## Intrinsic Viscosity of the Coiled Forms of Poly-L-Glutamic Acid and Poly-L-Lysine in Salt Solutions

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## Summary

Intrinsic viscosities of poly-L-glutamic acid at pH 8.8 and of poly-L-lysine at pH 3.2 in the presence of salts which are typically representative of lyotropic series are presented. Viscosities decrease continuously with increasing salt concentration and their temperature coefficients are negative. The ranking of cations and anions for decreasing the intrinsic viscosity of poly-L-glutamic acid and poly-L-lysine respectively, corresponds to the ranking for ion binding to isoelectric gelatin.

## INTRODUCTION

The effect of salts on both the denaturation and the solution properties of isoelectric proteins (1,2), uncharged peptides (3), and polyelectrolytes such as DNA (4) (the latter at sufficiently high concentrations where polyelectrolyte effects are effectively screened out) has been adequately studied and interpreted (1,3).

Recently (5) we investigated the denaturation of cationic tropocollagen at salt concentrations below 1 M, where polyelectrolyte effects are still conspicuous. The results were interpreted on the basis of the same mechanism of salt action as that established

* Contribution #32 from the Centro Virus Vegetali del C.N.R., Genova, Italy.

for isoelectric proteins (1) and uncharged model peptides (3). The principle used was that the order of effectiveness for ion binding to said solutes is the same under isoelectric, uncharged, and non-isoelectric conditions with the following provisions:

1. Given the series of anions and cations for increasing binding [for instance, to isoelectric gelatin (6)],

$$F^- < SO_4^{2-} < acetate^- < Cl^- < Br^- < NO_3^- < I^- < SCN^- \quad (1)$$

$$K^+ < Mg^{2+} < Na^+ < Cs^+ < Li^+ < Ca^{2+} \quad\quad\quad (2)$$
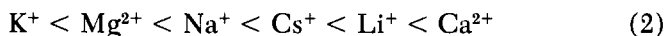
a strongly anionic polyelectrolyte will tend to repel the anions, and, consequently, the ranking of the latter according to (1) will not be manifested. Analogously, series (2) will not be effective for a strongly cationic polyelectrolyte when cations are electrostatically repelled.

2. Whereas ion binding to isoelectric or uncharged solutes promotes salting-in (i.e., increased solubility, decreased denaturation temperature, and often increased intrinsic viscosity), ion binding to polyelectrolytic solutes (at relatively low salt concentration) promotes the reverse behavior (i.e., salting-out).

In this paper we present a more thorough analysis of the above hypothesis, using two polyelectrolytes in their random coiled form, one strongly anionic (poly-L-glutamic acid at pH 8.8) and the other strongly cationic (poly-L-lysine at pH 3.2). The property investigated is the variation of the intrinsic viscosity with salt concentration $(C_s)$ extending from $\approx 0.2$ to $\approx 4$ $M$.

## EXPERIMENTAL

Two single samples of poly-L-glutamic acid (PGA) sodium salt and of poly-L-lysine (PL) hydrobromide were obtained from SIGMA Chemical Company (lot 125B-0400 for PGA and lot 115B-0700 for PL). According to the producer, approximate molecular weights were 68,000 for PGA and 150,000 for PL. Both polymers were readily soluble and were used without further purification. Solutions were prepared by mixing adequate amounts of a stock polymer solution in water with stock salt-water mixtures. The pH's of all primary solutions were adjusted to the desired values (8.8 for PGA and 3.2 for PL), readjusting, if necessary, the pH of the ternary solutions.

Viscosity measurements were performed at 25°C (±0.1) and, for PL, also at 35 and 50°, using suspended level capillary viscometers. Flow times were in all cases greater than 100 sec (±0.1 sec) and kinetic-energy corrections were negligible.

Salts of analytical grade were KSCN, KNO$_3$, KCl, LiCl, NaCl, and CaCl$_2$.

Reduced specific viscosities, $\eta_{sp}/c$, were measured at least at four different polymer concentrations, $c$, varying between 0.2 and 0.5 g/dl, in each salt solution of a given $C_s$. The customary extrapolation of reduced specific viscosity to zero polymer concentration yielded the intrinsic viscosity $[\eta]$. $\eta_{sp}/c$ decreased linearly with $c$; no anomalous trend with decreasing polymer concentration was observed for the systems investigated here. All data could be accurately reproduced.

Optical rotatory dispersion of typical solutions investigated yielded a value $[\alpha]_{233}^{25°} \approx -2000$, a value characteristic of randomly coiled conformations. Measurements were carried out with a Durrum-Jasco spectropolarometer.

## RESULTS

The variation of $[\eta]$ with $C_s$ for PGA and PL is illustrated in Fig. 1. In Fig. 2 the variation of $[\eta]$ of PL with temperature is shown. In the case of PGA, measurements in CaCl$_2$ are not reported, since the polymer precipitated even at low $C_s$. In all cases investigated, $[\eta]$ decreased with increasing $C_s$.

For the anionic PGA, no difference in the viscosities in KCl and KSCN is exhibited in spite of the considerable difference in the effects of the two anions in salting-in isoelectric proteins (1) [cf. series(1)]. Viscosities of PGA are greater in KCl than in NaCl and LiCl, just the opposite of what is observed for isoelectric gelatin (7). Values for the latter two salts are very similar, although they are slightly larger in NaCl than in LiCl.

In the case of cationic PL, no differences in the viscosities in KCl and CaCl$_2$ are exhibited when the $C_s$ values for the latter salt are multiplied by a factor 2 to obtain uniformity with respect to the Cl$^-$ concentration. Viscosities in KNO$_3$ are, however, smaller than in KCl. The temperature coefficient of $[\eta]$ for PL is more negative in 0.88 $M$ KCl than in 0.88 $M$ KNO$_3$.

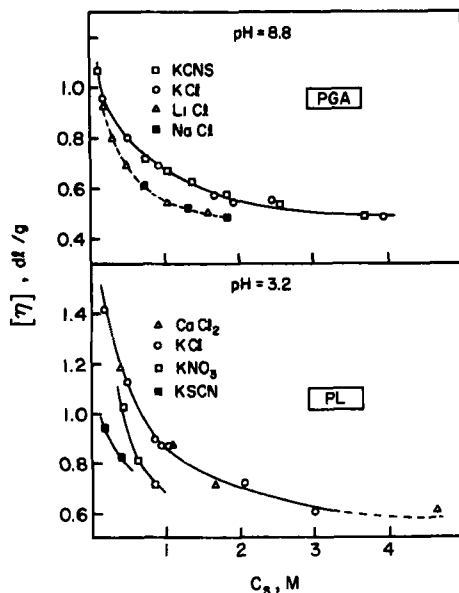**FIG. 1.** Variation of the intrinsic viscosity for PGA and PL with salt concentration. $CaCl_2$ molarities have been multiplied by a factor 2. $T = 25°C$.
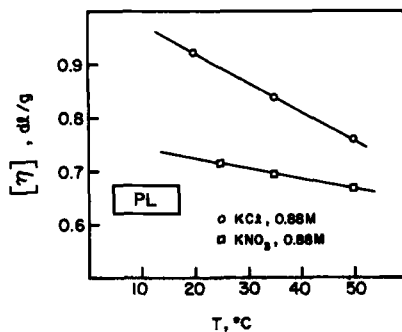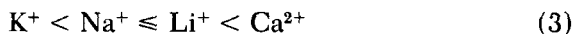


**FIG. 2.** Temperature variation of the intrinsic viscosity for PL in salt solutions at a constant $C_s$. pH 3.2.

## DISCUSSION

Considering the viscosity results and the insolubility of PGA in the presence of $CaCl_2$, the order for increasing salting-out for PGA is

$$K^+ < Na^+ \leqslant Li^+ < Ca^{2+} \tag{3}$$

$$SCN^- \approx Cl^- \tag{4}$$

and for PL

$$Cl^- < NO_3^- < SCN^- \tag{5}$$

$$Ca^{2+} \approx K^+ \tag{6}$$

It is readily seen that (3) and (5) are compatible, respectively, with (2) and (1), thus substantiating the hypothesis (cf. the introductory section) that increasing binding is responsible for increasing salting-out of polyelectrolytes. Moreover, (4) and (6) are in line with the expectation that differences in the intrinsic characteristics of anions or cations are largely inconsequential to the behavior of anionic or cationic polyelectrolytes, respectively.

The viscosity-temperature coefficient of PL appears to be more negative in the presence of the salt (KCl), which has poorer binding affinity, as found for isoelectric gelatin (7).

Iizuka and Yang (8) have also investigated the variation of $[\eta]$ with $C_s$ for PGA at pH 7.3 and found a decrease of $[\eta]$ with $C_s$ which is in line with that exhibited in Fig. 1. This decrease can be attributed to the normal polyelectrolyte behavior (9), which encroaches, at $C_s$ greater than at least 1 $M$, the salting-out region observed, at high $C_s$, even with normally salting-in agents for isoelectric gelatin (7). The ranking of ions according to (3), (4), (5), and (6) is not, perhaps, as surprising when $C_s < 1$ $M$ as when $C_s > 1$ $M$. A possible interpretation is that while the gross part of the polyelectrolyte effect is screened out at $C_s$ of a few tenths molar, a residual effect persists at considerably higher $C_s$ values. Iizuka and Yang (8) found that the empirical (9) $[\eta]^{-1}$ vs. $C_s^{1/2}$ plot yielded straight lines up to $C_s \approx 6$ $M$. However, the latter investigators did not observe a systematic difference between the viscosities in KF and LiBr.

Kono and Ikegami (10) found no evidence of binding of $Mg^{2+}$ to PGA; the interaction PGA-$Mg^{2+}$ was purely electrostatic. This is in line with the expectation from series (2) indicating that $Mg^{2+}$ does not possess a great tendency to bind [the interaction between collagen and $MgSO_4$ is also, in fact, described in terms of a generic diluent effect (1)]. Barone et al. (11) found that $Li^+$ has a considerable specific interaction with PGA, again in line with the expectation from series (2).

Considering data obtained for other polymers, Scruggs and Ross (12) found that the viscosity of anionic DNA in 1 $M$ salt solutions decreases with an order which is coherent with series (3). The denaturation behavior of cationic collagen (5) at pH $\approx$ 2 and $C_s < 1\ M$ was also in line with (5) and (6). As Robinson and Jencks (3) pointed out, the order for anion binding to quaternary ammonium exchange resins (13) (Dowex-2) is entirely consistent with (1). Finally, also in line with series (1), Fuoss and Strauss (9) noted that bromides are more effective than sulfates (at the same ionic strength) in reducing the intrinsic viscosity of a cationic synthetic polyelectrolyte.

The interaction described as a binding in the case of isoelectric proteins and uncharged model peptides is, very likely (3), an interaction of the ions with the dipoles of the peptide bond. The same interaction can be effective in the case of PGA and PL, while a more effective saturation of the charged groups of the polymers by the mobile ions also results in a reduction of the electrostatic free energy of the chain. As pointed out elsewhere (1,3), the behavior of the anions [series (1)] is probably much more general than that of cations. With the latter, specific alteration of series (2) from solute to solute could be observed.

It would be useful to explore the validity of the above conjectures in the region of typical polyelectrolyte behavior ($C_s < 0.2\ M$), where the problem of distinguishing between electrostatic and specific interactions is of considerable interest.

Finally, it is of interest to point out that in the case of PL in KSCN only viscosities for $C_s < 0.4$ are reported (Fig. 1), since when $C_s > 0.4$ the polymer is no longer in the coil form. In fact, it undergoes a new type of salt-induced coil-helix transformation which will be described in detail elsewhere (14).

## REFERENCES

1. A. Ciferri, L. Rajagh, and D. Puett, *Biopolymers*, 3, 461 (1965); D. Puett, R. Garmon, and A. Ciferri, *Nature*, 211, 1294 (1966); A. Ciferri, R. Garmon, and D. Puett, *Biopolymers*, 5 (1967).
2. P. H. Von Hippel and K. Y. Wong, *Science*, 145, 577 (1964); *J. Biol. Chem.*, 240, 3909 (1965).
3. D. R. Robinson and J. Jencks, *J. Am. Chem. Soc.*, 87, 2462 (1965).
4. K. Hamaguchi and E. P. Geiduschek, *J. Am. Chem. Soc.*, 84, 1329 (1962).
5. E. Bianchi, A. Ciferri, G. Conio, D. Puett, and L. Rajagh, *J. Biol. Chem.*, 242, 1361 (1967).
6. A. R. Docking and E. Heymann, *J. Phys. Chem.*, 43, 513 (1939).

7. L. Rajagh, D. Puett, and A. Ciferri, *Biopolymers*, 3, 421 (1965).

8. E. Iizuka and J. T. Yang, *Biochemistry*, 4, 1249 (1965).

9. R. M. Fuoss and V. P. Strauss, *Ann. N.Y. Acad. Sci.*, 51, 836 (1949).

10. N. Kono and A. Ikegami, *Biopolymers*, 4, 823 (1966).

11. G. Barone, V. Crescenzi, and F. Quadrifoglio, *Biopolymers*, 4, 529 (1966).

12. R. L. Scruggs and P. D. Ross, *Biopolymers*, 2, 593 (1964).

13. H. P. Gregor, J. Belle, and R. A. Marcus, *J. Am. Chem. Soc.*, 77, 2713 (1955).

14. D. Puett, A. Ciferri, E. Bianchi, and J. Hermans, Jr., *J. Phys. Chem.*, in press.

## Zusammenfassung

Die grundmolaren Viskositäten von Poly-L-Glutaminsäure bei pH 8.8 und von Poly-L-Lysin bei pH 3.2 in Gegenwart von Salzen, welche typische Vertreter einer lyotropen Reihe sind, wurden bestimmt. Die Viskositäten nehmen kontinuierlich mit zunehmender Salzkonzentration ab und die Temperaturkoeffizienten sind negativ. Die Reihe der Kationen und Anionen in der Anordnung zunehmender Herabsetzung der grundmolaren Viskosität von Poly-L-Glutaminsäure und Poly-L-Lysin entspricht der ionischen Bindungsstärke zu isoelektrischem Gelatin.

## Résumé

On présente les viscosités intrinsèques de l'acide poly-L-glutamique au pH 8.8 et de la poly-L-lysine au pH 3.2 en présence des sels typiquement représentatifs de séries lyotropes. Les viscosités diminuent continuèllement avec l'augmentation de la concentration des sels et leurs coëfficients de température sont négatifs. Le classement des cations et des anions selon l'abaissent de la viscosité intrinsèque de l'acide poly-L-glutamique et de la poly-L-lysine respectivement, correspond au classement de la fixation d'ions à la gelatine isoélectrique.