slightly more stable in water. The first is the shielding of the charge by solvent molecules which surround the carboxyl group, and the second is the conformational entropy.

It is of interest to apply this argument to other N-substituted molecules whose cis-trans equilibrium has been observed. For example, consider the difference between *N*-methyl-*N*-ethylformamide (40% with ethyl cis[6] to carbonyl oxygen) and *N*-methyl-*N*-ethylacetamide (51% with ethyl cis[6] to carbonyl oxygen). Since the attachment of a methyl group on the carbonyl carbon (in going from the formamide to the acetamide compound) would not be expected to change the barrier to rotation about the peptide bond[8] sufficiently to account for this difference, we attribute the difference to a similar entropic contribution, which arises from a steric effect as follows. Assuming that the barrier to rotation about the *N*-(ethyl CH_2) bond is small,[8] and that a CH_2 group is similar to a CH_3 group in steric behavior, the low-energy conformation (when the ethyl is trans to the carbonyl oxygen) would be one in which the ethyl CH_3 is pointed *away* from the acetyl CH_3 group of the acetamide compound to avoid steric interference. This restriction is not as severe when the ethyl is cis to the carbonyl oxygen. Hence, there is a smaller range available to the *N*-(ethyl CH_2) dihedral angle (hence, a smaller entropy) when the ethyl is trans to the carbonyl oxygen. In the formamide compound the restriction on the *N*-(ethyl CH_2) dihedral angle is less severe (than in the acetamide compound) when the ethyl is trans to the carbonyl oxygen, since formamide has an H atom in place of a methyl group; thus, a higher per cent of this trans conformation can occur in the formamide compound. We believe that this entropy effect accounts for the difference in behavior between these formamide and acetamide compounds.

Since the conformational entropy appears to be important for the relative stabilities of cis and trans peptides, we may now extend these arguments to explain the observed cis or trans preference for N-substituted peptides in naturally occurring polypeptides. The (small) enthalpy difference observed here for the cis $\rightleftarrows$ trans isomerization suggests that a cis peptide bond *could* exist in a polypeptide or protein at the L-prolyl or sarcosyl residue if the trans-preferred enthalpy and conformational entropy were overcome by other energetic and entropic factors. At present, it appears that only those naturally occurring molecules in which a covalent cyclic structure exists (*e.g.*, actinomycin D[32] which has two cis peptide bonds per ring) have sufficient constraints so that the above factors which favor trans may be overcome.

Acknowledgment. We are indebted to Mrs. Jane Derbenwick for help with the synthetic work.

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Conformational Energy Calculations. Thermodynamic Parameters of the Helix-Coil Transition for Poly(L-lysine) in Aqueous Salt Solution[1]

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ABSTRACT: The properties of the helix-coil transition of poly(L-lysine) at various degrees of ionization in aqueous salt solution are computed with the aid of empirical energy functions. The free-energy change in the process is considered to be a sum of a contribution (ΔG_0) from the neutral polymer in the aqueous medium and one (ΔG_e) from the electrostatic interactions between the charged side chains. ΔG_0 is obtained from an earlier calculation for poly(L-alanine) in water, and the effect of the lysine side chain is incorporated. ΔG_e is obtained by computing the partition function for the helix and coil at various degrees of ionization, the coil conformations being generated by a Monte Carlo procedure which yields a characteristic ratio of 8.69 for a neutral poly(L-lysine) chain of 40 residues in a Θ solvent. A Debye-Hückel screening potential was used in the computation of ΔG_e. The expansion factor, α, of the poly(L-lysine) coil due to electrostatic interactions between the side chains was computed and found to obey the relation $\alpha^5 - \alpha^3 \sim 1/C_s$ for salt concentrations $C_s \geq 0.1\ M$; however, the calculated numerical values of α were somewhat lower than experimental ones. The computed value of ($\Delta G_0 + \Delta G_e$) is zero at a degree of ionization of ~50%, compared to an experimental value of ~35%; the discrepancy may be due to the underestimate of the expansion of the coil. The stability of the poly(L-lysine) helix in 95% methanol at acid pH is attributed to ion-pair formation to the extent of about 35%.

It is well known that the conformation of a homopoly(amino acid) molecule with ionizable side chains depends on the pH of the solution. At high pH a poly(L-lysine) chain appears to be in an α-helical conformation, and at low pH adopts the conformation of a polylectrolyte random coil.[3] Zimm and Rice[4] have presented a statistical mechanical theory for this phenomenon, assuming a conformational transition from an α helix to a locally extended form. Several investigators[5-7] have applied conformational energy calculations to this problem, but these

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(2) (a) On leave from the Van't Hoff Laboratorium der Rijksuniversiteit, Utrecht, Netherlands, 1971-1972. (b) On leave from the Institute for Chemical Research, Kyoto University, Uji Kyoto-Fu, Japan, 1972.

(3) J. Applequist and P. Doty in "Polyamino Acids, Polypeptides and Proteins," M. A. Stahmann, Ed., Univ. Wisconsin Press, Madison, Wis., 1962, p 161.

(4) B. H. Zimm and S. A. Rice, *Mol. Phys.*, 3, 391 (1960).

(5) S. Krimm and J. E. Mark, *Proc. Nat. Acad. Sci. U. S.*, 60, 1122 (1968).

(6) W. A. Hiltner, A. J. Hopfinger, and A. G. Walton, *J. Amer. Chem. Soc.*, 94, 4324 (1972).

(7) A. J. Hopfinger, *Macromolecules*, 4, 731 (1971).

treatments involve several arbitrary assumptions: (1) a low value is used for the dielectric constant of the aqueous solution; (2) Coulomb's law is used without a screening potential, thereby neglecting the very important effect of the counterions and neutral salt on the conformation; and (3) a regularity condition (extended chain) is imposed on the random coil, thereby depriving it of its conformational entropy.

The present paper is an attempt to rectify these deficiencies, at least in first approximation, and thus to compute the properties of the helix-coil transition of poly(L-lysine) at various degrees of ionization in aqueous salt solution with the aid of empirical energy functions. The calculation is carried out in two parts; first, the difference in free energy between the neutral helical and coil forms *in water* is computed, making use of a similar earlier calculation for poly(L-alanine),[8] and then the electrostatic contribution is evaluated, using a Monte Carlo procedure to generate the random-coil and a Debye-Hückel screening potential to calculate the electrostatic free energies of the helix and coil.

I. General Formulation

The conformational free energy of a homopoly(amino acid) of N residues is a function of the dihedral angles $\{\phi_i\}$ and $\{\psi_i\}$ of the backbone (for planar trans peptide groups, and fixed bond lengths and bond angles) and $\{\chi_i^j\}$ of the j single bonds in each of the i side chains, where i varies from 1 to N. The partition function of a polymer in solution is given by

$$Z = Q_K(T)Q_C \tag{1}$$

where $Q_K(T)$ is the kinetic energy contribution, discussed previously[9] (and *assumed*[9] to depend only on temperature; hence, it can be neglected in a helix-coil transition), and Q_C is the contribution which depends on conformation (after averaging over the phase space of the solvent molecules, including the salt ions). The quantity Q_C may be expressed in terms of an empirical free energy[10] F_0 (which includes all contributions[11] except that part of the electrostatic free energy arising from the interactions between the charges on the side chains) and an electrostatic potential W_e to take account of the interaction between the charged side chains in the presence of salt; both F_0 and W_e depend on $\{\phi_i\}$, $\{\psi_i\}$, and $\{\chi_i^j\}$.

$$Q_C = \int\cdots\int \sum_{\substack{\text{charge}\\ \text{distribution}}} \exp(-\beta W_e - \beta F_0)\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\}\mathrm{d}\{\chi_i^j\} \tag{2}$$

where $\beta = 1/kT$, and W_e is the electrostatic energy of *one* charge distribution on *one* conformation.

In order to simplify the computations, we may express Q_C as a product of the term for the uncharged polymer and of the electrostatic term.

$$Q_C = Q_0Q_e \tag{3}$$

where

$$Q_0 = \int\cdots\int \exp[-\beta F_0(\{\phi_i\},\{\psi_i\},\{\chi_i^j\})]\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\}\mathrm{d}\{\chi_i^j\} \tag{4}$$

and Q_e is given in terms of the electrostatic partition function of a single conformation, q_e, by

$$Q_e = \langle q_e\rangle_C = \langle \sum_{\substack{\text{charge}\\ \text{distribution}}} \exp(-\beta W_e)\rangle_C \tag{5}$$

The symbol $\langle\cdot\cdot\cdot\rangle_C$ means that the summation is carried out for each conformation, and then averaged over all conformations, *i.e.*

$$Q_e = \frac{\int\cdots\int q_e \exp(-\beta F_0)\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\}\mathrm{d}\{\chi_i^j\}}{\int\cdots\int \exp(-\beta F_0)\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\}\mathrm{d}\{\chi_i^j\}} \tag{6}$$

The corresponding free-energy terms G_0 and G_e are computed as $-RT \ln Q_0$ and $-RT \ln Q_e$. Therefore, the free-energy change for the conversion of helix to coil, ΔG_{hc}, may be represented by two terms, one arising from the neutral polymer, ΔG_0, and the other from the change in electrostatic free energy, ΔG_e.

$$\Delta G_{hc} = \Delta G_0 + \Delta G_e \tag{7}$$

The term ΔG_0 includes contributions[11] from nonbonded interactions, torsional energies, electrostatic interactions between backbone amide dipoles, and hydrogen-bond interactions.[12]

The midpoint of the transition is taken as the pH (or degree of ionization) at which $\Delta G_{hc} = 0$, *i.e.*, where the helix content $\theta_h = 1/2$ for an infinite polymer; because of the existence of the nucleation parameter σ of the Zimm-Bragg theory[13] (or σ' of the Zimm-Rice theory[4]), $\theta_h < 1/2$ for a finite polymer when $\Delta G_{hc} = 0$.

A. Statistics of the Uncharged Homopolymer. The partition function of the uncharged poly(amino acid), Q_0, may in turn be expressed as a product of two factors

$$Q_0 = Q_bQ_s \tag{8}$$

where Q_b arises from interactions among the backbone atoms [including the C^β atom, but not the hydrogen atoms on the C^β atom (see Figure 1)] and Q_s arises from side-chain-backbone, intra-side-chain, and side-chain-side-chain interactions, with

$$F_0(\{\phi_i\}, \{\psi_i\}, \{\chi_i^j\}) = F_b(\{\phi_i\}, \{\psi_i\}) + F_s(\{\phi_i\}, \{\psi_i\}, \{\chi_i^j\}) \tag{9}$$

Thus,

$$Q_b = \int\cdots\int \exp[-\beta F_b(\{\phi_i\},\{\psi_i\})]\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\} \tag{10}$$

and

$$Q_s = \frac{\int\cdots\int \exp[-\beta F_s(\{\phi_i\},\{\psi_i\},\{\chi_i^j\})] \exp[-\beta F_b(\{\phi_i\},\{\psi_i\})]\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\}\mathrm{d}\{\chi_i^j\}}{\int\cdots\int \exp[-\beta F_b(\{\phi_i\},\{\psi_i\})]\mathrm{d}\{\phi_i\}\mathrm{d}\{\psi_i\}} \tag{11}$$

where Q_s is obtained by averaging the side-chain contribution over all backbone conformations. Therefore, ΔG_0 may be represented in terms of backbone and side-chain contributions, *i.e.*

$$\Delta G_0 = \Delta G_b + \Delta G_s \tag{12}$$

where

$$G_b = -RT \ln Q_b \text{ and } G_s = -RT \ln Q_s$$

In computing ΔG_0, we adopt the following approach. ΔG_b is assumed to be the same as that already evaluated

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[8] M. Gō, N. Gō, and H. A. Scheraga, *J. Chem. Phys.*, 54, 4489 (1971).

[9] N. Gō, M. Gō, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U. S.*, 59, 1030 (1968).

[10] Here, we distinguish between F_0, the free energy of *a particular conformation* of the neutral polymer (having averaged over the phase space of the solvent molecules), and G_0, the free energy of the *ensemble* of neutral polymer molecules.

[11] H. A. Scheraga, *Chem. Rev.*, 71, 195 (1971).

[12] In computing ΔG_0 in this manner, and adding it to ΔG_e as in eq 7, we are neglecting the electrostatic interaction between the charged side-chain ϵ-amino groups and the partial charges of the backbone amide groups. In other words, the procedure implied by the use of eq 7 is the neglect of differences in the conformational energy (ϕ,ψ) maps between charged and uncharged lysine residues, as far as the computation of ΔG_0 is concerned.

[13] B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, 31, 526 (1959).

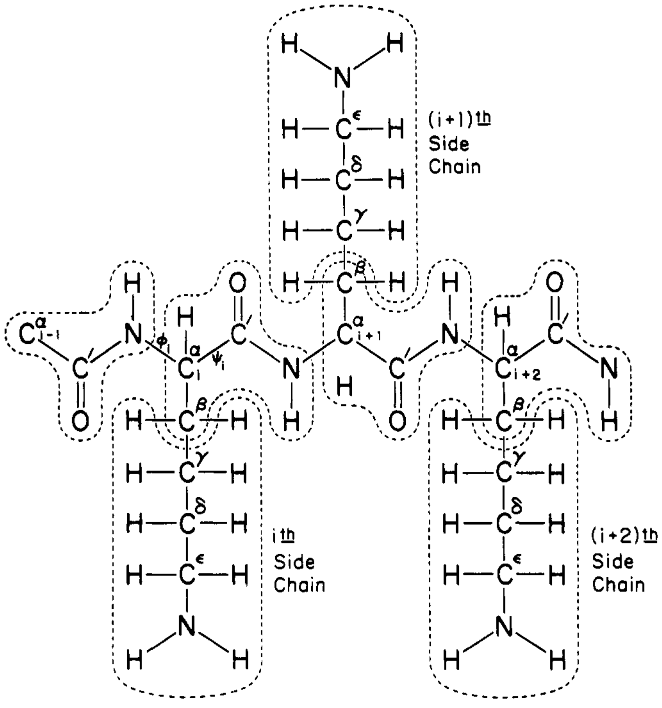

Figure 1. Diagram of poly(L-lysine) chain, showing grouping of backbone and side-chain portions. The dihedral angles ϕ_i and ψ_i are also indicated. $\{\chi_i^j\}$ is the set of j dihedral angles in the ith side chain.

for poly(L-alanine) *in water*.[8,14] For the longer side chain of *neutral* poly(L-lysine), the solvent is assumed to affect the free-energy contribution from the side chain to equal extents in the helix and coil forms; thus, no solvent effect will be taken into account in computing ΔG_s.

The enthalpy H for the helix and coil, respectively, can be obtained by differentiation of Q with respect to β. Thus, from eq 9, 10, and 11

$$H_b = \frac{\int \cdots \int F_b \exp(-\beta F_b) \mathrm{d}\{\phi_i\} \mathrm{d}\{\psi_i\}}{\int \cdots \int \exp(-\beta F_b) \mathrm{d}\{\phi_i\} \mathrm{d}\{\psi_i\}} \tag{13}$$

$$H_s = H_0 - H_b = \frac{\int \cdots \int F_0 \exp(-\beta F_0) \mathrm{d}\{\phi_i\} \mathrm{d}\{\psi_i\} \mathrm{d}\{\chi_i^j\}}{\int \cdots \int \exp(-\beta F_0) \mathrm{d}\{\phi_i\} \mathrm{d}\{\psi_i\} \mathrm{d}\{\chi_i^j\}} - \frac{\int \cdots \int F_b \exp(-\beta F_b) \mathrm{d}\{\phi_i\} \mathrm{d}\{\psi_i\}}{\int \cdots \int \exp(-\beta F_b) \mathrm{d}\{\phi_i\} \mathrm{d}\{\psi_i\}} \tag{14}$$

The value of ΔH_b is not computed, but taken directly from the data for poly(L-alanine);[8] only ΔH_s is computed here (from eq 14, for the helix and coil, respectively). ΔS_b is also taken from the data for poly(L-alanine) and ΔS_s is computed from

$$\Delta S_s = (\Delta G_s - \Delta H_s)/T \tag{15}$$

Therefore, in principle, we can compute ΔG_s, ΔH_s, and ΔS_s, and hence ΔG_0, ΔH_0, and ΔS_0 from eq 11, 13, 14, and 15. However, there are too many variables (dihedral angles) in this computation, and resort is had to some approximations in order to perform the integrations, as described below.

The first approximation, in order to evaluate Q_s for the coil state, is to assume that the energy is the sum of "dipeptide" energies, *viz*.

$$(Q_s)_{\text{coil}} = \left[\frac{\int \cdots \int \exp[-\beta F_s(\phi_i, \psi_i, \{\chi_i^j\})] \exp[-\beta F_b(\phi_i, \psi_i)] \mathrm{d}\phi_i \mathrm{d}\psi_i \mathrm{d}\{\chi_i^j\}}{\int \int \exp[-\beta F_b(\phi_i, \psi_i)] \mathrm{d}\phi_i \mathrm{d}\psi_i} \right]^N \tag{16}$$

The evaluation of $(Q_s)_{\text{coil}}$ by means of eq 16 can be carried out within a reasonable amount of computer time. However, this approximation is not accurate enough to evaluate the free energy of a side chain, G_s, for the coil because a long side chain can interact with the side chains and portions of the backbone other than those taken into account in eq 16. Therefore, additional approximations are required, in which longer range interactions are taken into account, as described in section IID.

The contribution of the side chains to the free energy of the helix $(G_s)_{\text{helix}}$ is obtained by integrating over $\{\chi_i^j\}$ (assuming that $\{\chi_i^j\}$ is the same for all i) at the values of ϕ_i^0 and ψ_i^0 corresponding to the minimum-energy (regular) conformation of poly(L-alanine)[8] (see section IIC). For this purpose, F_b was expanded about the minimum (where the first derivatives of F_b are zero) to obtain

$$(Q_s)_{\text{helix}} = \frac{\underset{\text{helix}}{\int \cdots \int} \exp[-\beta F_s(\phi_i, \psi_i, \{\chi_i^j\})] \mathrm{d}\phi_i \mathrm{d}\psi_i \mathrm{d}\{\chi_i^j\}}{\underset{\text{helix}}{\int \cdots \int} \mathrm{d}\phi_i \mathrm{d}\psi_i} \tag{17}$$

from eq 11. The calculations were performed by replacing the integration by a summation; *i.e.*, the average value of $\exp(-\beta F_s)$ around the minimum (ϕ_i^0, ψ_i^0) was computed at values of ϕ_i and ψ_i within 1° of ϕ_i^0 and ψ_i^0.

From the values of G_s, H_s, and S_s for the coil and helix, and the corresponding values for poly(L-alanine), the values of ΔG_0, ΔH_0, and ΔS_0 are obtained for the helix-coil transition of *neutral* poly(L-lysine) in water.

B. Electrostatic Free Energy of Helix and Coil. According to Rice and Nagasawa,[15] the free energy of a polyelectrolyte solution can be divided into three parts, G_1 being the free energy of the free small (counter) ions which can take part in binding and neutralization phenomena, G_2 being the free energy of the charges of the polyion (comprising the energy of interaction between the charges on the polymer and the entropy arising from the mixing of charged and uncharged groups on the polymer), and G_3 being the free energy of ionization (arising from both the binding of protons as well as salt ions). Since we will compare helix and coil conformations at equal net charge under identical solvent conditions, we shall assume that G_1 and G_3 are the same for helix and coil; this assumption is not completely justified because the coil will have a slightly higher degree of ionization than the helix during the transition,[4,16] but this effect will be neglected. The term G_2, designated here as $(G_e)_{\text{helix}}$ and $(G_e)_{\text{coil}}$, will be evaluated by assuming that the counter ions are either in solution (where they can be treated by the Debye-Hückel approximation) or they are so near to the charge on the polymer that they can be considered as bound in ion pairs. Recent results obtained by Manning[17] and MacGillivray[18] support this assumption. Ion pairs

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(14) Poly(L-alanine) contains three hydrogen atoms, and poly(L-lysine) two hydrogen atoms and the C^γ atom on the β carbon. The positions of all atoms bound to the C^β atom depend on χ_1, but that of the C^β atom is independent of χ_1. By taking ΔG_b as that for poly(L-alanine), we are neglecting the dependence of the side-chain interactions involving the C^β hydrogens on χ_1 in poly(L-alanine). In poly(L-lysine), the dependence of these interactions on χ_1 was taken into account in the term ΔG_s.

(15) S. A. Rice and M. Nagasawa, "Polyelectrolyte Solutions," Academic, New York, N. Y., 1961, p 307.

(16) M. Nagasawa and A. Holtzer, *J. Amer. Chem. Soc.*, **86**, 538 (1964).

(17) G. S. Manning, *J. Chem. Phys.*, **51**, 924 (1969); *Biopolymers*, **11**, 937 (1972); *Annu. Rev. Phys. Chem.*, **23**, 117 (1972).

(18) A. D. MacGillivray, *J. Chem. Phys.*, **57**, 4071 (1972).

will be treated as uncharged groups, the free energy of ion-pair formation being included in G_3 and assumed to be the same for helix and coil.

In order to take account of the counterions and neutral salt ions, we have calculated W_e using a Debye–Hückel screening potential in water, with a dielectric constant $D = 80$. Hence, for one charge distribution on one conformation

$$W_e = \sum_{i>j}(e^2/Dr_{ij}) \exp(-\kappa r_{ij}) \qquad (18)$$

where e is the (electronic) charge on the side chain, r_{ij} is the distance between charges i and j, and κ is the reciprocal of the thickness of the ionic atmosphere, given by

$$\kappa^2 = 4\pi ne^2/DkT \qquad (19)$$

where n is the number of univalent ions per unit volume. The correction for ionic size in the Debye–Hückel potential is omitted from eq 18 because conformations, in which the charges on the polymer are so near to each other that this correction would be important, will have a high electrostatic free energy and therefore a low probability of occurrence (see eq 25 and 26).

The value of W_e given in eq 18 pertains to a given charge distribution on a given conformation. The electrostatic partition function for a single conformation, q_e, is obtained according to eq 5 by summation over all possible charge distributions. Because of the nature of W_e in eq 18, q_e includes the interactions between the charges on the polymer as they "see each other" through a screen of counterions; the entropy of mixing of charged and uncharged groups on the polymer is included because the summation is taken over all possible charge distributions.[19]

In principle, the electrostatic term Q_e is then obtained according to eq 6 by averaging q_e over all conformations in the helix and the coil regions, respectively. In practice, the calculation of Q_e for the helix is based on only one conformation—that found by minimizing the energy of the uncharged polymer. The values for the inter-charge distances r_{ij} are taken as the distances between the ϵ-amino groups in this minimum-energy conformation.

For the calculation of Q_e for the coil, a Monte Carlo generation procedure is used to obtain a representative collection (200 in number) of coil conformations for an uncharged poly(L-lysine) chain under Θ conditions. The averaging indicated in eq 6 is then performed over these 200 conformations. The characteristic ratio, C, given by

$$C = \langle r_0^2\rangle/Nl^2 \qquad (20)$$

where $\langle r_0^2\rangle$ is the mean-square end-to-end distance and l is the virtual bond length per residue[20] (taken here as the distance between two successive C^α atoms; *i.e.*, $l = 3.75$ Å), of such a collection should be about 9.0 ± 0.5, on the basis of experimental[21] and theoretical[22,23] studies. When the coil is partially or fully charged, the weighting factor of eq 18 favors more extended conformations. We shall assume that the expansion of the coil dimensions beyond those under Θ conditions can be attributed solely to the electrostatic free energy of the charged side chains. The expansion of the coil because of other effects, such as excluded volume, solvent and electrostriction, is ignored. This is equivalent to the assumption, used previously,[24] that an aqueous salt solution acts as a Θ solvent for polylysine, except for the electrostatic interactions between the charges on the polymer.

Table I
Thermodynamic Parameters for the Side Chains of the Neutral Right-Handed α Helix[a] of Poly(L-lysine) in Water

Temp (°K)	$(G_s)_{\text{helix}}$ (kcal/Residue)	$(H_s)_{\text{helix}}$ (kcal/Residue)	$(S_s)_{\text{helix}}$ (eu/Residue)
280	−8.19	−9.84	−5.91
300	−8.14	−9.80	−5.53
320	−8.09	−9.75	−5.19
340	−8.02	−9.71	−4.88
360	−8.01	−9.66	−4.56

[a] For $\phi = -53.9°$ and $\psi = -51.7°$.

As will be shown, even though only 200 conformations are used in the Monte Carlo procedure, a reasonable value is obtained for the characteristic ratio. However, the number of conformations which contribute significantly to $(G_e)_{\text{coil}}$ is much less than 200 (because of the effect of W_e); therefore, our results here should be regarded as a first approximation, which is nevertheless an improvement over those obtained[5-7] without the use of a screening potential.

II. Computation of ΔG_0 for Poly(L-lysine)

A. Geometry and Energy Parameters. The geometry of the backbone and the energy parameters were the same as set A used for poly(L-alanine),[8] the C^β atom being regarded as part of the backbone. The geometry of the lysine side chain was taken from a recent compilation[25] of X-ray crystal data; in particular, τ(CCC) = 111°. The torsional energy for rotation around all C–C bonds of the side chain was taken as

$$E_{\text{tor}} = (E_\chi/2)(1 + \cos 3\chi) \qquad (21)$$

with $E_\chi = 3$ kcal/mol, and minima at ±60 and 180°. Since the electrostatic interaction between a neutral side-chain NH_2 group and the backbone peptide group is small in water, this electrostatic contribution was neglected. According to eq 12, ΔG_b (and the corresponding values of ΔH_b and ΔS_b) were taken to be the same as those computed for poly(L-alanine) in water.[8] Hence, only ΔG_s (and ΔH_s and ΔS_s) were computed.

B. Calculation Procedure. There are five single bonds in the side chain around which rotation can take place, *viz.*, C^α–C^β (χ_1), C^β–C^γ (χ_2), C^γ–C^δ (χ_3), C^δ–C^ϵ (χ_4), and C^ϵ–N (χ_5). In order to reduce the required computer time, χ_5 was fixed at 90°, the minimum-energy value found[25] for lysine, and only a few discrete values were selected for χ_1, χ_2, χ_3, and χ_4. For bonds remote from the backbone (with dihedral angles χ_3 and χ_4), only three values were taken for each bond, *viz.*, those of the minimum of the torsional potential (±60 and 180°); this was justified by demonstrating that the variation of the *total* energy (intra-side-chain plus side-chain backbone) with χ_3 and χ_4, respectively (for one backbone conformation, *viz.*, $\phi = -150°$ and $\psi = 110°$), was dominated by the torsional potential, with minima at ±60 and 180°. Since the C^α–C^β and C^β–C^γ bonds are closer to the backbone, it would probably have been advisable to select more values

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(19) For example, in the hypothetical case in which W_e is the same for all charge distributions in a given conformation of the polymer, q_e becomes equal to the product of $\exp(-\beta W_e)$ times the number of ways in which the charges can be distributed over the ionizable groups of the polymer in that conformation.

(20) P. J. Flory, "Statistical Mechanics of Chain Molecules," Interscience, New York, N.Y., 1969, p 277.

(21) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2788 (1965).

(22) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).

(23) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(24) T. V. Barskaya and O. B. Ptitsyn, *Biopolymers*, **10**, 2181 (1971).

(25) F. A. Momany, R. F. McGuire, and H. A. Scheraga, in preparation.

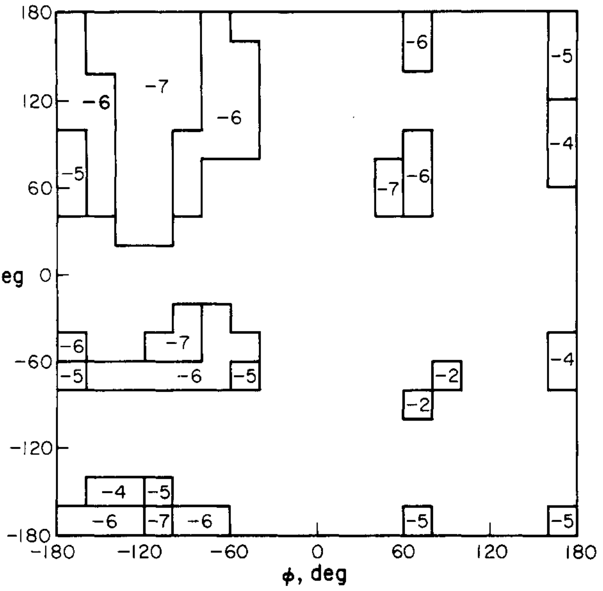

Figure 2. Values of F_s (in kcal/residue) at 300°K arising from side-chain backbone and intra-side-chain interactions in an L-lysine "dipeptide" for various values of ϕ_i and ψ_i.

for χ_1 and χ_2, say 9 each. However, because of the expense of computer time, 9 were taken for χ_1 (each torsional minimum-energy value and also values at ±20° from each minimum), but only 3 for χ_2. Thus, a total of 9 × 3 × 3 × 3 side-chain conformations were taken to evaluate the side-chain free energy for each backbone conformation, using eq 16 and 17 for the coil and helix, respectively. The temperature dependence of the thermodynamic parameters G_s, H_s, and S_s appears only in the quantity β.

C. $(G_s)_{\text{helix}}$. In order to compute the contribution of the side chain to the free energy per residue of the right-handed α helix, $(G_s)_{\text{helix}}$, the backbone was fixed at $\phi = -53.9°$, $\psi = -51.7°$, the minimum-energy conformation for poly(L-alanine) in water;[8] then $(G_s)_{\text{helix}}$ was computed from $(Q_s)_{\text{helix}}$, which in turn was calculated by means of eq 17. Similarly, $(H_s)_{\text{helix}}$ was obtained from eq 14, and $(S_s)_{\text{helix}}$ from the analog of eq 15. These results are shown in Table I.

D. $(G_s)_{\text{coil}}$. A "dipeptide" (ϕ_i,ψ_i) energy contour map for L-alanine (with no hydrogens on the C^β atom) was prepared,[26] taking into account the interaction of the C^β atom with all atoms in the grouping C^α-C′O-NH-C^αH-C′O-NH-C^α (see Figure 1); *i.e.*, the structure considered was that portion of the backbone between $C^\alpha{}_{i-1}$ and $C^\alpha{}_{i+1}$ and only the ith side chain. This map gave the values of $F_b(\phi_i,\psi_i)$ of eq 16. For each set of ϕ_i and ψ_i, $F_s(\phi_i,\psi_i,\{\chi_i^j\})$ was computed at various values of $\{\chi_i^j\}$ in order to obtain the contribution of the lysine side chain. After integrating over $\{\chi_i^j\}$ (eq 16), the values of F_s (shown in Figure 2) were obtained; similarly, the values of H_s and S_s, computed from eq 14 and 15 are shown in Figures 3 and 4, respectively. The maps in Figures 2-4 were used for the evaluation of $(G_s)_{\text{coil}}$, $(H_s)_{\text{coil}}$, and $(S_s)_{\text{coil}}$ by means of eq 16, 14, and 15, respectively. The blank areas in Figures 2-4 are regions of high backbone energy (low statistical weight). As can be seen from Figure 2, the contribution to the free energy of the dipeptide comes mainly from relatively extended backbone conformations, $\phi = -180°$ to $-50°$ and $\psi = 20$-$180°$; the same is observed in Figures 3 and 4 for the enthalpy and entropy, respectively. The free energy, energy and entropy (computed by averaging, according to eq 16, over all backbone regions) are given in Table II as the first approximation (including only intra-side-chain and side-chain–backbone interactions).

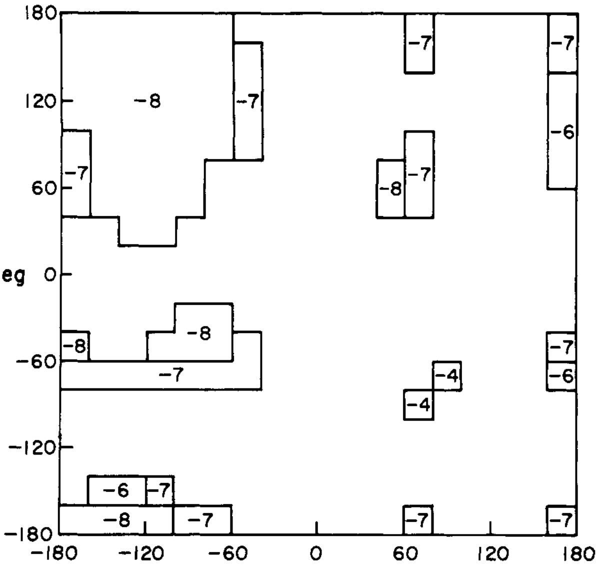

Figure 3. Same as Figure 2, but for H_s (in kcal/residue).

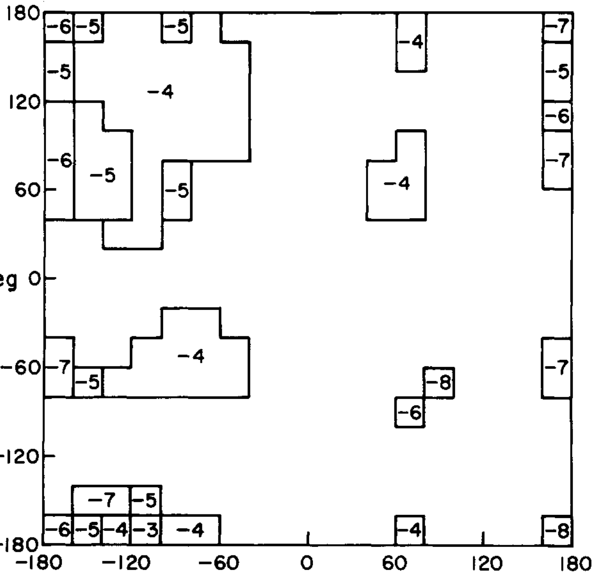

Figure 4. Same as Figure 2, but for S_s (in eu/residue).

To obtain a second approximation (see Figure 1), the structure used in the first approximation was augmented by inclusion of the additional backbone portion from $C^\alpha{}_{i+1}$ to $C^\alpha{}_{i+2}$ and also the $(i + 1)$th side chain. Since it would require too much computer time to cover the whole range of ϕ_{i+1}, ψ_{i+1}, and $\{\chi_{i+1}^j\}$, we carried out the computations by fixing ϕ_{i+1} and ψ_{i+1} at their preferred values (−150 and 150°, respectively, in the β region) and imposed the restriction that $\{\chi_{i+1}^j\} = \{\chi_i^j\}$. The dihedral angles of the ith residue were allowed to vary, and all interactions in this new structure were taken into account. Similarly, a third approximation was obtained (see Figure 1) by adding a CO-NH group to the backbone and the $(i + 2)$th side chain to the $C^\alpha{}_{i+2}$ atom, with ϕ_{i+2} taken the same as ϕ_{i+1} and the new side-chain conformation maintained the same as that of the other two; all interactions in this new structure were taken into account. The thermodynamic parameters in these two additional approximations, ob-

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[26] M. Gō, F. Th. Hesselink, N. Gō, and H. A. Scheraga, *Macromolecules*, to be submitted.

Table II

Thermodynamic Parameters for the Side Chains of the Neutral Coil of Poly(L-lysine) in Water

Temp (°K)	$(G_s)_{coil}$ (kcal/Residue)					$(H_s)_{coil}$ (kcal/Residue)					$(S_s)_{coil}$ (eu/Residue)				
	1st Approx	2nd Approx	3rd Approx	Reg[a] Structure	Reg[b] Structure	1st Approx	2nd Approx	3rd Approx	Reg[a] Structure	Reg[b] Structure	1st Approx	2nd Approx	3rd Approx	Reg[a] Structure	Reg[b] Structure
280	−6.68	−7.43	−7.53	−8.10	−8.08	−8.05	−8.86	−8.96	−9.46	−9.35	−5.44	−5.17	−5.13	−4.85	−4.54
300	−6.63	−7.39	−7.49	−8.07	−8.07	−8.00	−8.81	−8.91	−9.43	−9.29	−5.12	−4.83	−4.79	−4.54	−4.11
320	−6.59	−7.35	−7.46	−8.04	−8.04	−7.96	−8.77	−8.86	−9.40	−9.23	−4.82	−4.51	−4.47	−4.26	−3.72
340	−6.56	−7.33	−7.43	−8.02	−8.03	−7.92	−8.72	−8.82	−9.37	−9.17	−4.54	−4.21	−4.18	−3.99	−3.38
360	−6.53	−7.30	−7.41	−8.00	−8.02	−7.87	−8.68	−8.78	−9.35	−9.12	−4.27	−3.93	−3.90	−3.74	−3.06

[a] For $\phi = -120°$, $\psi = 150°$ in all nine residues. [b] For $\phi = -130°$, $\psi = 170°$ in all nine residues.

tained by averaging only over the major backbone regions by means of eq 16 (to conserve computer time), are also given in Table II. In both the second and third approximations,[26] the only variables are ϕ_i, ψ_i, and $\{\chi_i^j\}$, and the resulting average is regarded as the partition function, q_s, for one side-chain; hence Q_s is approximated as q_s^N, as in eq 16, and the results expressed as the free energy per residue. (The results in Table II for the regular structures will be considered in section E.)

E. ΔG_s. The values of ΔG_s, ΔH_s, and ΔS_s for the conversion from helix to coil in neutral poly(L-lysine) are shown in Table III for the various approximations. The values of ΔG_s (1.5 kcal/mol in the first approximation and 0.75 in the second approximation) are essentially independent of temperature, but give much higher values of ΔG_0 [after addition of the computed value[8] of 0.02 kcal/mol at 25° for poly(L-alanine)] than the experimental value[24,27] of 0.08 kcal/mol at 25°. This poor result clearly arises from not having taken enough interactions into accont in the coil, *i.e.*, the computed free energy of the coil is too high. Since longer range interactions are attractive, they would lower the free energy of the coil if they were taken into account; this effect can be seen from the results in Table III for the third approximation.[26,28]

Since the results of Table III indicate that the inclusion of longer range interactions in the coil lower the value of ΔG_s, a calculation was carried out for nine residues of two regular, extended structures ($\phi = -120°$, $\psi = 150°$ and $\phi = -130°$, $\psi = 170°$) as a limiting case for $(G_s)_{coil}$. These particular backbone dihedral angles were chosen because the previous computations in the dipeptide approximation indicated that $(G_s)_{coil}$ for these two backbone conformations were almost the same as the value of $(G_s)_{coil}$ which was obtained by averaging over the whole (ϕ,ψ) space. The values obtained for $(G_s)_{coil}$ for these two regular structures are shown in Table II; they arise from an additional ~0.5 kcal/mol in enthalpy and ~0.5 eu in entropy because of the longer range interactions (the entropy arising from the rotational freedom of the side chains). When the values of $(G_s)_{coil}$ for these two regular structures are averaged, the resulting values of ΔG_s (shown in Table III) become less than 0.1 kcal/residue. The corresponding values of ΔH_s and ΔS_s are ~0.5 kcal/residue and ~1 eu/residue, respectively (see Figure 5). We may thus use the values of ΔG_s, ΔH_s, and ΔS_s for the "regular" backbone structure of Table III for the helix-coil transition in *neutral* poly(L-lysine) in water.

F. ΔG_0. The value of ΔG_0 is obtained by adding the backbone contribution[8] ΔG_b to the computed values of ΔG_s shown in Table III. The temperature dependences of ΔG_b and ΔG_0 are shown in Figure 6, the difference between these two curves being the side-chain contribution ΔG_s. The calculated curve of ΔG_0 compares favorably with the experimental results;[24,27] the temperature dependences of the computed values of ΔH_0 and ΔS_0 are shown in Figure 5. At the transition temperature (~340°K or ~67°), $\Delta H_0 \approx 1$ kcal/residue and $\Delta S_0 \approx 2.9$ eu/residue are obtained from the computed curves of Figure 5; these values are in agreement with the experimental results.[24,27,29] While this agreement between the observed and calculated thermodynamic parameters for neutral poly(L-lysine) is good, the use of a regular conformation for the backbone of the coil state may be too drastic an approximation.

(27) J. Hermans, Jr., *J. Phys. Chem.*, 70, 510 (1966).

(28) It is worth noting that it is not clear what the range of interactions should be in the coil in order to simulate Θ conditions. This point is discussed elsewhere in connection with computations on the helix-coil transition in poly(L-valine) in water.[26]

(29) P. Y. Chou and H. A. Scheraga, *Biopolymers*, 10, 657 (1971).

Table III
Thermodynamic Parameters for the Side Chains in the Conversion of Helix to Coil in Neural Poly(L-lysine) in Water

Temp (°K)	ΔG_s (kcal/Residue) 1st Approx	2nd Approx	3rd Approx	Reg[a]	ΔH_s (kcal/Residue) 1st Approx	2nd Approx	3rd Approx	Reg[a]	ΔS_s (eu/Residue) 1st Approx	2nd Approx	3rd Approx	Reg[a]
280	1.51	0.76	0.66	0.10	1.79	0.98	0.88	0.44	0.47	0.74	0.78	1.22
300	1.51	0.75	0.65	0.07	1.79	0.99	0.89	0.44	0.42	0.71	0.75	1.21
320	1.50	0.74	0.63	0.05	1.79	0.98	0.89	0.44	0.37	0.68	0.73	1.20
340	1.47	0.70	0.60	0.03	1.79	0.99	0.89	0.44	0.34	0.67	0.70	1.19
360	1.48	0.71	0.60	0.01	1.79	0.98	0.88	0.43	0.29	0.63	0.68	1.16

[a] These values were obtained using the average of the two "regular" values in Table II.

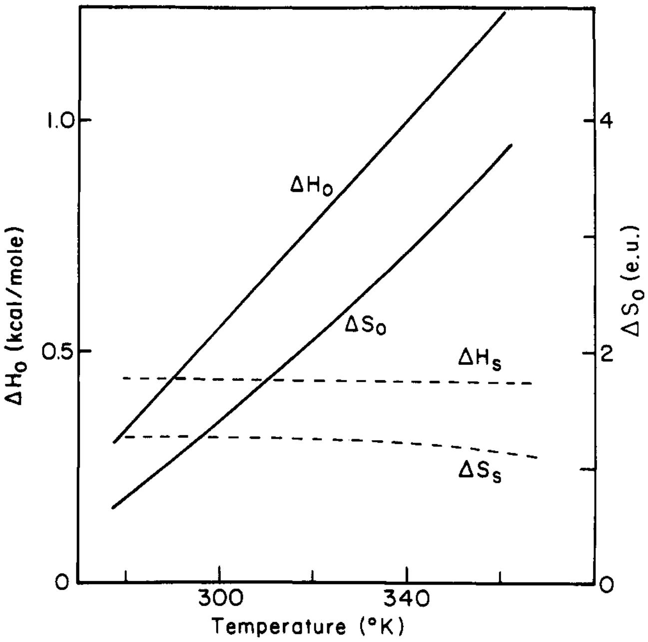

Figure 5. Computed temperature dependence of ΔH_0 and ΔS_0 for neutral poly(L-lysine) in water. ΔH_s and ΔS_s from Table III ("regular" ones) are shown by dashed lines. The values for ΔH_b and ΔS_b were taken from Table VIII of ref 8.

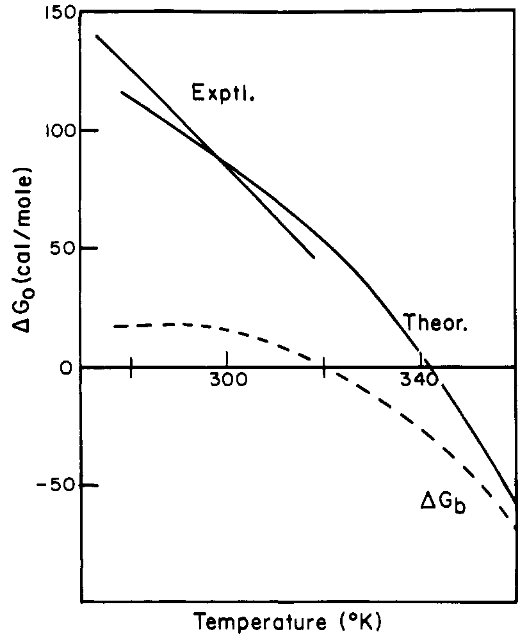

Figure 6. Comparison of experimental[24,27] and computed temperature dependence of ΔG_0 for neutral poly(L-lysine) in water. The backbone contribution ΔG_b [of poly(L-alanine), computed from the values of ΔH_b and ΔS_b of Table VIII of ref 8] is shown by a dashed line. The values of ΔG_s are the "regular" ones of Table III.

From these calculations on neutral poly(L-lysine) in water, it appears that the contribution from the side chain to ΔG_0, ΔH_0, and ΔS_0 is of approximately the same size as that of the backbone as can be seen from the curves in Figures 5 and 6.

III. Computation of ΔG_e for Poly(L-lysine)

The electrostatic free energy of the helix $(G_e)_{\text{helix}}$ was computed for a *regular* structure of 40 residues with $\phi = -61.3°$, $\psi = -43.4°$, $\chi_1 = -74.5°$, $\chi_2 = 116.1°$, $\chi_3 = -178.4°$, $\chi_4 = 176.8°$, and $\chi_5 = 90.5°$;[30] *i.e.*, we ignore the averaging of q_e over the *limited* conformational space in the helical region (indicated by eq 6), and use the approximation

$$(Q_e)_{\text{helix}} = (q_e)_{\text{helix}} \qquad (22)$$

The charges were placed on all nitrogen atoms of the ϵ-NH_2 groups for the fully charged helix. For the partially charged helix, the charges were distributed randomly as described below.

The electrostatic free energy of the coil, $(G_e)_{\text{coil}}$, must be calculated according to eq 6 by averaging the statistical weight of the charge distribution of a single conformation over all conformations. We circumvent this extremely tedious procedure by a Monte Carlo generation of a representative set of random-coil conformations. This Monte Carlo procedure is based on the conformational energy map for a neutral lysine residue, and therefore takes into account the different probabilities of occurrence of different conformations of each residue in the uncharged polymer. Thus, all generated conformations of a poly(L-lysine) chain have equal probabilities of occurrence because the preference of a residue for a particular conformation is accounted for in the generation of each chain. However, when the polymer is then charged (by placing charges

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(30) This conformation, which has been obtained as the low-energy one in an earlier calculation[31a] of neutral poly(L-lysine) with other energy parameters,[25] differs from that obtained for poly(L-alanine)[8] and used here for neutral poly(L-lysine). However, the differences in backbone dihedral angles between this conformation[31a] and the α-helical conformation found for poly(L-alanine),[8] and used in section IIC, do not result in appreciable differences in the geometry of the α helix. For example, this α helix[31a] has a pitch (rise per turn) of 5.36 Å and 3.61 residues/turn, whereas that for poly(L-alanine)[8] has a pitch of 5.40 Å and 3.63 residues/turn. Therefore, this difference in backbone dihedral angles would not be expected to affect any of our conclusions, especially since this second conformation[31a] is used only to evaluate $(G_e)_{\text{helix}}$.

(31) (a) A. Lewis, F. Th. Hesselink, and H. A. Scheraga, in preparation. (b) A map for a charged lysine residue with $D = 2$ and the same side-chain conformation is very similar to Figure 7 (unpublished results). Apparently, intra-residue electrostatic interactions between the charge on the terminal NH_3^+ group of the side chain and the charges on the backbone influence the conformation very little. The effect of this electrostatic interaction would be even smaller, if the screening by the counter ions and the solvent were included.

Table IV
Collection of 967 Conformations of an L-Lysine Residue

ϕ (deg)	ψ (deg)	χ_1 (deg)	Frequency of Occurrence
−160	−60	180	1
−160	80	180	5
−160	100	180	20
−160	120	180	23
−160	140	60	14
−160	140	180	15
−160	160	60	39
−160	180	60	4
−140	−60	180	1
−140	80	−60	1
−140	80	180	8
−140	100	−60	1
−140	100	180	28
−140	120	−60	1
−140	120	180	29
−140	140	−60	1
−140	140	60	8
−140	140	180	14
−140	160	−60	1
−140	160	60	15
−140	180	60	1
−120	−60	180	1
−120	60	−60	8
−120	80	−60	17
−120	80	180	6
−120	100	−60	12
−120	100	180	18
−120	120	−60	9
−120	120	180	15
−120	140	−60	11
−120	140	180	5
−120	160	−60	8
−100	−60	−60	1
−100	−60	180	3
−100	60	−60	14
−100	80	−60	46
−100	80	180	16
−100	100	−60	26
−100	100	180	40
−100	120	−60	15
−100	120	180	25
−100	140	−60	15
−100	140	180	7
−100	160	−60	10
−80	−60	−60	3
−80	−60	180	11
−80	−40	180	1
−80	60	−60	1
−80	80	−60	57
−80	80	180	35
−80	100	−60	36
−80	100	180	96
−80	120	−60	20
−80	120	180	57
−80	140	−60	18
−80	140	180	15
−80	160	−60	10
−60	−60	180	7
−60	100	180	8
−60	120	180	26
−60	140	180	7
60	60	−60	1

randomly on the nitrogen atoms of the ϵ-NH_2 groups as described below), the probability of occurrence of each conformation becomes a function of its electrostatic partition function q_e.

A chain of 40 residues was used in these computations. The conformations of the individual residues were obtained from neutral *N*-acetyl-*N'*-methyllysinamide as follows. The conformational energy, $E(\phi,\psi,\chi_1)$, for this molecule was calculated at 20° intervals in ϕ and ψ, with χ_1 having the values −60, 60, and 180°, and the rest of the side chain fixed in the extended conformation ($\chi_2 = \chi_3 = \chi_4 = 180°$, $\chi_5 = 90°$), with the geometry and energy parameters described elsewhere.[25] An energy contour map[31b] (Figure 7) was prepared from this 18 × 18 × 3 matrix of energy values by selecting the value of χ_1 which produced the lowest value of $E(\phi,\psi,\chi_1)$ at each value of (ϕ,ψ).

A. Monte Carlo Generation of Neutral Poly(L-lysine) Coil Conformations. The dimensions of a randomly coiled polypeptide chain depend mainly on the values of ϕ and ψ of a single residue.[32] Hence, the map of Figure 7 can be used to compute $\langle r_0^2\rangle/Nl^2$, where the relative probability of occurrence $P(\phi,\psi,\chi_1)$ of a specific $\{\phi,\psi,\chi_1\}$ combination is given by

$$P(\phi,\psi,\chi_1) = \exp\{-\beta E(\phi,\psi,\chi_1)\}/\sum_{\phi,\psi,\chi_1}\exp\{-\beta E(\phi,\psi,\chi_1)\} \qquad (23)$$

Using this equation, the 18 × 18 × 3 matrix for the conformational energies $E(\phi,\psi,\chi_1)$ is converted into an 18 × 18 × 3 matrix for the probabilities of occurrence $P(\phi,\psi,\chi_1)$, and a collection of 967 conformations is obtained by assigning each conformation a frequency of occurrence of $1000P(\phi,\psi,\chi_1)$. Less than 1000 conformations are obtained because $P(\phi,\psi,\chi_1)$ is less than 0.0005 for many conformations; also, some conformations are eliminated when the frequencies of occurrence are rounded off to obtain integral numbers. This collection of conformations is shown in Table IV, and was used in the Monte Carlo generation of poly(L-lysine) chains. The ordering of the conformations in Table IV determined the conformation assigned to a particular residue in the Monte Carlo procedure; *i.e.*, a conformation of a chain of 40 residues was obtained by first generating 40 random numbers between 1 and 967, the *i*th random number determining the conformation of the *i*th residue. For example, if the first random number were 4, then the conformation of the first residue was given by the fourth entry in Table IV, *viz.*, $\phi = -160°$, $\psi = 80°$, $\chi_1 = 180°$. Using this procedure, and appropriate transformation matrices,[33] 200 conformations were generated for a 40-mer of poly(L-lysine).

As a check on the validity of this procedure, the characteristic ratio, C, of eq 20 was computed for the 40-mer, by taking the average $(C_{40})_j$ for j conformations, where $1 \leq j \leq 200$. As can be seen in Figure 8, the value of $(C_{40})_j$ approaches the limiting value of 8.69 after $j \sim 120$. This may be compared with an experimental value[21] of 8.6 ± 0.9 found for poly(L-lysine) of DP = 550. Using an energy contour map similar to that of Figure 7, and an averaging procedure to obtain average transformation matrices, Brant and Flory[22,23] computed values of $C_\infty = 9.27$ and $C_{40} \sim 8.1$–8.2 for an infinite chain and a 40-mer, respectively, of poly(L-alanine). These theoretical values are somewhat dependent on the choice of parameters in the conformational energy calculations, and Flory adopts $C_\infty = 9.0 \pm 0.5$ for poly(amino acids) with a C^β atom. Consid-

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(32) Reference 20, p 252.

(33) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, 46, 4410 (1967).

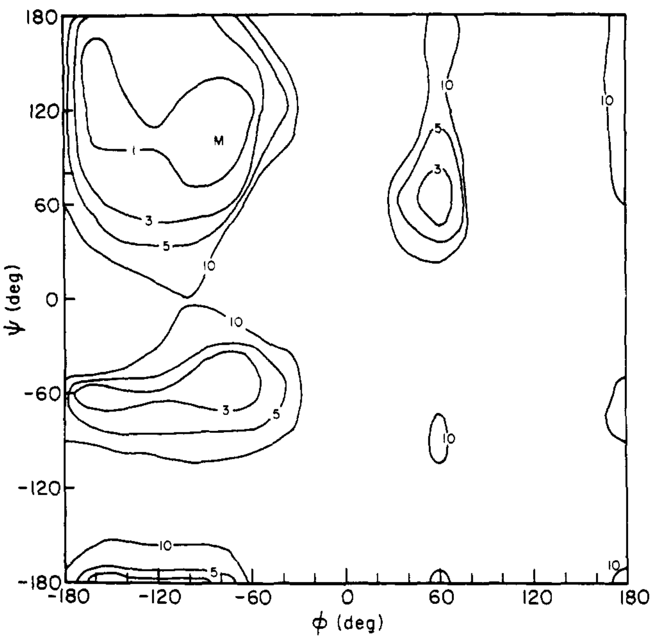

Figure 7. Energy contour map for neutral N-acetyl-N'-methyllysinamide with χ_1 corresponding to the lowest-energy conformation (of −60, 60, and 180°) at each value of (ϕ,ψ) with the rest of the side chain in the conformation $\chi_2 = \chi_3 = \chi_4 = 180°$ and $\chi_5 = 90°$. The energy is expressed in kcal/mol above the zero value taken at the minimum (M) at $\phi = -80°$, $\psi = 100°$, and $\chi_1 = 180°$.

ering these results, it appears that our use of a 40-mer and 200 conformations in the Monte Carlo procedure provides a valid representation of the random coil under Θ conditions.

B. Computation of G_e for Helix and Coil. In order to obtain the electrostatic free energy for the helix, $(G_e)_{\text{helix}}$, and coil, $(G_e)_{\text{coil}}$, the free energy, F_i, of the ith conformation, defined as

$$F_i = -\beta^{-1} \ln (q_e)_i \tag{24}$$

is calculated according to eq 5 and 18. In order to minimize end effects in our 40-mer in both the helix and coil conformations, the summation over i, j in eq 18 is performed as follows: i is chosen to run from 11 to 30, and the only interactions counted are those of the ith residue with the charges on the ϵ-NH_3^+ groups of ten residues to the left and ten residues to the right in the 40-mer. Thus, interactions between charges more than ten residues apart are neglected. This underestimate of G_e is in part compensated by the overestimate of G_e arising from the use of the Debye-Hückel screening potential since the screening by the counter ions is more effective[34] than that accounted for by the Debye-Hückel approximation at potentials higher than 25 mV. However, these problems are probably insignificant compared to that of the choice of the correct value of D. While we have taken $D = 80$, some results are given for $D = 33$ and $D = 56$. Further, eq 18 is not too sensitive to the choice of D, since its effect appears in both the numerator and denominator of eq 18. All calculations pertain to 25°.

The value of $(G_e)_{\text{helix}}$ is computed with the helix geometry described at the beginning of section III and divided by N (40 in this case) to obtain the electrostatic free energy per residue.

The value of $(G_e)_{\text{coil}}$ is obtained from the 200 generated conformations, by recognizing that the 200 conformations

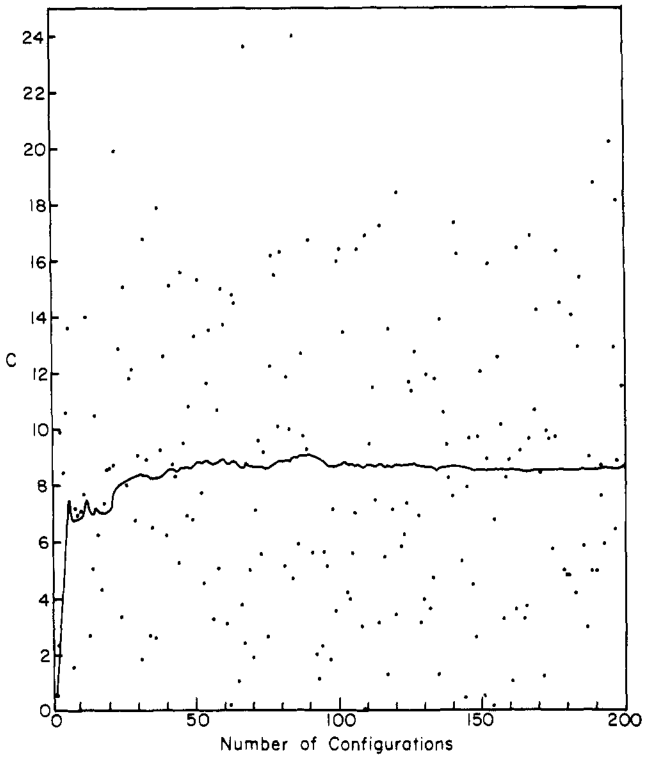

Figure 8. Dependence of C on the number of conformations used to compute the average. Each dot represents the individual value of r_0^2/Nl^2 for the particular random-coil conformation in the set of 200. The curve represents the cumulative average, $\langle r_0^2\rangle/Nl^2$, as each conformation is generated.

have an equal probability of occurrence only if the ϵ-amino groups are uncharged. If the side chains are charged, the probability of occurrence of the ith conformation, p_i, is determined by its electrostatic free energy, where

$$p_i = \exp(-\beta F_i)/\sum_{i=1}^{200} \exp(-\beta F_i) \tag{25}$$

Then, the electrostatic free energy per residue, $(G_e)_{\text{coil}}$, is given by

$$(G_e)_{\text{coil}} = (1/N)\sum_{i=1}^{200} p_i F_i \tag{26}$$

Thus, the averaging over all conformations is performed in eq 26, whereas the summation over all charge distributions is performed in the calculation of q_e (or F_i from q_e by eq 24); q_e includes the interaction between the charged side chains and also the entropy of mixing of charged and uncharged groups (see section IB).

We present the results first for the relatively simple case of completely charged helix and coil conformations, and then for the more interesting case of partially charged conformations.

C. Completely Charged Poly(L-lysine) Chains. When all the ϵ-amino groups carry a positive charge, the entropy of mixing of charged and uncharged groups is zero. Thus, $(G_e)_{\text{helix}}$ is given by

$$(G_e)_{\text{helix}} = (W_e)_{\text{helix}}/N \tag{27}$$

Values of $(G_e)_{\text{helix}}$ for various values of D and salt concentration are given in Table V. For the ith coil conformation, $F_i = [(W_e)_{\text{coil}}]_i$ and the values of $(G_e)_{\text{coil}}$, obtained after averaging according to eq 25 and 26, are also given in Table V, as are values of $\Delta G_e = (G_e)_{\text{coil}} - (G_e)_{\text{helix}}$.

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[34] C. Schildkraut and S. Lifson, *Biopolymers*, 3, 195 (1965).

Table V
Electrostatic Free Energy of Fully Charged Poly(L-lysine) Chains of 40 Residues at 25°

Dielectric Constant	Salt Concn (mol/l.)	$(G_e)_{\text{helix}}$	$(G_e)_{\text{coil}}$	ΔG_e	α
		kcal/Residue			
80	1	0.124	0.082	−0.042	1.02
	0.2	0.696	0.360	−0.336	1.12
	0.1	1.089	0.580	−0.509	1.19
	0.02	2.04	1.22	−0.82	1.26
56	1	0.101	0.072	−0.029	1.00
	0.2	0.745	0.375	−0.370	1.11
	0.1	1.257	0.648	−0.609	1.19
	0.02	2.63	1.53	−1.10	1.28
33	1	0.063	0.055	−0.008	0.99
	0.2	0.755	0.376	−0.379	1.05
	0.1	1.453	0.723	−0.730	1.18
	0.02	3.71	2.07	−1.64	1.29

These values obtained for ΔG_e are all negative, indicating that the electrostatic free energy of the coil is lower than that of the helix. This difference in free energy becomes especially important at salt concentrations at which the coil expands appreciably over its Θ dimensions. Our result of $\Delta G_e = -500$ cal/residue for $D = 80$ at 0.1 M salt and 25° is large compared to the value of ΔG_0 [+90 cal/residue (theoretical, Figure 6) and +80 cal per residue (experimental[24,27])]. Thus, the helix-coil transition will take place long before the whole molecule is charged; indeed, it is found[29] that the midpoint of the transition occurs when only about 35% of the ϵ-amino groups are charged.

Before turning to the case of partially charged poly(L-lysine) chains, it is of interest to compute the expansion of the fully charged random coil due to electrostatic interactions. For uncharged chains, the 200 conformations have equal probabilities of occurrence, and we find for C_{40}, the characteristic ratio of a 40-mer (see Figure 8)

$$C_{40} = \left(\sum_{i=1}^{200}(C_{40})_i\right)/200 = 8.69 \tag{28}$$

For fully charged chains, p_i is given by eq 25, and

$$\langle r^2\rangle/Nl^2 = \alpha^2 C_{40} = \sum_{i=1}^{200} p_i(C_{40})_i \tag{29}$$

The values of α computed from eq 29 are given in Table V and in Figure 9. Experimentally, Brant and Flory[21] found $\alpha = 1.33$ for poly(L-lysine) of DP = 550 in 1 M NaBr and Barskaya and Ptitsyn[24] found $\alpha = 1.45$ for poly(L-lysine) of DP = 360 in 0.2 M NaCl. In order to compare results for different DP and salt concentration, C_s, we use the following relation of Flory[35-37]

$$\alpha^5 - \alpha^3 = C_I(\text{DP})^{1/2}/C_s \tag{30}$$

where C_I can be regarded[37] as an empirical parameter. The data (for $D = 80$) of Table V are plotted according to eq 30 in Figure 9, and a reasonably straight line with $C_I = 0.011$ is found for $C_s \gtrsim 0.1$ M. This may indicate that eq 30 is valid only if the dimensions of the macromolecule are much larger than the thickness of the ionic atmosphere (κ^{-1}), a common limitation in polyelectrolyte theory. Applying eq 30 to the result of Brant and Flory,[21] we obtain $C_I = 0.077$, which gives $\alpha = 1.38$ for a poly(L-lysine) chain of 40 residues in 0.2 M salt, compared to our value of 1.12. Using the result of Barskaya and Ptitsyn,[24] we obtain $C_I = 0.035$ and $\alpha = 1.25$ for a poly(L-lysine) chain of 40 residues at the same salt concentration. Although the differences found between the various values of C_I cast doubt on the reliability[38] of eq 30, our value of $\alpha = 1.12$ seems too low; *i.e.*, our fully charged coil conformations are not extended enough. This may be due to the fact that our collection of 200 coil conformations is too small (even though it suffices for the calculation of C_{40} for the *neutral* polymer) because only 10–15 conformations contribute significantly to the calculated properties of the *fully charged* coil (*i.e.*, have values of $p_i > 0.01$). However, the situation is much better for the more interesting case of *partially* charged poly(L-lysine) chains because many more conformations contribute in this case.

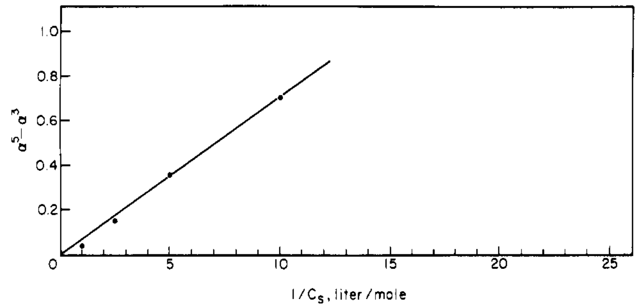

Figure 9. Plot of $\alpha^5 - \alpha^3$ *vs.* $1/C_s$, using the data of Table V (for $D = 80$). The values at 0.04 and 0.4 M were computed analogously but were not included in Table V, simply to avoid enlarging the table.

D. Partially Charged Poly(L-lysine) Chains. In section IIIC, we saw that ΔG_e for the completely charged chain is sufficient to overcome ΔG_0, thereby favoring the coil conformation. We now consider the extent to which the poly(L-lysine) chain must be charged so that $(\Delta G_e + \Delta G_0) = 0$, *i.e.*, we seek the degree of ionization at the midpoint of the pH-induced helix-coil transition.

The electrostatic partition function q_e of eq 5 should be calculated by summing over all possible charge distributions for each conformation. However, when n charges (with, say, n = 12, 20, 28, and 36) are to be distributed over 40 ϵ-amino groups, we have

$$g = 40!/\{(40 - n)!n!\} \tag{31}$$

different charge distributions; *e.g.*, for $n = 20$, $g = 1.4 \times 10^{12}$ distributions, which is too large a number even for high-speed computers. Therefore, q_e is calculated by an approximate procedure in which the n charges are distributed over the 40 ϵ-amino groups by a random number generator, and the electrostatic free energy, $(W_e)_j$, of this jth distribution is calculated. This process is repeated R times for both the helix and *each* of the 200 coil conformations, usually with $R = 100$. Hence[40]

$$q_e = (g/R)\sum_{j=1}^{R} \exp[-\beta(W_e)_j] \tag{32}$$

The values of $(G_e)_{\text{coil}}$ were then calculated by eq 18, 24,

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[35] P. J. Flory, *J. Chem. Phys.*, 21, 162 (1953).

[36] P. J. Flory and J. E. Osterheld, *J. Phys. Chem.*, 58, 653 (1954).

[37] Reference 15, p 506.

[38] The dependence of $\alpha^5 - \alpha^3$ on $(\text{DP})^{1/2}$ seems especially doubtful, as discussed by Hawkins and Holtzer.[39]

[39] R. B. Hawkins and A. Holtzer, *Macromolecules*, 5, 294 (1972).

[40] This procedure was checked by calculating Q_e from eq 5 and 32 for a poly(L-lysine) chain of 16 residues in the α-helical conformation at various degrees of ionization (f = 1/8, 2/8, 3/8, · · ·, 8/8), and the results differed by less than 0.5%, indicating the reliability of eq 32.

Table VI
Electrostatic Free Energy of Partially Charged Poly(L-lysine) Chains at 25°

Solvent	Fraction, *f*, of Side Chains Charged	$(G_e)_{\text{helix}}$	$(G_e)_{\text{coil}}$ kcal/ Residue	ΔG_e
$D = 80$	0.3	−0.273	−0.291	−0.018
$C_s = 0.1 M$	0.5	−0.193	−0.265	−0.072
	0.7	+0.073	−0.100	−0.173
	0.9	+0.607	+0.265	−0.342
	1.0	+1.089	+0.580	−0.509
$D = 33$	0.3	−0.219	−0.250	−0.031
$C_s = 0.02 M$	0.5	+0.160	−0.094	−0.254
	0.7	+0.913	+0.272	−0.641
	0.9	+2.345	+1.211	−1.134
	1.0	+3.710	+2.067	−1.643

25, 26, 31, and 32 [and analogously for $(G_e)_{\text{helix}}$], and the results are shown in Table VI for two different solvent conditions. The negative values of $(G_e)_{\text{helix}}$ and $(G_e)_{\text{coil}}$ at low values of the degree of ionization *f* arise from the entropy of mixing of charged and uncharged groups; the free energy becomes positive at higher values of *f* because of the repulsive interactions between the side chains. The values of $\Delta G_e = (G_e)_{\text{coil}} - (G_e)_{\text{helix}}$ are also given in Table VI.

IV. pH-Induced Helix-Coil Transition in Poly(L-lysine)

The midpoint of the pH-induced helix-coil transition is taken as the value of the degree of ionization, *f*, at which $\Delta G_0 + \Delta G_e = 0$. In Figure 10, we have plotted ΔG_e as a function of *f* for 0.1 *M* salt solution at 25° (with $D = 80$). The experimental[24,27] (+80 cal/mol) and theoretical (+90 cal/mol, from Figure 6) values of ΔG_0 are also shown; both lead to a predicted value of $f \sim 0.5$, compared to an experimental value[29,41] of 0.35.

The data of Table VI for $D = 33$ and 0.02 *M* salt may be used to discuss the helix-coil transition in 95% aqueous methanol since the dielectric constant of this solvent is 33. Yaron *et al.*[42] found that the poly(L-lysine) helix is about 200 cal/residue more stable in 50% aqueous methanol than in water. Extrapolating to 95% aqueous methanol, we might expect ΔG_0 to be about 500 or 600 cal per residue in 95% methanol at 25°, compared to 80 cal/residue in water. If this result is combined with $\Delta G_e = -1.64$ kcal/residue at $f = 1$ (Table VI), we would conclude that the completely charged coil is about 1 kcal/residue more stable than the completely charged helix in 95% methanol. This conclusion is in disagreement with ORD experiments[43] indicating that poly(L-lysine) is completely helical in methanol (>90%) at neutral pH and with nmr data[44] indicating that the polymer is charged under these conditions. However, the nmr experiment probably does not distinguish between bound counterions and ions of the ionic atmosphere (Debye-Hückel model), and it is possible that in methanol some of the counterions are so near to the charged groups that they behave as ion pairs. This might explain why Liem *et al.*[45] could not fit the titration curve of poly(L-lysine) in 95% methanol using the Debye-Hückel screening potential. If we assume that ΔG_0 is ~600 cal/residue in 95% methanol, then the data of Table VI would suggest that $\Delta G_e = -600$ cal/residue at $f \sim 0.65$, implying a degree of ion-pair formation of at least 35% for the solvent conditions (low pH) used by Joubert *et al.*[44]

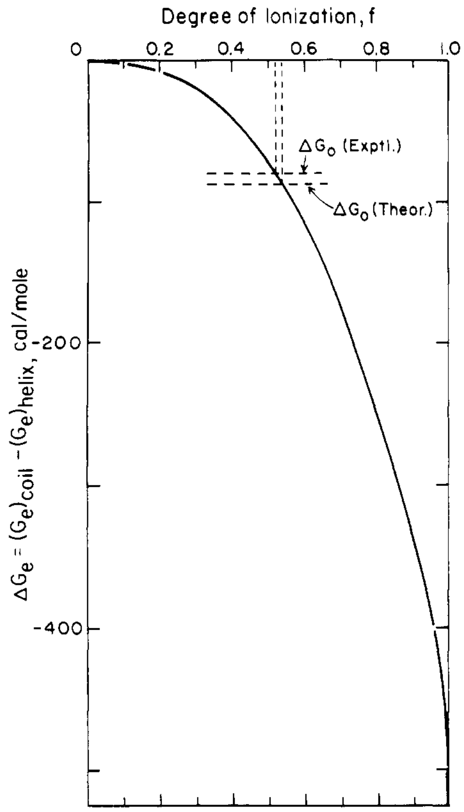

Figure 10. Dependence of ΔG_e on the degree of ionization *f* for 0.1 *M* salt solution at 25°. The experimental[24,27] and theoretical values of ΔG_0 are also included, together with the predicted values of *f* at the midpoint of the helix-coil transition.

V. Discussion

The major features of the pH-induced helix-coil transition for poly(L-lysine) in aqueous salt solution have been accounted for. The value of ΔG_0 is compatible with experimental data, and is of comparable magnitude to ΔG_e so that a variation of ΔG_e with pH leads to a transition in a range of the degree of ionization that is also compatible with experiment. Further, the Monte Carlo generation of the random coil has led to a value of the characteristic ratio (under Θ conditions) that is in agreement both with experiment and with other theoretical procedures for computing this quantity. It is therefore of interest to reconsider some of the approximations made in the calculations.

In the calculation of ΔG_0, all of the effect of water has been included in the poly(L-alanine) portion of the poly(L-lysine) molecule. Thus, possible hydrophobic bonding between lysine side chains has been neglected. As with many other effects, some cancellation of this possible interaction arises from its possible presence in *both* the helix and coil forms. In addition, dipole-dipole interactions between neutral ϵ-NH_2 groups and between these groups and backbone amide groups have been omitted. In computing the conformational entropy of the side chains, only a limited number of conformations was used. Fur-

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(41) If, as a limiting case, ΔG_e is recalculated using the extended β structure instead of the Monte Carlo generated coil (but retaining the Debye-Hückel screening potential), the value of *f* at the transition point is reduced to 0.35 in 0.1 *M* salt, in better agreement with the experimental value.[29] It could be that, in a larger Monte Carlo collection of *charged* coil conformations, extended β conformations might predominate.

(42) A. Yaron, E. Katchalski, A. Berger, G. D. Fasman, and H. A. Sober, *Biopolymers*, **10**, 1107 (1971).

(43) R. F. Epand and H. A. Scheraga, *Biopolymers*, **6**, 1383 (1968).

(44) F. J. Joubert, N. Lotan, and H. A. Scheraga, *Physiol. Chem. Phys.*, **1**, 348 (1969).

(45) R. K. H. Liem, D. Poland, and H. A. Scheraga, *J. Amer. Chem. Soc.*, **92**, 5717 (1970).

ther, the higher approximations showed the need for including longer range interactions in the calculation of the free energy of the coil form. While it was a severe approximation (especially in restricting the entropy of the side chain) to take a regular extended backbone structure for the coil form (in the calculation of the free energy of the side chain), it appears that the resulting free energy may be a limiting one which would be approached if longer range interactions were taken into account in a proper treatment of the neutral coil; also, the preference for extended forms, when the coil is charged, makes the use of this approximation for the neutral coil more valid when the resulting ΔG_0 is to be combined with ΔG_e in a treatment of poly(L-lysine).

In the Monte Carlo generation of the neutral coil, based on a collection of 967 conformations of a single residue (obtained from an empirical energy map) a reliable value (8.69) of the characteristic ratio is obtained. This result is equivalent to that obtained with average transformation matrices[22,23] (in which all possible conformations of a residue are taken into consideration); *i.e.*, the Monte Carlo method used here can generate a representative set of the energetically important conformations, and is effective for a polypeptide with long side chains. This method may be used to improve those of Scott *et al.*[46,47] and Tanaka and Nakajima[48] who obtained low values of the characteristic ratio[49] (*e.g.*, $C_{40} \lesssim 4$, instead of the correct value[21-23] of about 8).

The calculation of the expansion of the coil due to electrostatic interactions yielded rather low values for the expansion factor α, probably because of the use of too small a number of coil conformations and perhaps too small a chain length. However, the fact that the value for α calculated from the data of Brant and Flory is appreciably higher than that based on the results of Barskaya and Ptitsyn may be an indication of the need to include a nonelectrical contribution to the expansion of the coil in aqueous salt solution. Further calculations based on a larger collection of coil conformations for longer chains could shed light on this problem.

The values of ΔG_e are of the right order of magnitude since they predict the correct transition range. The somewhat higher predicted degree of ionization f_{tr} at the midpoint of the transition is probably related to the underestimate of α, since a more expanded coil would have a lower value of $(G_e)_{coil}$; hence, ΔG_e would be greater in absolute value (see Figure 10) for a more expanded coil, leading to a lower predicted value for the degree of ionization at the midpoint of the transition. Of course, as has been stated for the success of the Zimm–Rice theory[4] of the helix-coil transition, the correctness of the predicted value of ΔG_e may be due to a fortuitous cancelation of errors, but it is not apparent which errors would be involved in this cancelation.

The use of an unscreened Coulomb potential with a very low dielectric constant[5-7] leads to values of ΔG_e which are at least an order of magnitude too high, *i.e.*, -20 kcal/residue for completely charged chains[6] compared to our result of about -0.5 to -1.0 kcal per residue. The high electrostatic repulsion, implied by an unscreened potential, would prevent the formation of helix, if the degree of ionization exceeds only a few per cent.

In conclusion, the present treatment of the electrostatic interactions, which did not contain adjustable parameters or an arbitrary assumption about the dielectric constant, seems to be a promising approach for the treatment of the influence of electrostatic interactions on the conformation of macromolecules. It indicates that, in treating electrostatic interactions between charged groups on the surface of proteins, cognizance must be taken of the screening effect of the counterions; *i.e.*, the electrostatic interactions between charged groups on a protein in water are much lower than those that would be computed by ignoring the screening effect.[50]

(46) H. E. Warvari, K. K. Knaell, and R. A. Scott, *J. Chem. Phys.*, **55**, 2020 (1971).

(47) H. E. Warvari and R. A. Scott, *J. Chem. Phys.*, **57**, 1146 (1972).

(48) S. Tanaka and A. Nakajima, *Macromolecules*, **5**, 708 (1972).

(49) These low values arise because of an underestimate[46-48] of the contribution from the large β region of the dipeptide map. On the other hand, these procedures[46-48] can make allowance for excluded volume effects.

(50) In order to reduce the electrostatic interactions between ionizable side chains on the surface of a protein (in contact with water), *i.e.*, to simulate the screening effect, Platzer *et al.*[51] took the groups as uncharged in computing the structure of chymotrypsin and its interaction with substrates.[51]

(51) K. E. B. Platzer, F. A. Momany, and H. A. Scheraga, *Int. J. Peptide Protein Res.*, **4**, 201 (1972).