the latter, a study of the effect of polydispersity is required.

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Polyelectrolytes in Salt Solutions. Quantitative Separation of Binding and Electrostatic Effects for Poly(L-ornithine) and Poly(L-lysine)

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ABSTRACT: Potentiometric titrations for poly(L-ornithine) (Orn$_n$), calorimetric measurements for Orn$_n$ and poly-L-lysine (Lys$_n$), and optical rotatory dispersion data for Orn$_n$ and Lys$_n$ are presented. Measurements were performed in the presence of variable quantities (up to 2 M) of KSCN and KCl. In agreement with previous findings, KSCN causes stabilization of the helical conformation for Lys$_n$, even when the polymer is fully ionized (pH 3). Negligible helix formation is instead observed for Orn$_n$. KCl is unable to induce helix formation for both Lys$_n$ and Orn$_n$ (pH 3). These results have been quantitatively interpreted in terms of an extension of existing formalisms of the electrostatic free energy of polyions in salt solutions. The modification introduced accounts for a reduction of the fixed charge on the polymer caused by a binding reaction between charged ϵ-amino residues and SCN$^-$ ions. The binding reaction is formulated in terms of multiple equilibria theory. Classical effects of the ionic strength on the slopes of pK_{app} *vs.* Z plots and on intrinsic equilibrium constants were retained. The satisfactory agreement between experimental data and theoretical predictions strongly supports the validity of the model based on the simultaneous occurrence of specific (binding) and nonspecific (electrostatic) interactions for polar solutes in salt solutions.

Salts affect the conformational properties of polyelectrolytes by contributing to the charge-dependent and to the charge-independent part of the free energy. When the charge density of the polymer is high, a prevailing contribution of the electrostatic free energy is expected. If the surface potential of the macroion can be estimated, salt effects may be predicted according to different models.

Simple electrostatic effects, *i.e.*, charge screening according to the Debye–Hückel concept, have been theoretically described for a variety of macroion models.[1] The concept of ion condensation[2,3] has been recently introduced in a model for the conformational transition of rodlike polyelectrolytes possessing high charge density.[4] According to this theory, some counterions must "condense" on the surface of the rod when the charge density reaches a critical value, specifically during the random coil → helix transition.

On the other hand, it has been shown that salts may affect conformational stability by interactions which are not readily interpreted in terms of charge screening, or ion condensation effects.[5] For instance, Puett *et al.*[6-8] have reported that a conformational transition from coil to helix is observed for fully charged poly(L-lysine) when KSCN is added to the solution (the transition was not induced in the presence of comparable amounts of KCl).

Further work[9] has shown that the role of ion type is evident for every value of the degree of ionization. This result parallels the observed deviations (from the traditional model) of the colligative properties of acidic polyelectrolytes in the presence of certain divalent cations.[2] These effects cannot be rationalized by purely electrostatic models and require consideration of other specific properties of the counterions.

The ion binding concept has been often advocated in order to describe specific properties of ions.[5] Ion binding effects should occur simultaneously with screening effects associated with "free" (*i.e.*, unbound) coions and counterions. We have often stressed[10,11] that the ability which some ions (*i.e.*, SCN$^-$, Li$^+$, etc.) have to bind to polar substrates (*i.e.*, peptide bonds) should also be manifested with polyelectrolytic solutes. However, quantitative verifications of the validity of the ion binding mechanism for altering the conformational stability of polyelectrolytes (when multiple equilibria effects cannot be neglected) have not been forthcoming.

In the present paper, we attempt to describe quantitatively the titration and the calorimetric behavior of poly-(L-ornithine) and poly(L-lysine), and the KSCN-induced transition for the latter, in terms of binding and screening effects.

Method

Potentiometric Titrations. Our purpose is that of showing that the KSCN-induced coil → helix transition observed[6-8] at pH 3 for poly(L-lysine) $(CONHCH(CH_2)_4NH_2)_n$ (Lys_n), when the polyelectrolyte is fully ionized $(\alpha = 0)$, can be quantitatively described in terms of: (1) binding of SCN^- ion which reduces the fixed electrostatic charge on the polymer, and (2) an electrostatic effect of the ionic atmosphere due to mobile K^+ and SCN^- ions. In addition to the investigation of the potentiometric and calorimetric behavior of Lys_n, some complementary experiments on the closely related compound poly(L-ornithine) $(CONHCH(CH_2)_3NH_2)_n$ (Orn_n) have also been carried out. Indeed, the analysis of the conformational transitions induced by binding phenomena requires the experimental evaluation of the separate binding effects on the helical and random-coiled conformers. This is difficult to achieve for the system Lys_n–KSCN, because the conformational transition occurs at very low salt concentration. Orn_n, on the contrary, retains the random-coil conformation up to 1 M KSCN at pH 3 owing to the low intrinsic stability of the helix.[12] Moreover, Orn_n and Lys_n possess similar polyelectrolytic properties, as shown by the close values of the intrinsic ionization constants K_0[7,12] and of the electrostatic free energies at any value of α. This enables us to determine the association constants for the random-coil state by investigating the potentiometric and calorimetric behavior of Orn_n at pH 3 between 0 and 1 M KSCN.

On the basis of the results which have already been presented,[6-13] we then assume that: (1) SCN^- is able to bind to the side-chain amino groups of both Lys_n and Orn_n, (2) no binding of Cl^- occurs to the side chain of both Orn_n and Lys_n, and (3) no coil to helix transition occurs for Orn_n–KSCN and Orn_n–KCl at pH 3 up to 1 M salt. Using the latter system we determine those parameters (*i.e.*, intrinsic equilibrium constants for the dissociation of the hydrogen ions, and slopes of pK_{app} *vs.* Z plots) which should depend upon ionic strength but not upon the fixed electrostatic charge on the polymer.[1] The latter is determined at any KSCN concentration by assuming a formal equivalence between the dissociation of an H^+ and the association of a SCN^- ion. Details are as follows.

The ϵ-amino group of Orn_n (and Lys_n) is considered to be involved both in the dissociation equilibrium of H^+ ions and in the association equilibrium of SCN^- ions according to the reactions

$$R-NH_3^+ \rightleftarrows R-NH_2 + H^+ \tag{1}$$

$$R-NH_3^+ + SCN^- \rightleftarrows R-NH_3^+SCN^- \tag{2}$$
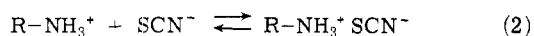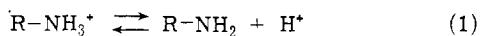
The corresponding equilibrium constants may be written as

$$K = [-NH_2][H^+]/[-NH_3^+] =$$
$$\eta\alpha a_H/\eta(1-\alpha)(1-\gamma) = \alpha a_H/(1-\alpha)(1-\gamma) \tag{3}$$

$$K^b = [-NH_3^+SCN^-]/[-NH_3^+][SCN^-] =$$
$$\eta(1-\alpha)\gamma/\eta(1-\alpha)(1-\gamma)a_s = \gamma/(1-\gamma)f_\pm C_s \tag{4}$$

where η is the total concentration of ϵ-amino groups (mole/liter); α the degree of dissociation; γ the degree of association (*i.e.*, the concentration of $NH_3^+SCN^-$ divided by $\eta(1-\alpha)$, the concentration of sites available for SCN^- binding); a_s, C_s, and $f_\pm$ are the activity, the molar concentration, and the mean activity coefficient of salt, respectively.

In order to account for the interdependence of the reactions on the η sites, multiple equilibria theory yields[1]

$$K_{app} = \alpha a_H/(1-\alpha)(1-\gamma) =$$
$$K_0 e^{2\omega Z} = K_0 e^{2w(1-\alpha)(1-\gamma)} \tag{5}$$

$$K^b_{app} = \gamma/(1-\gamma)C_s f_\pm = K^b_0 e^{2\omega Z} = K^b_0 e^{2w(1-\alpha)(1-\gamma)} \tag{6}$$

where K_0 is the intrinsic equilibrium constant for the dissociation of the last H^+, when $Z = 0$; Z is the electrostatic charge per mole of polymer; and ω is equal to the ratio A/RT where A occurs in the general expression[1,7] for the electrostatic free energy $W_{el} = AZ^2$. The parameter w differs from ω because it includes the degree of polymerization P. In fact, when the substitution $Z = \alpha P$ can be made, the value of w can be determined from the slope of an experimental pK_{app} *vs.* α plot according to the equation

$$\text{slope} = 0.868AP/RT = 0.868w \tag{7}$$

In the case in which binding is negligible (*i.e.*, in the presence of KCl) eq 5 yields

$$K'_{app} = \alpha a_H/(1-\alpha) = K_0 e^{2w(1-\alpha)} \tag{8}$$

In other words, it is stipulated that occurrence of ion binding only alters the value of Z; K_0 and w (which depend upon salt concentration) being independent of salt type, at a given salt concentration.

Equations 5 and 8 yield

$$pH - \log \frac{\alpha}{(1-\alpha)(1-\gamma)} =$$
$$pK_0 - 0.868w(1-\alpha)(1-\gamma) \tag{9}$$

$$pH' - \log \frac{\alpha}{1-\alpha} = pK_0 - 0.868w(1-\alpha) \tag{10}$$

Subtracting eq 10 from eq 9 at constant C_s and α

$$pH - pH' + \log(1-\gamma) = 0.868w(1-\alpha) -$$
$$0.868w(1-\alpha)(1-\gamma) \tag{11}$$

Equation 11 is at the basis of our method for determining γ. In fact, if titration curves (pH *vs.* α) are obtained for Orn_n at a given salt concentration of both a binding salt (KSCN) and a salt unable to bind (KCl), one can determine the corresponding pH–pH' difference at a given α value (at the given α, it is required that Orn_n maintains the same conformation in KCl and KSCN). Since the w value can be determined from a pK_{app} *vs.* α plot for KCl at the given C_s, eq 11 may be solved for γ. The knowledge of γ, along with that of w values, allows the calculation of K^b_{app} and K^b_0 using eq 6. Although the above-indicated determination of K^b_0 is accomplished using data at finite α values, K^b_0, representing the intrinsic equilibrium constant for binding of the last SCN^- ion when $Z = 0$, retains its value when $\alpha = 0$. Under this condition, eq 6 becomes

$$K_{app}^b = \gamma/(1-\gamma)C_s f_\pm = K^b_0 e^{2w(1-\gamma)} \tag{12}$$

Thus, the knowledge of K^b_0 and of w values (again determined at any given C_s in KCl) allows the determination of γ and K^b_{app} as a function of C_s, at $\alpha = 0$.

It is known[12,13] that while Orn_n generally maintains the random-coil conformation during the titration from the charged to the uncharged state, Lys_n undergoes a random coil → helix transition[7] when $\alpha \sim 0.6$ (KCl, 0.1 M, 25°). Data in the literature[7] have shown that while w depends, as expected,[1] on conformation, K_0 is unaffected by it. On the basis of these results, eq 12 can be applied to the determination of K^b_{app} for both the random-coiled and the helical conformation provided w values appropriate to the

two conformations are used. For the analysis of our data, the value of $K^b{}_0$ determined for Orn_n in KSCN has also been used for Lys_n (random coil and helical forms).

Once the values of $K^b{}_{app}$ for the pure random-coiled and helical conformations have been determined, it is possible to verify how the KSCN-induced random coil → helix transformation observed for Lys_n at pH 3^{6-8} can be predicted. For this purpose, we calculate the electrostatic free energy for residue W_{el} (at $\alpha = 0$) from the equation (*cf.* eq 7)

$$W_{el} = wRT(1 - \gamma)^2 \qquad (13)$$

from both random-coiled (RC) and helical (H) Lys_n as a function of C_s, and we write for the free-energy change during the random coil → helix transition

$$\Delta G = W_{el}{}^H - W_{el}{}^{RC} + \Delta G_0 \qquad (14)$$

where ΔG_0 is the free-energy change for the transition between uncharged conformers. The latter, according to Hermans,[14] is equal to -80 cal/mol. The way in which ΔG changes with KSCN concentration determines the corresponding variation of the helical fraction, f_H, with C_s through the equation[15]

$$f_H = 0.5\left\{1 + \frac{(S' - 1)}{[(1 - S')^2 + 4\sigma S']^{1/2}}\right\} \qquad (15)$$

where, according to Zimm and Rice

$$S' = e^{-\Delta G/RT} \qquad (16)$$

and the σ factor is of the order of[16-19] 5×10^{-3}.

Calorimetry. Equation 4 can be rearranged to give (the product $K^b C_s$ remains the same whether $f_\pm$ is introduced or not)

$$\gamma = \frac{K^b C_s}{1 + K^b C_s} \qquad (17)$$

The calorimetric method applied to the system Orn_n–KSCN yields the heat associated with reaction 2, ΔH, which may be expressed in cal/mol of peptide residue. The degree of association can be related to ΔH through the equation

$$\gamma = \Delta H/\Delta H^\circ \qquad (18)$$

where ΔH° is the heat associated with the formation of 1 mol of the $-NH_3{}^+SCN^-$ product. ΔH° is expected to vary with salt concentration, owing to the large effect of C_s on the partial molar enthalpy of the salt which appears on the equation

$$\Delta H^\circ = \bar{H}^{product} - \bar{H}^{polymer} - \bar{H}^{salt} \qquad (19)$$

Introducing the partial molar enthalpy of the salt $\bar{H}_0{}^s$ at infinite dilution, and neglecting heat effects associated with the dilution of product and polymer,

$$\Delta H^\circ = \Delta H^{\circ\circ} - (\bar{H}^s - \bar{H}_0{}^s) \qquad (20)$$

where $\Delta H^{\circ\circ}$ refers to infinite dilution of the salt, and the second term on the right-hand side can be experimentally determined. Equations 18-20 are not strictly correct since the role of charge interaction (giving rise to the exponential factor in eq 6) is neglected. However, the assumption of a constant $\Delta H^{\circ\circ}$ value may be justified on the basis of experimental findings. In fact, the heat of ionization of poly(L-glutamic acid) (in the pure random-coiled and pure helical forms) was found to be independent of the degree of ionization.[20] Preliminary work carried out in our laboratory also indicates that the heat of ionization for Lys_n and Orn_n remains constant in a wide range of α.

From eq 17, 18, and 20, we obtain

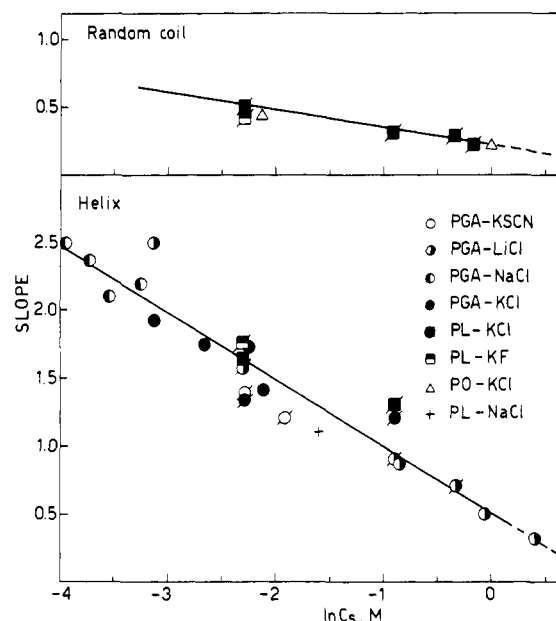$$\Delta H^{\circ\circ} - (\bar{H}^s - \bar{H}_0{}^s) = \Delta H(1 + K^b C_s)/K^b C_s \qquad (21)$$

Figure 1. Slopes of pK_{app} *vs.* α plots for Lys_n (PL), Orn_n (PO), and Glu_n (PGA) in several salt solutions of the indicated molarity. All data refer to 25°. A bar across the symbol indicates data of Ciferri *et al.*,[7] a cross indicates a value of Ptitsyn.[23]

Provided $\Delta H^{\circ\circ}$ is known, eq 21 allows the determination of K^b from the experimental quantities ΔH, C_s, and $\bar{H}^s - \bar{H}_0{}^s$. $\Delta H^{\circ\circ}$ may be obtained at a single value of C_s by assigning to K^b the value of $K^b{}_{app}$ determined from titration data for random-coiled Orn_n (pH 3). The ΔH *vs* C_s dependence can then be converted into the $K^b{}_{app}$ *vs.* C_s curve. With the same procedure used and in connection with the titration data, a $K^b{}_{app}$ *vs.* C_s curve for binding to the pure helical form can also be calculated.

The experimental variation of ΔH with KSCN concentration for Lys_n (pH 3) reflects the binding effect to both conformers plus the conformational transition which, as indicated by ORD data,[6,12] occurs between ~ 0.1 and $\sim 0.9\,M$. Thus, we can write for ΔH

$$\Delta H = (1 - f_H)\gamma_{RC}\Delta H^\circ + f_H\gamma_H\Delta H^\circ + f_H\Delta H_T \qquad (22)$$

where γ_{RC} and γ_H indicate the degree of association to the random-coiled and to the helical form, respectively, and ΔH_T is the heat of transition from an uncharged coil residue to an uncharged helical one. For the latter, we take the value of -885 cal/mol as determined by Hermans[14] and by Davidson and Fasman[21] (for other relevant determinations of ΔH_T, *cf.* Chou and Scheraga,[22] and Rialdi and Hermans[20]). Thus, with the knowledge of γ values for pure random coil and pure helical forms, eq 22 can be used for calculating the dependence of ΔH upon KSCN concentration. Comparison with the direct experimental determination of ΔH for Lys_n affords a method for testing the internal consistency of the binding model.

Results

Potentiometric Titrations. In Figure 1 we collect the values of slopes of pK_{app} *vs.* α plots measured, for Orn_n and Lys_n, at several concentration of salts (KCl, NaCl, and KF) unable to bind. Included in Figure 1 are published data[7,9] obtained for poly(L-glutamic acid) (Glu_n) in salt solutions where unusual salt effects were not observed. Slopes obtained in the region of stability of random-coiled conformation are plotted separately from slopes obtained for the helical conformation. The precision on the determination of the slopes decreases on increasing C_s when slopes are very small. The data support
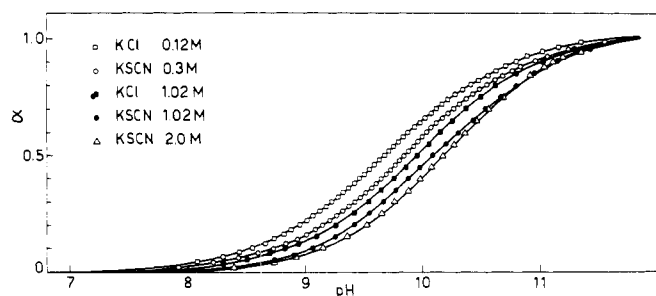
Figure 2. Potentiometric titration, α *vs.* pH curves, for Orn_n in solution of KSCN and KCl; $T = 25°$.

the contention that for a variety of polyelectrolyte–salt systems, when binding effects need not to be considered, w (= slope/0.868) is an univocal function of C_s, depending upon polymer conformation. The data can be directly used, as indicated in the preceding section, in order to determine the proper value of w to be used, for the random-coiled or the helical form, at each value of C_s.

Typical titration curves for Orn_n in KSCN and KCl are collected in Figure 2. The data were used to calculate the degree of association γ according to eq 11. The pH shift was determined using the pH values determined at $\alpha = 0.2$ for equal concentrations of KCl and KSCN. The relevant w values were obtained from Figure 1. From the value of γ (measured γ varied between 0 and 0.3), the constant $K^b{}_{app}$ was calculated, and from the latter the value of $K^b{}_0$ was derived according to the eq 6, again using the w data reported in Figure 1. The value of $K^b{}_0$ was found to be 0.23 M^{-1}, essentially independent of salt concentration.

Apparent binding constants for Orn_n–KSCN, calculated at $\alpha = 0$ according to eq 12, using the above value of $K_0{}^b$ and the w values of Figure 1, are plotted as a function of C_s in Figure 3. Our previous work[12] and the presently obtained ORD data for Orn_n (*cf.* Figure 4) have indicated that Orn_n is essentially unable to assume the helical conformation even in KSN solution. The fractional helical content (Figure 4) is, in fact, zero up to $C_s \sim 1.1$ M and is only about 0.1 at $C_s = 2$ M. Accordingly, the lower curve in Figure 3 can be regarded as a good approximation for the binding of KSCN to randomly coiled Orn_n and to the (hypothetical) randomly coiled Lys_n. The upper binding curve in Figure 3 was calculated using the above indicated value of $K^b{}_0$ (0.23) and the w values deduced from Figure 1 for helical Lys_n. Accordingly, the curve refers to the binding of KSCN to fully helical Lys_n. It is seen that $K^b{}_{app}$ for the helical form is consistently greater than that for the random coiled form. This is a consequence of the greater contribution of the w parameter of the helical form. The $K^b{}_{app}$ values determined for the two forms converge to a common value (= $K^b{}_0$) on increasing C_s. It is evident that the largest effect of C_s is observed when $C_s < 0.5 M$.[23]

Using the values of $K^b{}_{app}$ reported in Figure 3, the W_{el} terms (*cf.* eq 13) for both helical and random-coiled form can be determined as a function of KSCN concentration. The results are collected in Table I. Also included in Table I are the values of W_{el} calculated in the absence of binding. The latter data appear in good agreement with a recent theoretical evaluation.[24] The examination of W_{el} data for KSCN and KCl reveals the large binding contribution to the decrease of W_{el} with increasing C_s.

The W_{el} values allow determination of the free-energy change accompanying the transformation according to eq 14. From the latter, the fractional helical content was determined using eq 15. The results of the calculation are represented by the solid line in Figure 4 and are compared
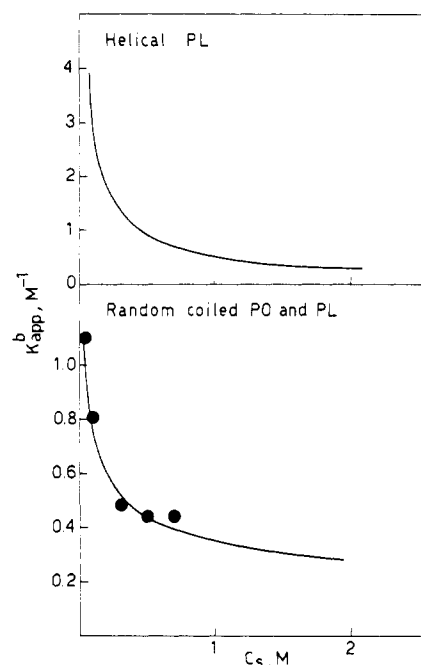

Figure 3. Apparent binding constants of SCN^- to the pure random-coiled form of Lys_n and Orn_n, and to the pure helical form of Lys_n, plotted as a function of KSCN concentration: $\alpha = 0$; $T = 25°$. The solid line represents potentiometric data ($K^b{}_0 = 0.23$). Black point obtained from calorimetric data.
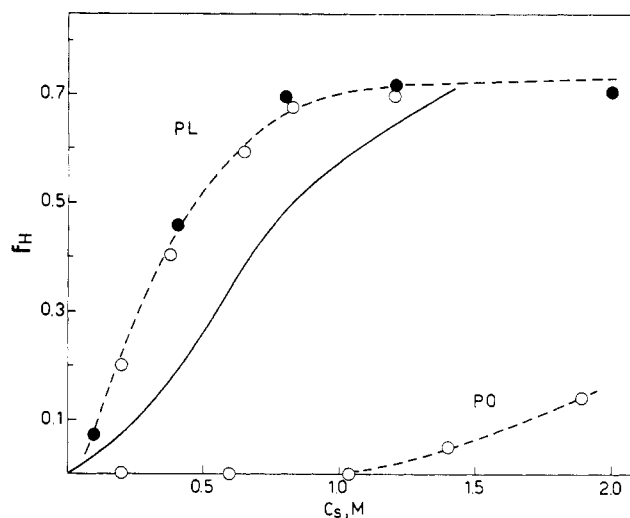

Figure 4. Fraction of residues in the helical conformation for Lys_n and Orn_n as a function of KSCN concentration ($\alpha = 0$, pH 3, $T = 25°$). Open points: results of ORD determination of this work. Black point: results of ORD determination of previous work.[6] Solid line: calculated using eq 15, along with eq 14, and $K^b{}_0 = 0.23$; $\sigma = 5 \times 10^{-3}$.

with the direct determination of helical content by ORD for Lys_n in KSCN solutions at $\alpha = 0$. The agreement between the experimental and the calculated data is regarded as a satisfactory one. Possible causes of discrepancy between the solid curve and the experimental points in Figure 4 may be related to uncertainties in the theoretical calculation which are discussed under Concluding Remarks. We note that the ORD data indicate a 70% limit to the helical content which Lys_n can assume in KSCN solutions. Viscosity data[6] are also in line with an incomplete conversion to the helical form. The origin of this effect is not clear. A competition between binding and screening effects at high C_s, and binding of SCN^- to the peptide bond may be involved.

Table I
Electrostatic Free Energies per Residue for the Pure Helical and Random Coiled Conformations of Lys_n and Orn_n in KSCN and KCl Solutions; $T = 25°$

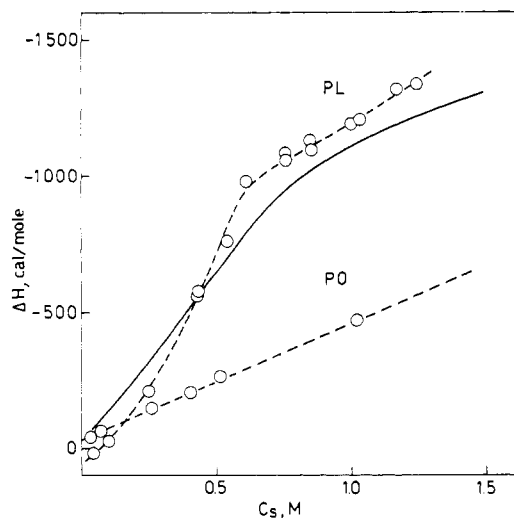| C_s (mol/l.)	Binding (KSCN), $\alpha = 0$, $K^b_0 = 0.23$			No Binding (KCl), $\alpha = 0$		
	W_{el}^H (cal/mol)	W_{el}^{RC} (cal/mol)	ΔW_{el} (cal/mol)	W_{el}^H (cal/mol)	W_{el}^{RC} (cal/mol)	ΔW_{el} (cal/mol)
0.05	752.0	388.1	363.9	1339	421.5	917.5
0.10	599.4	309.4	290.0	1108	353.5	754.5
0.30	373.8	196.6	177.2	741.0	258.3	482.7
0.50	274.4	145.0	129.4	577.8	210.7	367.1
0.70	214.4	121.8	92.6	463.9	191.0	272.9
1.00	152.6	84.2	68.4	341.0	150.0	191.0

Figure 5. Corrected heat of reaction for the systems Lys_n-KSCN and Orn_n-KSCN at 25°, pH 3. The solid line was calculated according to eq 22 (*cf.* text).

Calorimetry. The heat of reaction is measured when a polymer solution is mixed with a salt solution. Before the proper ΔH is obtained, correction for the heats of dilution of salt and of the polymer have to be made. The former is automatically accomplished, by electrical compensation, and the latter can be independently measured. At the polymer concentration used (~ 1 g/100 ml) the correction for the dilution of the polymer is about -45 cal/mol. An additional correction appears to be necessary in order to obtain the ΔH value which includes only the heat of binding and of helix $\rightarrow$ coil transition. In fact, for the systems Orn_n + KBr (pH 3), when neither binding nor transition occur, we measured a heat absorption of about $+85$ cal/mol (corrected for polymer dilution), essentially independent of salt concentration. This heat is associated with nonspecific interactions between KBr and the polymeric substrate. Accordingly, we have subtracted the latter value from the heat determined for the Orn_n-KSCN and the Lys_n-KSCN systems. The final ΔH values obtained are plotted in Figure 5. The error affecting ΔH is within 2–5%.

For both systems investigated there is an evolution of heat. However, while the heat evolved increases slowly with increasing C_s for Orn_n, in the case of Lys_n the heat evolved rises sharply in the same range of KSCN molarity in which the coil $\rightarrow$ helix transformation is revealed by ORD data (*cf.* Figure 4). Since for Orn_n no helix formation occurs, the corresponding ΔH can be used for calculating binding constants (reaction 2) according to the method based on eq 21. The correction term $\bar{H}^s - \bar{H}_0^s$ (*cf.* eq 20) was evaluated by straightforward application of Gibbs–Duhem relationship to experimental data of the heat of dilution of KSCN. The correction amounted to ~ 230 cal/mol at $C_s = 1$ M and was ~ 70 cal/mol at $C_s =$

0.4 M. The value of $\Delta H°°$ (determined by one point fit of K^b_{app} obtained from titration data at $C_s = 0.5$) was -1800 cal/mol. Using the latter $\Delta H°°$ value, equilibrium constants K^b_{app} from calorimetric data were calculated for the Orn_n-KSCN system. The results are plotted in Figure 3 along with the corresponding potentiometric determination. The agreement in the dependence of K^b_{app} from C_s obtained with the two methods is quite good.

Using the calculated variation of helical fraction with KSCN concentration (solid line in Figure 4), along with the corresponding γ_{RC} and γ_H values calculated from the binding constants, the experimental variation of ΔH with C_s can be predicted on the basis of eq 22 (*cf.* Methods). The solid line included in Figure 5 represents the result of this calculation. The agreement between the direct experimental results and the calculated ones is gratifying.

Concluding Remarks

A satisfactory agreement has been found between the experimental data and the calculations based on SCN^- binding. The occurrence of a conformational transition between 0 and 1 M KSCN is correctly predicted. The comparison between the experimental and theoretical helix content at any salt concentration is however to some extent dependent on the choice of the helix initiation parameter σ which is affected by an uncertainty of one order of magnitude.[16-19] Further, owing to the small ΔpH associated with binding equilibrium, the value of the intrinsic binding constant K^b_0 is obtained within $\pm 20\%$. By introducing in our relationships $K^b_0 = 0.5$ and $\sigma = 5 \times 10^{-3}$ a complete agreement with the experiment could be obtained.

In spite of limitations associated to the exact values of K^b_0 and σ, the close evaluation of the salt molarity at which the free-energy difference between the charged conformed becomes zero, and the correct prediction of the dependence of the calorimetric binding constants on C_s, strongly support the validity of the model based on the separate contributions of binding and electrostatic effects to the electrostatic free energy of polyelectrolytes in salt solutions. Specific ion effects are also known to occur with polar (uncharged) solutes and isoelectric proteins in aqueous solutions.[5] For instance, the denaturation temperature of isoelectric collagen in water is greatly depressed by KSCN, while it is essentially unaffected by KCl. These results have been quantitatively explained in terms of binding and nonspecific effects.[11] The ability of some ionic species to strongly interact with polar substrates is also manifested in the absence of water. For instance, a large depression of the melting temperature of polycaproamide is observed when small amounts of LiCl are mixed with the polymer.[25] Thus, the binding model which has been considered in this paper is coherent with a general interpretation of specific salt effects for a variety of systems.

Some comments about the assumptions made in connection with our method are in order. In line with existing

formalisms for the electrostatic effect,[1] we have considered the slope of pK_{app} *vs.* Z plots to be dependent only upon ionic strength and conformation. Yet, in previous work,[7] the slope of a pK_{app} *vs.* α plot obtained for helical Lys_n in KSCN solution was found to be significantly smaller than the slope obtained at the same concentration of KCl. In fact, from the observed decrease of slope, a binding constant in the range of 1–10 M^{-1} (in line with the present results) was estimated. The dependence of the slope on salt type is, however, an apparent effect due to the incorrect substitution of the charge Z with the degree of dissociation when binding occurs. If the proper substitution involving the term $1 - \gamma$ is made (according to eq 9), it can be verified that the slopes of pK_{app} *vs.* Z plots do no longer depend upon salt type. A small effect of salt type on K_0 was also observed.[7] However this effect may, again, be related to the inappropriate substitution of Z with the degree of dissociation when binding occurs.

A second assumption concerns the role of salts on the intrinsic binding constants. According to existing formalism, K_0 should depend upon C_s. The effect, which was discussed and quantitatively determined for Lys_n in KSCN and KCl solutions,[7] could cause about a 3% alteration in the values of pK_0 when C_s is changed from ~0.1 to ~2 M. Any alteration of the value of $K^b{}_0$ with C_s would be of a comparable order of magnitude. The latter would introduce only a negligible indetermination in our calculations. For instance, a change of $K^b{}_0$ from 0.28 to 0.18, which is of one order of magnitude larger than expected, would cause the equilibrium helix content to decrease from 30 to ~20% at 0.5 M salt. The assumption of equal values of $K^b{}_0$ for Orn_n and Lys_n may be justified on the basis of the close chemical structure. Titration data for Lys_n[7] and Orn_n[12] reveal, in fact, only a small difference (~3%) between K_0 values for the two polymers. Finally, the assumption that the same value of $K^b{}_0$ applies to the helical and random-coiled conformations seems largely justified by an appreciation of the physical significance of $K^b{}_0$. The invariance of K_0 with polymer conformation adequately supports the latter assumption.

Experimental Section

Materials. Poly(L-ornithine hydrobromide) (lot O-30) and poly(L-lysine hydrobromide) (lot L-115) were obtained from Pilot Chemicals Div., Boston, Mass., and had a reported molecular weight of 80,000 and 150,000, respectively. All salts used were of reagent grade.

Potentiometric Titrations. The titrations were carried out at 25° with a Radiometer type 4 pH meter, as previously described.[7,12,26] Experiments were performed on solutions containing 37 mg of Orn_n in 10 ml of solution, corresponding to 0.019 molar residue.

Calorimetry. Calorimetric measurements were performed at 25° with an LKB 10700-2 flow calorimeter,[27] calibrated both electrically and by heat neutralization of HCl with excess NaOH.[28] Flow calibrations of the peristaltic pumps (I and II) were performed. The base line of each experiment was obtained by pumping (I) HBr in water pH 3, and (II) KSCN solutions. Heat of binding was measured pumping (I) Orn_n or Lys_n solutions.

Optical rotatory disperison curves for Orn_n and Lys_n were obtained with a Jasco ORD/UV 5 recording spectropolarimeter (cell path lengths from 5 to 10 mm). The treatment of data is similar to that described before.[7,12] Owing to the absorption of KSCN in the far-ultraviolet region, the per cent helix was determined, in the wavelength range between 350 and 600 mμ, from the b_0 parameter of the Moffitt–Yang equation.[29]

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