temperature determined by our probe (relaxation frequency 10^{11} Hz) agrees well with those determined by slow thermal relaxation techniques. The answer to this question lies in the fact that molecular and submolecular motions to which the probe is sensitive contribute to the macroscopic behavior of the polymer. In addition, the steady-state fluorescence measurements average out the macrodynamic behavior of the polymer chains.

Basically, two principal findings have emerged. One is the dependence of the fluorescence yield of molecular rotors on the tacticity of the polymer matrix. The other is the sensitivity of the fluorescence of molecular rotors to dynamic changes in the conformation of polymers over wide temperature ranges. One must remember that different relaxation techniques applied to polymeric materials respond with different sensitivity to the various types of molecular motion. The fluorescence probe method complements many of the well-established methods of polymer material research.

Acknowledgment. We are in debt to Drs. S. Sundararajan and T. Smith for many useful discussions.

Registry No. 1, 58293-56-4; atactic PMMA, 9011-14-7; isotactic PMMA, 25188-98-1.

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Effect of Different Shielding Groups on the Polyelectrolyte Behavior of Polyamines

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ABSTRACT: The stepwise protonation of three polymeric amines, containing a different shielding group between the amine moieties of the repeating units, has been studied from a thermodynamic standpoint. This was done for comparison purposes with structurally related poly(amido amines) whose monomeric units had been previously found to behave independently toward protonation. This study was performed by potentiometric and calorimetric techniques, and specific methods for the treatment of either "sharp" or "apparent" thermodynamic functions in polyelectrolyte having more than one basic group in the repeating unit have been developed.

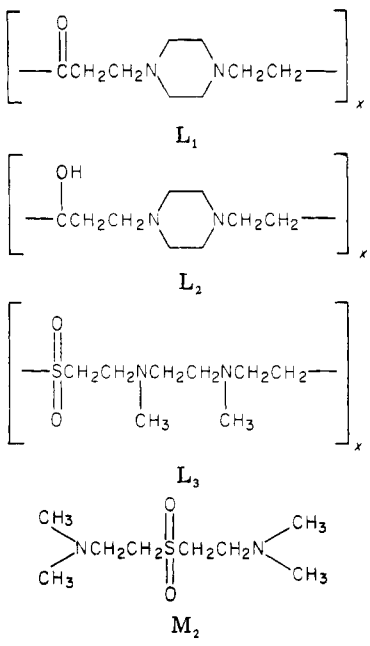
Introduction

The thermodynamic functions for the protonation of polymeric bases usually depend on the degree of protonation of the macromolecule (i.e., on its total charge).[1] Up to now, in only two classes of polymers, poly(amido amines)[2] and poly(macrocycles),[3] have the monomeric units behaved independently toward protonation, thus giving rise to "sharp" thermodynamic functions.[4] ("Sharp" means that the thermodynamic functions do not depend on the degree of protonation (α) of the whole macromolecule, and "apparent" means that they do.)

In order to gain further insight into the structural reasons of this unusual trend, we have modified the monomeric units of poly(amido amines) in several respects[2,5-8]

0024-9297/83/2216-0456$01.50/0

Chart I



but never succeeded in obtaining a "typical" polyelectrolyte behavior. This behavior has been attributed to the fact that the tertiary amino groups of each repeating unit are separated from those of neighboring units by rigid bisamidic structures, which act as effective shielding groups.[2] Therefore, we thought it interesting to study the effect of different shielding groups on the polyelectrolyte behavior of polymeric amines structurally related to poly(amido amines). For this purpose, we have studied from a general thermodynamic standpoint the stepwise protonation of three polymers, L_1, L_2, and L_3, whose structures are reported in Chart I.

In order to better ascertain the effect of macromolecularity, we have also synthethized and studied a low molecular weight model, M_2 (Chart I).

This study concerns polyelectrolytes having two basic groups in the repeating unit and was performed by potentiometric and calorimetric techniques. It required the development of specific methods for the treatment of either sharp or apparent thermodynamic functions. The strategy we followed to deal with these problems and the computer programs purposely written for the numerical treatment of experimental data will be also described. On the grounds of the results obtained in this and previous studies[2,4,7,9] some general conclusions will be drawn about the structural reasons determining the presence of sharp or apparent thermodynamic functions in the protonation of a given polymeric amine.

Experimental Section

Materials. The synthesis of polymers L_1[10] and L_2[11] has been previously described. The polymer L_3 was synthesized by polyaddition of 1.00 g of divinyl sulfone (Aldrich) to 0.740 g of *N,N'*-dimethylethylenediamine in a 1:1 molar ratio according to the following scheme:

$$CH_2CHSO_2CHCH_2 + CH_3HNCH_2CH_2NHCH_3 \xrightarrow[7\ \text{days}]{H_2O,\ \text{room temp}} L_3$$

At the end of the reaction the mixture was evaporated to dryness in vacuo, and the polymer was purified by dissolving in chloroform and reprecipitating with ether; the product was then dried under high vacuum to constant weight (yield 85%). The intrinsic viscosity (in chloroform at 30 °C) was 0.19 dL/g. Anal. Calcd for $C_8H_{18}N_2O_2S$: C, 46.6; H, 8.79; N, 13.59; S, 15.58. Found: C, 45.9; H, 8.88; N, 13.46; S, 15.34. Model M_2 was obtained in similar way starting from 1.00 g (8.43×10^{-2} mol) of divinyl sulfone and 0.775 g (1.69×10^{-1} mol) of dimethylamine in a 1:2 molar ratio, according to the following scheme:

$$CH_2CHSO_2CHCH_2 + 2HN(CH_3)_2 \xrightarrow[5\ \text{days}]{H_2O,\ \text{room temp}} M_2$$

At the end of the reaction the mixture was evaporated to dryness in vacuo, and the model was obtained by dissolving the product in ethanol and reprecipitating with HCl as the dihydrochloride, mp 254 °C (yield 95%). Anal. Calcd for $C_8H_{20}N_2O_2S{\cdot}2HCl$: Cl, 25.2. Found: Cl, 25.2. All the compounds are soluble in water except for L_1, which is soluble only in acid solution and reprecipitates in weakly basic solution.

Other Reagents. A CO_2-free NaOH solution was prepared, stored, and standardized as described elsewhere.[12] Stock solutions of 0.1 M NaCl were prepared from sodium chloride (Erba, ACS grade) and used without further purification as the ionic medium for potentiometric and calorimetric measurements.

Electromotive Force Measurements. All potentiometric measurements were carried out at 25 °C in 0.1 M NaCl.

Potentiometric titrations were performed by using a digital PHM-84 Radiometer potentiometer, an Ag–AgCl reference electrode, an Orion 91-01-00 glass electrode, and a salt bridge containing 0.1 M NaCl solution. All the titration operations (the amount of the titrant liquid to add step by step, the total amount of titrant to add, the number of millivolt voltage readings at each step) were automatically governed by a Rockwell AIM 65 minicomputer. The obtained data (the milliliters of titrant added at each step and the corresponding average of output voltages) were automatically printed and stored on a floppy disk for further processing.

The titrant vessel was thermostated at 25.0 ± 0.1 °C. A stream of nitrogen presaturated with water vapor by bubbling through a 0.1 M NaCl solution was passed over the surface of the solution to be titrated. For the titrations, the NaOH or HCl solutions were dispensed from a Metrohm 655 Dosimat piston buret governed by the minicomputer. Buret and $E°$ calibrations were performed before and after each titration. The concentration of hydrogen ion was calculated from the emf values (in millivolts) by means of the formula

$$[H^+] = \exp(E - E°)/25.693$$

The effects due to the ligand junction potential and to the potential drift of the electrode during the measurements were shown to be negligible for the range of pH under consideration; the maximum values of these effects were of the same order as the experimental error of the potentiometer.

No attempt was made to correct the data to zero ionic strength or to apply activity coefficient corrections since under the conditions used the bases do not contribute very much to the ionic strength, and ionic strength is quite high. The basicity constants were first computed for each titration by the APPARQ program described in the next section and operating on the Rockwell minicomputer. Only sharp basicity constants were then refined by using the program MINIQUAD 76 A[12] and by utilizing all the points of several titration curves. The experimental details are reported in Table I.

Calorimetric Measurements. The calorimetric titrations were typically done by using a Tronac Model 1250 calorimeter operating in the isothermal mode, with a 25-mL stainless steel reaction vessel. The reaction vessel was charged with 25 mL of polymer (or model) solution in 0.1 M NaCl (Table I), and then 2.50 mL of titrant was added at a constant rate after thermal equilibration. All the titrations were done at 25 ± 0.1 °C. At least two complete titrations were carried out for each base. All operations were governed by a North Star CCP 930 computer, connected to the instrument by use of the program ISOTHERM delivered by Tronac Inc. The enthalpy values were computed by the FITH program described in the next section. Some experimental details are given in Table II.

Method of Calculation

Basicity Constants. The program APPARK (written in Basic) used directly utilizes as input the milliliters of titrant added at each step and the corresponding output

Table I. Experimental Details of the Potentiometric Measurements at 25 °C in 0.1 M NaCl

compd	reaction	α range	pH range	log K°	n	Δn(99%)	R	T_L	C_{H^+}	points (n)
L_1	$L + H^+ \rightleftharpoons LH^+$	0.49–0.73	9.20–7.98	9.11 (4)	0.96 (1)	1.77	0.992	0.1736	0.1052	6
		0.50–0.80	9.09–7.31	9.084 (5)	3.03 (18)	0.55	0.9998	0.1730	0.1043	26
		0.55–0.79	8.74–7.46	9.07 (1)	2.94 (35)	1.04	0.9993	0.1742	0.1043	10
	av			9.088 (0.02)	2.64 (51)	1.16				
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	0.15–0.63	5.33–3.38	3.869 (7)	2.22 (15)	0.59	0.998	0.1736	0.1052	13
		0.11–0.70	5.32–2.93	3.614 (4)	2.00 (4)	0.12	0.998	0.1730	0.1043	109
		0.16–0.67	5.12–3.03	3.634 (3)	2.13 (5)	0.14	0.9998	0.1742	0.1043	60
	av			3.706 (5)	2.12 (8)	0.28				
	$\log K_1^a = 9.09 + 1.64 \log [(1-\alpha)/\alpha]$; $\log K_2^a = 3.706 + 1.12 \log [(1-\alpha)/\alpha]$									
L_2	$L + H^+ \rightleftharpoons LH^+$	0.11–0.74	8.80–7.02	7.636 (4)	1.35 (2)	0.05	0.999	0.0816	0.0883	16
	av			7.636 (4)	1.35 (2)	0.05				
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	0.13–0.71	4.28–2.80	3.316 (7)	1.25 (3)	0.09	0.998	0.2208	0.1111	17
		0.25–0.51	4.04–3.36	3.399 (3)	1.37 (2)	0.11	0.9999	0.2208	−0.1638	5
		0.14–0.64	4.24–2.91	3.290 (5)	1.30 (3)	0.08	0.998	0.08164	0.0883	23
	av			3.335 (5)	1.31 (3)	0.09				
	$\log K_1^a = 7.64 + 0.35 \log [(1-\alpha)/\alpha]$ $\log K_1^b = 7.72$ (1); $\log K_2^a = 3.335 + 0.31 \log [(1-\alpha)/\alpha]$ $\log K_2^b = 3.34$ (3)									
L_3	$L + H^+ \rightleftharpoons LH^+$	0.14–0.74	5.66–7.38	6.277 (3)	1.36 (1)	0.04	0.9999	0.1607	0.1086	11
		0.15–0.79	5.67–7.30	6.306 (2)	1.28 (3)	0.11	0.9991	0.2145	0.1092	22
		0.11–0.77	5.57–7.67	6.278 (5)	1.43 (2)	0.06	0.9992	0.3185	0.1115	30
		0.19–0.78	5.62–7.30	6.247 (3)	1.36 (2)	0.05	0.9998	0.2145	0.1092	13
	av			6.277 (3)	1.36 (2)	0.07				
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	0.12–0.58	2.51–3.41	2.47 (3)	0.97 (6)	0.15	0.9798	0.2145	0.1092	31
		0.10–0.43	2.48–3.21	2.33 (3)	0.95 (5)	0.14	0.9793	0.3185	0.1115	16
		0.11–0.28	2.93–3.35	2.58 (1)	0.85 (2)	0.10	0.9984	0.1607	0.1086	10
	av			2.46 (2)	0.92 (4)	0.13				
	$\log K_1^a = 6.277 + 0.36 \log [(1-\alpha)/\alpha]$; $\log K_2 = 2.46$									
M_2	$L + H^+ \rightleftharpoons LH^+$	2.30–8.32						0.3545	0.1172	90
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	2.28–8.16						0.2534	0.1172	150
	$\log K_1^b = 7.230$ (4); $\log K_2^b = 6.344$ (8)									

[a] $\log K_i = \log K^\circ + (n-1) \log [(1-\alpha)/\alpha]$ (eq 6a). [b] Obtained by the MINIQUAD 76 A program with 254 points (L_2) or 240 points (M_2).

Table II. Experimental Details of the Calorimetric Measurements at 25 °C in 0.1 M NaCl

compd	reaction	log K	α range	pH range	$-\Delta H^\circ$, kcal mol^{-1}	C_{H^+}, M	buret, mL min^{-1}	T_L, mmol	points (n)
L_1	$L + H^+ \rightleftharpoons LH^+$	8.487	0.67–0.74	8.25–8.11	6.35 (4)	0.1052	0.083307	0.1724	
		8.229	0.74–0.80	7.95–7.80	5.14 (5)				
		7.961	0.80–0.87	7.59–7.39	5.35 (9)				
		7.525	0.87–0.93	7.12–6.80	5.81 (5)				
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	4.512	0.13–0.19	5.19–4.98	5.69 (15)				
		4.380	0.17–0.23	5.02–4.85	5.11 (17)				
		4.215	0.23–0.29	4.77–4.63	4.39 (10)				
		4.023	0.29–0.35	4.53–4.40	3.96 (6)				
		3.944	0.35–0.41	4.30–4.19	3.87 (9)				
		3.823	0.41–0.47	4.06–3.95	3.78 (9)				
		3.725	0.46–0.52	3.84–3.74	3.83 (10)	0.1052	0.083307	0.1724	
		3.628	0.51–0.57	3.63–3.54	3.62 (11)				
		3.529	0.56–0.61	3.44–3.35	3.58 (9)				
		3.447	0.60–0.65	3.30–3.22	3.52 (9)				
		3.383	0.64–0.68	3.18–3.10	3.48 (9)				
		3.317	0.67–0.70	3.06–2.99	3.60 (10)				
		3.270	0.69–0.72	2.95–2.88	3.57 (12)				

L_2	$\log K_1$	9.09[a]	% species	7.95–2.99	6.1 (0.9)			25
	$\log K_2$	3.706[a]	95.4–17.3	7.95–2.99	4.9 (1.9)			
	$L + H^+ \rightleftharpoons LH^+$	7.64[a]	82.7–0	7.69–3.44	5.77 (9)	0.0882	0.08331	0.08517	19
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	3.34[a]	50.8–97.9	7.69–3.44	3.81 (15)				
			0–44.0	8.21–4.16	5.74 (4)	0.0882	0.08331	0.1010	25
L_3	$L + H^+ \rightleftharpoons LH^+$	7.64[a]	24.3–83.7	8.21–4.16	3.94 (15)				
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	3.34[a]	0–16.3	2.72–7.08	6.63 (7)	−0.1885	0.08331	0.238	22
		6.277[a]	64.3–13.4	2.72–7.08	2.05 (9)				
		2.46[a]	35.7–0	6.35–5.47	6.35 (4)	0.1043	0.08330	0.1591	18
		6.277[a]	45.5–86.2	6.71–5.59	6.48 (10)	0.1040	0.08330	0.2702	21
M_2	$L + H^+ \rightleftharpoons LH^+$	6.277[a]	29.4–82.7	9.6–1.89	6.80 (8)	0.2080	0.08330	0.03361	25
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	7.230	0.39–98.0	9.6–1.89	6.76 (14)				
		6.344	0–99.0						

[a] Values calculated at $\alpha = 0.5$ or obtained by the MINIQUAD 76A program.

voltages, together with the analytic data. Polyelectrolytes with more than one basic group in the monomeric unit can be analyzed in this way if the stepwise protonation constants K_i are different enough that each species LH_{i-1} (where L, in the case of a polymeric ligand, indicates the repeating unit) undergoes protonation in a separate pH range (i.e., the intrinsic protonation constants $K_i°$ differ by at least 2 orders of magnitude from $K_{i-1}°$). In such circumstances, the ith step can be considered independent from the others and corresponds to the protonation of a monoprotic base (automatically performed by the program) by subtracting $(i - 1)$ times the equivalent volume from the total volume of the added titrant. $\log K_i$ is then computed at each pH value by the well-known Henderson–Hasselbach equation:

$$\log K_i = \mathrm{pH} + \log [\alpha/(1 - \alpha)] \tag{1}$$

where α is the degree of ionization, i.e.

$$\alpha = \frac{[LH_i]}{[LH_i] + [LH_i - 1] + \ldots} \simeq \frac{[LH_i]}{C_L} \tag{2}$$

and C_L is the analytical concentration of the L species. The situation at each point of the titration is equivalent to a buffer solution of LH_i and LH_{i-1}, with analytical concentration C_i and C_{i-1}, respectively, given by

$$C_i = C_{H^+} \tag{3}$$

$$C_{i-1} = C_L - C_{H^+}$$

for titrations with acids and

$$C_i = C_L - C_{OH^-} \tag{3'}$$

$$C_{i-1} = C_{OH^-}$$

for titrations with bases; $[LH_i]$ at the equilibrium is then given by[14]

$$[LH_i] = C_H - [H^+] + [OH^-] \tag{4}$$

for titrations with acids and

$$LH_i = C_L - C_{OH^-} - [H^+] + [OH^-] \tag{4'}$$

for titrations with bases; both cases can be unified in the compact formula

$$[LH_i] = C_{H^+} - [H^+] + [OH^-] + C_L\delta_{OH} \tag{5}$$

by using $-C_{H^+} = C_{OH^-}$ and $\delta_{OH} = 1$ when the titrant is a base and $\delta_{OH} = 0$ when the titrant is an acid. The simplest way to apply the above treatment to "typical" polyelectrolytes (i.e., having apparent basicity constants) is to rewrite eq 1 in the form

$$\mathrm{pH} = \log K_i° + n \log [(1 - \alpha)/\alpha] \tag{6}$$

where $\log K_i° = \mathrm{pH}$ at $\alpha = 0.5$ and $n = 1$ in the case of sharp basicity constants.

Only values of α between 0.15 and 0.85 are taken into account by the program in order to minimize the effect of simultaneous protonation of bases other than the considered one. The linear regression fit of pH vs. $\log [(1 - \alpha)/\alpha]$ gives $\log K_i$ and n as the intercept and the slope, respectively. The standard deviations of the two parameters, the confidence limits of n $(n - \Delta n, n + \Delta n)$ with a probability of 99%, and the correlation coefficient R are also computed.[15] The quantity $|n - 1|$ defined by eq 6a (the difference between eq 1 and 6) then gives a measure of the deviation of the studied compound from the "normal" behavior of a small molecule (i.e., showing sharp basicity constants).

$$\log K_i = \log K_i° + (n - 1) \log [(1 - \alpha)/\alpha] \tag{6a}$$

The program APPARK has been tested with reference to the MINIQUAD 75 A[13] program by using several compounds having sharp basicity constants (see, for instance, the results for polymer L_2 reported in Table I).

Protonation Enthalpies. When the basicity constants are apparent, the protonation enthalpies have been obtained in a first instance by the program APPARQ operating on a North Star CCP 930 computer, which utilizes the heats of a whole calorimetric titration stored on a floppy disk by the program ISOTHERM. Also in this case (as in APPARK) the different protonation steps are treated independently. The amount of heat liberated (q_R) during the course of the protonation reaction in which n moles of product are produced is

$$q_R = -n\Delta H^\ominus$$

$\Delta H_i^\ominus$ is evaluated for each pair of points (p, q) near enough that the corresponding basicity constant remains nearly unchanged. The $\Delta H_i^\ominus$ values are obtained from

$$\Delta_{p,q} = \Delta_{dil} + \Delta_w + ([LH_i]_q - [LH_i]_p)\Delta H_i^\ominus \quad (7)$$

where $\Delta_{p,q}$ is the difference of the stored heats at point (p,q), Δ_{dil} is the corresponding difference of dilution heats, and Δ_w is the heat due to the formation of water. The pH and the $[LH_i]$ needed for the computation of $\Delta H_i^\ominus$ are computed by the program from the previously determined constants (see the section on basicity constants) for each point.

When two enthalpies do not change with pH, all the $\Delta H_i^\ominus$ values are refined by the program FITH, which takes into account all the equilibria at the same time.

Sharp protonation enthalpies have been obtained by the program FITH purposely written in Basic for the North Star CCP 930 computer connected to the Tronac calorimeter. The program utilizes the heats previously stored on a floppy disk by the program ISOTHERM. Then the difference $\Delta_{p,q}$ between the measured heats at points (p, q) is given by

$$\Delta_{p,q} = \Delta_{dil} + \Delta_w + \sum_{i=1}^{N_T}([LH_i]_q - [LH_i]_p)\Delta H_i^\ominus \quad (8)$$

with the same notation as in the preceding section. In the next step the point q becomes the first point of the pair (q, $q + 1$) and so on. The whole set of N_T equations (N_T being the number of different species) relative to the unknown $\Delta H_i^\ominus$ values for all N measured points ($N > N_T$) is solved by the least-squares method, which also gives the different standard deviations σ_i as the square roots of the diagonal elements of the variance–covariance matrix. The concentrations of the different species LH_i at each point were previously obtained by solving the mass balance equations for the ligand L and the proton H with the Newton–Raphson method[16] according to

$$C_L = [L] + \sum\beta_i[L][H]^i$$

and

$$C_{H^+} = [H] + \sum i\beta_i[L][H]^i \quad (9)$$

where the analytical concentrations C_L and C_{H^+} are computed by the program from the analytic data and the buret rate. The concentrations of all the other species at the equilibrium are then computed according to

$$[LH_i] = \beta_i[L][H]^i \quad (10)$$

Titrations with both acids and bases can be treated by the simple device of indicating the OH^- ion as $[H^+]^{-1}$ with formal analytical concentration $C_{H^+} = -C_{OH^-}$ and formation constant $\beta_{-1} = K_w$.

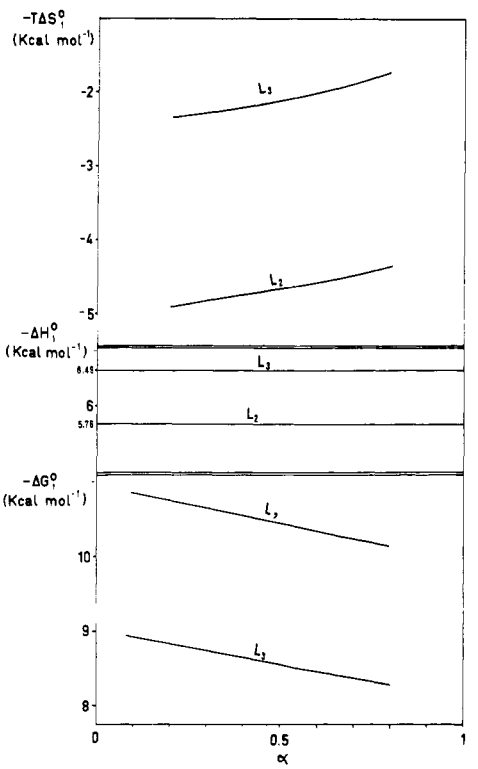


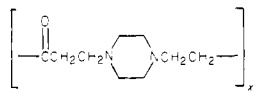
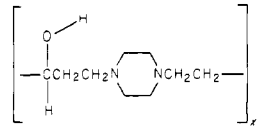
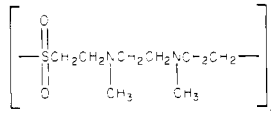
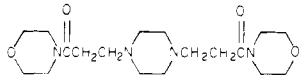
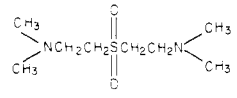
Figure 1. Thermodynamic parameters (kcal/mol) for protonation of L_2 and L_3 at 25 °C in 0.1 M NaCl ($\alpha = [LH^+]/C_L$).

The case of apparent basicity constants (but sharp protonation enthalpies) can be treated exactly in the same way changing point by point the values of β_i, according to the coefficients of the modified Henderson–Hasselbach equation previously obtained by program APPARK. In its present version the program FITH can deal with 30 points and 6 contemporaneous equilibria.

Results and Discussion

The basicity constants and the enthalpy values of all the polymers and models studied in the present work are reported in Table III, together with the corresponding $\Delta S^\ominus$ values obtained from $\Delta S^\ominus = (\Delta H^\ominus - \Delta G^\ominus)/T$. In the case of polymer L_1 the first basicity constant was calculated by using only a few points, owing to its limited water solubility when in the form of free base. For comparison purposes, the thermodynamic functions previously found for a related nonmacromolecular model M_1[17] are given in the same table. It can be seen that the three polymers behave in quite a different manner toward protonation, although the number of basicity constants is equal to the number of aminic nitrogen present in the repeating unit. In particular, both the basicity constants of L_1 are undoubtedly apparent while the basicity constants of L_2 and L_3 can be considered on the borderline between sharp and apparent, with the exception of the second basicity constant of L_3, which is undoubtedly sharp. This means that in all cases but one, the ($n - 1$) values in the modified Henderson–Hasselbach equation (6a) are higher than zero. In particular for L_1 they are higher than 1 while for L_2 and L_3 they approach zero. In fact, the approach of the incoming proton becomes more and more difficult as the ionization degree (α) of the whole macromolecule increases (Figures 1 and 2). As a general trend, the second basicity constants are, so to speak, more sharp than the first ones, as the values of ($n - 1$) are nearer to zero. In fact, as pointed above, the ($n - 1$) value for the second constant of L_3 is equal to zero within the experimental error. Such an effect can be explained by considering that the conformational

Table III
Thermodynamic Values of Protonation of Some Polymers with Different Shielding Groups and Their Corresponding Nonmacromolecular Models at 25 °C in 0.1 M NaCl

compd	reaction	log K_i[a]	$-\Delta H^{\ominus}$,[a] kcal mol^{-1}	$\Delta S^{\ominus}$,[a] cal K^{-1} mol^{-1}
 L_1	$L + H^+ \rightleftharpoons LH^+$	9.09 (2) + 1.64 (51) × log [(1 − α)/α]	≃6	
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	3.706 (5) + 1.12 (8) × log [(1 − α)/α]	3.8 (α = 0.5)	4.2 (α = 0.5)
 L_2	$L + H^+ \rightleftharpoons LH^+$	7.636 (4) + 0.35 (2) × log [(1 − α)/α]	5.76 (6)	15.6 (α = 0.5)
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	3.335 (5) + 0.31 (3) × log [(1 − α)/α]	3.88 (15)	2.2 (α = 0.5)
 L_3	$L + H^+ \rightleftharpoons LH^+$	6.277 (3) + 0.36 (2) × log [(1 − α)/α]	6.49 (7)	6.95 (α = 0.5)
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	2.46 (2)	2.06 (9)	4.4 (3)
 M_1[b]	$L + H^+ \rightleftharpoons LH^+$	7.124 (2)	5.50 (10)	14.1 (3)
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	3.289 (8)	4.00 (10)	1.6 (4)
 M_2	$L + H^+ \rightleftharpoons LH^+$	7.230 (4)	6.80 (8)	10.3 (3)
	$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	6.344 (8)	6.75 (14)	6.3 (5)

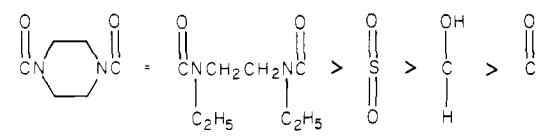
[a] $\log K_i = \log K_i^{\circ} + (n-1)\log[(1-\alpha)/\alpha]$. The values in parentheses are standard deviations. [b] Reference 17.

freedom of the macromolecule decreases upon protonation, so that during the second protonation step the different monomeric units can interact with each other less effectively than in the first one. This process occurs gradually, and therefore, a conformational change at a specific α value can be excluded, as previously seen, for instance, in the case of poly(iminoethylene) (PEI).[18] It may be further observed that the "polyelectrolyte" behavior of polymers L_1 and L_3 is mainly due to the entropy term. The protonation enthalpies of L_2 and L_3 are undoubtedly sharp (Table II) while the $\Delta H_2^{\ominus}$ of L_1 is constant in a significant pH range (Figure 1). It is also interesting to note that other polymeric amines behave differently. For instance, poly(vinylamine)[18] shows a different behavior toward protonation: the protonation enthalpy remains almost constant in a wide range of α (0–0.5) and then sharply decreases by further increasing α. This behavior was explained by assuming that a stiff structure occurs at the intermediate pH owing to interactions between neighboring ammonium and amine groups via H bonding.[19]

The thermodynamic functions of the polymers L_1 and L_2 are similar to each other with the exception of the first basicity constant of L_1 and not very different from those obtained for both the analogous nonmacromolecular compound M_1 (see Table II) and the corresponding polymer.[6]

Furthermore, the thermodynamic data of both model M_2 and polymer L_3 show that the SO_2 moiety is a very efficient shielding group, as the two basicity constants (corrected for the statistical effect) and protonation enthalpies of M_2 are equal, and the second basicity constant and protonation enthalpy of L_3 are sharp.

On the grounds of the results obtained in this as well as in previous studies[2,4-9,17] we can conclude that the shielding effectiveness of different groups decreases in the order



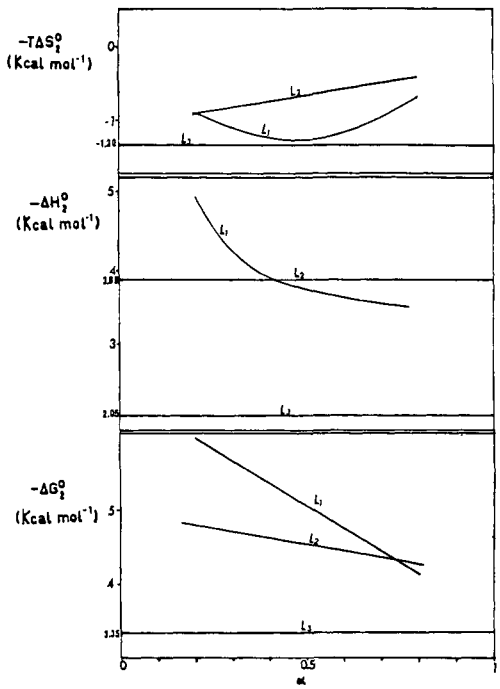


Figure 2. Thermodynamic parameters (kcal/mol) for protonation of L_1, L_2, and L_3 at 25 °C in 0.1 M NaCl ($\alpha = [LH_2^{2+}]/C_L$).

The effectiveness of the first two groups is due to their size and stiffness, while the effectiveness of SO_2 is probably related to its quite large size. Finally, the difference found on this respect between alcoholic and carbonylic functions might be due to their different degrees of solvation as also revealed by the much higher water solubility of L_2. In fact, a strongly coordinated shell of water molecules exists around the OH group,[20] and this is probably responsible for its relatively high shielding ability.

Acknowledgment. We thank M. Porcù for technical assistance and the Italian CNR for partial support.

Registry No. L_1 copolymer, 79536-27-9; L_3 copolymer, 84280-01-3; M_2, 59406-68-7; L_1 SRU, 79536-61-1; L_3 SRU, 84280-00-2; divinylsulfone, 77-77-0; dimethylamine, 124-40-3.

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α-Helix-to-Random-Coil Transition of Two-Chain, Coiled Coils. Theory and Experiments for Thermal Denaturation of α-Tropomyosin at Acidic pH

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Received July 9, 1982

ABSTRACT: New data are presented for the α-helix content of non-cross-linked and cross-linked α-tropomyosin at acidic pH as a function of temperature (20–80 °C) over a 1000-fold range of protein concentration. Experimental thermal denaturation curves at acidic pH are independent of concentration for both non-cross-linked and cross-linked samples, unlike the case of neutral pH, where it was previously shown that the non-cross-linked protein data are concentration dependent. The α helix in the non-cross-linked case is more stable than in the cross-linked case at low pH and both are considerably more stable than at near-neutral pH. The realized theory developed earlier is applied to these new data. The theory shows that even noninteracting helices are considerably more stable at acidic pH because of substantial increases in the helix propagation parameter $s(T)$ for aspartic and glutamic acids over those for aspartate and glutamate, respectively. The theory for interacting helices fits the data well with an interaction parameter $w(T)$ that is only slightly larger than that required to fit the data at neutral pH. Thus, most of the enhanced tropomyosin helix stability in acid has its origin in enhanced short-range interactions [$s(T)$] for its acidic residues. The insensitivity of $w(T)$ to pH implies that the salt linkages, which cannot be present in acid, do not contribute appreciably to the helix–helix interaction even when present at neutral pH. The theory also reveals that dissociation to monomers is entirely negligible at low pH over the full range of conditions employed, explaining the lack of dependence of the observed helix content on protein concentration. The reduced helix content of the actual cross-linked dimers, as compared with both theory and experiment for non-cross-linked dimers, confirms an earlier conclusion (drawn less directly from the data on the partially dissociated system at neutral pH) that the actual disulfide cross-link has a locally adverse effect on the helix-stabilizing interactions.

I. Introduction

In preceding papers in this series a statistical mechanical theory is described that treats the α-helix-to-random-coil transition of two-chain, coiled coils[1] and the theory is applied to extant experiments on a synthetic model polypeptide and to new experiments on α-tropomyosin.[2,3] The theory is implemented by use of the extensive compilation by Scheraga et al. of the helix initiation (σ) and propagation [$s(T)$] parameters that characterize the short-range interactions for each type of amino acid residue.[4] Fitting the theory to experimental data for the fraction helix (Φ_h) vs. temperature determines a temperature-dependent parameter $w(T)$ that characterizes the helix–helix interaction in the coiled coil. In this manner, the theory makes possible a partial dissection into its constituent parts of the free energy responsible for the overall coiled coil stability.

Tropomyosin is an important muscle protein whose native molecular structure has for some time been known to be that of a double α-helical, coiled coil.[4] This molecular architecture is certainly a result of the remarkable pseu-

0024-9297/83/2216-0462$01.50/0