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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neutralized with sodium hydroxide so as to obtain the sodium salt form with a neutralization degree $\alpha' = 1$, as previously described.[1] Poly(sodium galacturonate) with $\overline{DP}_n \sim 50$ and $\alpha' = 1$ (PG_{50}) was a fraction of a polymer purchased from Nutritional Biochemicals Corp. and purified in our laboratory.[11] The κ-carrageenan sodium salt ($\alpha' = 1$) was supplied by Sigma and purified[12] ($\bar{M}_w \sim 180\,000$; $\overline{DP}_w \sim 440$).

All solutions were prepared at a concentration 2×10^{-2} equiv·L^{-1} as previously described[1] except for the carrageenan which gives solutions too viscous to be prepared at high concentrations and thus was prepared only at 5×10^{-3} equiv·L^{-1}.

Conductimetry and Potentiometry. All conductimetric and potentiometric measurements were performed in a thermostated cell at 25 ± 1 °C containing initially 40 cm^3 of a 10^{-4} equiv·L^{-1} solution of one polymer. A solution of 2×10^{-2} equiv·L^{-1} of the other polymer was then added stepwise in order to vary the value of ρ (ρ is the ratio between total concentration, expressed in equiv/L, of [Lys] residues and [P], where P is the polyanion considered; $\rho = [\mathrm{Lys}]/[\mathrm{P}]$ when $(\mathrm{Lys,HBr})_m$ is added to polyanion and $\rho = [\mathrm{P}]/[\mathrm{Lys}]$ for the reverse sequence of addition).

Measurements of Br$^-$ ion activity were made with a Sargent potentiometer equipped with a set of Orion electrodes, one of which was a double-junction electrode and the other specific for Br$^-$. Specific conductivities were obtained by means of a conductivity cell adapted to a Tacussel CD 78 conductimeter.

Circular Dichroism Measurements. CD spectra were recorded with a Jobin-Yvon Mark III dichrograph at room temperature, using a 1-mm quartz cell in the region of the characteristic absorption bands of the amide groups, between 190 and 250 nm. The difference between the extinction coefficients for left- and right-handed circularly polarized light, $\Delta\epsilon$, was normalized to a 2×10^{-4} equiv·L^{-1} concentration of peptide residues. ρ was varied by mixing variable amounts of two solutions of both polymers.

Results and Discussion

When $(\mathrm{Lys,HBr})_m$ is added to a polyanion sodium salt, the mechanism of interaction can be schematically described, as previously proposed,[1] by

$$\begin{gathered} \mathrm{P^-Na^+ + P'^+Br^- \rightleftharpoons PP' + Na^+Br^-} \\ (1 - \rho x) + \rho(1 - x) \rightleftharpoons \rho x + \rho x \end{gathered} \qquad (1)$$

in which x is the degree of complexation. When the complex is neutral with a stoichiometry 1/1, as when we use $(\mathrm{Glu,ONa})_{30}$, the reaction is not governed by a classical equilibrium law but is always complete ($x = 1$).[1] The complex is uncharged and adopts a β-pleated sheet structure. We now are interested in studying the influence of the chain length and the nature of the polyanion on reaction 1. We limit our study to conductivity, Br$^-$ activity, and CD measurements.

1. Conductimetric Study. Figures 1 and 2 give the variations in the specific conductivities when $(\mathrm{Lys,HBr})_m$ is progressively added to $(\mathrm{Glu,ONa})_n$ = 1, 3, 5, and 6, to $(\mathrm{PG})_{50}$, and to κ-carrageenan sodium salt. The reverse sequence of addition is also shown and for each case there is an intercept located at $\rho = 1$, indicating an equivalent mechanism for both modes of addition.

A. (Glu,ONa) Monomer. For (Glu,ONa) monomer, the values of the conductivity are still higher than those expected from a simple additivity relationship (dotted line in Figure 1). For this case we observe an exchange of counterions in the condensed fraction of Br$^-$ counterions on $(\mathrm{Lys,HBr})_m$. Thus, for each ρ value, when equilibrium is attained, the measured specific conductivity (χ) can be expressed as

$$\chi/KC_\mathrm{P} = (\lambda_\mathrm{P} + \lambda_\mathrm{Na})(1 - x_\rho) + (\lambda_\mathrm{P}' + \lambda_\mathrm{Br})\rho\Phi_\mathrm{Br} + (\lambda_\mathrm{Na} + \lambda_\mathrm{Br})x_\rho \qquad (2)$$

where λ_P, λ_Br, λ_Na, and λ_P' are the limiting equivalent conductivities of the different ions considered, Φ_Br is the free fraction of ions of $(\mathrm{Lys,HBr})_m$ ($\Phi_\mathrm{Na} = 1$ for the monomer), x_ρ is the fraction of P$^-$ ions exchanged with Br$^-$ condensed for a given ρ value, K is a constant including experimental parameters, and C_P is the concentration of the polyanion (equiv·L^{-1}). Considering now a simple additivity when no reaction occurs, χ/KC_P becomes

$$\chi'/KC_\mathrm{P} = (\lambda_\mathrm{P} + \lambda_\mathrm{Na}) + (\lambda_\mathrm{P}' + \lambda_\mathrm{Br})\rho\Phi_\mathrm{Br} \qquad (3)$$

and the difference between χ and χ' gives

$$(\chi - \chi')/KC_\mathrm{P} = x_\rho(\lambda_\mathrm{Br} - \lambda_\mathrm{P}) \qquad (4)$$

Relation 4 explains quite well the conductimetric behavior when only exchange of counterions occurs. Such a situation exists only for the monomer, suggesting that complexation is predominant in the other cases. For $\rho = 1$, assuming λ_P 31 Ω^{-1}·equiv^{-1}·cm^2 and λ_Br 78 Ω^{-1}·equiv^{-1}·cm^2, we find $x_\rho \simeq 0.20 \pm 0.03$.

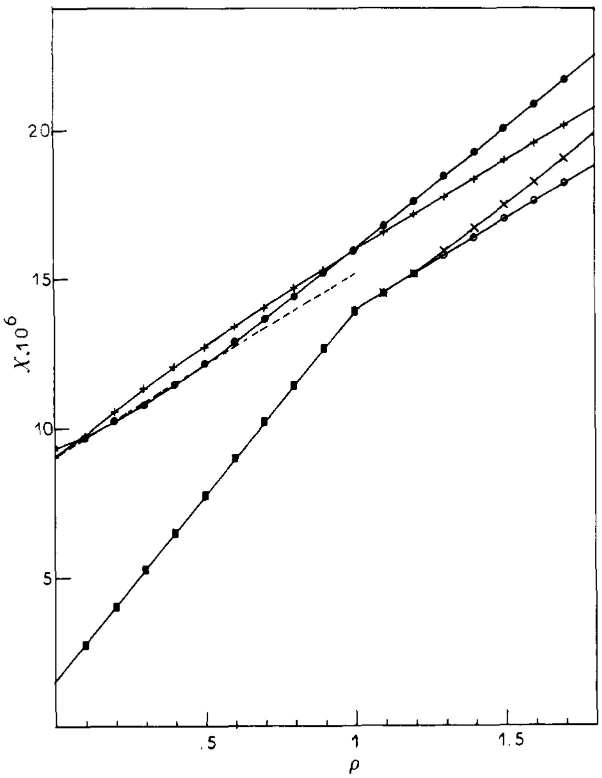

Figure 1. Variation in the conductance for additions of $(\mathrm{Lys,HBr})_m$ to 10^{-4} equiv·L^{-1} (Glu,ONa) (+), (Glu,ONa) to 10^{-4} equiv·L^{-1} $(\mathrm{Lys,HBr})_m$ (○), NaBr to pure water (concentration = $\rho \times 10^{-4}$ equiv·L^{-1}) (■), and (Glu,ONa) (×) and $(\mathrm{Lys,HBr})_m$ (○) to 10^{-4} equiv·L^{-1} NaBr (concentration = $(\rho - 1) \times 10^{-4}$ equiv·L^{-1}). Simple additivity of $(\mathrm{Lys,HBr})_m$ to 10^{-4} equiv·L^{-1} (Glu,ONa) (- - -).

B. Other Polyanions. When $\rho = 1$, the difference in conductivity between the experimental values for the intercept and values for addition of NaBr in pure water at the same concentration C_P (corresponding to complete complexation) decreases progressively with increasing DP and becomes zero with $(\mathrm{Glu,ONa})_6$ (Figure 2C). This shows that the complex is formed with a 1/1 stoichiometry and uncharged as soon as the DP reaches 6 (thus corresponding to $x = 1$). Another proof of the 1/1 stoichiometry is that for DP ≥ 6 no change occurs upon further addition of $(\mathrm{Lys,HBr})_m$ or polyanion above $\rho = 1$, considering that the curves (Figure 2) are quite equivalent to those obtained when the considered polyion is added to NaBr with concentration C_P. Furthermore, when the complex is uncharged (in the situation when we add a polyanion to $(\mathrm{Lys,HBr})_m$), for each $\rho < 1$, the conductivity of the solution must only correspond to that of residual polycation

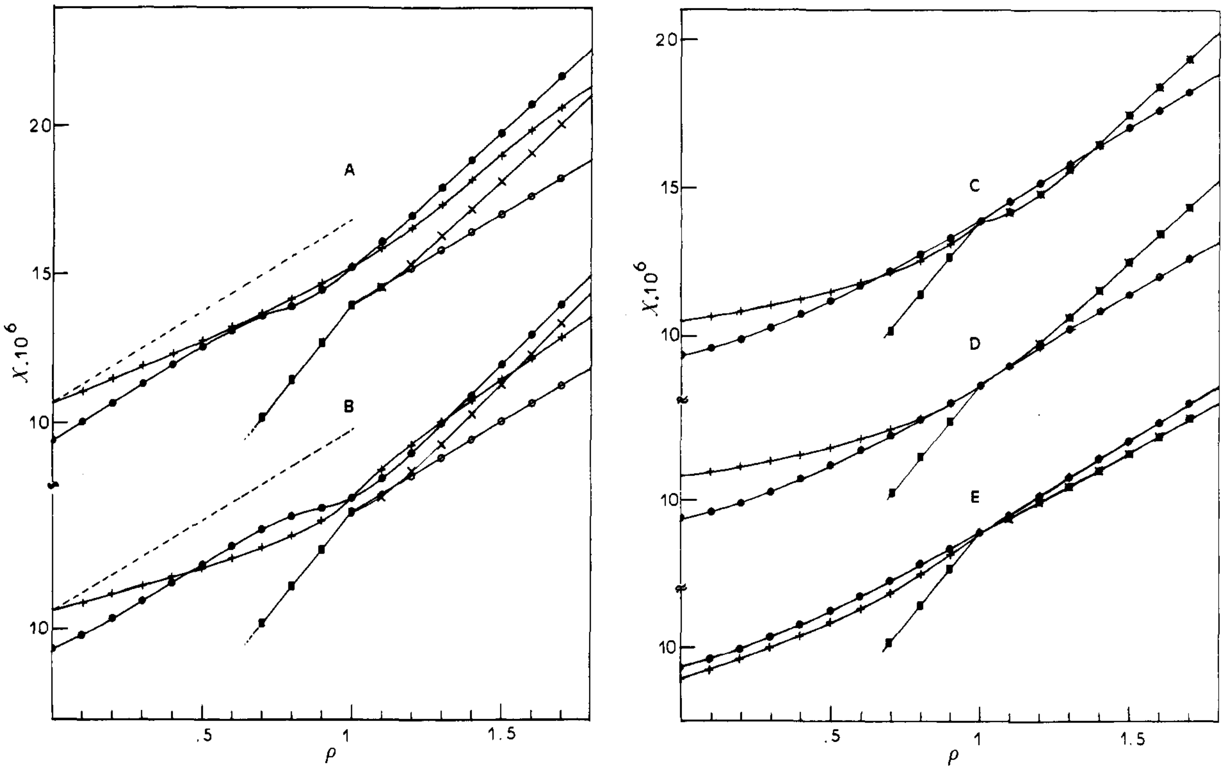

Figure 2. Variation in the conductance for additions of $(\mathrm{Lys,HBr})_m$ to 10^{-4} equiv·L $(\mathrm{Glu,ONa})_n$ with $n = 3$ (A), $n = 5$ (B), and $n = 6$ (C), to PG_{50} (D), and to κ-carrageenan (E) (+), polyanions A–E to 10^{-4} equiv·L $(\mathrm{Lys,HBr})_m$ (●), NaBr to pure water (concentration $= \rho \times 10^{-4}$ equiv·L^{-1}), and polyanions A–E (×) and $(\mathrm{Lys,HBr})_m$ (○) to 10^{-4} equiv·L NaBr (concentration $= (\rho - 1) \times 10^{-4}$ equiv·L^{-1}). Additivity of $(\mathrm{Lys,HBr})_m$ to the polyanion considered to be 10^{-4} equiv·L^{-1}.

$(1 - \rho)$ and to NaBr (ρ) released by complex formation and, as a consequence, must be independent of the specific conductance of the polyanion considered. This result is obtained for $(\mathrm{Glu,ONa})_n$ with $n \geq 6$ and with both other polyanions investigated if we notice that for these cases the conductimetric curves are all superposable (compare curves C, D, and E in Figure 2).

Considering relation 1, the specific conductivity of the solution, assuming additivity of conductivities of free ionic species, can be expressed as follows for addition of $(\mathrm{Lys,HBr})_m$:

$$\chi/KC_P = (\lambda_P + \lambda_{Na})\Phi_{Na}(1 - \rho x) + (\lambda_P' + \lambda_{Br})\Phi_{Br}\rho(1 - x) + (\lambda_{Na} + \lambda_{Br})\rho x \quad (5)$$

If no complex is formed at all $(x = 0)$ (corresponding to dotted lines in Figure 2 for polymers A and B), the additivity of the conductivities leads to

$$\chi_0/KC_P = (\lambda_P + \lambda_{Na})\Phi_{Na} + (\lambda_P' + \lambda_{Br})\Phi_{Br}\rho \quad (6)$$

On the other hand, when total complexation occurs $(x = 1)$, we have

$$\chi_1/KC_P = (\lambda_P + \lambda_{Na})\Phi_{Na}(1 - \rho) + \rho(\lambda_{Na} + \lambda_{Br}) \quad (7)$$

For $\rho = 1$, relation 7 becomes equivalent to the specific conductivity corresponding to a NaBr solution at the same concentration C_P. Considering relations 5–7, we have

$$[(\chi - \chi_1)/KC_P]_{\rho=1} = (1 - x)[(\lambda_P + \lambda_{Na})\Phi_{Na} + (\lambda_P' + \lambda_{Br})\Phi_{Br} - (\lambda_{Na} + \lambda_{Br})] \quad (8)$$

and

$$[(\chi - \chi_1)/KC_P]_{\rho=1} = [(1 - x)/KC_P](\chi_0 - \chi_1)$$

For each polymer at $\rho = 1$, the conductivity χ allows one to deduce the degree of complexation x by taking as a reference the conductivities χ_0 and χ_1 (Figure 2). We find 0.55 and 0.85 for the fraction of $(\mathrm{Glu,ONa})_n$ complexed with $n = 3$ and 5, respectively. In fact we have assumed that Φ_{Na} and Φ_{Br} are independent of ρ. This is certainly not true since, for these cases, the complex is charged. A more rigorous treatment would require the determination of their variation with ρ.

For a reverse sequence of addition, starting from a solution of $(\mathrm{Lys,HBr})_m$, equation 7 becomes

$$\chi_1'/KC_P = (\lambda_P' + \lambda_{Br})\Phi_{Br}(1 - \rho) + \rho(\lambda_{Na} + \lambda_{Br}) \quad (7')$$

As Φ_{Br} is constant for a total complexation, this relation explains quite well the appearance of a conductimetric curve independent of the polyanion added.

Above $\rho = 1$ two situations must be considered according to the sequence of addition.

(1) If an excess of polyanion is added to $(\mathrm{Lys,HBr})_m$, the conductimetric curves can be described as

$$\chi/KC_P = (\lambda_P + \lambda_{Na})\Phi_{Na}(\rho - x) + (\lambda_P' + \lambda_{Br})\Phi_{Br}(1 - x) + (\lambda_{Na} + \lambda_{Br})x \quad (9)$$

When the addition of PNa to a solution of NaBr with concentration C_P is considered, the conductivity is given by

$$\chi'/KC_P = (\lambda_P + \lambda_{Na})\Phi_{Na}(\rho - 1) + (\lambda_{Na} + \lambda_{Br}) \quad (10)$$

and it follows that

$$(\chi - \chi')/KC_P = (1 - x)[(\lambda_P + \lambda_{Na})\Phi_{Na} + (\lambda_P' + \lambda_{Br}) - (\lambda_{Na} + \lambda_{Br})] \quad (11)$$

Experimentally (Figure 1), this increment increases at first and then becomes constant for $(\mathrm{Glu,ONa})_n$ monomer. It is always constant for $n = 3$ and $n = 5$ and zero for the other polyanions (Figure 2). If the difference is zero, it implies $x = 1$ and shows that when an uncharged complex is formed with 1/1 stoichiometry, this stoichiometry is not modified by further addition of polyanion. If the difference is constant, it implies certainly that $x < 1$ and constant;

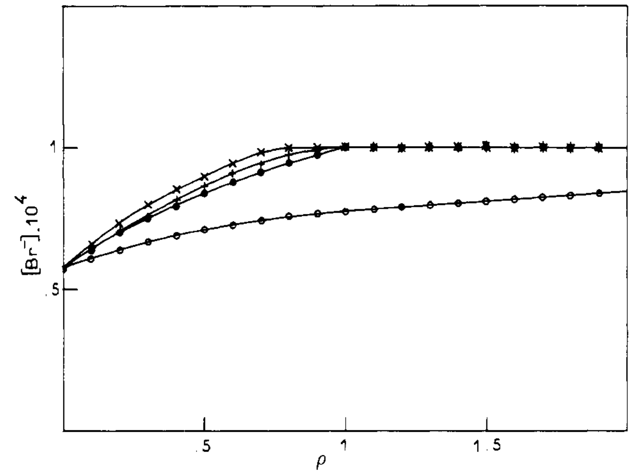

Figure 3. Variation in [Br$^-$] concentration for additions of $(\mathrm{Glu,ONa})_n$ with $n = 1$ (○), $n = 3$ (×), and $n = 5$ (+) to 10^{-4} equiv·L^{-1} $(\mathrm{Lys,HBr})_m$. (●) Shows data for $n = 6, 10, 16$, and 23, PG$_{50}$, and κ-carrageenan.

i.e., for $n = 3$ and $n = 5$ the complex is stable for this sequence of addition.

(2) When an excess of polycation is added, the conductimetric curves are described by relation 5 and the addition of $(\mathrm{Lys,HBr})_m$ to a solution of NaBr with concentrations C_P corresponds to

$$\chi''/KC_\mathrm{P} = (\lambda_\mathrm{P} + \lambda_\mathrm{Br})\Phi_\mathrm{Br}(\rho - 1) + (\lambda_\mathrm{Na} + \lambda_\mathrm{Br}) \quad (12)$$

The difference becomes

$$(\chi - \chi'')/KC_\mathrm{P} = (1 - \rho x)[(\lambda_\mathrm{P} + \lambda_\mathrm{Na})\Phi_\mathrm{Na} + (\lambda_\mathrm{P}' + \lambda_\mathrm{Br})\Phi_\mathrm{Br} - (\lambda_\mathrm{Na} + \lambda_\mathrm{Br})] \quad (13)$$

This difference is constant for (Glu,ONa) monomer (Figure 1) and certainly no modification occurs. However more details about the variation of Φ_Br are necessary to reach a definitive conclusion. For $n = 3$ and $n = 5$ (Figure 2) this difference increases and thus ρx corresponding to the fraction of complexed polyanion decreases accompanied by a decrease of Φ_Br and an increase of Φ_Na. For the other polyanions this difference remains zero and we find again that the fraction of complexed polyanion is always constant and equal to one.

Thus the conductimetric measurements give important information about the mechanism of interaction but a potentiometric study of Br$^-$ activity is important for each case.

2. Potentiometric Study of Br$^-$ Activity. In Figure 3, we show the variations of [Br$^-$] concentration when a polyanion is progressively added to $(\mathrm{Lys,HBr})_m$. This variation is given for a reverse sequence of addition in Figure 4.

A. (Glu,ONa) Monomer. For the (Glu,ONa) monomer, the results reflect the exchange of Br$^-$ by Glu$^-$ in the condensed fraction of counterions. In Figures 3 and 4 at $\rho = 1$, we find 0.2 Br$^-$ released; thus $x_\rho = 0.2$ and this confirms quite well the value deduced from conductimetric measurements (relation 4).

In Manning's theory of polyelectrolytes,[13] which gives the fraction of condensed counterions $(1 - 1/\lambda)$ with λ, the charge parameter, the free fraction of Br$^-$ during exchange can be expressed as

$$\Phi_\mathrm{Br} = \Phi_0 + (1 - 1/\lambda)/(1 + \rho) \quad (14)$$

in which Φ_0 is the free fraction in the absence of (Glu,ONa). When $\rho = 1$, using $\lambda = 1.97$, we deduce that $\Phi_\mathrm{Br} - \Phi_0 = 0.246$, which is relatively near the experimental value of 0.2.

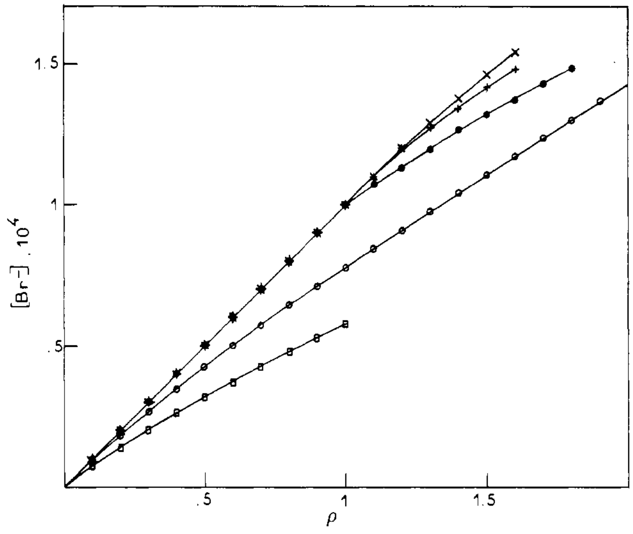

Figure 4. Variation in [Br$^-$] concentration for addition of $(\mathrm{Lys,HBr})_m$ to $(\mathrm{Glu,ONa})_n$ with $n = 1$ (○), $n = 3$ (×), and $n = 5$ (+). (●) Shows data for $n = 6, 10, 16$, and 23, PG$_{50}$, and κ-carrageenan. $(\mathrm{Lys,HBr})_m$ in pure water (□).

Above $\rho = 1$ (Figure 3) in an excess of (Glu,ONa), Φ_Br increases and we find, for instance, $\Phi_\mathrm{Br} = 0.85$ for $\rho = 2$. For an excess of $(\mathrm{Lys,HBr})_m$ (Figure 4), the release of Br$^-$ remains constant and as a consequence Φ_Br is a constant. These results are in good agreement with a purely electrostatic interaction and reflect quite well the conductimetric curves in Figure 1.

B. Other Polyanions. In Figure 4, we observe a total release of Br$^-$ for each ρ value ≤ 1 when $(\mathrm{Lys,HBr})_m$ is added to a polyanion as a consequence of the strong polyanion-polycation interactions. For $(\mathrm{Glu,ONa})_n$ with $n = 3$ and $n = 5$, the total release of Br$^-$ ions must be related to a significant decrease of charge density of the polycation. Thus, it is possible to get $\Phi_\mathrm{Br} = 1$ even when a charged complex is formed. For these low DP values, the interaction must occur randomly and x, the complexed fraction, can be deduced from conductimetric measurements; for $\rho = 1$, taking into account $\Phi_\mathrm{Na} = \Phi_\mathrm{Br} = 1$, relation 5 becomes

$$\chi/KC_\mathrm{P} = (1 - x)(\lambda_\mathrm{P} + \lambda_\mathrm{P}') + \lambda_\mathrm{Na} + \lambda_\mathrm{Br}$$

Using the exact value for Φ_Br, one determines degrees of complexation 0.88 and 0.96 for $n = 3$ and $n = 5$, respectively. These results demonstrate the importance of combining potentiometric and conductimetric measurements.

For the reverse sequence of addition (Figure 3), all the curves are superposable except for $(\mathrm{Glu,ONa})_n$ with $n = 3$ and $n = 5$ for $0 < \rho < 1$. This confirms the conductimetric results. The curves corresponding to an uncharged 1/1 complex ($n \geq 6$) can be deduced easily by assuming that for this situation, each addition of polyanions is accompanied by a release of Br$^-$ such as

$$[\mathrm{Br}^-]/C_\mathrm{P} = (1 - \rho)\Phi_\mathrm{Br} + \rho \quad (15)$$

where C_P is the concentration of the initial lysine sites and Φ_Br is deduced from a curve giving the variations of [Br$^-$] as a function of the concentration of $(\mathrm{Lys,HBr})_m$ (Figure 4) when added in pure water. The curve so computed is quite superposable with the experimental one and confirms the formation of a neutral 1/1 complex at each ρ value. For the interaction with $(\mathrm{Glu,ONa})_n$ ($n = 3$ and $n = 5$) the release of Br$^-$ is higher than when an uncharged 1/1 complex is formed with $n \geq 6$. In this last case, whenever $\rho \leq 1$, the Φ_Br of the polycation remaining in solution is

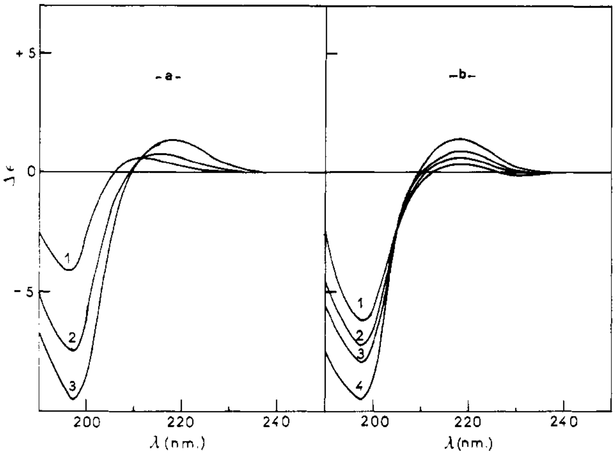

Figure 5. CD spectra obtained when $(Lys,HBr)_m$ is added to 5×10^{-4} equiv·L^{-1} $(Glu,ONa)_n$ (a) with $n = 1$ for (1) $\rho = 0$, (2) $\rho = 0.5$, and (3) $(Lys,HBr)_m$ alone and (b) with $n = 3$ for (1) $\rho = 0$, (2) $\rho = 0.5$, (3) $\rho = 1$, and (4) $(Lys,HBr)_m$ alone.

constant. When a random interaction occurs ($n = 3$ and $n = 5$), Φ_{Br}, corresponding to free ionic sites on the polycation, increases largely due to a decrease of the net charge of polycation.

Above $\rho = 1$, for an excess of polyanion added, Φ_{Br} remains always equal 1 (Figure 4) regardless of the value of ρ. As Φ_{Br} is constant, the only condition for $\chi - \chi'$ being constant (relation 11) is that x remains constant, implying no modifications of the complex for ρ above 1, whatever the polyanion. For an excess of polycation added when an uncharged complex ($x = 1$) is formed, the curves are all superposable and are the same as when $(Lys,HBr)_m$ is directly added in pure water. The difference $\chi - \chi''$ (relation 13) is zero and implies $\rho x = 1$. Thus no modification of the complex is observed at any ρ. With $(Glu,ONa)_n$, $n = 3$ and $n = 5$, we observe a decrease of Φ_{Br} (Figure 4) and $\chi - \chi''$ (relation 13) increases (Figure 2). This must correspond to a decrease of ρx, as a consequence of a progressive reorganization of the complex toward a decrease of the average degree complexation of the cationic sites. In conclusion, the conductimetric and potentiometric studies are sufficient to interpret completely the mechanisms of interaction in polyanion–polycation complex formation.

3. Circular Dichroism Study. In a previous paper[1] we had shown the occurrence of a β structure for a $(Glu,ONa)_n$ with $\overline{DP}_n = 30$. It was important here to study the influence of degree of polymerization and of the nature of the polyanion on the structure of the complex. For $(Glu,ONa)_n$ oligomers with DP = 1 and DP = 3 (Figure 5), at each ρ value, the resulting CD spectra correspond to the simple additivity of the spectra of both species taken separately, with relative concentrations corresponding to the ρ considered. This result is in agreement with a low interaction situation, leading to no modification of the conformation of each interacting molecules. For DP = 5 to DP = 16 (Figure 6a), whatever the value of ρ, we obtain spectra similar to that obtained with the corresponding $(Glu,ONa)_n$. Consequently, the $(Lys,HBr)_m$ adopts the same conformation as the oligomer complexed. With this series of oligomers, it is necessary to attain $\overline{DP}_n = 23$ in order to form pleated β sheets as previously found for $\overline{DP}_n = 30$, with an amount of β structure for each ρ equal to the fraction of complexed peptide residues. This β structure is induced by the strong electrostatic interaction and also results from the fact that $(Glu,ONa)_n$ in a range of DP between 20 and 50 exists as rodlike molecules.[14,15]

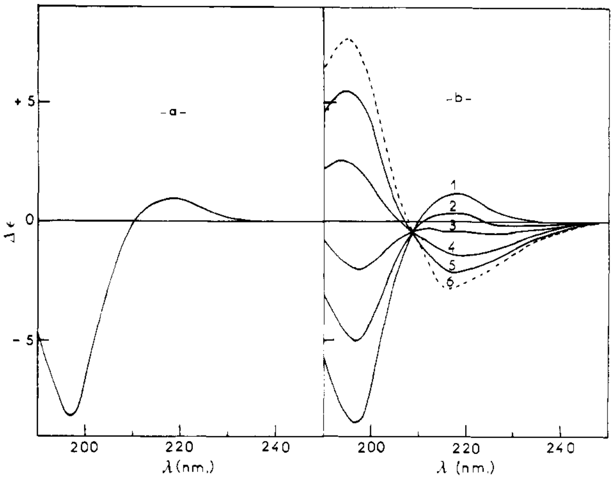

Figure 6. CD spectra obtained when $(Lys,HBr)_m$ is added to 5×10^{-4} equiv·L^{-1} $(Glu,ONa)_n$ (a) with $n = 16$ for all ρ values between 0 and 1 and (b) with $n = 23$ for (1) $\rho = 0$, (2) $\rho = 0.125$, (3) $\rho = 0.250$, (4) $\rho = 0.5$, (5) $\rho = 0.750$, and (6) $\rho = 1$ (extrapolated).

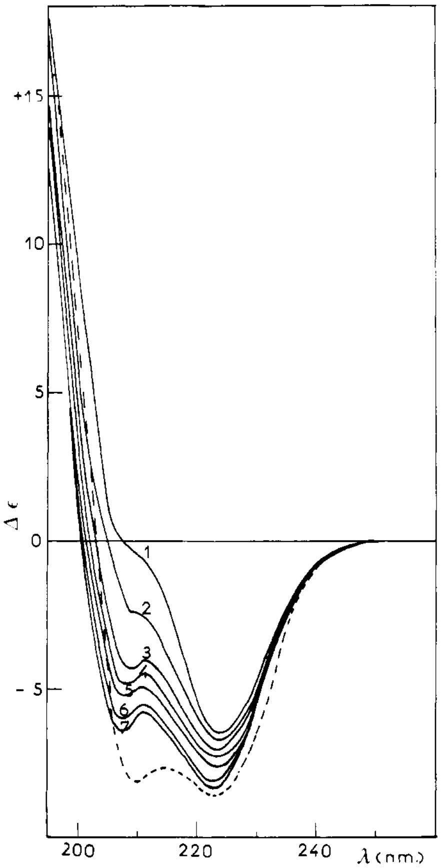

Figure 7. CD spectra obtained when $(Lys,HBr)_m$ is added to poly(sodium galacturonate) for (1) $\rho = 0.125$, (2) $\rho = 0.250$, (3) $\rho = 0.375$, (4) $\rho = 0.5$, (5) $\rho = 0.625$, (6) $\rho = 0.750$, and (7) $\rho = 0.875$ and CD spectrum for a pure α helix (---).

For κ-carrageenan the CD spectra of $(Lys,HBr)_m$ are not modified whatever the value of ρ (Figure 5a, 3). Further X-ray diffraction studies might suggest a complexed carrageenan conformation.

With poly(sodium galacturonate) (Figure 7), CD spectra show that $(Lys,HBr)_m$ adopts an α-helical structure. Blackwell et al.[2,6] obtained similar results for interactions of this polycation with other polysaccharides. The spectra reflect the contribution of the α helix and also that of the comformation adopted by the polysaccharide. The latter may be helical in order to form an helix around the peptide chain, but up to now no classical reference spectra[16] could be used to interpret these CD results completely.

Conclusion

In this extension of our previous paper[1] we show that the mechanism of interaction in the polyanion–polycation complex formation can be relatively well interpreted by means of simple techniques such as conductimetry, potentiometry, and circular dichroism.

It is demonstrated that as soon as the DP of the glutamic acid of oligomer equals 6, a 1/1 uncharged complex is formed. With a DP $\geq$ 23 a β structure is cooperatively obtained, as shown by CD measurements. In fact, the problems of conformation of the complex can be solved easily when both interacting species are oligopeptides. The lack of information concerning the conformation of nonpeptide polyanions necessitates further investigation.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. 19. Isoleucine Parameters from Random Poly[(hydroxypropyl)glutamine-*co*-L-isoleucine][1]

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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-isoleucine and N^5-(3-hydroxypropyl)-L-glutamine, and the thermally induced helix–coil transitions of these copolymers in water, are described. The incorporation of L-isoleucine was found to increase the helix content of the polymers in water at all temperatures in the range of 0–70 °C. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-isoleucine) in water were deduced from an analysis of the copolymer melting curves in the manner described in earlier papers. The computed values of s indicate that L-isoleucine stabilizes helical sequences at all temperatures in the range of 0–60 °C. The two nonpolar residues which are branched at their C^β atoms (L-isoleucine and L-valine) differ markedly in their helix–coil stability constants.

The "host–guest" technique is being used for the determination of the helix–coil stability constants for the naturally occurring amino acids in water. This paper is a continuation of the series of earlier papers[3-20] and extends the technique to L-isoleucine. In the "host–guest" technique, a water-soluble, α-helical host homopolymer with nonionizable side chains is selected, and a guest amino acid is incorporated at various compositions to form random copolymers. The Zimm–Bragg[21] helix–coil parameters σ and s for the guest residue are determined from the thermally induced helix–coil transitions of these copolymers by examining the effect of the guest residue on the helix–coil transition properties of the *host* homopolymer in water. These copolymers are prepared by incorporating L-isoleucine with the host N^5-(3-hydroxypropyl)-L-glutamine.

There has been no reported experimental determination of the helix–coil stability constants for L-isoleucine in aqueous solution, although the properties of homo- and copolymers involving this residue have been examined by a variety of techniques.[22-29] The present study indicates that L-isoleucine in water promotes helix–coil boundaries and enhances helix growth at all temperatures in the range of 0–60 °C.

The synthesis of water-soluble random copolymers of L-isoleucine with N^5-(3-hydroxypropyl)-L-glutamine is described in section I. The experimental characterization of these copolymers and their melting behavior in water are presented in section II. The data are analyzed in section III by means of an appropriate form of the theory[3] to determine the helix–coil stability parameters of L-isoleucine in water. The theory is based on evidence[30,31] that short-range interactions dominate in determining the local conformation of a polypeptide or protein. The parameters for L-isoleucine are compared with empirical observations on the behavior of this residue in proteins and with a theoretical analysis of these quantities.

I. Experimental Section

Preparation and Characterization of the Copolymers. With sodium methoxide as an initiator, the copolymers were synthesized by first copolymerizing L-isoleucine *N*-carboxyanhydride (NCA) and γ-benzyl L-glutamate NCA in dioxane. The γ-benzyl blocking groups were subsequently exchanged by reaction

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