Enthalpy Changes upon Dilution and Ionization of Poly(L-glutamic acid) in Aqueous Solutions

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The enthalpy changes accompanying the dilution and ionization of poly(L-glutamic acid) in water have been measured at 25 °C for two degrees of polymerization (DP = 115 and DP = 480) at various degrees of ionization, α, for a concentration range from about 0.2 to 0.002 monomol/L. The heat of dilution displays an unusual dependence on the degree of ionization, which is in sharp contrast to the behavior of other weak carboxylic polyelectrolytes, such as poly(acrylic acid). The exothermic heat effects observed at low values of α become endothermic for the region where the helix−coil transition is most pronounced, and for high degrees of ionization, they are exothermic again. Evidently, an endothermic heat effect, produced by an additional conformational transition in the dilution process, is superimposed on the exothermic enthalpy of dilution, and it overweighs the latter in the region of α where the conformational transition is prevailing. The calorimetric titration curve, which gives the dependence of the heat of ionization, ΔH_i, on α, has a maximum and is typical for poly(carboxylic acids) which undergo pH-induced conformational transition, such as poly(methacrylic acid). The values of ΔH_i obtained at two polymer concentrations indicate that the enthalpy of ionization depends on the polypeptide concentration.

Introduction

Poly(L-glutamic acid) (PGA) is a poly(α-amino acid), which can be classified among weak synthetic polyelectrolytes.[1] The functional groups of this polypeptide are known to be in water solution at low pH practically un-ionized.[1−3] As the degree of ionization is increased (by adding strong base to a solution), the poly(glutamate anion) gradually displays remarkable properties of highly charged polyions. Furthermore, the increased repulsive forces between the ionized carboxylic groups on the polyion can induce eventual conformational changes. Since the work of Zimm and Rice,[4] synthetic polypeptides have been extensively studied, as they are simple analogues of proteins. Therefore, they are excellent models for interpretations of the physicochemical studies of these natural polyelectrolytes as well as the model systems for protein folding and unfolding. Pure poly(L-glutamic acid) is characterized by a rigid α-helical conformation in solutions at low degrees of ionization.[2] By altering the solution conditions such as pH,[5−10] temperature,[8,11,12] ionic strength,[6,9,13,14] solvent composition,[15−19] and counterion species[19−21], various investigators have observed a rather sharp transformation of the helical structure of the macroion into the flexible coiled conformation, typical for most synthetic polyelectrolytes. This conformational transition has been reflected also in measured thermodynamic properties,[22] such as the mean activity coefficient, osmotic coefficient, and apparent molar volume, as well as in transport properties, such as the molar conductivity[23] and transport number.[22]

The conformational changes of PGA and its derivatives in pure water or in mixtures of various solvents and in the presence of added simple salts have been studied also by direct calorimetry.[24−29] Inspection of literature data has disclosed that, although PGA has been one of the most frequently studied weak polyelectrolytes, no data exist for the enthalpy of dilution of this polyacid. This thermodynamic property is interesting not only per se, but it has also been considered as a very rigorous test of existing theories on solutions of various solutes.[3] The object of the present work was to determine the enthalpies of dilution of aqueous solutions of PGA as functions of the polymer concentration and degree of ionization for two samples with different degrees of polymerization. Our particular interest was directed to the region where conformational changes of PGA take place. Parallel to the dilution experiments, we performed also calorimetric titration studies at two PGA concentrations (0.1 and 0.002 monomol/L). The results showed that the enthalpy of ionization only slightly depends on the concentration and that the enthalpy change for the helix−coil transition, brought about by diluting the polymer, amounted to only about 10% of the overall enthalpy change accompanying the conformational transition.

Experimental Section

Materials. Two samples with different degrees of polymerization (DP = 480 and DP = 115, Sigma Chemical Co.) of the sodium salt of poly(L-glutamic acid), NaPG, were used without further purification. Diluted aqueous solutions of both samples of NaPG were passed through a cation exchange column in the H^+ form (Amberlite IR 200, Merck). The resulting solutions of poly(L-glutamic acid) were neutralized with CO_2-free solutions of NaOH from a pH of about 4 (α = 0) to the desired degree of neutralization, α. The neutralization was performed immediately after ion exchange, since it has been known that precipitation occurs[6,7] in pure PGA solutions upon storage.

The concentration of freshly prepared solutions of PGA was determined by potentiometric titration with NaOH under a nitrogen atmosphere. The concentration, *c*, is expressed in

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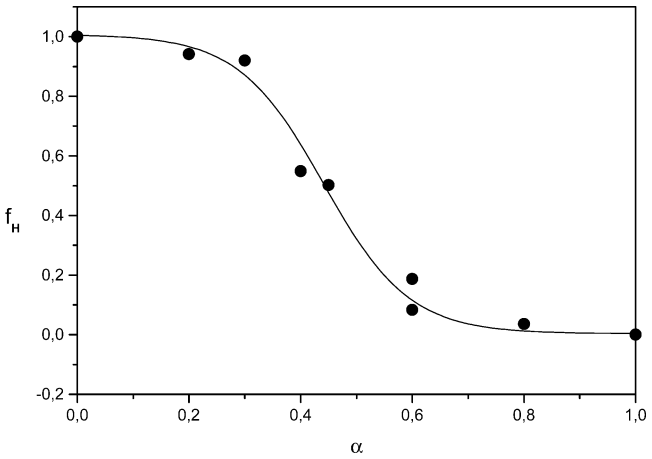

Figure 1. Fraction of the helical content, f_H, of poly(L-glutamic acid) in water solution at 25 °C as a function of the degree of ionization, α. Values of f_H were deduced from the molar ellipticities at 222 nm.

monomol dm^{-3}, that is, in mol of monomer units (or carboxylic groups) per dm^3.

Double distilled water with an electrical conductivity of less than 1×10^{-6} Ω^{-1} cm^{-1} was used throughout.

Apparatus. Heats of dilution measurements were carried out at 25 °C in an LKB (Sweden) 100700-2-batch microcalorimeter with golden reaction cells. The same volumes of a polymer solution and water were mixed. The final solution from each experiment was used as the initial solution for the subsequent dilution. By summing up the heat effects accompanying successive dilutions, the concentration dependence of the enthalpy of dilution over a larger concentration range was obtained. Some measurements were performed also in a TAM 2277 calorimeter (Thermometric AB, Sweden) by mixing equal amounts of a PGA solution with water in the flow-mix measuring cup. The heat of ionization measurements were carried out in a TAM 2277 calorimeter using the titration cell. Aqueous solutions of PGA (2 mL of 0.002 monomol dm^{-3}, α = 0, or 0.1 monomol dm^{-3}, α = 0.15, solutions) were titrated by a NaOH solution of an appropriate concentration, placed in a 250 μL syringe. The measured exothermic heat effects were corrected for the heat of protonation of water[30,31] (−55.80 kJ mol^{-1}) and for the heats of dilution of NaOH[30] and PGA. The latter two corrections were found to be practically negligible.

We followed by circular dichroism (CD) measurements the conformational transition of PGA from the helix to the random coil. They were performed at 25 ± 0.1 °C by an AVIV 62A DS circular dichroism spectrometer. The polymer concentrations in these measurements were around 1.5×10^{-3} mol of COO$^-$ dm^{-3}. From the observed ellipticity, Θ_λ (deg), we calculated the molar ellipticity, $[\Theta]_\lambda$ (deg cm^2 mol^{-1}) ($=\Theta_\lambda/lc$, where l is the optical path of the cell in cm and c is the concentration in mol of COO cm^{-3}), as a function of the wavelength, λ, for various degrees of ionization, α. We estimated the fraction of the helical content, f_H, presented in Figure 1, from the observed values of the molar ellipticities at 222 nm, $[\Theta]_{222}$, according to the relationship

$$f_H = \frac{[\Theta]_{222} - [\Theta]^C_{222}}{[\Theta]^H_{222} - [\Theta]^C_{222}} \quad (1)$$

where the superscripts H and C refer to completely helix (α < 0.2) and completely coil (α > 0.8) conformations, respectively.

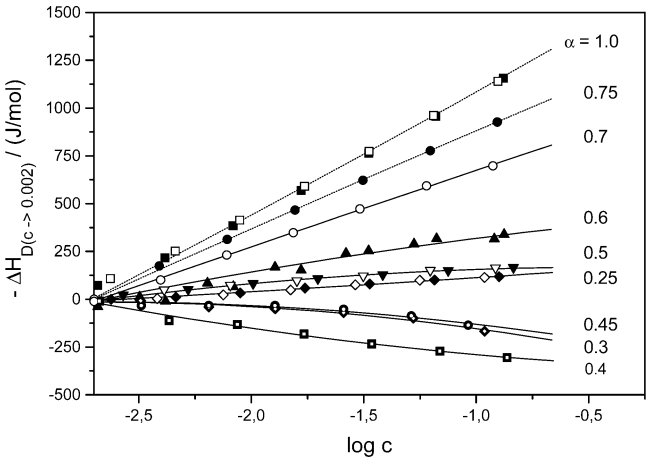

Figure 2. Enthalpies of dilution of poly(L-glutamic acid) in water at 25 °C for two degrees of polymerization (DP = 115 (open symbols) and DP = 480 (solid symbols)) and for nine degrees of ionization, α, as a function of polyelectrolyte concentration. Dashed lines: theoretical values calculated for α = 1 and α = 0.75 from the cell model (eqs 2 and 4) with the structural value of the parameter λ.

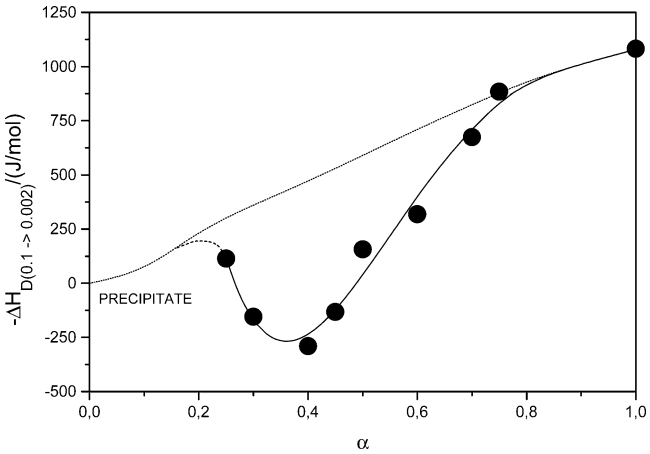

Figure 3. Enthalpy of dilution for the concentration range from 0.1 to 0.002 monomol/L as a function of the degree of ionization. The doted line represents the electrostatic contribution to ΔH_D calculated from the cell model.

Results and Discussion

The results of the calorimetric measurements are presented in Figure 2. The intermediate enthalpy of dilution, $\Delta H_{D(c\rightarrow cf)}$, is defined as the enthalpy change upon dilution from the initial concentration, c, to the final concentration, c_f (in the present case 0.002 mol of COO dm^{-3}). Various aspects of the experimental results are noticeable. We can see that ΔH_D is approximately a linear function of the logarithm of concentration. Furthermore, experimental results for the degrees of ionization 0.25, 0.5, and 1 indicate that it practically does not depend on the degree of polymerization, a finding observed also with other polyelectrolytes of moderate and high molecular weights.[32,33] The most remarkable feature of the present results is the unusual dependence of ΔH_D on the degree of ionization. It is in sharp contrast to the behavior of other weak carboxylic polyelectrolytes, such as poly(acrylic acid). Whereas the heat effects for solutions containing the poly(acrylate anions) are exothermic for all values of α and increase with the increasing degree of ionization,[34] for solutions of PGA, the exothermic heat effects observed at low values of α became endothermic for the region where the helix−coil transition is most pronounced, and for high degrees of ionization, they are exothermic again. This observation is more clearly presented in Figure 3,

where the experimental ΔH_D for the concentration range from 0.1 to 0.002 monomol/L is plotted against α.

In Figures 2 and 3 are presented also theoretically predicted values of ΔH_D. For polyelectrolyte solutions containing no simple salts, two theories have been used frequently. The first is the infinite line-charge theory of Manning,[35] which predicts limiting laws for various thermodynamic properties and has successfully explained the behavior of polyelectrolyte solutions at low concentrations. For a wider concentration range, the infinite rodlike polyion theory of Lifson and Katchalsky,[36] based on the cell model,[37] has proved to be useful. According to this theory, the electrostatic enthalpy of a polyelectrolyte solution is given by the expression[34,38]

$$H^e = \frac{\alpha z_1 RT}{z_2 \lambda}\left[(1+\beta^2)\gamma + \ln\frac{(1-\lambda)^2-\beta^2}{1-\beta^2} + \lambda\right] \times \left(1 + \frac{\mathrm{d}\ln\epsilon}{\mathrm{d}\ln T}\right) + \frac{\alpha z_1 RT}{2 z_2 \lambda}\left[1-\beta^2 - \frac{2\lambda e^{2\gamma}}{e^{2\gamma}-1}\right]\left(\frac{\mathrm{d}\ln V}{\mathrm{d}\ln T} - 2\frac{\mathrm{d}\ln a}{\mathrm{d}\ln T}\right) \quad (2)$$

The principle parameter of the theory is the charge-density parameter λ defined by

$$\lambda = \alpha z_1 z_2 e_0^2/\epsilon kTb \quad (3)$$

All symbols have the same meaning as defined previously.[1,34,36]

The observed enthalpy of dilution, ΔH_D, is related to the electrostatic contribution, ΔH_D^e, by

$$\Delta H_D = \Delta H_D^e + \Delta H_D^0 \quad (4)$$

where ΔH_D^0 is the nonelectrostatic contribution to the dilution enthalpy.

The parameter λ appears in theoretical expressions for all thermodynamic properties and usually has to be properly adjusted in order to bring theory and experiment into agreement. It has been found that the ratio between this effective λ and the structural λ, given by eq 3, is always $\lambda_{effective}/\lambda_{structural} \geq 1.0$ and that this ratio is proportional to the extent of coiling of the polyion. Thus, it has been found that the ratio $\lambda_{effective}/\lambda_{structural}$ is 1 for the truly rodlike polyelectrolyte DNA,[39] 1−1.3 for rather stiff poly(styrenesulfonates),[38,40,41] and about 2 for flexible-chain polyelectrolytes, poly(phosphates), poly(methacrylates), and poly(acrylates).[42]

In computations of H^e and ΔH_D^e, the following values have been chosen. For parameters characteristic for solvent, the values for water at 25 °C were used:[43] the dielectric constant $\epsilon = 78.36$, $\mathrm{d}\ln\epsilon/\mathrm{d}\ln T = -1.368$, and $\mathrm{d}\ln V/\mathrm{d}\ln T = 0.0767$. The parameters necessary for specifying the polyion dimensions are the distance between the ionizable groups on the polyion, *b*, and the radius of the polyion, *a*, needed to obtain the relation[36,42] between the parameter γ and concentration. We have estimated these values from structural parameters[44,45] for polypeptides in their respective conformations, and they are consistent with literature data.[46−48] It has to be pointed out that the value of *a*, for which some scattering can be observed in the literature,[46,48] does not influence significantly the theoretically predicted values. We used for *a* the values 0.65 and 0.58 nm for the helix and coil conformational states, respectively. With $b_H = 0.15$ nm and $b_C = 0.36$ nm for the helical and coiled conformations, respectively, we calculated from eq 3 the structural values of the charge-density parameter: $\lambda_H = 4.76\alpha$ and $\lambda_C = 2.04\alpha$.

Comparison of the theoretically predicted curves for degrees of ionization of 1.0 and 0.75, presented in Figure 2 as dotted lines, with the experimental points shows a good agreement. It has to be emphasized that the theoretical curves have been calculated with the structural value of the parameter λ, indicating that the poly(glutamate anion) is a stiff polyion even in the coiled conformation. As pointed out above, it can be seen from Figure 2 that the measured ΔH_D values do not show any regularity with respect to the degree of ionization. This observation is demonstrated by Figure 3, constructed from Figure 2, where the intermediate enthalpy of dilution between concentrations of 0.1 and 0.002 mol/L is plotted against α. It can be seen that the heat effects are exothermic from $\alpha = 1$ to about $\alpha = 0.5$, for $0.5 > \alpha > 0.27$ they are endothermic, and below $\alpha \approx 0.27$ they became exothermic again. Such a dependence of ΔH_D clearly indicates that an endothermic heat effect is superimposed on the exothermic enthalpy of dilution and that it overweighs the latter in the region of α where the conformational transition is prevailing.

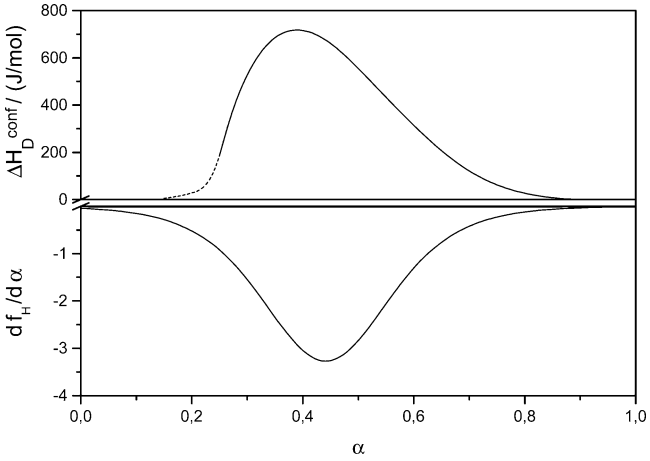

Figure 4. Comparison of the nonelectrostatic contribution to ΔH_D with the derivative of the fraction of the helical content, $df_H/d\alpha$.

In view of a good agreement between experimental and calculated results for completely coiled PGA ($\alpha = 1$), we decided to estimate the electrostatic contribution to the enthalpy of dilution, ΔH_D^e, also for the transition region where both conformational states are present. In these computations, we applied the additivity rule for *a* and *b*: $a = a_H f_H + a_C(1 - f_H)$ and $b = b_H f_H + b_C(1 - f_H)$, where f_H is the fraction of the helical content (Figure 1) and the subscripts H and C at *a* and *b* refer to completely helix and completely coil conformations, respectively. The calculated ΔH_D^e is presented in Figure 3 as the dotted line. According to eq 4, the difference between the experimental and calculated curves in Figure 3 yields the nonelectrostatic contribution, ΔH_D^0, to the dilution enthalpy. This contribution is presented in Figure 4 together with the first derivative of f_H. A good coincidence of the course of the two curves undoubtedly indicates that the nonelectrostatic contribution to ΔH_D is the consequence of an additional conformational transition in the dilution process. Evidently, the extent of coiling is concentration dependent at a given degree of ionization. Due to the fact that this additional conformational contribution, ΔH_D^{conf}, is endothermic, one may conclude that the extent of coiling increases in the dilution process. This increase is, however, small. The overall conformational enthalpy change caused by dilution from the initial concentration 0.1 monomol/L to the final concentration 0.002 monomol/L (obtained from the area below the ΔH_D^{conf} curve) is about 220 J/mol, whereas the reported[7] value of the overall enthalpy change for the α-helix−coil transition, derived from the potentiometric titration curve,[7] is 4720 J/mol in 0.1 M KCl. The value $\Delta H_D^{conf} = 220$ J/mol,

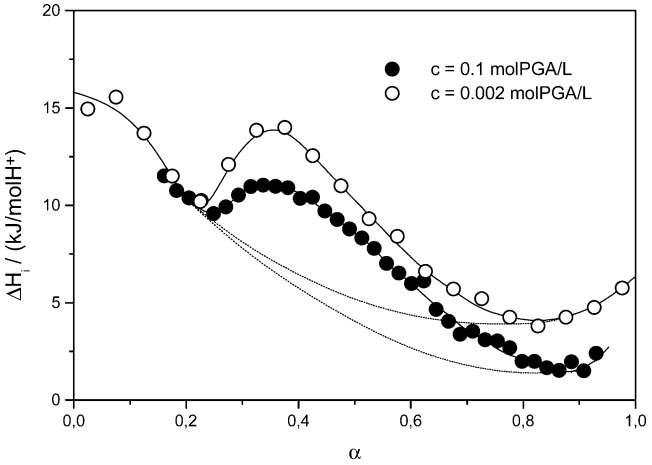

Figure 5. Enthalpy of ionization of poly(L-glutamic acid) in water at 25 °C for two polymer concentrations as a function of the degree of ionization.

related to the present dilution experiments, represents thus only about 5% of the total enthalpy change caused by the conformational transition.

To estimate the enthalpy change accompanying the helix−coil transition by direct calorimetry, we performed calorimetric titration of PGA solutions with NaOH at the initial and final concentrations (0.1 and 0.002 monomol dm^{-3}, respectively) of the intermediate enthalpy of dilution presented in Figure 3. From the calorimetric titration measurements, the resulting enthalpy of ionization, ΔH_i (see the Experimental Section), is plotted in Figure 5 against α. As expected, the curves clearly show that the ionization behavior of PGA is not normal and that ΔH_i depends on concentration. If the pH-induced helix−coil transition did not occur, ΔH_i would vary monotonically with α, similarly as observed with other weak polyelectrolytes which show no conformational change.[49] Instead, the experimental curves are similar to those found for polyelectrolytes undergoing globule−coil transition, such as poly(methacrylic acid).[50] It can be seen that, within the α range where the conformational transition has been observed, the endothermic heat effects are superimposed on the enthalpy of ionization curves. The normalized areas (dotted baselines) under the peaks can be thus considered to represent the overall enthalpy changes caused by the helix−coil transition. From the two areas, we get for ΔH_{conf} the values 2.0 ± 0.1 and 1.7 ± 0.1 kJ/mol for 0.002 and 0.1 monomolar solutions, respectively. These values are lower than the value 4720 J/mol, derived from the potentiometric titration curve.[7] Since the conformational transition is spread over a wide α range, the values of ΔH_{conf} cannot be determined with a high precision. Anyhow, the difference between the two values (0.3 kJ/mol) roughly agrees, within the range of the experimental error, with the more reliable value 220 J/mol, derived above from the heat of dilution measurements.

Conclusion

The enthalpy change accompanying the conformational transition of poly(L-glutamic acid) from helix to coil, ΔH_{conf}, has been determined from calorimetric titration curves, in which the dependence of the heat of ionization on the degree of ionization has been determined. The results of the measurements show that the enthalpy of the conformational transition depends on the polypeptide concentration. The values derived for 0.002 and 0.1 monomolar solutions are $\Delta H_{conf} = 2.0 \pm 0.1$ and 1.7 ± 0.1 kJ/mol, respectively. This finding has been supported by the heat of dilution measurements which display an unusual dependence on the degree of ionization, in sharp contrast to the behavior of other weak carboxylic polyelectrolytes. An endothermic heat effect has been detected in the dilution process, which is superimposed on the exothermic enthalpy of dilution and which overweighs the latter in the region of α where the conformational transition is prevailing. For the dilution from the initial concentration 0.1 monomol dm^{-3} to the final concentration 0.002 monomol dm^{-3}, the value of this endothermic heat effect is 220 J/mol, which is in rough agreement with the difference between the two values of ΔH_{conf} (0.3 kJ/mol) given above.

References and Notes

(1) Armstrong, R. W.; Strauss, U. P. In *Encyclopedia of Polymer Science and Technology*; Mark, H. F., Gaylord, N. G., Bikales, N. M., Eds.; Interscience: New York, 1969; Vol. 10, pp 781−861.
(2) Fasman, G. D. *Poly-α-amino Acids*; Arnold (Publishers) LTD: London, 1967.
(3) Harned, H. S.; Owen, B. B. *The Physical Chemistry of Electrolytic Solutions*; Reinhold: New York, 1958.
(4) Zimm, B. H.; Rice, S. A. *Mol. Phys.* **1960**, *3*, 391−407.
(5) Wada, A. *Mol. Phys.* **1960**, *3*, 409.
(6) Nagasawa, M.; Holtzer, A. *J. Am. Chem. Soc.* **1964**, *86*, 538−543.
(7) Hermans, J., Jr. *J. Phys. Chem.* **1966**, *70*, 510−515.
(8) Warashima, A.; Ikegami, A. *Biopolymers* **1972**, *11*, 529−547.
(9) Nakamura, H.; Wada, A. *Biopolymers* **1981**, *20*, 2567−2582.
(10) Reda, T.; Hermel, H.; Höltje, H. D. *Langmuir* **1996**, *12*, 6452−6458.
(11) Santiago, G.; Maroun, R. C.; Hawkins, E. R.; Mattice, W. L. *Biopolymers* **1981**, *20*, 2181−2194.
(12) Doty, P.; Wada, G.; Yang, J.; Blout, E. R. *J. Polym. Sci.* **1957**, *23*, 851−861.
(13) McDiarmid, R.,; Doty, P. *J. Phys. Chem.* **1966**, *70*, 2620−2627.
(14) Bychkova, N. E.; Ptitsyn, O. B.; Barskaya, T. N. *Biopolymers* **1971**, *10*, 2161−2179.
(15) Hermans, J., Jr. *J. Am. Chem. Soc.* **1966**, *88*, 2418−2422.
(16) Conio, G.; Patrone, E. *Biopolymers* **1969**, *8*, 57−68.
(17) Morcellet, M.; Loucheux, C. *Biopolymers* **1980**, *19*, 2177−2190.
(18) Daoust, H.; St-Cyr, D. *Polym. J.* **1982**, *14*, 831−838.
(19) Kimiyama, J.; Miyoshi, M.; Katayama, D.; Satoh, M.; Iijima, T.; Uedaira, H. *Polym. Commun.* **1986**, *27*, 235−237.
(20) Barone, G.; Crescenzi, V.; Quadrifoglio, F. *Biopolymers* **1966**, *4*, 529−538.
(21) Satoh, M.; Fujii, Y.; Kato, F.; Komiyama, J. *Biopolymers* **1991**, *31*, 1−10.
(22) Ise, N.; Okubo, T. *Macromolecules* **1969**, *2*, 401−407.
(23) Bordi, F.; Cametti, C.; Paradossi, G. *J. Phys. Chem.* **1992**, *96*, 913−918.
(24) Kagemoto, A.; Fujishiro, R. *Biopolymers* **1968**, *6*, 1753−1758.
(25) Teramoto, A.; Norisuye, T. *Biopolymers* **1972**, *11*, 1693−1700.
(26) Jeremic, K.; Karasz, F. E. *Biopolymers* **1985**, *24*, 1823−1840.
(27) Paradosi, G.; Pispisa, B.; Rizzo, R. *Biopolymers* **1986**, *25*, 1249−1258.
(28) Daoust, H.; St-Cyr, D. *Biopolymers* **1988**, *27*, 1267−1281.
(29) Roles, K. A.; Xenopoulos, A.; Wunderlich, B. *Biopolymers* **1993**, *33*, 753−768.
(30) Vanderzee, C. E.; Swanson, J. A. *J. Phys. Chem.* **1963**, *67*, 2608−2612.
(31) Hale, J. D.; Izatt, R. M.; Christensen, J. J. *J. Phys. Chem.* **1963**, *67*, 2605−2608.
(32) Dolar, D.; Špan, J.; Isaković, S. *Biophys. Chem.* **1974**, *1*, 312−317.
(33) Oman, S. *Makromol. Chem.* **1977**, *178*, 475−484.
(34) Škerjanc, J. *Biophys. Chem.* **1974**, *1*, 376−380.
(35) Manning, G. S. *J. Chem. Phys.* **1969**, *51*, 924−933.
(36) Lifson, S.; Katchalsky, A. *J. Polym. Sci.* **1954**, *13*, 43−55.
(37) (a) Fuoss, R. M.; Katchalsky, A.; Lifson, S. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 579−589. (b) Alfrey, T., Jr.; Berg, P. W.; Morawetz, H. *J. Polym. Sci.* **1951**, *7*, 543−547.
(38) Škerjanc, J.; Dolar, D.; Leskovšek, D. *Z. Phys. Chem.* **1967**, *56*, 207−217; **1970**, *70*, 31−38.
(39) Auer, H. E.; Alexandrowicz, Z. *Biopolymers* **1969**, *8*, 1−20.
(40) Dolar, D. In *Polyelectrolytes*; Sélégny, E., Mandel, M., Strauss, U. P., Eds.; Reidel: Dordrecht, The Netherlands, 1974; pp 97−113.
(41) Vesnaver, G.; Rudež, M.; Pohar, C.; Škerjanc, J. *J. Phys. Chem.* **1984**, *88*, 2411−2414.
(42) Katchalsky, A.; Alexandrowicz, Z.; Kedem, O. In *Chemical Physics of Ionic Solutions*; Conway, B. E., Barradas, R. G., Eds.; Wiley: New York, 1966; pp 295−346.

(43) Owen, B. B.; Miller, R. C.; Milner, C. E.; Cogan, H. L. *J. Phys. Chem.* **1961**, *65*, 2065−2070.

(44) Pauling, L. *The Nature of the Chemical Bond*; Cornel University Press: London, 1948.

(45) Elliott, A. In *Poly-α-Amino Acids*; Fasman, G. D., Ed.; Arnold: London, 1967; pp 14−38.

(46) Zhang, L.; Takematsu, T.; Norisuye, T. *Macromolecules* **1987**, *20*, 2882−2887.

(47) Paoletti, S.; Cesàro, A.; Arce Samper, C.; Benegas, J. C. *Biophys. Chem.* **1989**, *34*, 301−309.

(48) Nilsson, S.; Zhang, W. *Macromolecules* **1990**, *23*, 5234−5239.

(49) Crescenzi, V.; Delben, F.; Quadrifoglio, F.; Dolar, D. *J. Phys. Chem.* **1973**, *77*, 539−544.

(50) Crescenzi, V.; Delben, F.; Quadrifoglio, F. *J. Polym. Sci., Part A: Polym. Chem.* **1972**, *10*, 357−368.