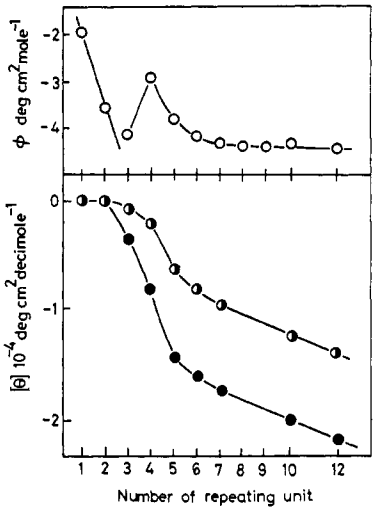

Figure 6. Change of the total molar rotation at the sodium D line and the ellipticities of the CD bands at 207 and 222 nm with the repeating unit in TFE: (○) total molar rotation; (◑) ellipticity of the band at 222 nm; (●) ellipticity of the band at 207 nm.

clude that the onset of helicity begins at the 12-peptide for this series of peptides. Another interesting fact is that the total molar rotation increases once at the 12-peptide (n = 4) and decreases again gradually to reach a constant value at the 21-peptide (n = 7). In accordance with this change of the total molar rotation, the ellipticities of the bands at 207 and 222 nm decrease once greatly to the 15-peptide (n = 5) and then decrease gradually from n = 5 to n = 7 followed by a constant decreasing at the 21-peptide. We think that the constant value of the total molar rotation and the constant decreasing of the ellipticities of the bands at 207 and 222 nm at the 21-peptide suggest the formation of a stable α-helical conformation at this peptide length. It should be noted that the 21-peptide is the critical peptide length for the formation of the α helix in the solid state obtained by the slow phase transformation.

In conclusion, we deduce a relationship between the conformations of these peptides in solution and those in the solid state. Though the α helix is first formed at the 12-peptide in TFE, it is not stable enough to be maintained during the phase transformation to the solid state. The α helix is more stabilized in a longer peptide, the 15-peptide (n = 5), as is shown by increasing ellipticity of the band at 222 nm. At this peptide length the α helix is maintained during the rapid phase transformation to the solid state during reprecipitation. However, the α helix at this peptide length is still not stable enough to be maintained and transformation to the β structure occurs during the slow phase transformation via a concentrated solution state. Finally, an α helix which is stable enough to be maintained even in the slow phase transformation process is formed at the 21-peptide.

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Comparative Conformational Studies of Polypeptides Containing a High Percentage of Proline

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ABSTRACT: In the search for models of a proline-rich protein isolated from human parotid saliva we were led to synthesize and study H-[Gly-(Pro)$_x$]$_n$-OH (x = 3, 4). Preliminary results concerning these polypeptides have already been reported[1] and suggest that in aqueous solution these peptides adopt a polyproline II (PPII) conformation in addition to some other structures (probably unordered). In this paper we present a new synthesis of these products and a more complete conformational study comparing the relative stabilities of the PPII helix adopted by these polymers.

Synthesis

The thermal polycondensation method previously set forth by Goodman et al.[2] is reported to be rapid when compared to solution techniques and to give higher yields and molecular weights.[3] The "monomers" used in the work reported here, H-Gly-(Pro)$_x$-OC$_6$H$_5$Cl$_5$·HCl (x = 3, 4), have already been described.[1] To carry out the thermal polycondensation they are mixed with a known quantity of Celite and desiccated by repeated evaporation in vacuo of a mixture of DMF and dioxane (1:3 v/v). Then they are heated in vacuo for 5 h at 40 °C and 3 days at 125 °C, extracted with acetic acid (5% in aqueous solution), and

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dialyzed against water. Molecular weights are approximately 5000 in each case as determined by gel filtration on a previously calibrated Bio-Gel P10 column (50.0 × 2.2 cm), using water as eluent. The amino acid composition of a sample hydrolyzed for 24 h was $Pro_{2.92}Gly_{1.05}$ for H-[Gly-(Pro)$_3$]$_n$-OH and $Pro_{3.95}Gly_{1.07}$ for H-[Gly-(Pro)$_4$]$_n$-OH.

The yields are 18% for H-[Gly-(Pro)$_3$]$_n$-OH and 15% for H-[Gly-(Pro)$_4$]$_n$-OH. Though they are rather low, they are twice as good as when working in DMF with triethylamine. The low yields previously obtained by us when using solution polycondensation can be explained by the precipitation of the polymers during the reaction, thus preventing these molecules from reacting further. On the other hand, in the case of thermal polycondensation Celite enhances removal of the pentachlorophenol during reaction, which enables higher yields for polypeptides. However, our results are still lower than the values published by Goodman in the case of poly(tripeptides). This can be attributed to the length of our "monomers", since it is known that tripeptide esters polymerize readily by thermal condensation whereas this is not the case for tetrapeptide esters.[4] These low yields can also be explained by the sequence of our "monomers": proline is a bulky molecule and its presence as a carboxyl terminal amino acid can perturb the polymerization process. However, molecular weights for H-[Gly-(Pro)$_x$]$_n$-OH (x = 3, 4) obtained either by polycondensation in solution or by thermal polymerization on a matrix were of the same order of magnitude, thus allowing us to compare the results obtained by ^{13}C NMR with the ones obtained by circular dichroism and ^{1}H NMR.

^{1}H NMR Study

This study was conducted on a Cameca NMR spectrometer operating at 250 MHz in the FT mode. Solution concentrations in D_2O were 10 mg/mL and spectra were recorded at room temperature. Chemical shifts are given in ppm relative to internal DSS.

It has been shown[5] that in D_2O at 22 °C the resonances of the α protons of prolyl residues are located either at 4.40 ppm in the case of polyproline I (Pro-Pro bonds cis) or at 4.70 ppm in the case of polyproline II (Pro-Pro bonds trans). Some differences are also visible in the δ-proton region of the spectra: PPII is characterized by two peaks (3.60 and 3.80, respectively) whereas PPI gives only a rather broad resonance located at 3.60 ppm. Moreover, the destruction of a polyproline conformation by an added salt, such as calcium chloride, to the solution can be easily followed by NMR,[5] the main changes being seen in the α- and δ-proton regions. Therefore, we undertook this ^{1}H NMR study in order to ascertain whether or not a PPII conformation was adopted by our polymers in D_2O.

Figure 1 presents the region of the spectrum corresponding to the α-Gly and δ-Pro protons recorded for H-[Gly-(Pro)$_3$]$_n$-OH in D_2O and shows its evolution with added $CaCl_2$. If we consider the α-proton region we can distinguish an AB spectrum centered at 4.08 ppm. Its position was attributed to a trans Gly-Pro bond.[1,6] The resonance corresponding to the other Gly residues overlaps those of the δ-Pro residues, as shown by normalization of peak areas. On the other hand, the δ-Pro proton region presents two broad peaks just as polyproline II does. However, adding $CaCl_2$ to the solution leads to some changes in the spectrum. The main difference observed concerns the AB spectrum attributed to the Gly residues, which becomes narrower whereas the ^2J coupling constant does not vary. Some differences can be seen in the δ-Pro region too: the area of the high-field δ resonance becomes larger than that corresponding to the downfield δ resonance, which can be used as a probe of the appearance of cis X-Pro bonds.[5] These changes can be interpreted as reflecting the PPII → unordered transition.

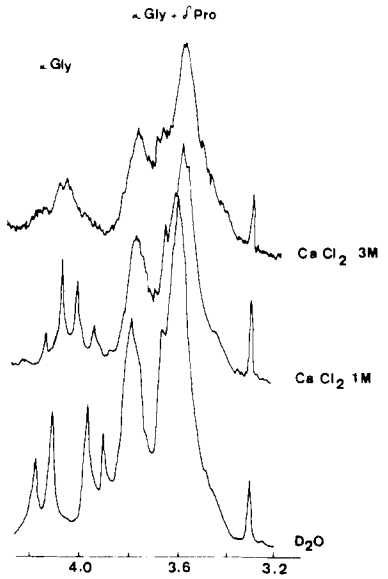

Figure 1. 250-MHz ^{1}H NMR spectra in D_2O and $CaCl_2$ of H-[Gly-(Pro)$_3$]$_n$-OH: α-Gly and δ-Pro proton region.

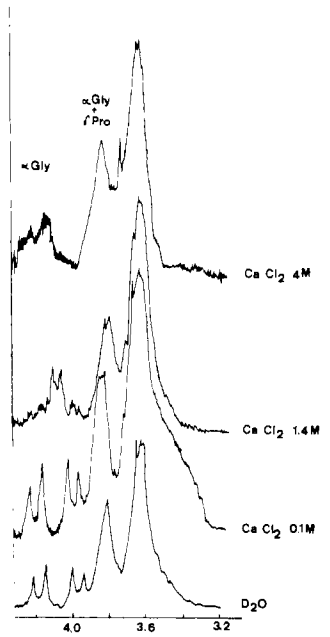

Figure 2. 250-MHz ^{1}H NMR spectra in D_2O and $CaCl_2$ of H-[Gly-(Pro)$_4$]$_n$-OH: α-Gly and δ-Pro proton region.

The ^{1}H NMR spectrum recorded for H-[Gly-(Pro)$_4$]$_n$-OH in D_2O and its evolution with added $CaCl_2$ are presented in Figure 2. These spectra are not very different from the ones observed for H-[Gly-(Pro)$_3$]$_n$-OH. The evolution of the spectrum with added $CaCl_2$ is quite the same in both cases, which leads us to think that the two synthetic polypeptides have nearly the same conformational stability.

^{13}C NMR Study

The spectra were recorded at room temperature on a Perkin-Elmer R32 pulse FT spectrometer operating at 22.63 MHz. The polymers were dissolved in D_2O at a concentration of 40 mg/mL. Chemical shifts are given in ppm relative to external Me_4Si.

Table I
^{13}C NMR Chemical Shifts Obtained for H-[Gly-(Pro)$_x$]$_n$-OH (x = 3, 4) in D_2O and $CaCl_2$ Solutions[a]

	H-[Gly-(Pro)$_3$]$_n$-OH					H-[Gly-(Pro)$_4$]$_n$-OH						
		$CaCl_2$					$CaCl_2$				PPI[b]	PPII[b]
assignment	D_2O	0.1 M	1 M	2 M	4 M	D_2O	0.1 M	1 M	2 M	4 M	D_2O	D_2O
C_γ Pro				23.7	24.0	24.2			23.2	23.5	23.2	
C_γ Pro	25.8	25.9	26.0	26.4	26.6	26.0	26.1	25.9	26.1	26.4		25.5
C_β Pro	29.3	29.4	29.5	29.8	30.0	29.5	29.4	29.4 29.3	29.6 29.7	29.8		28.9
C_β Pro	30.6	30.7	30.8	31.0 31.2	31.2	30.8		30.9	30.7		31.8	
C_α Gly	42.7	42.8	43.0	43.7	43.7	43.2	42.8	43.3	42.6	43.2		
C_δ Pro	48.2	48.5	48.6	49.0		48.2						
C_δ Pro	48.9	49.0	49.2	49.3 49.5	49.9	49.1	49.0	49.1	49.3	49.2	48.6	48.6
C_α Pro	59.9	60.0	60.2	60.6	61.2	59.5	59.8				59.5	59.5
C_α Pro	61.6	61.7	62.0	62.3	62.5 63.7	60.1	60.0	60.0	60.2	60.1		
C_α Pro						61.9		61.8	61.9	62.2		
C=O Gly	169.7	169.8	169.9	170.2	170.4	169.8	169.8	169.7	169.8	170.2		
C=O Pro	173.0	173.1	173.2	173.4		173.1	173.2	173.1	173.2	173.0	172.7	172.7
C=O	173.5	173.6	173.7	173.9		173.7	173.4	173.7	173.6	173.4		
C=O Pro	175.6	175.7	175.8	176.0	176.1	175.8	176.0	175.9	175.8	175.9		

[a] External Me_4Si reference. [b] Reference 7.

^{13}C NMR spectroscopy has been shown to be the method of choice for distinguishing between cis and trans X-Pro bonds.[7] The values of C_β and C_γ chemical shifts are very different in the case of cis or trans bonds: 31.8 (C_β) and 23.2 ppm (C_γ) in the case of polyproline I; 28.9 (C_β) and 25.5 ppm (C_γ) in the case of polyproline II. Bovey et al.[8] used this difference in chemical shifts to study the influence of $CaCl_2$ in disrupting the PPII conformation. Upon adding this salt, they observed an increase of the relative intensities C_β cis/C_β trans and C_γ cis/C_γ trans and a broadening of the peaks due to the higher viscosity of the solution. They interpreted these changes in the spectra as reflecting a transition from PPII to an unordered structure.

We too studied the evolution of the ^{13}C NMR spectra recorded for H-[Gly-(Pro)$_x$]$_n$-OH (x = 3, 4) when salt was added to the solution. Figure 3 shows the spectra obtained for the two polypeptides in D_2O solution. They look nearly the same; chemical shifts are given in Table I (assignments were made by comparison with previous studies).[7,9] The positions of some peaks are very near the ones observed by Bovey[7] in the case of polyproline II. Therefore we may conclude from this point alone that a high percentage of each polypeptide adopts a PPII conformation. To confirm this hypothesis, we recorded the evolution of the spectra with added $CaCl_2$. We observed an increase of the bands characteristic of cis C_β and C_γ and a broadening of the peaks. The evolution of the spectra is the same as that observed by Bovey et al.[8] for the case of polyproline II. The values of the chemical shifts for both polypeptides are reported in Table I and show that, even in concentrated salt solution, no polyproline I conformation is present. Instead, the addition of $CaCl_2$ leads to a polyproline II → unordered structure transition.

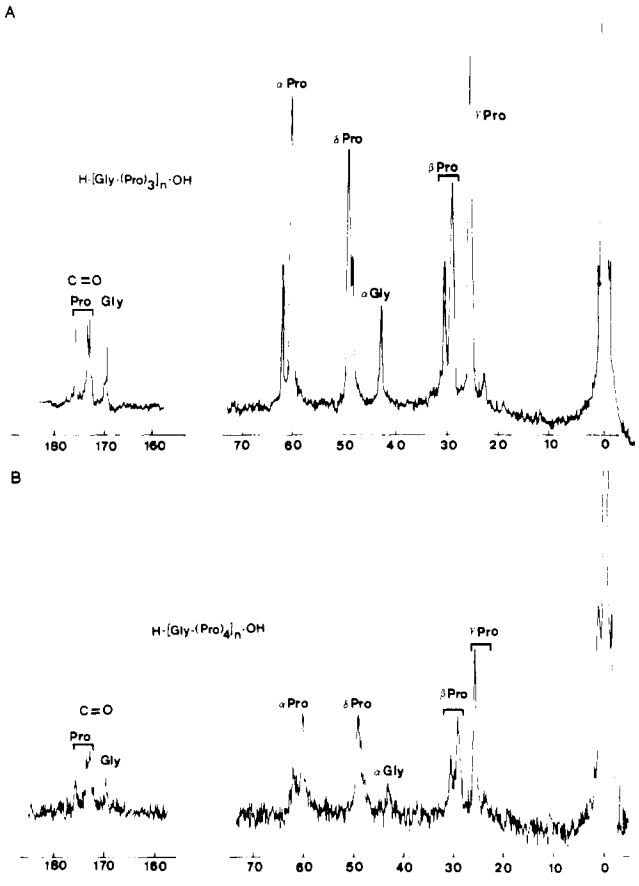

Figure 3. 22.63-MHz ^{13}C NMR spectra in D_2O of (A) H-[Gly-(Pro)$_3$]$_n$-OH and (B) H-[Gly-(Pro)$_4$]$_n$-OH.

Discussion

Our preliminary results[1] led to the conclusion that our products adopt mainly a polyproline II conformation together with some other unelucidated structures. Therefore we had to use ^{13}C NMR spectroscopy in order to obtain further, more precise conformational information on these polymers. This technique has recently[10] been used for studying small linear oligoprolines and their conformational evolution when adding concentrated $CaCl_2$ to aqueous solutions of these products. Chao and Bersohn[10] concluded from their work that a high salt concentration ($CaCl_2 \geq 4$ M) induces a conformational randomization, the percentage of trans Pro-Pro isomers being nearly the same (0.7–0.8) for the short-chain oligomers as for polyproline. The spectra presented here in Figure 3 look nearly the same for both polymers and lead us to think that a high percentage of each polypeptide adopts a PPII conformation (85% trans Gly-Pro bonds in H-[Gly-(Pro)$_3$]$_n$-OH and 75% trans Gly-Pro bonds in H-[Gly-(Pro)$_4$]$_n$-OH from ^1H NMR measurements; 92% and 88%, respectively, from ^{13}C

NMR estimations). This conclusion was essentially drawn from the fact that the chemical shifts exhibited by our polypeptides are not very different from the ones observed by Bovey[7] in the case of polyproline II. Comparing the evolution of chemical shifts with added $CaCl_2$ for our polymers in D_2O (Table I) shows that this variation is not so important for H-[Gly-(Pro)$_4$]$_n$-OH as for H-[Gly-(Pro)$_3$]$_n$-OH. This fact leads us to think that the PPII helix of H-[Gly-(Pro)$_4$]$_n$-OH is more stable than that of H-[Gly-(Pro)$_3$]$_n$-OH. However, the percentage of trans X-Pro isomers obtained in 4 M $CaCl_2$ in both cases is of the same order of magnitude as that observed by Chao and Bersohn (75% in H-[Gly-(Pro)$_3$]$_n$-OH and 68% in H-[Gly-(Pro)$_4$]$_n$-OH from ^{13}C NMR measurements). Therefore the evolution of spectra with added $CaCl_2$ can be interpreted as reflecting the formation of random sequences of cis and trans peptide bonds in concentrated salt solutions, though the mechanism of such an interconversion is still discussed. On the other hand, we are still unable to propose any hypothesis concerning the other structures adopted by those polymers. Even further CD study accompanying the evolution of spectra with pH as observed by ^{13}C NMR spectroscopy is no help in determining these structures. The only conclusion we can draw from this study is that a small percentage of cis X-Pro (X = Gly or Pro) bonds is present in both polypeptides when dissolved in D_2O (nearly 10%). However, our ^{13}C NMR studies indicate the existence of PPII structures adopted by both polypeptides, this conformation being more stable in the case of H-[Gly-(Pro)$_4$]$_n$-OH.

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Polyelectrolyte Complexes: Interaction between Poly(L-lysine) and Polyanions with Various Charge Densities and Degrees of Polymerization

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ABSTRACT: Ionic polymer–polymer interaction was studied in dilute aqueous solutions of poly(L-lysine) hydrobromide [(Lys,HBr)$_m$] and (1) a series of poly(L-glutamic acid) oligomers [(Glu,ONa)$_n$] and (2) two polysaccharides, poly(galacturonic acid) and κ-carrageenan. The charge density and the nature of charged sites were taken as variables. The polyanions were all of the sodium form. With the (Glu,ONa)$_n$ series, a neutral 1/1 complex was obtained as soon as the degree of polymerization was equal to 6 and an ordered β structure was formed for DP ≥ 20. With both polysaccharides considered, a stable 1/1 complex was also formed, but the first polyanion induced an α-helical conformation of (Lys,HBr)$_m$ in the complex whereas the second did not modify the initial conformation of the (Lys,HBr)$_m$.

Introduction

In a previous paper[1] we discussed the formation of complexes obtained when (Lys,HBr)$_m$ and (Glu,ONa)$_n$ (with degree of polymerization n = 30) were mixed in dilute aqueous solution. We particularly emphasized the stoichiometry and conformational changes resulting from the variation of the composition for several degrees of neutralization α' of this low molecular weight polymer of glutamic acid.

In order to complete this study, it seemed interesting to consider the influence of the chain length n of (Glu,ONa)$_n$ on the stoichiometry and conformation of the complex and, in particular, to determine the critical size necessary to form the β structure (observed with a degree of polymerization n = 30). The study of the influence of the nature of the polyanion on complex formation also appeared to us to be important.

In this work we study the interaction of (Lys,HBr)$_m$ first with (Glu,ONa)$_n$ of degrees of polymerization (DP) 1, 3, 5, 6, 10, 16, and 23 and secondly with two different polysaccharides, a poly(sodium galacturonate) with $\overline{DP}$ = 50 and a κ-carrageenan sodium salt with $\overline{DP}$ = 440. The interaction of these polyelectrolytes with (Lys,HBr)$_m$ has not yet been studied by others. The interaction of poly(Lys,HBr) with other polyanions has been extensively studied recently.[2-9]

Experimental Section

Materials. (Lys,HBr)$_m$ was purchased from Pilot (lot L-112) ($\bar{M}_w \sim 100000$; $m \sim 478$). It was purified as previously described.[1] Poly(L-glutamic acid) oligomers were well-fractionated samples with degrees of polymerization 1, 3, 5, 6, 10, 16, and 23, previously obtained by ion-exchange chromatography except for the monomer, which was end-protected by synthesis.[10] They were all

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