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Polyelectrolytic aspects of the titration curve

pH-induced conformational transition of poly(L-glutamic acid): the semi-flexible model *

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The cooperative conformational transition of poly(L-glutamic acid) induced by pH is monitored by the titration curves from literature. The polyelectrolytic approach described in the preceding article (A. Cesàro, S. Paoletti and J.C. Benegas, Biophys. Chem. 39 (1991) 1) is used to fit the experimental curves under various conditions of ionic strength and temperature, with the sole assumption that each polymeric state is characterized by a proper conformational flexibility. The helix-coil transition of the system becomes molecularly defined by the balance between the non-ionic conformational energy and the repulsive electrostatic energy of the two forms. Implications of the results of the theoretical model on the energetics of the cooperative order-disorder transition are discussed.

1. Introduction

The helix-coil transition in biopolymers has been extensively studied using a variety of experimental approaches and theoretically treated in detail by statistical mechanical methods (see, for instance, ref. 1). Various approaches have also been outlined to describe the conformational transition of charged biopolymers. In many cases, however, only the phenomenological aspects of the cooperativity are emphasized and the molecular parameters of the conformational transition are not elucidated, since adjustable parameters of the model are often used to fit the experimental data. In some other cases, sophisticated calculations have even been carried out by making a number of assumptions aimed at a quantitative definition of the stability of different conformations.

Some theoretical treatments have been specifically devoted to finding the most stable conformation of charged polyamino acids. In these systems the calculations entail the problem of taking into account the role of long-range electrostatic interactions and their dependence on other physical variables. As a result, they always refer to the minimum energy state [2–4] and not to the whole conformational space accessible to the chain. A recent approach has been applied to the specific case of charge polypeptides by Vila [5], who used an approximate potential of the Kac type for the electrostatic repulsion.

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The scope of this work is to reconsider the cooperative conformational transition of poly(L-glutamic acid) (PLGA; fig. 1), induced in aqueous solution by changes in pH in the range from about 3.5 to neutrality. In a previous paper [6], the theoretical description of the apparent dissociation constant, pK_a, of PLGA was attempted. It was shown that a modified polyelectrolyte approach [7], based on that proposed by Manning [8], was able to describe satisfactorily the observed conformational stability of the α-helical form as a function of the ionic strength. Yet, it was altogether acknowledged that the quantitative agreement between the experimental pK_a curves and the theoretical predictions was not very satisfactory. Although the absence of any adjustable parameter in the theoretical approach was emphasized in comparison with other semi-empirical fittings, it was also believed that some of the hypotheses of the original model proposed by Manning could have been relaxed, thus also improving the physical meaning of the charged line model.

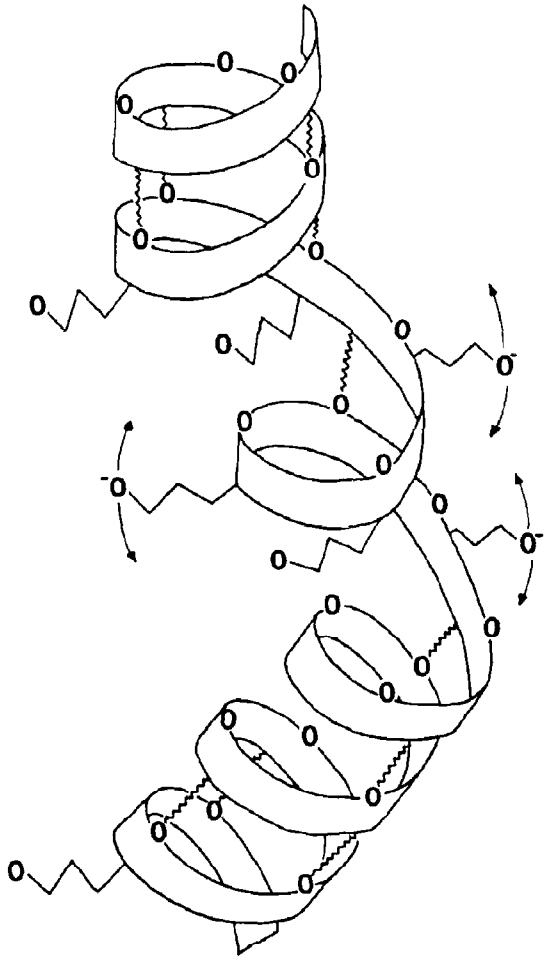

Fig. 1. Schematic representation of the helix-coil transition in poly(L-glutamic acid).

An extension is now presented which takes into account some previous issues [6] and which, in particular, results in a better fitting of the experimental data. The approach has been developed specifically to take into account the distribution of distances between charged groups as it reasonably arises from chain conformational flexibility. This approach has been tested with semi-flexible polypeptides in the preceding paper [9], and previously developed for a spring-like ionic polysaccharide [10].

The mechanistic treatment of the multiple ionization equilibria of weak polyacids was greatly improved by Strauss [11] and was set forth also to describe the conformational transition [12]. Our treatment mainly differs from his because it derives from a molecular view of the contribution to charge fluctuations, which stems principally from the conformational freedom of the linear chain.

2. Results and discussion

2.1. Theoretical backgrounds

Poly(L-glutamic acid) is known to take on a helical conformation at low pH, when the carboxylic groups are uncharged, and to undergo a cooperative transition to a disordered conformation upon ionization. Indeed, it has also been inferred that the disordered state is made by sequences of conformations predominantly in the 'extended' form [2]. Electrostatic interactions (of the Debye-Hückel type) are responsible for the conformational stability and therefore trigger the transition that occurs upon the proton dissociation of this polymer.

Theoretical approaches to the transition usually assume two definite conformational states, one

α-helical and the other conventionally referred to as a single disordered thermodynamic state, defined as a coil. In our approach the process has been treated, indeed, as a transition between two distinct populations of conformational states, each defined by a suitable conformational flexibility with average 'monomeric structural distance', b_0. The average structural parameters for the two limiting forms are obtained from the known conformational geometries and are 1.5 and 3.4 Å, respectively [1–3].

In this framework, the free energy change that determines the state of the system is given by:

$$\Delta G = \Delta G(\mathrm{h} \rightarrow \mathrm{c}) = \langle G_{\mathrm{h}}^{\mathrm{ion}}(\alpha)\rangle - \{\langle G_{\mathrm{c}}^{\mathrm{ion}}(\alpha)\rangle + G^{\mathrm{conf}}\} \quad (1)$$

where $\langle G_{\mathrm{h,c}}^{\mathrm{ion}}(\alpha)\rangle$ is the (averaged) ionic free energy of the helix (or coil) conformation.

Following the methodology previously outlined [9], the thermodynamic averaging is done on a polymer-segment population, for which the end-to-end distribution function is obtained by assuming a hookian conformational free energy. The free energy, therefore, is a function of the distance b between the chargeable groups

$$G(b) = k^0(b - b_0)^2 \quad (2)$$

and the fitting of the experimental data is obtained with a suitable 'Hooke' constant k^0 for each of the two conformations (helix and coil). The individual terms of the ionic contribution can be calculated following the procedure used to describe the titration curves of poly(DL-glutamic acid) [9].

The non-ionic term G^{conf} is the overall conformational energy difference between the two states (helix and coil) of the polymer, in the zero-charge limit, and is available experimentally by an integration procedure of the pK_a curves [13,14].

At any given point of the titration curve, the system contains a fraction f_h of the polymer in the helical conformation and $(1 - f_h)$ as a coil. Assuming the additivity rule, any property of the system is obtained by summing the contributions of both conformational states. For instance:

$$G^{\mathrm{ion}} = f_{\mathrm{h}} G_{\mathrm{h}}^{\mathrm{ion}} + (1 - f_{\mathrm{h}}) G_{\mathrm{c}}^{\mathrm{ion}} \quad (3)$$

where the ionic free energy can be calculated for each conformation by the following expressions [6]:

(i) case $\xi \leq 1$

$$g^{\mathrm{ion}} = G^{\mathrm{ion}}/n_{\mathrm{p}}RT = -\xi \ln(1 - \exp(-kb)) \quad (4)$$

and

(ii) case $\xi > 1$

$$g^{\mathrm{ion}} = \Big[-1/\xi \ln(1 - e^{-kb}) + (1 - 1/\xi) \ln\{(1 - 1/\xi)/V_{\mathrm{p}}C_{\mathrm{p}}(1 + R')\} + (R' + 1/\xi) \ln\{(R' + 1/\xi)/(1 - V_{\mathrm{p}}C_{\mathrm{p}})(R' + 1)\} + R' \ln\{1/(1 - V_{\mathrm{p}}C_{\mathrm{p}})\} + (1 - 1/\xi)\Big] \quad (5)$$

where

$$V_{\mathrm{p}}C_{\mathrm{p}} = \Big[(1 - e^{-kb})^2(1 - 1/\xi) \times e^{(1 + kb/2(e^{kb} - 1)(2R'\xi + 1))}\Big][R' + 1/\xi]^{-1}$$

and k is the Debye-Hückel parameter (see ref. 9).

Imposing the same reference state for the uncharged helix and the uncharged coil, we write

$$\Delta pK_{\mathrm{a}} = f_{\mathrm{h}} \Delta pK_{\mathrm{a_h}} + (1 - f_{\mathrm{h}}) \Delta pK_{\mathrm{a_c}} \quad (6)$$

where the apparent dissociation constant pK_a is related to the ionic free energy (eq. 3 of ref. 9).

In order to evaluate the actual fraction of polymers in each state, the cooperativity of the process has to be taken into account. According to the theory of cooperative transitions [15], it suffices to define a cooperative parameter σ, associated with the excess free energy of the initiation of a given (helical) state. For infinitely long chains, the value of σ is correlated with that of $N(\sigma \approx N^{-1/2})$, operatively defined as the number of monomers in the cooperative unit. Following this approximation, the fraction of helical states can be therefore defined by a Boltzmann equation which contains N-times the free energy changes between the two states:

$$f_{\mathrm{h}}(\alpha) = \frac{1}{Z} \exp - \frac{N\Delta G(\alpha)}{RT} \quad (7)$$

Z being the partition function of the two-state system.

As a consequence of the above discussion, the fitting of the experimental titration curve(s) data of PLGA is achieved by considering not only the proper flexibility but also the cooperativity of the transition.

2.2. *Ionic strength dependence of the transition*

The theoretical approach developed above is used to describe the titration curve of PLGA and its dependence on the ionic strength and temperature, by comparison with the literature data of Nagasawa and Holtzer [13]. Fig. 2 shows the typical behavior of the pK_a curve of the PLGA undergoing the conformational helix-coil transition. The titration curves for a pure helical and a pure coil polymer calculated within the flexible model approach serve as asymptotes of the actual (experimental) curve at $C_s = 0.01$ M [13]. In fig. 2 we also report the fraction, f_h, of polymers in the helical conformation as a function of α, calculated according to the eq. 7, with a cooperativity factor $N = 10$. The simulated pK_a curve follows from the use of eq. 6.

The effect of the salt concentration on the titration curves is shown in fig. 3, where experimental and calculated pK_a curves are plotted for the polymer concentration $C_m = 0.0188$ M, with

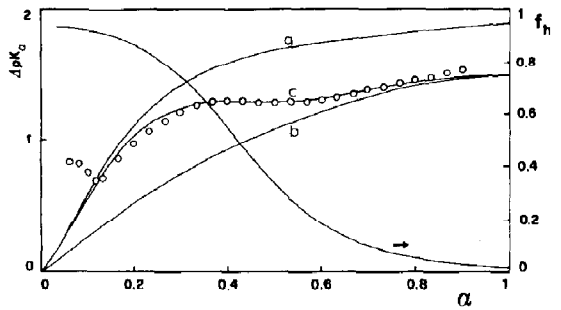

Fig. 2. Titration curve of PLGA in 0.01 M aqueous salt solution. Calculated curves for helix (a) and coil (b) conformations and transition curve (c) calculated according to the text with $k_h^0 = 0.7$ kcal mol^{-1} Å^{-2}, $k_c^0 = 0.34$ kcal mol^{-1} Å^{-2}, $N = 10$ and G^{conf} as in fig. 7. The fraction of polymer in helical conformations, f_h, is given by the ordinate on the right. Experimental data (○) are from ref. 13.

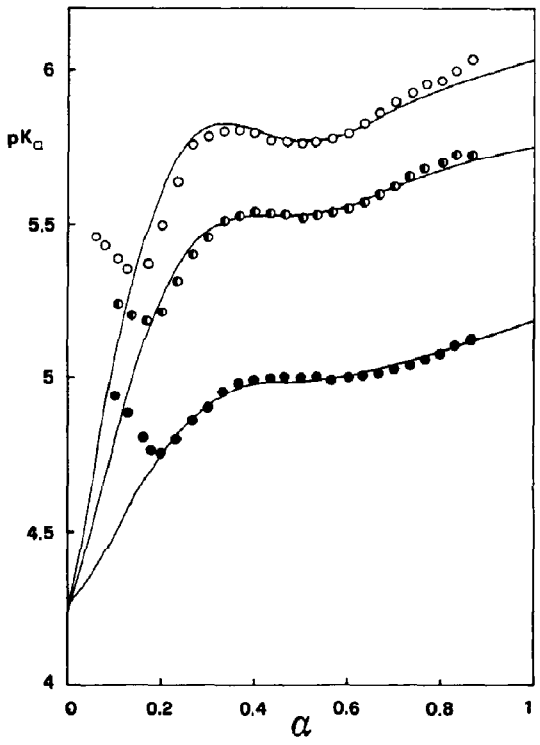

Fig. 3. Ionic strength dependence of the titration curves. Data from ref. 13 with $C_p = 0.0188$ M and $C_s = 0.005$ M (○), $C_s = 0.02$ (◐) and $C_s = 0.2$ M (●), respectively. Curves calculated with parameter values as in fig. 2.

salt concentrations $C_s = 0.005$, 0.01 and 0.2 M, respectively. The quantitative agreement between the experimental and theoretical portions of the pK_a curves has been obtained with stiffness constants $k_h^0 = 0.7$ kcal mol^{-1} Å^{-2} and $k_c^0 = 0.34$ kcal mol^{-1} Å^{-2} for the helical and coiled conformation, respectively. The helical conformation has a stiffness constant about twice that of the coiled conformation. Furthermore, the latter value is consistent with those obtained for the random conformation of poly(DL-glutamic acid) and for poly(L-aspartic acid) [9].

In order to assess the consistency of the theoretical methods, other experimental titration curves from literature have been considered and fitted. The results of these calculations are reported in fig. 4 in comparison with the data of McDiarmid and Doty [16]. In all cases, a satisfactory agree-

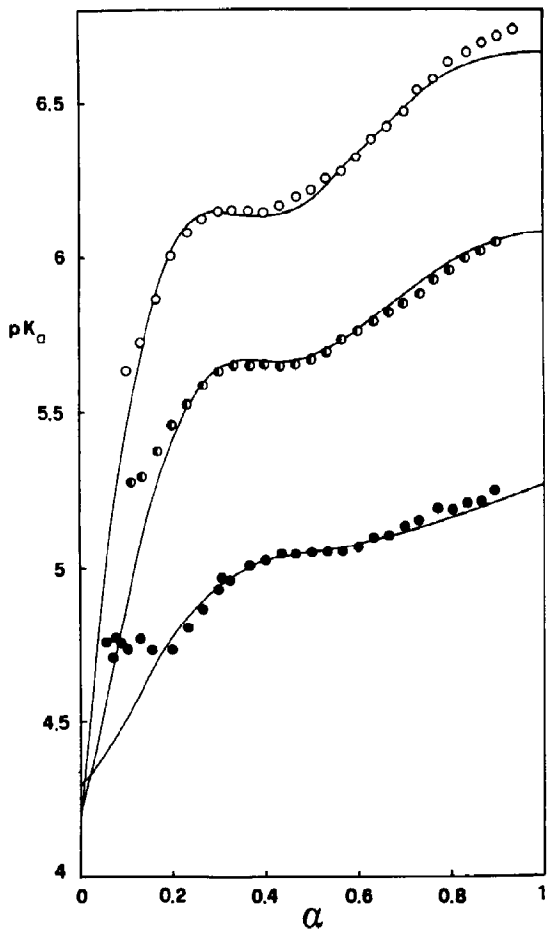

Fig. 4. Ionic strength dependence of the titration curves. Data of ref. 16 with $C_p = 0.003$ M and $C_s = 0$ M (○), $C_s = 0.01$ M (◐) and $C_s = 0.2$ M (●). Curves calculated with parameter values as in fig. 2.

ment has been found by using the same values of the stiffness constants k_c^0 and k_h^0.

Having developed a procedure for simulating the titration curve of the polypeptide undergoing a helix-coil conformational transition, it is worthwhile calculating the fraction f_h of polymer in helical conformation as a function of the degree of ionization, and deriving the dependence of the degree of ionization at the mid-point of the transition, α_{tr}, on the ionic strength. Literature data unambiguously show that the transition point shifts to higher α values (i.e., pH) upon increasing the salt concentration, and that the cooperativity apparently decreases. These findings were already recognized in a previous paper [6], where the original model of a rigid polymer chain was used. Fig. 5 reports the calculated dependence of the degree of ionization of the transition point, α_{tr} as a function of the salt concentration. This value is independent of the cooperativity parameter introduced in eq. 7, since it is determined by the crossing point of the electrostatic free energy curves calculated for the coil and helical forms, provided that an appropriate value of G^{conf} is taken for the non-ionic free energy difference between the two conformations in the uncharged state (see fig. 4 of ref. 6). Fig. 5 also includes the results obtained from the straightforward application of the counterion condensation theory to a rigid polymer with two values of the non-ionic free energy difference ($G^{conf} = 0.22$ and 0.27 kcal/mol, respectively) to show the sensitivity of α_{tr} to the value of G^{conf} used.

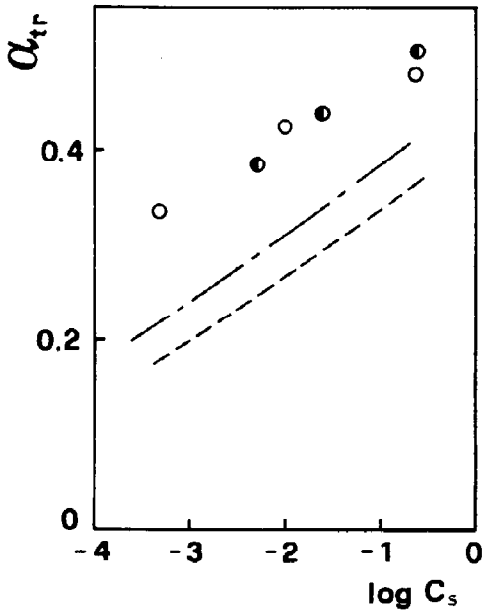

Fig. 5. Dependence of the degree of dissociation at the transition point ($f_h = 0.5$) on the total concentration of small ions in solution. Calculations and experimental data correspond to fig. 3 (◐) and fig. 4 (○). For comparison the results of the rigid model [6] for $G^{conf} = 0.22$ (- - - - - -) and $G^{conf} = 0.27$ (— · —) are also shown.

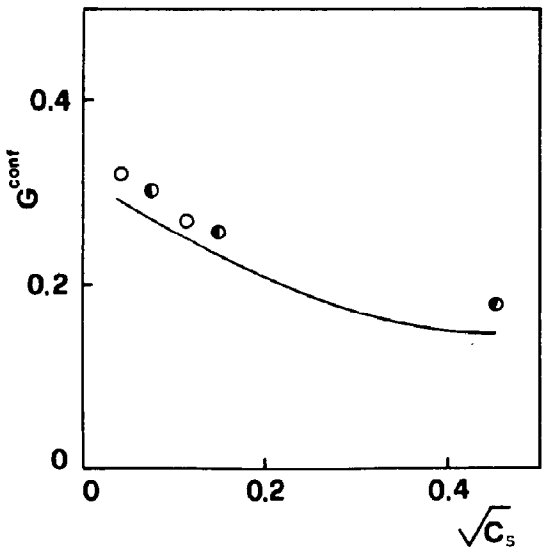

Fig. 6. Dependence of G^{conf} on C_s, for data of fig. 3 (◐) and fig. 4 (○). The line is from fig. 5 of ref. 13.

Consideration has to be made about the fact that the G^{conf} values of the literature are obtained by the integration of the experimental curves pK_a, and are subjected to the uncertainties of the experimental extrapolation to $\alpha = 0$, as already discussed in the literature [13,16–19]. We may resort to the present fitting of the experimental pK_a curves to obtain G^{conf}, and its dependence on the thermodynamic variables, from eq. 1. The dependence of the calculated G^{conf} with ionic strength is shown in fig. 6 for the experimental data of figs 3 and 4. The claimed trend toward higher values of G^{conf} for more dilute salt solutions [13] is therefore confirmed also by the present calculations that reproduce the experimental data. The small, systematic difference is well within the experimental and calculational uncertainties.

2.3. *Temperature dependence of the transition*

In order to assess the ability of the theory to explain other aspects of the process, the temperature dependence of the pH-induced transition has been considered. Experimental titration curves of PLGA at three different temperatures, 0.6, 25 and 48 °C, respectively, were available from the literature [19].

Agreement between experimental curves and those calculated was obtained by using the literature values of G^{conf} [19] and taking the number of cooperative units, N, as the only adjustable parameter. The agreement found in this way is satisfactory. Despite this fact, we let the values of G^{conf} vary with T. An even better agreement is found with values of G^{conf} that are by about 50 cal/mol systematically lower than those proposed by Olander and Holtzer [19]. These deviations are not dramatic, in consideration of the already discussed uncertainties in the determination of G^{conf} from the extrapolation of the experimental pK_a values to $\alpha = 0$. The excellent agreement between the calculated pK_a curves and the corresponding experimental data is shown in fig. 7. Consistency of the theoretical approach has been checked through the evaluation of G^{conf} by the integration of the area between the simulated pK_a curve and that calculated for the pure coil conformation (within an error less than 5%). No appreciable difference is found for the value of ΔH (975 ± 50 cal/mol residue) from the van't Hoff plot.

More interestingly, we find that in addition to the expected dependence of the free energy of the transition on the ionic strength and temperature, the cooperativity of the transition is also a function of temperature, as measured by the factor N which multiplies the free energy change per residue. We find that N varies with T, being 14, 11

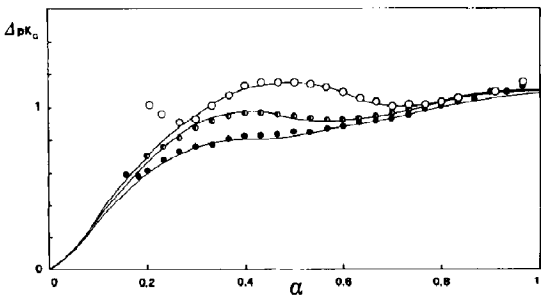

Fig. 7. Temperature dependence of the titration curves. Experimental data of ref. 19 for $T = 0.6$ °C (○), $T = 25$ °C (◐) and $T = 48$ °C (●). Curves calculated with parameter values same as in fig. 2, except $N = 14$, 11 and 8, respectively.

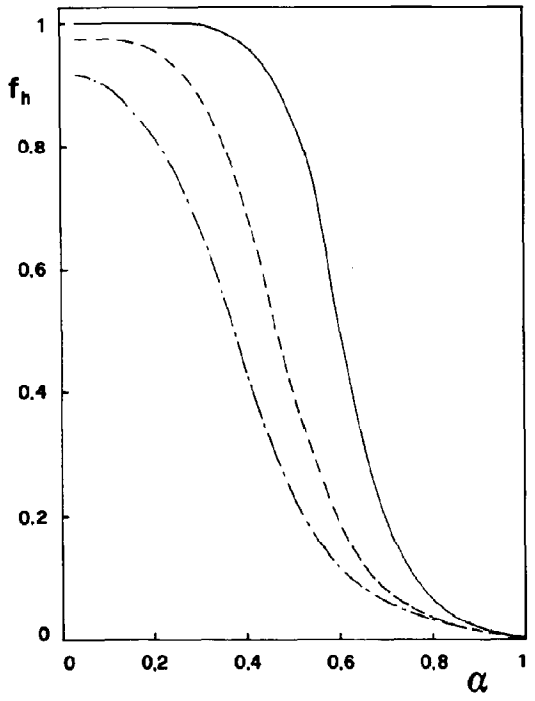

Fig. 8. Calculated fraction of polymer in helical conformation as a function of the degree of dissociation, for the data represented in fig. 8, for $T = 0.6\,^{\circ}C$ (———), $T = 25\,^{\circ}C$ (- - - - - -) and $T = 48\,^{\circ}C$ (· – · – ·).

and 8 for 0.6, 25 and 48 °C, respectively. This feature is demonstrated in fig. 8, where the fraction of polymer in helical conformation is plotted as a function of α. It is also noteworthy to report that the van der Waals interaction energy calculated for a α-helical polypeptide as a function of the chain length reaches 90% of the asymptotic value for a chain of approx. 10 residues. A major contribution to the stabilizing energy comes from the dipole-dipole interactions, as firstly outlined by Brant [20]. The values of N reported above are therefore in line with both the independent results of the conformational energy calculations and with the older hypothesis on the origin of the cooperativity.

From the results of the temperature dependence of the length of the cooperative unit one may calculate an enthalpic contribution of $\Delta H_\sigma \approx$ 3 kcal/mol to the statistical weight σ of the Zimm and Bragg theory. This is in contrast to the older assumption that the statistical weight σ is of merely entropic origin, but is consistent with conformational calculations which assign an important enthalpic contribution to σ (see, for instance, ref. 20).

3. Conclusions

The aim of the present paper has been to verify that a quantitative description can be obtained for the titration curve of a polypeptide undergoing a pH-induced conformational transition, under different physico-chemical conditions. Our approach contains two main assumptions:

(i) the electrostatic interactions are considered in the framework of the counterion condensation theory of linear polyelectrolytes, and

(ii) the polymer in solution is considered as semiflexible; i.e., rather than a single rigid conformation, there exists a distribution of conformational states (due to both backbone and side chain conformational freedom) contributing to a statistical value of the charge distribution.

An important direct consequence of the last assumption is that a measured property is correctly evaluated only as the thermodynamic average of the corresponding physical variable over the appropriate distribution of the end-to-end distance of the polymer segments [9].

A wide body of experimental data has been satisfactorily reproduced, including titration curves at different ionic strengths and temperatures. In all cases the conformational transition occurring upon ionization of the polypeptide is simply related to the balance of the non-ionic and the electrostatic free energy of the two conformations.

We also note that all experimental curves have been fitted with the same conformational model (e.g., the coil form is that used in the description of the titration curve of PDLGA [9]). Considering the simplicity, plausibility and good results of our approach, we believe that it deserves to be extended in order to take into account other phenomena in which the semi-flexibility of polymers in solution should play an important role.

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