Polyelectrolyte Complexes. Interaction of Poly(L-lysine)-Poly(L-glutamic acid) in Dilute Aqueous Solution

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ABSTRACT: Electrostatic interaction between two polyelectrolytes of opposite charge, poly(L-lysine) hydrobromide and poly(L-glutamic acid), was investigated at various degrees of neutralization α' of the glutamic acid, with sodium as counterion. Because both polymers have equal distances between ionic sites and a high conformational stability, this system is well suited to the study of structural order in polyelectrolyte complexes. It was found by using various techniques, especially gel permeation chromatography, that the binding process is strongly cooperative for all α' and all polymer mixing ratios, involving strong electrostatic interaction between the COO^- and NH_3^+ groups.

Since the first results presented by Katchalsky in 1954,[1] the studies of polyanion-polycation interactions have been little developed, in spite of the interest that this field represents for the understanding of a great number of biological mechanisms, as, for example, the organization of intercellular tissues. These interactions may be responsible for the formation of soluble complexes or may induce phase changes. An application of coacervation thermodynamics has been proposed, for instance, by Veis et al.[2] for gelatin-DNA complexes; this approach has been reviewed recently by Veis.[3] Polyanion-polycation interactions are also implied for the preparation of complex membranes, widely used in ultrafiltration. We mention the work of Michaels et al.[4-6] and the more recent important contributions of two groups, Tsuchida et al.[7-10] and Zezin et al.[11-14]

From previously published results, it appears that only a few general rules can be proposed[11] and it seems that the mechanism of such interactions depends on the type of polymer, on the degree of polymerization, on the repartition of ionic sites along the backbone, and on the nature of the reactive functions.

Experimental Section

Materials. (Lys, HBr)$_m$ was purchased from Pilot (Lot L-112) ($M_w \sim 100\,000$, $m \sim 478$). It was purified by dissolution in water and then filtered and freeze-dried. The resulting product was thoroughly washed with acetone and ether and then dried. Poly(L-glutamic acid) was a carefully fractionated sample with a degree of polymerization of 30, previously obtained by ion-exchange chromatography.[15]

Solutions were deliberately prepared at concentrations higher than needed and then diluted into the reacting media in order to avoid volume corrections. (Lys, HBr)$_m$ was first diluted in distilled water at a concentration close to 2×10^{-2} equiv·L^{-1}. The final concentration was adjusted after a potentiometric titration of Br^- ions by 0.1 N $AgNO_3$, using a set of silver and calomel electrodes equipped with a KNO_3 bridge. For (Glu)$_n$, the polymer initially in the acidic form was dissolved in 0.04 N NaOH (concentration $C_p \sim 10^{-3}$ equiv·L^{-1}). The solution was passed through an ion-exchange column (IR 120 H^+ Amberlite) and then adjusted to $\alpha' = 0.3$, 0.6, or 1 by pH titration. The solutions were then concentrated to about 2×10^{-2} equiv·L^{-1} and adjusted by a new titration. For $\alpha' = 0$, the solution could not be concentrated in the same way because of precipitation of the acidic form; hence, direct preparation of a 10^{-4} or 2×10^{-3} equiv·L^{-1} solution was carried out.

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 = [Lys]/[Glu], the ratio between total concentrations of both residues, [Lys] and [Glu], respectively).

The measurements of pBr were made with a Sargent S300000 potentiometer equipped with a set of Orion electrodes, one of which was a double-junction electrode and the other specific for Br^-.

The pH was measured with a Tacussel Minisis 6000 potentiometer equipped with glass and calomel Tacussel electrodes standardized with a Sargent pH 4 buffer.

Specific conductivities were obtained by means of a conductivity cell adapted to a Wayne-Kerr B-642 conductimeter.

Gel Permeation Chromatography (GPC). To 10-cm^3 solutions containing various concentrations of (Glu)$_n$ was added stepwise (Lys, HBr)$_m$ up to a concentration of 10^{-4} equiv·L^{-1} in order to vary ρ and to always maintain [COO^-] larger than [NH_2], hence avoiding possible Lys-gel interactions. Whatever the eluent ionic strength, the solutions were kept salt free. They were injected by means of a 7-cm^3 injection loop; simultaneous conductimetric and refractometric detections[16] were adopted. For $\alpha' = 1$, 75-, 150-, 1250-, and 3000-Å-porosity Spherosil gels from Saint-Gobain were used, mixed in equal weights and filled in a glass column of 147-cm length and 1.5-cm diameter under conditions previously described.[16] For $\alpha' = 0$, 0.3, and 0.6, three 120-cm-length, 0.9-cm-diameter glass columns packed with a 6000-Å Spherosil gel were used instead.

Circular Dichroism Measurements (CD). CD spectra were recorded on a DC III dichrograph (Jobin-Yvon) with a 1-mm quartz cell at room temperature in the region of the characteristic electronic 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 concentration of peptide residues of 2×10^{-4} equiv·L^{-1}. ρ was varied by mixing variable amounts of two solutions of 2×10^{-4} equiv·L^{-1} of each polymer.

Results and Discussion

When poly(L-lysine) is added to a partially neutralized poly(glutamic acid), the mechanism of interaction can be schematically described by eq 1, where P^- represents a

$$\begin{aligned} &P^-X^+ + P'^+,Br^- \rightleftarrows PP' + X^+Br^- \\ &(\alpha_T - \rho x) + \rho(1 - x) \rightleftarrows \rho x + \rho x \end{aligned} \quad (1)$$

dissociated glutamic site having a Na^+ and/or H^+ counterion, depending on α', P'^+,Br^- is the hydrobromide form, PP' is the corresponding complexed form of a lysine site, and x represents the complexed fraction (x = [PP'] formed/[P'^+,Br^-] added). [P'^+] and [Glu] are the total concentrations of lysine and glutamic residues, respectively, and α_T is the apparent total degree of dissociation; hence α_T[Glu] represents the concentration of carboxyl sites able to be complexed with [P'^+].

Measurements of the ionic activity or conductivity seem to be peculiarly adapted for monitoring complex formation. However, the following effects have to be taken into ac-

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Table I
Comparison between Experimental and Calculated Values of Specific Conductance at ρ_{eq} for Different α'

	$(Glu)_n$ + $(Lys, HBr)_m$ (Figure 1)			$(Lys, HBr)_m$ + $(Glu)_n$ (Figure 2)		
α'	ρ_{eq}	$(1/R)_{exptl}$	$(1/R)_{calcd}$	ρ_{eq}	$(1/R)_{exptl}$	$(1/R)_{calcd}$
0	0.425	16.50	16.50	0.476	36.90	37.80
0.3	0.55	13.05	13.30	0.6	24.60	25.10
0.6	0.7	11.30	11.30	0.745	16.40	17.00
1	1.0	13.30	13.30	1.0	13.30	13.30

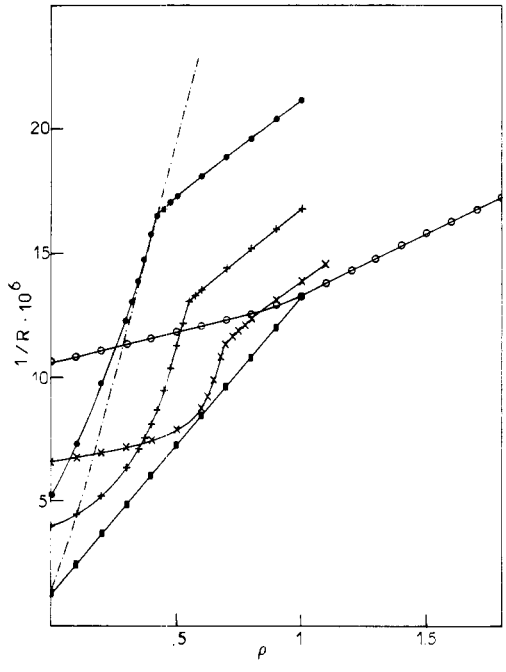

Figure 1. Variation of the conductance when a solution of (Lys, HBr)$_m$ is added to 10^{-4} equiv·L^{-1} (Glu)$_n$ for varying degrees of neutralization of (Glu)$_n$: (○) 1; (×) 0.6; (+) 0.3; (●) 0. (-·--) and (■) are for the addition of HBr and NaBr in pure water.

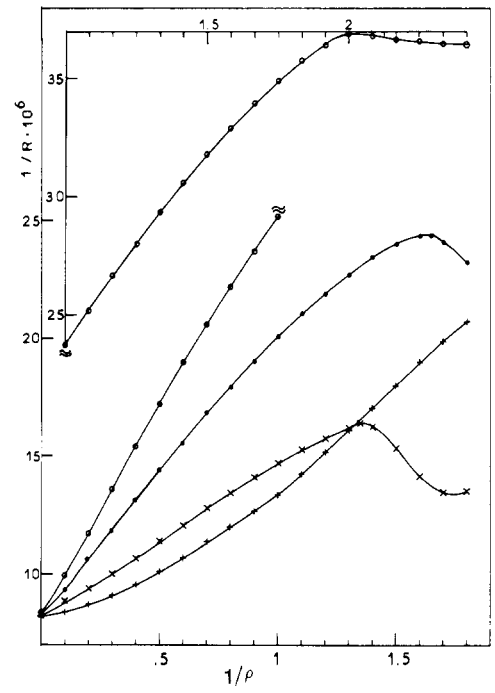

Figure 2. Variation of the conductance when a solution of (Glu)$_n$ is added to 10^{-4} equiv·L^{-1} (Lys, HBr)$_m$ for varying degrees of neutralization of (Glu)$_n$: (+) 1; (×) 0.6; (●) 0.3; (○) 0.

count. Both polymers, because of their polyelectrolyte behavior, have to be characterized by two variables, ϕ_{P^-} and ϕ_{P^+}, representing the activity coefficient of counterions for (Glu)$_n$ and (Lys, HBr)$_m$, respectively. In addition, the acidic glutamic function is weak, and the value of the degree of neutralization α' has to be corrected in order to take into account the autodissociation. Finally, for conductimetric studies, the average equivalent mobilities λ_{P^-} and λ_{P^+} for a monomer which is assumed to be fully dissociated may have to be considered as variables, depending on their mechanism of interaction.

Furthermore, at the low concentrations adopted to avoid precipitation during the complex formation, it is important to follow the pH variations due to (Lys, HBr)$_m$ hydrolysis and chiefly due to H^+ release during the polymer complexation.

In this work we have studied both the stepwise addition of poly(glutamic acid) into poly(lysine) solutions and the inverse. Some authors have shown that the two procedures can lead to different results.[4]

(1) Conductimetric Studies. Figure 1 shows the variation of specific conductivity when (Lys, HBr)$_m$ is progressively added to (Glu)$_n$ for initial degrees of neutralization of poly(glutamic acid) of 1, 0.6, 0.3, and 0. The case for reversed addition is given in Figure 2.

All curves in Figures 1 and 2 show a break in slope at equivalent ρ values, ρ_{eq}, as given in Table I. For $\alpha' = 0$, the conductivity values $(1/R)$ at ρ_{eq} (0.425 (Figure 1) and 0.476 (Figure 2)) are quite similar to those obtained with HBr solutions at corresponding concentrations (0.425×10^{-4} and 0.476×10^{-4} equiv·L^{-1} HBr). For $\alpha' = 1$, the values of $1/R$ for $\rho_{eq} = 1$ (Figures 1 and 2) are identical and also identical with that of the 10^{-4} equiv·L^{-1} NaBr solution. In both cases (Figures 1 and 2) all is going on as if independent of the sense of addition when the two polymers are associated; no free ionic species other than the counterions are released during the complexation: H^+ and Br^- in the first case and Na^+ and Br^- in the second. Thus, it seems that a complex with 1/1 stoichiometry is formed in which each NH_3^+ is exactly associated with one glutamic ionic site. In both cases, reaction 1 is completely displaced to the right and, consequently, $x = 1$. Furthermore, the composition of the complex at these equivalent points, defined as the ratio $\{[Lys]/[Glu]\}_{eq}$ included in the complex (expressed in monomole concentration), becomes equal to α_T, with $\alpha_T > \alpha'$ corresponding to the fraction of complexed carboxyl sites. For α' values between 0 and 1, $(1/R)_{eq}$ can be calculated by using relationship 2, where $(1/R)$[NaBr]

$$(1/R)_{eq} = \alpha'\frac{1}{R}[NaBr] + (\rho_{eq} - \alpha')\frac{1}{R}[HBr] \qquad (2)$$

and $(1/R)$[HBr] are the respective conductances of NaBr and HBr solutions at an equivalent concentration equal to [Glu] in monomoles. The results reported in Table I show good agreement between the experimental values of $(1/R)_{eq}$ and the values deduced from relation 2 for each pair of α' and ρ_{eq}.

For $\alpha' = 0$, the acidic function of (Glu)$_n$ is weak[15] ($pK_0 = 4.45$ at 10^{-3} equiv·L^{-1}) and only a few carboxyl sites are dissociated and hence accessible to form a complex. Two parameters can lower this initial dissociation: a decrease of pH (obtainable by addition of a strong acid) or an in-

Table II
Comparison between Experimental and Calculated Values of H^+ Concentration Released at ρ_{eq} for Different α'

	$(Glu)_n$ + $(Lys, HBr)_m$ (Figure 4)				$(Lys, HBr)_m$ + $(Glu)_n$ (Figure 5)			
			$10^5[H^+]$, equiv·L^{-1}				$10^5H^+]$, equiv·L^{-1}	
α'	ρ_{eq}	pH	exptl	calcd	ρ_{eq}	pH	exptl	calcd
0	0.425	4.37	4.26	4.25	0.476	5.02	9.55	10.0
0.3	0.55	4.62	2.40	2.5	0.6	4.32	4.79	4.98
0.6	0.7	5.04	0.98	1.0	0.745	4.78	1.66	1.94
1	1.0	5.65			1.0	5.65		

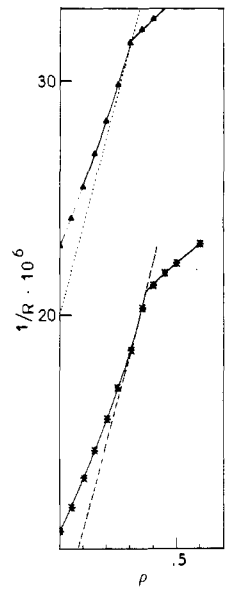

Figure 3. Variation of the conductance when a solution of $(Lys, HBr)_m$ is added to 10^{-4} equiv·L^{-1} $(Glu)_n$, for $\alpha' = 0$ with an initial excess of HBr: (*) 1.6×10^{-5}; (▲) 5×10^{-5} equiv·L^{-1}. (---) and (···) are for the addition of HBr in water containing the corresponding initial excess of HBr.

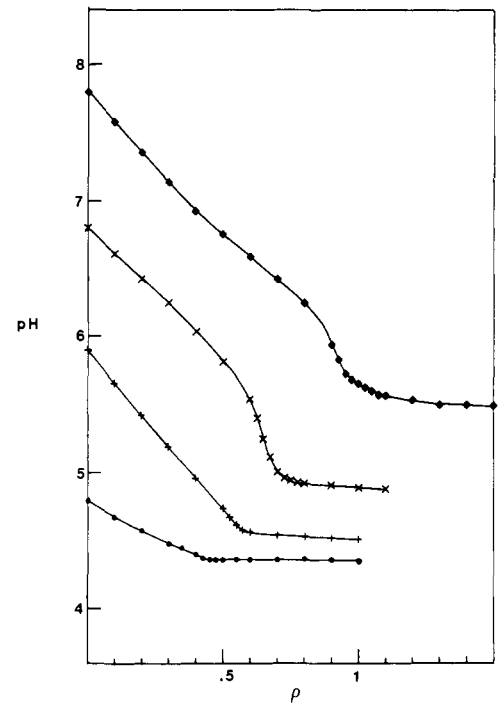

Figure 4. Variation of the pH when a solution of $(Lys, HBr)_m$ is added to 10^{-4} equiv·L^{-1} $(Glu)_n$ for varying degrees of neutralization of $(Glu)_n$: (♦) 1; (×) 0.6; (+) 0.3; (●) 0.

crease of the polymer concentration. Therefore, starting from $(Glu)_n$ solutions, ρ_{eq} decreases with increasing excess of HBr (decreasing pH), as shown in Figure 3 and the values of the conductance satisfy relation 2; consequently, complex interaction does not take place with an undissociated carboxylic site.

Below the equivalence point, the solutions are stable whatever α', but above, they slowly precipitate. When $\alpha' = 1$, the complex is formed irreversibly, for all solvent conditions, including even highly alkaline or acidic media. This is certainly due to the fact that for a $(Glu)_n$ of $DP_n = 30$, the interaction with $(Lys, HBr)_m$ is very strong; in addition, for $\alpha' = 1$ the complex is neutral because of complete association of COO^- with NH_3^+. On the other hand, for $\alpha' < 1$, the complex is destroyed at sufficiently low pH values.

(2) pH Measurements. Figures 4 and 5 show pH variations for conditions identical with those given in Figures 1 and 2, respectively.

For all α' values, we again observe a transition near ρ_{eq}. At this point—which we call pH_{eq}—the corresponding concentration $[H^+]_{eq}$, released as HBr, can be calculated from eq 3.

$$[H^+]_{eq} = (\rho_{eq} - \alpha')[Glu] \qquad (3)$$

Equation 3 is similar to relation 2, [Glu] being the total concentration of glutamic residues introduced in the medium. As demonstrated in Table II, release of H^+ is well predicted by relation 3 at the equivalence point ρ_{eq}. For $\alpha' = 1$, pH_{eq} equals that of distilled water, which is in good agreement with the conductimetric results.

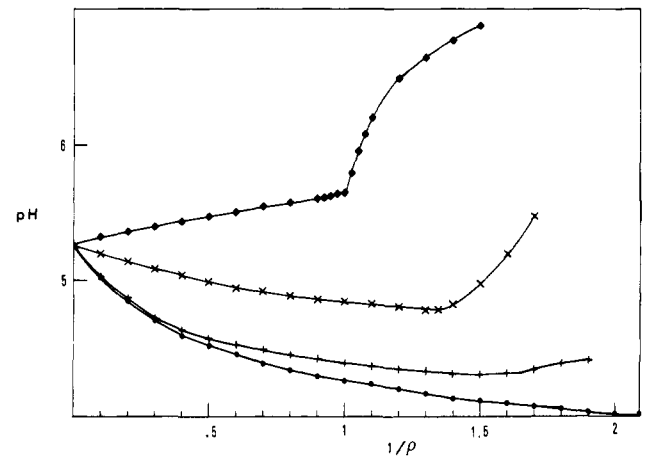

Figure 5. Variation of the pH when a solution of $(Glu)_n$ is added to 10^{-4} equiv·L^{-1} $(Lys, HBr)_m$ for varying degrees of neutralization of $(Glu)_n$: (♦) 1; (×) 0.6; (+) 0.3; (●) 0.

(3) Potentiometric Study of pBr. It would be interesting to study the variations of the free Na^+ and Br^- concentrations during complexation. Unfortunately, because of the low concentrations prescribed by the experimental conditions and because of the important ρ dependence of the pH, such activity measurements of free Na^+ ions are not feasible. On the other hand, they are possible for Br^-, especially in a pH range between 4.6 and 9 since Br^--specific electrodes can be used.

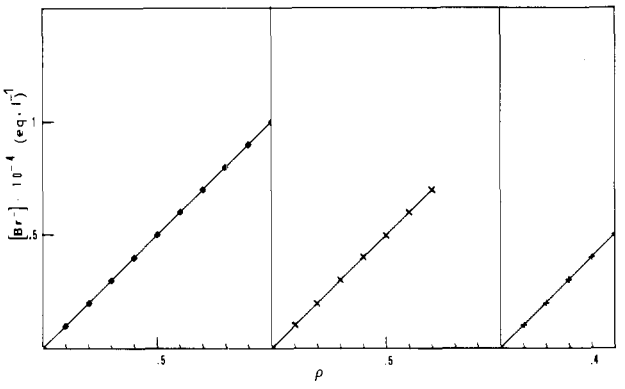

Figure 6. Variation of [Br^-] concentration when a solution of $(Lys, HBr)_m$ is added to 10^{-4} equiv·L^{-1} $(Glu)_n$ for varying degrees of neutralization of $(Glu)_n$: (♦) 1; (×) 0.6; (+) 0.3.

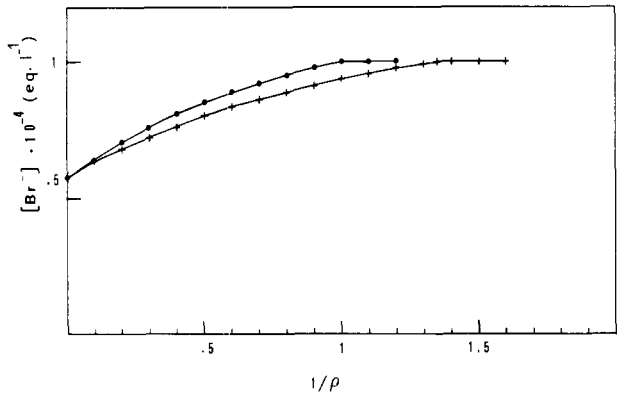

Figure 7. Variation of [Br^-] concentration when a solution of $(Glu)_n$ is added to 10^{-4} equiv·L^{-1} $(Lys, HBr)_m$ for varying degrees of neutralization of $(Glu)_n$: (●) 1; (+) 0.6.

The results are given in Figures 6 and 7 as obtained under conditions similar to those mentioned above. When $(Lys, HBr)_m$ is added to poly(glutamic acid) (Figure 6) the poly(lysine) always releases all its Br^- counterions. This is in good agreement with the above results and shows, in addition, that, at any time, all lysine sites are involved in a complex interaction with an equivalent amount of carboxyl sites; thus x always equals 1 and reaction 1 is always out of equilibrium. In the reverse process (see Figure 7), starting from the value of the activity (ϕ_{Br}[Lys]) for the solution $(Lys, HBr)_m$, the concentration of Br^- ions increases progressively and reaches a constant value which is equal to the total initial concentration of [Lys], i.e., 10^{-4} equiv·L^{-1}. This is so even above the equivalence point and confirms the results obtained by the other techniques. However, below this point, interpretation is difficult because the [Br^-] variation reflects not only the complex formation but also the modification of the equilibrium NH_2, HBr $\rightleftarrows$ $NH_3^+ + Br^-$ which depends simultaneously on pH, polymer concentration, ionic strength, and variation of charge density of the free remaining lysine sites.

The curves obtained above the equivalence point (Figure 1) do not depend on whether $(Glu)_n$ or $(Lys, HBr)_m$ is added to distilled water containing NaBr or HBr at concentrations defined by relations 2 and 3. In addition, they confirm that no further interaction can occur in the reaction mixture, if no dissociated sites are left.

All the above results are in good mutual agreement. They allow the conclusion that, whatever α', a stable complex is formed for $\rho_{eq} = \alpha_T$ with a 1/1 charge stoichiometry and a molar composition equal to the ratio $[P^+]/[Glu] = \alpha_T$.

(4) Chromatographic Study by Gel Permeation (GPC). The silica gels used in this study exhibit a weak negative charge; therefore in order to avoid retention on the gel by electrostatic interaction, we have always operated without an excess of $(Lys, HBr)_m$. The experimental conditions are otherwise identical with those given in Figure 1.

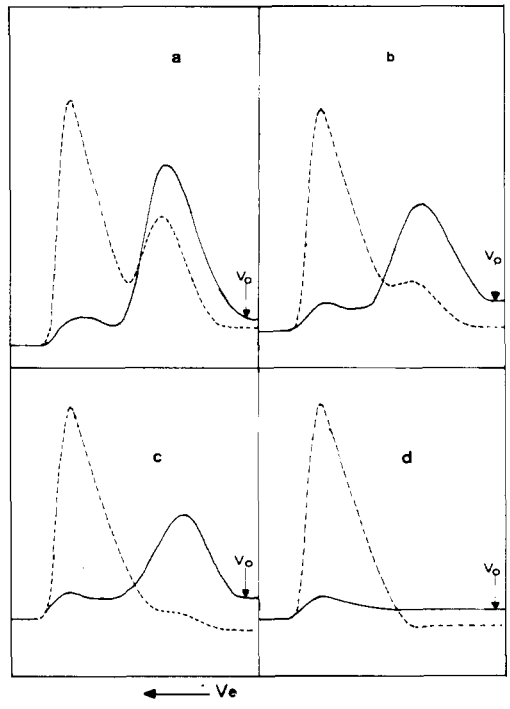

Figure 8. Chromatograms obtained when $(Glu)_n$ is added to 10^{-4} equiv·L^{-1} $(Lys, HBr)_m$ in pure water as eluent for degree of neutralization of $(Glu)_n$ = 1: (a) ρ = 0.31; (b) ρ = 0.5; (c) ρ = 0.57. (d) 10^{-4} equiv·L^{-1} NaBr. (---) and (—) are for conductimetric and refractometric signals, respectively.

α' = 1. For α' = 1, and in pure water as eluent, two conductimetric ($\Delta\chi$) and two refractometric (Δn) peaks are obtained (Figure 8). Neither position nor $\Delta\chi/\Delta n$ values of the first signal (in decreasing order of elution volumes) depend on ρ; they are also identical with the signal when a solution of 10^{-4} equiv·L^{-1} NaBr is injected separately. This confirms not only the total release of the Br^- counterions but also a simultaneous liberation of an identical amount of Na^+ ions coming from glutamic sites.

The second peak corresponds to the elution of the whole polypeptide. The $\Delta\chi/\Delta n$ ratio decreases with increasing ρ and with decreasing elution volume. Consequently, in this peak, variously charged species are eluted, the apparent average charge of which lowers with increasing ρ. On the other hand, the maximum of the refractometric peak is displaced toward low elution volumes with ρ increases. This can be interpreted as an increase of the apparent molecular weight (even if the electrostatic exclusion decreases[17]).

In 5×10^{-4} equiv·L^{-1} NaBr as eluent, the electrostatic exclusion effects are substantially decreased[16] and a better molecular weight resolution for the polypeptide can be expected. We obtain (Figure 9) two refractometric peaks but only one conductimetric signal. A calibration with successive injections of $(Glu, ONa)_n$ at different concentrations proves that the first peak originates from the elution of free poly(glutamic acid) sodium salt ($\Delta\chi/\Delta n$ and the elution volumes are identical). This allows the amount of uncomplexed $(Glu, ONa)_n$ to be deduced; the complexed form can then be obtained by subtraction from the total glutamic acid injected; it is independent on ρ and equal to the initially added $(Lys, HBr)_m$ (assuming $\Delta n_{Lys} \equiv \Delta n_{Glu}$).

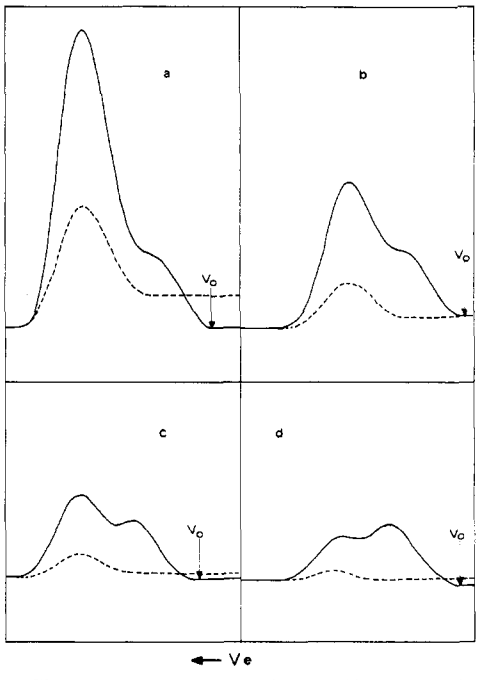

Figure 9. Chromatograms obtained when $(Glu)_n$ is added to 10^{-4} equiv·L^{-1} $(Lys, HBr)_m$ in 5×10^{-4} equiv·L^{-1} NaBr as eluent for degree of neutralization of $(Glu)_n$ = 1: (a) $\rho = 0.1$; (b) $\rho = 0.2$; (c) $\rho = 0.286$; (d) $\rho = 0.4$. (- - -) and (—) are for conductimetric and refractometric signals, respectively.

The second peak is due to the elution of the complex formed. Its area is constant and equals twice that of the complexed (Glu, ONa)$_n$. The absence of a corresponding conductimetric signal shows that the eluted material under this peak is uncharged; assuming again $\Delta n_{Lys} = \Delta n_{Glu}$, it follows that for $\alpha' = 1$ and all ρ values, an uncharged complex with 1/1 stoichiometry is formed between the antagonist ionic sites in a cooperative process. These results confirm the above data on ρ_{eq} and allow recalculation of the complete conductivity curve. Indeed, α_T in this case always equals 1 and the specific conductance $(1/R)_\rho$ can be expressed, for all ρ and glutamic acid concentration C_p, by

$$(1/R)_\rho = \{(\phi_p\lambda_p)(1-\rho) + (\phi_p\lambda_{Na}) \times (1-\rho) + \rho(\lambda_{Na} + \lambda_{Br})\}C_p + (1/R)_\rho[H^+, OH^-] \equiv (\phi_p C_p \lambda_{P,Na})(1-\rho) + \rho C_p(\lambda_{NaBr}) + (1/R)_\rho f(pH) \quad (4)$$

The first two terms of the right-hand side are deduced from the (Glu, ONa)$_n$ and NaBr concentration dependence of $1/R$, respectively; the last term is deduced from pH and $(1/R)_\rho[H^+]$ curves. Relation 4 is in very good agreement with the experimental curve of Figure 1 for $\alpha' = 1$.

When $\rho > 0.4$ the second peak progressively disappears, $\Delta\chi$ always remains equal to zero, and a retention of the complex at the top of the column occurs. This phenomenon indicates a precipitation due to the relatively large ionic strength of the eluent and to the pH of the injected solution. However, the peak of (Glu, ONa)$_n$, when in excess, is normally eluted, permitting a further interpretation of the chromatograms in this case. The elution volume of the complex formed under these conditions is identical with the exclusion volume of the column, indicating that the average molecular weight of the complex is at least equal to 10^7.

$\alpha' = 0$, 0.3, and 0.6. Figure 10 shows some examples of chromatograms obtained for an eluent with an ionic strength of 2×10^{-4} equiv·L^{-1} for $\alpha' = 0$, 0.3, and 0.6. When

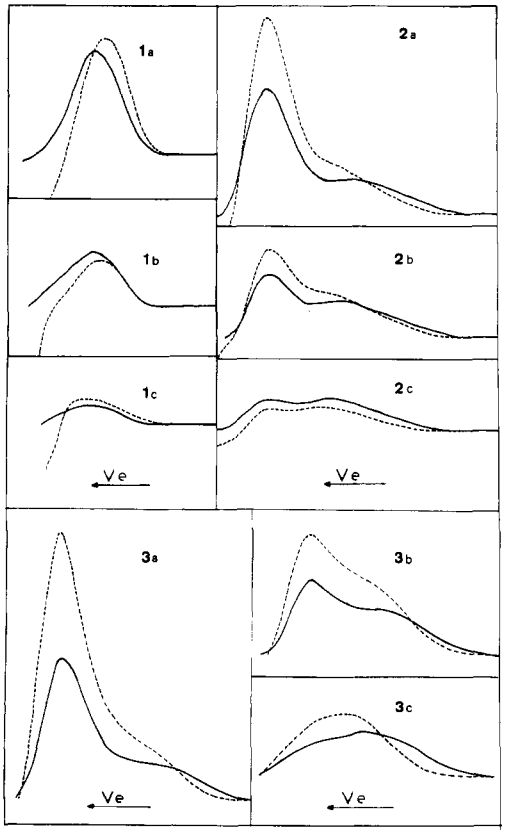

Figure 10. Chromatograms obtained when $(Glu)_n$ is added to 10^{-4} equiv·L^{-1} $(Lys, HBr)_m$ in 2×10^{-4} equiv·L^{-1} NaBr. (1) $\alpha' = 0$: (a) $\rho = 0.1$; (b) $\rho = 0.133$; (c) $\rho = 0.2$. (2) $\alpha' = 0.3$: (a) $\rho = 0.133$; (b) $\rho = 0.2$; (c) $\rho = 0.308$. (3) $\alpha' = 0.6$: (a) $\rho = 0.133$; (b) $\rho = 0.2$; (c) $\rho = 0.308$. (- - -) and (—) are for conductimetric and refractometric signals, respectively.

$\alpha' = 0.3$ and 0.6, two peaks are observed: the first peak (in decreasing order of elution volume) is due to an excess of $(Glu)_n$, whereas the second, as a shoulder on the first one, is due to a part of the complex. Using a series of injections of $(Glu)_n$ for standardization, we show that the height of the peak can be used to deduce the amount of $(Glu)_n$ in excess and, by subtraction, how much is involved in the complex. Some of the complex is always retained at the top of the column, and the remainder which elutes is weakly charged. The corresponding $\Delta\chi/\Delta n$ increases with elution volume. Although the complex does not contain free lysine sites, as demonstrated by the Br^- release (Figure 6), it exhibits a distribution of partially dissociated sequences of $(Glu)_n$. For $\alpha' = 0$, only the $(Glu)_n$ in excess is eluted, but the treatment above remains applicable.

Working with an eluent of lower ionic strength (10^{-4} M), some of the previously retained complex is eluted since there is no charge implying retention. The elution of those molecules near the void volume of the column (V_0) confirms that they have very high molecular weights.

Thus we can deduce at every condition the composition of the complex, the average DP_n of complexed $(Glu)_n$, and the percent of uncomplexed $(Glu)_n$. The results are listed in Table III.

When $\alpha' = 1$, [Glu]/[Lys] is equal to 1, whatever ρ. When α' decreases, the [Glu] content within the complex increases; one might speculate that the fraction of the uncharged [Glu] given by GPC forms side chains which are not complexed with [Lys]. When ρ increases, for a given α', the ratio [Glu]/[Lys] decreases since the complex is formed in the presence of a decreasing amount of free $(Glu)_n$ and of an increasing concentration of free H^+ (see pH measurements). For $\rho^* = \rho_{eq}$, as obtained from con-

Table III
Parameters Deduced from GPC Study for $\alpha' = 0$, 0.3, and 0.6

		ρ								
	α'	0.1	0.2	0.308	0.4	0.425[a]	0.5	0.55[a]	0.615	0.7[a]
[Glu]/[Lys] in the complex	0	5	4.2	3.26		2.35				
	0.3	3	2.5	2	1.88		1.82	1.82		
	0.6	2.6	2.13	2.13	2		1.63		1.45	1.43
av DP of complexed $(Glu)_n$	0	6	7.10	9.2		12.8				
	0.3	10	12	15	16		16.5	16.5		
	0.6	11.5	14.1	14.1	15		18.4		20.7	21
% free $(Glu)_n$	0	50	16	0		0				
	0.3	70	50	38.5	25		6	0		
	0.6	74	58	35	20		19		9	0

[a] Values from conductivity experiments.

ductimetry, the ratio [Glu]/[Lys] is equal to $1/\alpha_T$ and confirms the above data.

When $\alpha' = 0$ the obtained values of the average degree of polymerization of the glutamic segment complexed implies that H^+ ions are released after each addition of lysine. For $\alpha' = 0.3$ and 0.6, the H^+ release occurs at $\rho > 0.1$ and $\rho > 0.45$, respectively, and the amount of released H^+ increases with ρ; these results explain very well the conductimetric curves for $\alpha' = 0.6$, especially the variation of the slope, which is like that for $\alpha' = 1$ for small ρ, and its abrupt change above $\alpha_T = 0.4$.

Thus, GPC appears to be the only technique which is able to give the mean composition of the complex for all α'.

(5) Conformational Study by Circular Dichroism. The CD spectra obtained for $\alpha' = 0$, 0.3, 0.6, and 1 at different values of ρ are given in Figure 11.

For $\alpha' = 0$, and increasing ρ we progressively pass from an α-helical structure for single-stranded $(Glu)_n$ to a more complex structure which shows a positive CD maximum near 195 nm and a negative one at 223 nm. These latter maxima do not correspond to any well-known structure.[18]

For $\alpha' = 1$, when ρ varies from 0 to 1, the CD spectra exhibit an isodichroic point near 209 nm and pass from features typical for an extended structure to others very similar to a classical β structure[18] (positive maximum at 196 nm and negative maximum near 217 nm). If we call γ the ratio between the complex concentration and the concentration of total peptide residues, $\Delta\epsilon$ is a linear function of γ for all investigated wavelengths. This is in good agreement with the above results and also shows that there is only a single process of complexation when $\alpha' = 1$.

If we consider now the case for $\alpha' = 0.6$ at increasing ρ, we simultaneously observe complex formation and a progressive retrogradation of the free $(Glu)_n$, first into a random coil form (spectrum 2) and then into a helix form. The limiting spectrum at high ρ is intermediate between those at $\alpha' = 1$ and $\alpha' = 0$.

Finally, for $\alpha' = 0.3$, the retrogradation of an α helix is peculiarly well indicated by the lowering of $\Delta\epsilon$ at 222 nm on spectrum 2. The limiting spectrum is also in between those at $\alpha' = 1$ and $\alpha' = 0$.

This study shows that the complex can adopt two different forms at $\alpha' = 0$ and 1 and that the complexes obtained for intermediates α' values might be a mixture of at least these limiting forms, the relative proportion varying with α' and ρ. The form obtained for $\alpha' = 1$ might consist of an alternating superposition of β sheets of $(Glu)_n$ and $(Lys)_m$ kept together by electrostatic interaction.

Conclusion

It is shown in this paper that, whatever the degree of neutralization α', the interaction of $(Glu)_n$ with $(Lys, HBr)_m$ leads to the formation of a cooperative complex with complete association of the lysine sites. The interaction process is not governed by classical equilibrium laws, but the reaction is always complete, as demonstrated very well by the potentiometric study of $[Br^-]$ release.

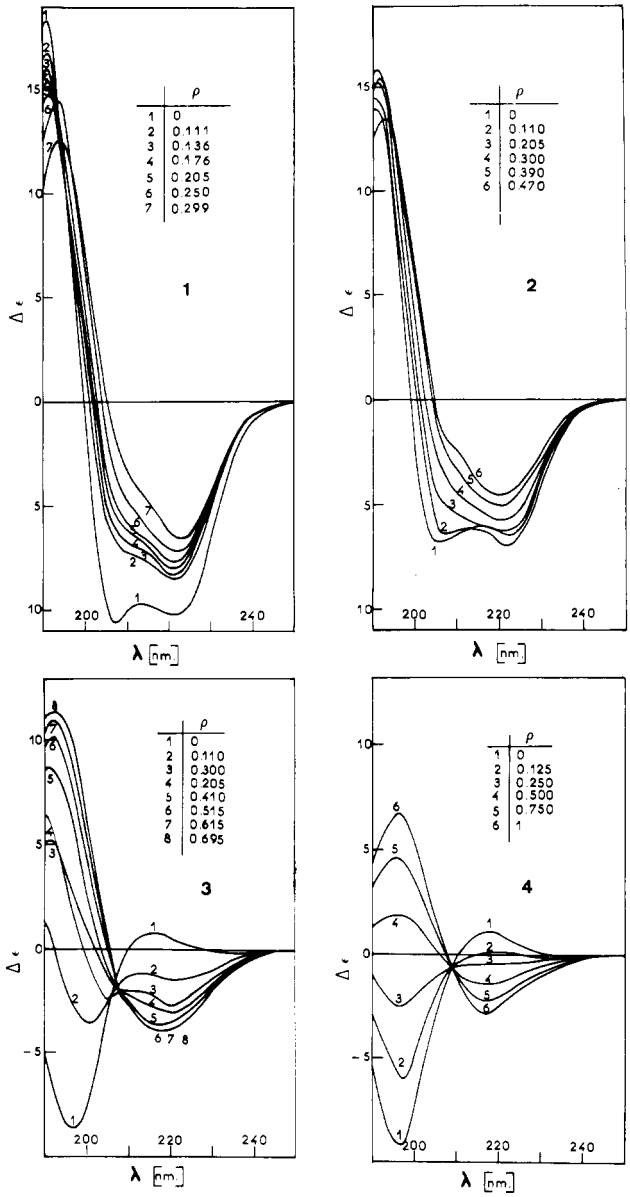

Figure 11. CD spectra obtained when $(Lys, HBr)_m$ is added to $(Glu)_n$ for different degrees of neutralization of $(Glu)_n$: (1) $\alpha' = 0$; (2) $\alpha' = 0.3$; (3) $\alpha' = 0.6$; (4) $\alpha' = 1$.

GPC studies permit a complete interpretation of the mechanism for $\alpha' = 1$ at [Lys]/[Glu] = 1 in the complex,

whatever the initial concentration ratio ρ of both components is. In addition, GPC gives the mean complex composition for all ρ and for the different α' studied; it is found that [Lys]/[Glu] equals the fraction of dissociated carboxylic sites α_T.

Finally, CD experiments confirm the one-way process for $\alpha' = 1$, resulting in a structure certainly similar to the β-pleated sheet. X-ray diffraction work in progress will presumably yield more detailed structural information.

One remaining problem to be solved concerns the question why, for $\alpha' \neq 1$, the complexation is stopped at a certain ρ value ρ_{eq}. Polyelectrolyte behavior must be responsible for the lowering of the attractive strength when the charge density decreases; nevertheless, for ρ_{eq}, the [Glu] sites not associated to a lysine group are undissociated; this can be explained only by an important enhancement of pK_a due to a local modification of the dielectric constant as a consequence of the formation of ordered structures.

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Averaged Principal Moments of the Inertia Tensor for Unperturbed Poly(hydroxybutyl-L-glutamine) As It Passes through the Helix-Coil Transition

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Received December 6, 1979

ABSTRACT: Averaged principal moments, $\langle L_1^2 \rangle \geq \langle L_2^2 \rangle \geq \langle L_3^2 \rangle$, of the inertia tensor have been obtained for polypeptides undergoing a helix-coil transition. Polypeptide chains containing 101, 201, 401, and 801 amino acid residues were studied. Conformational energy surfaces and Zimm-Bragg statistical weights used are those appropriate for aqueous poly(hydroxybutyl-L-glutamine) when it is unperturbed by long-range interactions. At all degrees of polymerization studied, $\langle L_1^2 \rangle$ and the mean square radius of gyration pass through a minimum during the transition from random coil to α helix. In contrast, the qualitative behavior of the remaining two principal moments is strongly dependent on the degree of polymerization. These two principal moments experience monotonic changes at a low degree of polymerization, but at a high degree of polymerization they pass through both a minimum and a maximum. Principal moment ratios $\langle L_2^2 \rangle / \langle L_1^2 \rangle$ and $\langle L_3^2 \rangle / \langle L_1^2 \rangle$ experience monotonic changes even when the changes in all three $\langle L_i^2 \rangle$ are not monotonic. Behavior of the principal moments is related to the presence of short helical segments at low average helicity and long helical segments at high helicity.

Measurement of the molecular weight dependence of the mean square radius of gyration or intrinsic viscosity is a classic method for establishing whether a polypeptide in a given solvent is in the random coil state or exists instead as an α helix.[1] These same parameters are also used to follow a helix-coil transition induced in a certain polypeptide by alteration of solvent composition or temperature. However, the experimentally measured parameter is not necessarily found to be a monotonic function of the perturbing influence. In the case of the pH-induced helix-coil transition of aqueous poly(L-glutamic acid), for example, the intrinsic viscosity at the midpoint of the transition is substantially lower than that of either the helix or the completely disordered form.[2] This result implies the mean square radius of gyration passes through a minimum as the polypeptide undergoes the helix-coil transition.

Using matrix methods,[3] Miller and Flory[4] obtained theoretical verification that the characteristic ratio passes through a minimum during the helix-coil transition. The characteristic ratio is defined here as $\langle r^2 \rangle_0 / n_p l_p^2$, where $\langle r^2 \rangle_0$ is the mean square unperturbed end-to-end distance for a polypeptide containing $n_p + 1$ amino acid residues and l_p is the distance (3.80 Å) between neighboring C^α atoms. Classification of amino acid residues as helical or nonhelical was achieved by Zimm-Bragg treatment.[5] This procedure assigns a statistical weight of unity to each nonhelical amino acid residue, while the statistical weights of helical amino acid residues are σS or S, depending on whether the amino acid residue initiates or propagates,

0024-9297/80/2213-0904$01.00/0