Titration of α-Helical Poly-L-lysine in 95% Methanol. A Study of the Range of the Electrostatic Potential in Polypeptides[1]

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Abstract: The titration curves of *n*-butylamine and poly-L-lysine in 95% aqueous methanol were obtained at 25°. Since the conformation of the polymer is α helical over the whole range of the titration curve, *without* the occurrence of a helix-coil transition, the two titration curves differ by the electrostatic free energy of the charges on the polymer. Using both a Debye–Hückel and also an empirical pair-electrostatic potential, and a matrix method to evaluate the partition function and average quantities derived therefrom, it was possible to compute the titration curves of the polymer for various values of a quantity μ (up to $\mu = 6$), which is essentially a measure of the range of the electrostatic interactions taken into account in the computations. By extrapolating these data to infinite μ, agreement was obtained with the experimental titration curve for poly-L-lysine. An assessment of the range of the electrostatic potential in 95% methanol is obtained from the rate of convergence of the curves for μ = 0–6 to that for infinite μ. The Debye–Hückel potential is also used to obtain a rough comparison between the range of the potential in 95% methanol and in water.

In conformational energy calculations on polypeptides and proteins,[4,5] electrostatic interactions (which are known to be of long range) must be included since they influence the structure[6–8] of the macromolecule. For example, fully charged poly-L-lysine in water exists in the random coil form, whereas the uncharged polymer is α helical[6] in this solvent. Therefore, it is important to have some knowledge of the form and the range of the electrostatic potential in charged polyamino acids.

Many useful treatments have been reported for the computation of the electrostatic potential in order to account for the properties of solutions of polyelectrolytes. While most theories have dealt with uniformly charged spheres[9–11] or cylinders,[12–15] matrix methods, which involve the use of discrete charges, have been applied to the study of electrostatic interactions in polyelectrolytes;[16,17] however, in the latter methods, it was necessary to truncate the potential beyond a very small range in order to be able to use a matrix of manageable size. For example, Zimm and Rice[16] used a matrix method, with a Debye–Hückel pair-interaction electrostatic potential, to treat the helix-coil transition in charged polyamino acids, but restricted the range of the potential to only four neighboring charges.

In the treatment of Zimm and Rice,[16] two phenomena were present simultaneously, *viz.*, the equilibrium between charged and uncharged groups, and the equilibrium between helical and coil states. In order to study the range of the electrostatic potential, it would be preferable to examine the equilibrium between charged and uncharged groups in a system in which no change of conformation occurs as the degree of ionization varies. For this purpose, it may be noted that the α-helical form of some charged polyamino acids is known to be stable in methanol[18–20] and in other alcohols.[21,22] In particular, Epand and Scheraga[18] showed that poly-L-lysine seems to be completely helical in methanol (>90%), even at neutral pH, and Joubert, *et al.*,[23] showed that this polymer is charged at neutral pH even at this high a concentration of methanol. Thus, poly-L-lysine in methanol solution would seem to be a good system for the study of the equilibrium between charged and uncharged groups in a polymer of known (α-helical) geometry, without the accompaniment of a helix-coil transition. Therefore, in this paper we report titration data for α-helical poly-L-lysine, and its model compound *n*-butylamine, in 95% methanol. The titration curve of the model compound reflects the behavior of an isolated amine group at the end of a hydrocarbon chain (like the ϵ-amine group of lysine), and departures of the titration curve of poly-L-lysine from that of *n*-butylamine are assumed to arise because of intramolecular electrostatic interactions in the polymer. A matrix treatment of the equilibrium between charged and uncharged groups is applied to the data in order to determine the range of the electrostatic potential, rather than truncate it beyond four charged groups as Zimm and Rice did. In this treatment, the solution is assumed to be suf-

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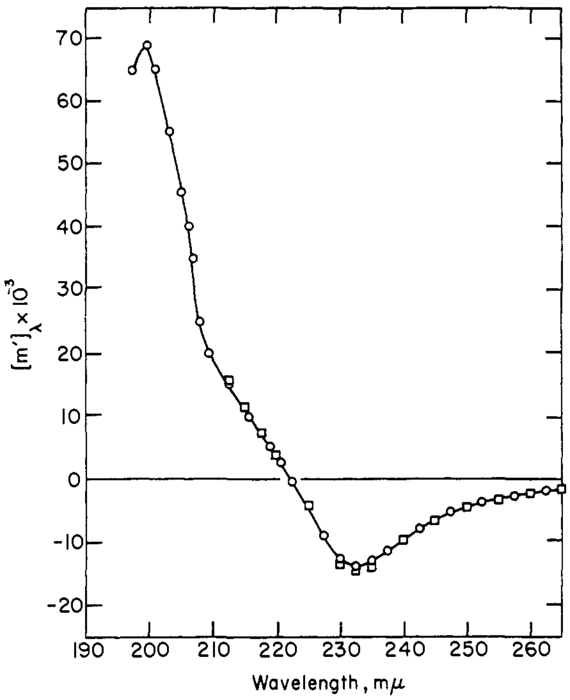

Figure 1. Values of $[m']_\lambda$ as a function of wavelength for poly-L-lysine hydrobromide in 95% aqueous methanol. The solid curve was obtained at 26° and $pa_H^* = 6.5$: ○, selected points at 26° and $pa_H^* = 12$; □, selected points at 11° and $pa_H^* = 6.5$.

ficiently dilute, so that interactions between different poly-L-lysine (or *n*-butylamine) molecules may be neglected.

Experimental Section

Materials. Poly-L-lysine hydrobromide (Lot L-90) was obtained from Pilot Chemicals Inc., Watertown, Mass. Methanol, spectranalyzed, from Matheson Coleman and Bell, was used without further purification. Lithium chloride was from Baker and Adams, and dried at 110° before using. The Karl Fischer reagent (single stabilized solution) was obtained from Matheson Coleman and Bell. Ammonium oxalate and oxalic acid were of reagent grade and obtained from Mallinckrodt; *n*-butylamine was a Fischer Scientific reagent.

Preparation of Solutions. All solutions were made up in 95% v/v (93.7% w/w) methanol. Poly-L-lysine solutions were made up by dissolving the polymer first in water and then adding methanol. Lithium chloride was added to the alcohol solutions, since other salts were found to be insoluble in solutions containing a large percentage of methanol. However, small concentrations of lithium chloride (0.02 *M*) had to be used, since larger amounts caused the polymer to salt out. Concentrations of the polymer were determined by a micro-Kjeldahl method. The percentage of water in the solutions was determined with the Karl Fischer reagent.

Viscosity. The specific viscosity of a 1% solution of poly-L-lysine hydrobromide in salt-free water was determined by using an Ubbelohde dilution viscometer at 25.0°. The degree of polymerization was determined by using the calibration curve of Yaron and Berger.[24]

Optical Rotatory Dispersion. ORD measurements were carried out on a Cary-60 recording spectropolarimeter. Measurements were made at pa_H^* (defined in eq 2) of 6.5 and 12 at 11 and 26° from 265 to 195 mμ to check for any change in conformation that might occur in the pH range of interest. A water-jacketed quartz cell of 0.1-cm pathlength was used. This cell was thermostatted with a Haake constant temperature bath. The reduced mean residue rotation, $[m']_\lambda$, was calculated by eq 1 where *n* is the refractive index of the solvent, M_0 is the residue molecular weight, and $[\alpha]_\lambda$ is the specific rotation.

$$[m']_\lambda = \frac{3M_0}{(n^2 + 2)100}[\alpha]_\lambda \qquad (1)$$

Titrations. pH measurements were made with a Radiometer type 4 pH meter with the use of a glass electrode type G222B and a calomel electrode type K400. Since the salt concentration was very low, the glass electrode error was negligible. The titrations were carried out on solutions containing about 20 mg of poly-L-lysine hydrobromide in 20 ml of 0.02 *N* LiCl in 95% methanol with both NaOH and HCl (to show reversibility) delivered from a microburet. The temperature was maintained at 25.0° in a water-jacketed closed vessel. To eliminate the undesirable effect of carbon dioxide, prepurified nitrogen, saturated with solvent, was passed over the solution during the course of the titrations.

The meter readings of pH were calibrated with six NBS buffers (dissolved in water) in 1-pH unit intervals over the pH range of 6–11. These pH meter readings were then converted to units expressing acidity in methanol by the following relation[25]

$$pa_H^* = \mathrm{pH} - \bar{E}_j + \log {}_m\gamma_H = \mathrm{pH} - \delta \qquad (2)$$

where a_H^* is the quantity that designates the activity of H^+ in the equilibrium constant expression in the medium, $\bar{E}_j$ is the residual liquid-junction potential expressed in pH units, and ${}_m\gamma_H$ is a measure of the medium effect. The values of the constant δ as a function of composition are available.[26] Values of pa_H^* were therefore obtained by subtracting the quantity δ from the pH meter reading. The value of δ for 95% methanol was checked by measuring the pH, and hence the pa_H^* of the oxalate buffer described by deLigny and Rehbach,[27] and was found to be in agreement with their value. After corrections were made for the titration of the solvent, the curve of α (the degree of dissociation of protons from RNH_3^+) *vs.* pa_H^* was easily obtained.

Water Determinations. Since the constant δ depends strongly on the concentration of the methanol in solution (independent of salt concentration),[26] it was necessary to determine the water concentrations of the solutions before and after a titration. This was done by using the Karl–Fischer reagent, standardized against a water–methanol solution of known composition. The concentrations were found to be constant within 0.1% over the whole titration.

Results

Viscosity. The specific viscosity of a 1% solution of the hydrobromide form of the polymer in salt-free water was found to be 9.6 ± 0.2. Using the calibration curve of Yaron and Berger,[24] this was found to correspond to a degree of polymerization of 1620 or a molecular weight of 360,000.

Optical Rotatory Dispersion. The ORD data of poly-L-lysine hydrobromide in 95% methanol at pa_H^* 6.5 and 12 at 26 and 11° are shown in Figure 1. As can be seen, there is hardly any noticeable change in conformation over this temperature and pH range. The values of $[m']_{233}$ and $[m']_{198}$ are identical within experimental error with the values reported by Epand and Scheraga[18] for this polyamino acid in 98% methanol at neutral pH. These results indicate that the titration of poly-L-lysine in 95% methanol involves only an equilibrium between charged and uncharged side chains of an α-helix, with no transformation to the coil state.

Titration Curves. The curves for α *vs.* pH and $-(pa_H^* - \log \alpha/(1 - \alpha) - pK_0)$ (from eq 3) *vs.* α for *n*-butylamine and poly-L-lysine are shown in Figures 2 and 3, respectively. From Figure 2, $\alpha = 0.5$ at $pa_H^* = 10.1$ for *n*-butylamine; a value of 10.08 has been reported[28] for the p*K* value of *n*-butylamine in 94.17% w/w methanol at 25° for an NaCl concentration of 5.77 ×

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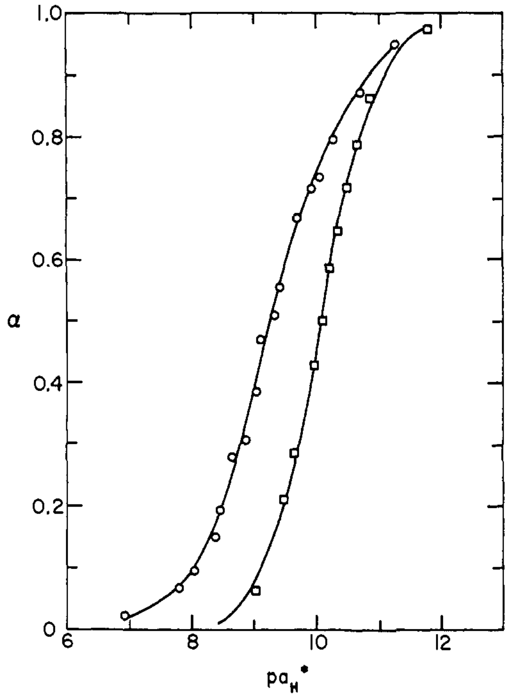

Figure 2. Experimental titration curves for: □, *n*-butylamine; and ○, poly-L-lysine in 95% methanol at 25° in 0.02 *M* LiCl.

10^{-5}. The difference in the curves for *n*-butylamine and poly-L-lysine presumably arises because of the electrostatic interactions on the poly-L-lysine chain.

The potentiometric titration curve of a polyion is given by eq 3[29] where pK_0 is the intrinsic dissociation

$$\mathrm{pH} = pK_0 + \log \alpha/(1-\alpha) - 0.434\epsilon\langle\psi\rangle/kT \quad (3)$$

constant, $\langle\psi\rangle$ is the electrostatic potential on the surface of the polyion, and ϵ is the magnitude of the electronic charge. The term $-0.434\epsilon\langle\psi\rangle/kT$ is also designated ΔpK, and can be found from a comparison of the titration curves of *n*-butylamine (for which $\Delta pK = 0$) and poly-L-lysine, *i.e.*, by subtracting the curve for *n*-butylamine from that for poly-L-lysine. This quantity is plotted *vs.* α in Figure 3. It can be seen from the curve that the quantity $\epsilon\langle\psi\rangle/kT$ is in fact greater than unity for most values of α. For the Debye–Hückel condition to hold, it is necessary that $\epsilon\langle\psi\rangle/kT \ll 1$. As we will discuss later, the fact that $\epsilon\langle\psi\rangle$ is greater than kT leads to a limitation in the Zimm–Rice treatment.

Theory

In this section, we develop the theory for interpreting the titration data. We base our discussion on the assumptions that: (1) intermolecular interactions are negligible in comparison with intramolecular interactions; (2) the conformations of the backbone and side chains are independent of the degree of protonation (α-helical backbone and fully extended side chains); and (3) the sole source of intramolecular interactions is the (screened) coulombic interaction between charged side chains. These assumptions are discussed in a later section.

We consider first the equilibrium

$$\mathrm{RNH_3^+} \rightleftharpoons \mathrm{RNH_2} + \mathrm{H^+} \quad (4)$$

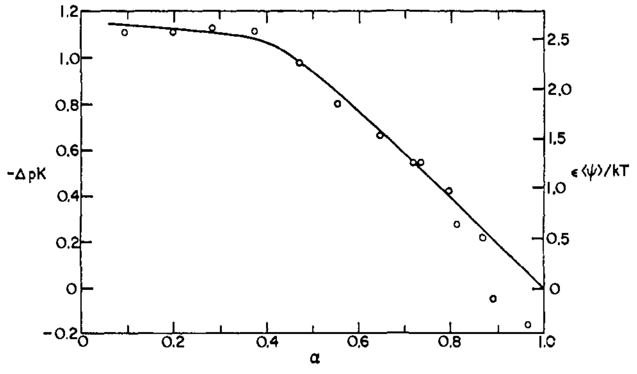

Figure 3. Data for $-(pa_H^* - \log \alpha/(1-\alpha) - pK_0)$ and $\epsilon < \psi >/kT$ *vs.* α for poly-L-lysine in 95% methanol at 25° (data of Figure 2).

in the absence of any perturbing interactions, *e.g.*, as in *n*-butylamine. The change in the chemical potential, $\Delta\mu$, for reaction 4 is given by eq 5, where

$$\Delta\mu = \Delta\mu^\circ + RT \ln a_H^* \quad (5)$$

a_H^* is the activity of hydrogen ion in 95% methanol and $\Delta\mu^\circ$ is the value of $\Delta\mu$ for reactants and products in their standard state. It is convenient to define the quantity ζ (the relative activity), which may be expressed in terms of the quantities pK_0 and pa_H^*

$$\zeta = e^{\Delta\mu/RT} = 10^{pK^\circ - pa_H^*} \quad (6)$$

Turning next to the polymer containing N amine side chains, we must consider all possible states of protonation, assign an appropriate statistical weight to each state, and then sum the statistical weights of all possible states. Thus, we must construct the grand partition function

$$\Xi = \sum_{M=0}^{N} Q(N,M)\zeta^M \quad (7)$$

where M is the number of bound protons, ζ represents the statistical weight for binding a proton in the absence of perturbing interactions, and $Q(N,M)$ is the statistical weight that takes into account the electrostatic interactions between charged side chains. The latter is given by

$$Q(N,M) = \exp\left[-\Phi(N,M)\right] \quad (8)$$

where $\Phi(N,M)$ is the sum over all pair interactions, ϕ_j, representing the electrostatic interaction of two charged side chains i and $i + j$

$$\Phi(N,M) = \sum_{i=1}^{N} \sum_{j=i+1}^{N} \sum_{\eta_i,\eta_j} \eta_i\eta_j\phi_j \quad (9)$$

with the constraint

$$\sum_{i=1}^{N} \eta_i = M \quad (10)$$

The quantity ϕ_j is defined as $\epsilon\psi_j/kT$, where ψ_j is the coulombic pair potential, and η_i is an index (1 or 0) representing the charged and uncharged states, respectively, of the side chains.

Having defined α as the degree of dissociation in reaction 4, the more conveniently computed quantity, $1 - \alpha$, the degree of association, is given in standard fashion by

$$1 - \alpha = \frac{1}{N}\frac{\partial \ln \Xi}{\partial \ln \zeta} \quad (11)$$

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(29) A. Katchalsky, N. Shavit, and H. Eisenberg, *J. Polym. Sci.*, 13, 69 (1954).

Matrix Representation of Ξ. Alternatively, Ξ can be formulated by giving the following set of rules. (1) Each side chain can exist in one of two states, h (uncharged) or h$^+$ (charged). (2) All h states are assigned the factor 1. (3) All h$^+$ states are assigned the factor ζ. (4) An h$^+$ state is assigned the additional factor q_j, which is given by

$$q_j = \exp(-\phi_j) \tag{12}$$

if it is followed by another h$^+$ state j residues away from it. The partition function Ξ is then formed by allowing each residue to exist in either the h or h$^+$ state and summing over all possible states, assigning statistical weights according to the above rules. The sum is conveniently given by forming a matrix product; the matrix correlates the states of a given residue with those of all the other N residues in the chain

$$\Xi(N,\zeta) = \mathbf{e}\mathbf{W}(N,\zeta)^N\mathbf{e}^+ \tag{13}$$

where $\mathbf{e}$ and $\mathbf{e}^+$ are the appropriate end vectors (reflecting the fact that the first and last residues in the chain cannot be preceded or followed, respectively, by charged side chains). Equation 13 represents an exact solution for the model assumed; however, the matrix required to allow all side chains to interact with all others is $2^{N-1} \times 2^{N-1}$ where N is the chain length (about 1600 in this work). Such matrix sizes are unmanageable even for today's computers; hence we must resort to an approximate evaluation of Ξ.

The approximation which we use is not to allow each side chain to interact with all others, but to truncate the extent of interactions to μ residues away ($\mu = 1$ corresponds to nearest neighbor interaction, $\mu = 2$ to next nearest neighbor, etc.). In this approximation, the total electrostatic potential for M charges is

$$\Phi(N,M,\mu) = \sum_{i=1}^{N} \sum_{j=i+1}^{i+\mu} \sum_{\eta_i,\eta_j} \eta_i\eta_j\phi_j \tag{14}$$

The matrix size now required for the formulation of Ξ is $2^\mu \times 2^\mu$

$$\Xi(N,\zeta,\mu) = \mathbf{e}\mathbf{W}(\mu,\zeta)^N\mathbf{e}^+ \tag{15}$$

The values μ = 1–6 lead to matrices of sizes 2×2 to 64×64, all of which can easily be handled by computer. One can vary μ over the range μ = 1–6 to examine the effect of truncating the range of the electrostatic potential. The results can then be extrapolated to $\mu = N$.

As an example, the matrix for $\mu = 3$, giving the statistical weight of the ith residue in terms of the states of residues $i + 1$, $i + 2$, and $i + 3$ is

$$\begin{array}{ccc|cccccccc}
 & & i+3 & h & h^+ & h & h^+ & h & h^+ & h & h^+ \\
 & & i+2 & h & h & h^+ & h^+ & h & h & h^+ & h^+ \\
 & & i+1 & h & h & h & h & h^+ & h^+ & h^+ & h^+ \\
i & i+1 & i+2 & & & & & & & & \\
\hline
h & h & h & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
h & h & h^+ & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\
h & h^+ & h & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 \\
h & h^+ & h^+ & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 \\
h^+ & h & h & \zeta & \zeta q_3 & 0 & 0 & 0 & 0 & 0 & 0 \\
h^+ & h & h^+ & 0 & 0 & \zeta q_2 & \zeta q_2 q_3 & 0 & 0 & 0 & 0 \\
h^+ & h^+ & h & 0 & 0 & 0 & 0 & \zeta q_1 & \zeta q_1 q_3 & 0 & 0 \\
h^+ & h^+ & h^+ & 0 & 0 & 0 & 0 & 0 & 0 & \zeta q_1 q_2 & \zeta q_1 q_2 q_3
\end{array} \tag{16}$$

Since the chain length of our sample of poly-L-lysine is very long ($N \sim 1600$), we may use the infinite-chain approximation (neglect of end effects) in which the grand partition function is expressed in terms of the maximum eigenvalue $\lambda_1(\zeta,\mu)$ of the matrix $\mathbf{W}(\zeta,\mu)$, *viz.*

$$\Xi(N,\zeta,\mu) = \lambda_1(\zeta,\mu)^N \tag{17}$$

Average quantities are then given by

$$1 - \alpha = \frac{1}{N}\frac{\partial \ln \Xi}{\partial \ln \zeta} = \frac{\zeta}{\lambda_1}\frac{\partial \lambda_1}{\partial \zeta} \tag{18}$$

for the average degree of association, and

$$\langle\phi\rangle = \sum_{j=1}^{\mu} \phi_j \frac{\partial \ln \lambda_1}{\partial \ln q_j} \tag{19}$$

for the average electrostatic energy per residue divided by RT. These average values can be calculated with a computer as a direct matrix product, without having to take any derivatives numerically, using the method of Jernigan.[30] We note that this method can be applied to finite chains, including the effect of the terminal carboxyl and amino groups; in such a case, special end matrices would be required.

The case in which all perturbing electrostatic interactions are ignored, *i.e.*, $\mu = 0$, can be taken to be identical with that of the reference compound, *n*-butylamine. For this case of independent side chains, $Q(N,M)$ is given simply by the binomial coefficient

$$Q(N,M) = \frac{N!}{(N-M)!M!} = \binom{N}{M} \tag{20}$$

which gives the number of ways of placing M protons on N independent side chains. Inserting $Q(N,M)$ of eq 20 in eq 7 for Ξ gives

$$\Xi(N,\zeta) = \sum_{M=0}^{N} \binom{N}{M} \zeta^M = (1 + \zeta)^N \tag{21}$$

The degree of association becomes

$$1 - \alpha = \zeta(1 + \zeta) \tag{22}$$

which is independent of N (*i.e.*, a polymer of N independent side chains has the same titration curve as the isolated side chain or the reference compound *n*-butylamine).

Form of $\phi(\mathbf{r})$. The simplest potential that one might use for a polyelectrolyte is based on the Debye–Hückel model, which was used by Zimm and Rice.[16] In this model, the polyelectrolyte is regarded as being surrounded by an atmosphere of small ions which screen the interactions between the neighboring charges on the

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[30] This method is explained in detail in the Appendix of the paper by Poland and Scheraga.[31]

[31] D. Poland and H. A. Scheraga, *Biopolymers*, 7, 887 (1969).

macroion. Hence, the electrostatic interaction potential is simply a screened Coulombic potential, and the quantity ϕ_j may be written as

$$\phi_j = \frac{\epsilon^2}{D r_j kT} e^{-\kappa r_j} \tag{23}$$

where r_j is the distance between the ith and $(i + j)$th charged side chains (each of charge ϵ), D is the dielectric constant, and $1/\kappa$ is the Debye–Hückel screening length

$$\kappa^2 = \frac{4\pi}{DkT} \sum n_i(z_i\epsilon)^2 = \frac{8\pi\epsilon^2}{DkT} I \tag{24}$$

for n_i species of charge $z_i\epsilon$ in 1 l., where the sum is evaluated only over the small counterions and co-ions; I is the ionic strength.

The values of r_j used here were calculated from coordinates obtained from the bond angles and bond distances for the extended lysine side chain in the right-handed α-helical conformation.[4] The distances between one charge and its first six neighbors are: r_1 = 12.2, r_2 = 16.0, r_3 = 9.5, r_4 = 7.8, r_5 = 16.5, and r_6 = 16.8 Å. These values of r_j and q_j are plotted as a function of j in Figure 4. The periodicity in these quantities, which arises from the helical arrangement of the charges, damps out at large j.

We would expect the Debye–Hückel potential to give unsatisfactory results since it arises from a linearization of the Poisson–Boltzmann equation, which in turn is valid only if $\phi \ll 1$, a condition which is not satisfied here over most of the range of ionization (see Figure 3). In addition, since the local dielectric constant is an unknown quantity, one is forced to use the bulk dielectric constant in the calculations. However, the results of Mukerjee and Ray[32] seem to suggest that this might not be a bad approximation for aqueous methanol solutions. Further, the local ionic strength is assumed to be independent of α and equal to that of the bulk medium. In spite of these deficiencies (in addition to a truncation of the potential at μ = 3), Zimm and Rice obtained surprisingly good agreement between their theoretical calculations and the experimental data of Wada[8] for poly-L-glutamic acid in aqueous dioxane–salt solutions. However, this may be due to a fortuitous cancellation of errors in the poly-L-glutamic acid system, which also involves a helix-coil transition (the truncation at μ = 3 may compensate the overestimate of the potential by the Debye–Hückel theory; also, the potential may be weaker in the random coil form, since the charges are farther apart, and therefore may not be of importance beyond a few charged groups). In view of the success which Zimm and Rice obtained with the Debye–Hückel potential, we will also apply it here to the poly-L-lysine titration data, but allow for larger values of μ than Zimm and Rice did.

However, since we will find that, as expected, the Debye–Hückel potential leads to unsatisfactory results, we will try to overcome these difficulties by using an alternative electrostatic interaction potential. The justification comes from the fact that many counterions are attracted to the immediate neighborhood of the charges on the polyelectrolyte because of its high charge density.[14,15] At a given total salt concentration, the fraction of counterions condensing around the charged polyelectrolyte decreases with an increase in the degree of dissociation; hence, the local ionic strength would vary with α. Since it is assumed in the matrix procedure that the ionic strength remains constant throughout the titration, we may take account of this problem by using an empirical alternative to the κ calculated from the bulk ionic strength. Such an empirical alternative to κ will also take account of differences in the dielectric constant inside and outside the macroion, as discussed by Harris and Rice.[33] In addition to modifying the κ of eq 24, there is also some basis for modifying the pre-exponential term; for example, Record[34] reduced the charge ϵ by a shielding factor, in his treatment of electrostatic effects on polynucleotide transitions. For all of the above reasons, we will assume that ϕ has the following empirical form

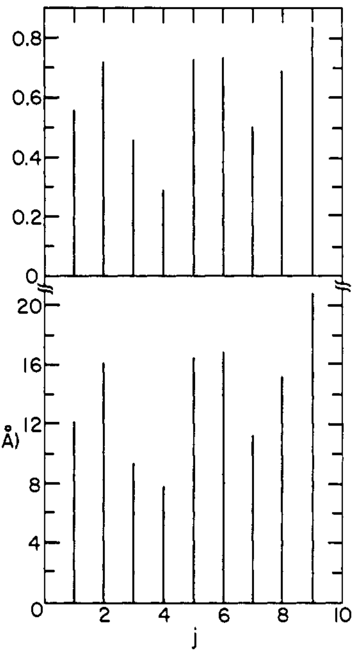

Figure 4. Plot of r_j and q_j as a function of j; q_j is the electrostatic statistical weight, computed from the Debye–Hückel potential with I = 0.025 M and D = 33.5.

$$\phi(r) = (A/rkT)\exp(-Br) \tag{25}$$

where A and B are adjustable parameters, to be determined by the procedure described in the Discussion, and the electrostatic statistical weight q_j of eq 12 is then given in terms of $\phi(r)$ of eq 25. It should be noted that even this empirical form is not entirely satisfactory, since it does not allow for a dependence of ionic strength on α or for intermolecular interactions. While Ise and Hoseno[35] criticized the neglect of intermolecular electrostatic interactions in dilute solutions of polyelectrolytes, the calculations of Scheraga, *et al.*,[15] indicate that the dependence of ΔpK on polymer concentration is small; hence, the neglect of intermolecular interactions is a reasonably good approximation.

Discussion

The experimental data of Figure 2, together with the theoretical curves (based on a Debye–Hückel potential)

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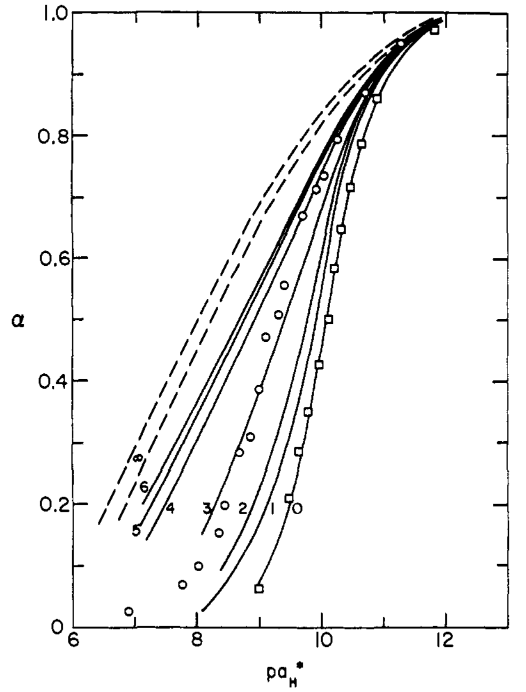


Figure 5. Experimental data of Figure 2, together with theoretical curves at 25.0° for various values of μ, based on the Debye–Hückel potential with $D = 33.5$ and $\kappa = 0.0796$ Å^{-1} ($I = 0.025\ M$). Dashed curves are the extrapolated ones for $\mu \rightarrow \infty$: □, n-butylamine; ○, poly-L-lysine.

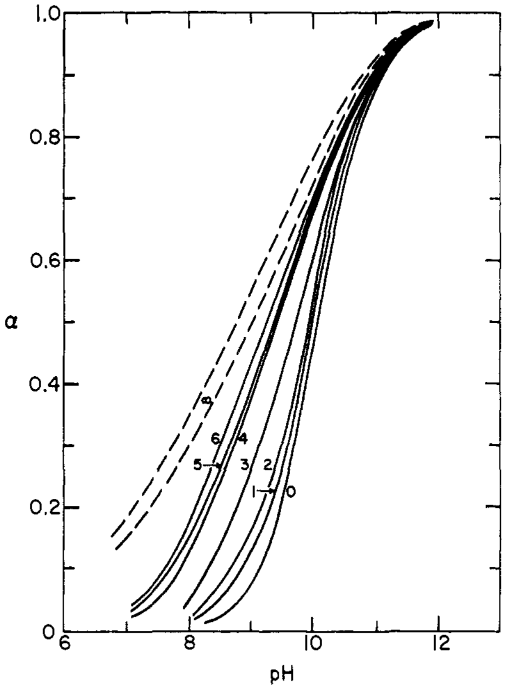


Figure 7. Theoretical curves for poly-L-lysine in water at 25.0° for various values of μ, based on the Debye-Hückel potential with $D = 80$ and $I = 0.025\ M$ ($\kappa = 0.0515$ Å^{-1}).

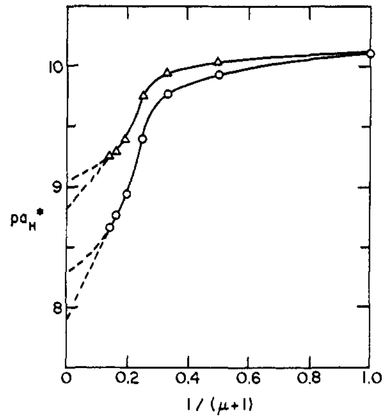


Figure 6. Curve of pa_H^* *vs.* $1/(\mu + 1)$ at $\alpha = 0.5$, based on a Debye–Hückel potential. The extrapolation to $\mu \rightarrow \infty$ is also shown: △, for water ($D = 80$); ○, for 95% methanol ($D = 33.5$).

for $\mu = 1$–6, as well as for the case where $\mu = 0$, *i.e.*, no perturbing electrostatic potential, are shown in Figure 5. Calculations not shown here indicate that the variation in ionic strength, which occurs during the titration, does not affect the theoretical curves significantly. We have also plotted in Figure 5 two dashed curves which represent the limiting range (because of errors in extrapolation) of the extrapolated curve for $\mu \rightarrow \infty$, obtained by plotting pa_H^* *vs.* $1/(\mu + 1)$ at different values of α; such an extrapolation is shown in Figure 6 for $\alpha = 0.5$. While the curves for $\mu = 3$ or 4 give the best agreement between theory and experiment, we cannot accept this result since the electrostatic interactions are long range and all curves for finite μ should lie on the high pa_H^* side of the experimental curve for poly-L-lysine, *i.e.*, the experimental curve should correspond to the extrapolated $\mu \rightarrow \infty$ curve. The curve for $\mu = 0$, corresponding to no electrostatic interactions, of course fits the data for n-butylamine very well. The behavior observed in Figure 5 may arise from the fact that the Debye–Hückel theory overestimates the potential.

Since we are interested in comparing the range of the electrostatic potential in 95% methanol and in water, we have also computed the theoretical titration curves for poly-L-lysine using the dielectric constant of water, *i.e.*, $D = 80$. The curves for various values of μ, as well as the extrapolated ones (for the range of error in the extrapolation) for $\mu \rightarrow \infty$, are shown in Figure 7. For the purpose of comparison with Figure 5, we have used $pK_0 = 10.1$ (the value in 95% alcohol) instead of 10.4 (the value in water). By comparing Figures 5 and 7, we see that the curves for $\mu \rightarrow \infty$ for poly-L-lysine lie closer to the curve for n-butylamine in water than in 95% methanol; this indicates that truncation at $\mu = 6$ would be a better approximation for water than for 95% methanol. Further comparisons are made in Figures 6 and 8. Figure 6 compares the extrapolations of pa_H^* *vs.* $1/(\mu + 1)$ at $\alpha = 0.5$ for 95% methanol and for water; Figure 8 shows a comparison of the Debye–Hückel potentials in these two solvents. From both figures, we note that the electrostatic potential is smaller in water than in 95% methanol. Although this fact should not be very surprising, since water has a higher dielectric constant, it is important to point it out here since it means that we can truncate the potential after fewer interactions in water than we can in 95% methanol, although the range of the potential will not be directly proportional to the dielectric constant.

Since we regard the inadequacy of the results of Figure 5 for poly-L-lysine as arising from the inapplicability of the Debye–Hückel potential, we consider now the use of the empirical potential of eq 25. Since ϕ

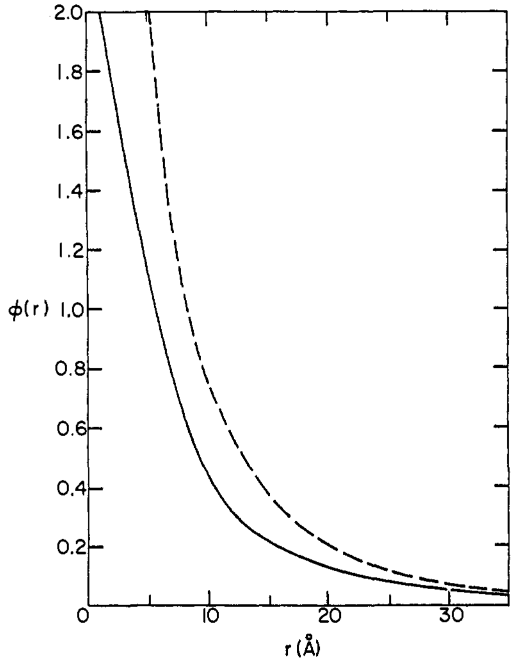


Figure 8. Plot of $\phi(r)$ *vs.* r, from the Debye–Hückel theory, for $D = 33.5$ (dashed curve) and $D = 80$ (solid curve), and $I = 0.025\ M$

depends on two parameters A and B, a "best" fit of the experimental curve can be obtained with a range of values of A and B. In order to determine A and B, it was required that the experimental data be fit by the theoretical curve for the largest value of μ used, *viz.*, $\mu = 6$. The range of values of A and B, which provide good agreement, with $\mu = 6$, is shown in Figure 9. If we tentatively ascribe to A and B the interpretation of the Debye-Hückel model, *viz.*

$$A = \epsilon^2/D \tag{26}$$

and

$$B^2 = \frac{4\pi}{DkT}\sum n_i(z_i\epsilon)^2 = 8\pi\epsilon^2 I/DkT \tag{27}$$

where the dielectric constant D, and ionic strength I, may now be regarded as adjustable parameters, and if we assign to D the value of 33 (close to that of 95% methanol), we find that $I = 0.087\ M$, which is approximately four times the bulk ionic strength. Such a high value is not unreasonable, in view of the high charge density on the polyelectrolyte. Using the values of A and B, corresponding to these values of D and I, the curves of Figure 10 were drawn. The data of Figure 10 were extrapolated to $\mu \rightarrow \infty$ (as in Figure 6) to yield the range of values shown by the dashed curves of Figure 10. The good agreement for the poly-L-lysine data arises, of course, from the proper adjustment of A and B. However, we also note that the curve for $\mu = 6$ is closer to that of $\mu \rightarrow \infty$ than it was when the Debye–Hückel potential was used and that truncation at $\mu = 4$ would have led to no serious error.

A possible way of resolving the problem of selecting a unique set of values of A and B is to measure the enthalpy change for the reaction

$$n\mathrm{RNH_3^+} \longrightarrow (\mathrm{RNH_3^+})_n \tag{28}$$

and compare it with the value of $\langle\phi\rangle$ computed by means of eq 19. However, although the values of

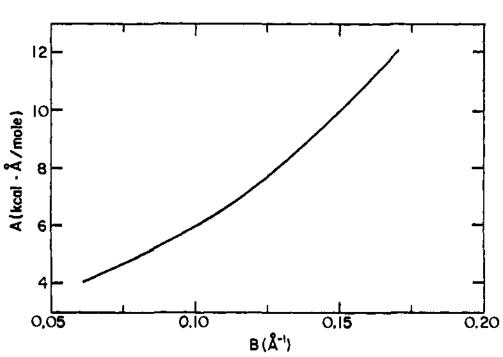


Figure 9. Range of values of A and B which provide good agreement between experimental data for poly-L-lysine and the theoretical curve for $\mu = 6$.

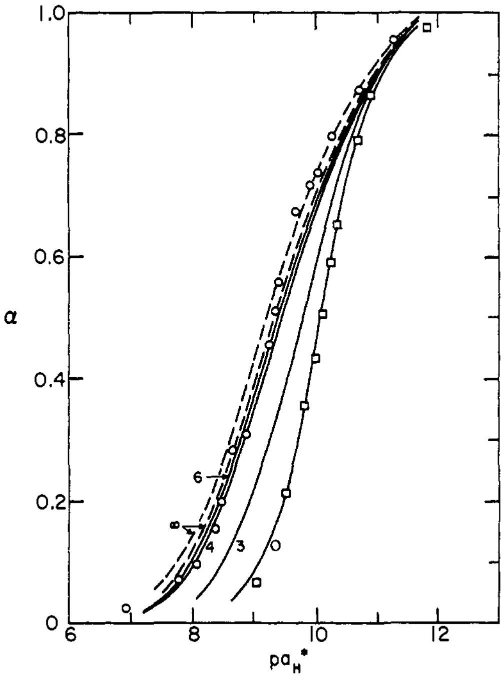


Figure 10. Experimental data of Figure 2 together with theoretical curves (at 25°) for $\mu = 0$, 3, 4, and 6 based on the empirical potential of eq 25 with $A = 10$ kcal Å/mol and $B = 0.15$ Å^{-1}. Dashed curves are the extrapolated ones for $\mu \rightarrow \infty$: □, n-butylamine; ○, poly-L-lysine.

$RT\langle\phi\rangle$, the average electrostatic energy per residue, are quite large (of the order of 1 kcal), the variation in $RT\langle\phi\rangle$, due to the allowed variation in A and B, is in fact quite small (100 or 200 cal) and within the experimental error of calorimetric measurements.

Conclusion

Even though we have not been able to resolve the problem inherent in the proper choice of the parameters for the empirical potential function, we see that it is incorrect to truncate the potential at $\mu = 3$, for poly-L-lysine in 95% methanol, as Zimm and Rice did for poly-L-glutamic acid in water. The range of the potential, up to $\mu = 6$, has been taken into account by explicit matrix multiplication which can be carried out with a computer, without having to resort to the perturbation method of Zimm and Rice. For larger investments of computing time, the calculations can be extended to higher values of μ—to see how rapidly the results are converging to their limit for infinite μ. Thus, the matrix method (with the extrapolation to $\mu \rightarrow \infty$) provides information about the relative contributions of nearest neighbor, next nearest neighbor, etc. interactions.

We have also compared the potential function of eq 23 for water and 95% methanol, by varying the dielectric constant. Because of the higher dielectric constant of water, the potential has a shorter range than in methanol. This may be one reason why the truncation of the potential at $\mu = 3$ (for poly-L-glutamic acid in water) did not introduce an error into the procedure of Zimm and Rice. In treating electrostatic interactions in aqueous solutions of proteins, it will be necessary to obtain a reasonable estimate of the local dielectric constant in order to compute the electrostatic interaction energy; from the results obtained here, we have a basis for selecting an empirical potential for such computations.

It should be noted that there is not necessarily a contradiction between the assertion that the electrostatic potential is of shorter range in water than in methanol, and the observation that poly-L-lysine is α helical in methanol but randomly coiled in water; the helical conformation in methanol may arise from other factors (possibly strengthened hydrogen bonds) than the electrostatic repulsion between the charged ϵ-amino groups.

Acknowledgment. We would like to thank Mr. Hua Tjan for performing the nitrogen analyses to determine the concentrations of the poly-L-lysine solutions.

Communications to the Editor

Photochemistry of Cyclobutanone. Trapping of the Initial Ring-Opened Intermediate with Butadiene

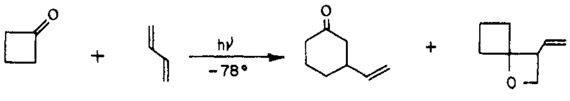
Sir:

Carbon–carbon cleavage at the carbonyl carbon (Norrish type I cleavage) is a photochemical reaction of considerable importance and current interest.[1] Particular attention has been focused on cyclic ketones and the question as to whether the various fragmentation and intramolecular disproportionation products are formed through an intermediate diradical[2] or *via* concerted pathways.[3] The need for understanding the fragmentation modes of cyclic ketones in mass spectrometry has further increased the significance of this area of photochemistry.

Support for the intermediacy of diradicals in the photochemical scission of cyclic ketones has been presented recently in the isomerization studies of appropriately substituted cyclohexanones,[4] cyclopentanones,[5] and 17-keto steroids.[6] However, that which is known of the chemical reactivity of the proposed diradicals is limited to clues provided by intramolecular reactions since such intermediates have never been trapped by external reagents. We have discovered a unique instance of an apparent trapping reaction and present here the experimental evidence and the unusual features of this reaction.

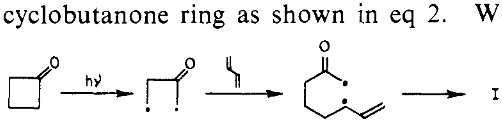
The photolysis of cyclobutanone has been studied in the gas phase[2,7] and in methanol solution.[8] From the former studies it has been concluded that the excited singlet state leads to fragmentation into ethylene and ketene. In accord with these findings we have observed that photolysis of cyclobutanone in liquid butadiene at 10° in a sealed tube yields acetic acid (2,2,4,4-cyclobutanone-d_4 yields dideuterio acetic acid) (19%), ketene dimer (32%), the adduct of ketene and butadiene[9a] (5%), as well as an oxetane (13%).[9b]

When the photolysis is run at −78°, a striking change occurs. None of the products derived from ketene are observed; instead one observes 3-vinylcycohexanone (I) as a major product of the reaction (32%).[10] Oxetane[9b] is also formed under these conditions (32%).

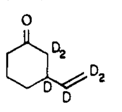


I (1)

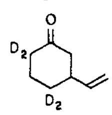
It seemed possible *a priori* that the 3-vinylcyclohexanone might be formed following a β cleavage (II) of the cyclobutanone ring as shown in eq 2. We have been able to rule out this possiblity completely. Photolysis of cyclobutanone in butadiene-d_6 yielded 3-vinylcyclohexanone-d_6 (III) which exchanged two atoms of deu-



II (2)



III



IV

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