/_4$ helix of poly(β-L-aspartate)s appears to be negligible. This is a striking result that strongly contrasts with the behavior described for poly(α-peptide)s. However, the stability of the helix was found to depend critically on the stereochemistry of the polymer. Whereas no ordered structure is detected for D,L-stereocopolymers with a random microstructure, those consisting of configurationally homogeneous sequences display helix−coil transitions at conditions that depend upon the length of the stereoblocks.

The α-helix-random-coil transition occurring in proteins and synthetic poly(peptide)s is a phenomenon of exceptional relevance. Poly(β-L-aspartate)s constitute the first system other than poly(α-amino acid)s for which such a transition is observed. Hopefully, discovery of α-helixlike structures in poly(β-peptide)s and more recently in oligo-β-peptides will increase rapidly interest in these systems. The conformational transition characterized in poly(β-aspartate)s will be then a useful reference for further research in this field.

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