

Ionization of Poly(ethylenimine) and Poly(allylamine) at Various pH's

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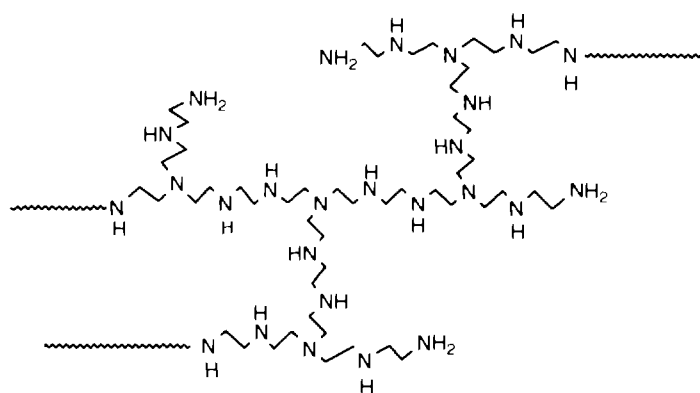
Titration curves were obtained for poly(ethylenimine) (PEI), lauryl-containing PEI, and poly(allylamine) (PAA). Since each polymer contains more than 1000 amino groups, the pK_a values of individual amino groups are needed for rigorous analysis of the titration curves. Instead, the titration curves are empirically analyzed by defining the deprotonation quotient Q as the ratio of the fraction of unprotonated amino groups and the fraction of protonated ones and by assuming that $\log Q$ is linearly related to pH. Very low basicity of the amines at acidic pH's is attributed to the electrostatic suppression of the protonation of amines in cationic microenvironments. Introduction of lauryl groups to PEI lowers basicity of the amines further, which agrees with unfavorable protonation of amines in hydrophobic domains. PAA, a linear polyamine, manifests basicity comparable to or smaller than those of PEI. This is taken to suggest that the hydrophilic portions and the hydrophobic portions of PAA form separate clusters. Implications of the results to various biomimetic functional molecules built on PEI or PAA derivatives are discussed. © 1994 Academic Press, Inc.

INTRODUCTION

Poly(ethylenimine) (PEI) has been intensively exploited as backbones of artificial enzymes (1–10). PEI contains ethylamine as the repeating unit and, thus, is highly soluble in water. The molecular weight of the PEI used in construction of artificial enzymes is ca. 60,000, corresponding to 1400 monomer residues. Among the 1400 nitrogens present in PEI, 25, 50, and 25% are primary, secondary, and tertiary amines, respectively. The tertiary amines represent branching points on the polymer backbone, and PEI is a highly branched and globular polymer. The amino nitrogens of PEI can be easily modified by alkylation, acylation, or imine formation, allowing incorporation of various catalytic elements to the backbone (Scheme 1).

In devising artificial enzymes, it is necessary to incorporate catalytic principles exploited by enzymes in relatively small spaces on synthetic molecules. Furthermore, in order to achieve cooperative catalytic participation of several functional groups in the chemical transformation of the bound substrate, fine alignment of convergent catalytic groups is needed. Creation of such active sites on molecular skeletons provided by relatively small synthetic hosts would not be easy. For this

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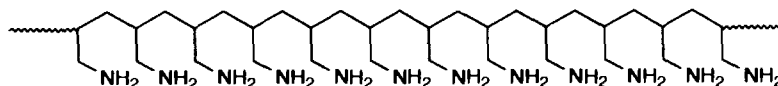
**PEI**

SCHEME 1

reason, various structural features have been added to PEI in attempts to design highly effective biomimetic catalysts.

PEI has been also used as a macromolecular spacer to crosslink preorganized metal complexes to design biomimetic metal-sequestering agents (11). In addition, the resistivity to thermoinactivation or denaturing conditions of chymotrypsin has been enhanced by a multiple attachment of the enzyme to PEI and its derivatives (12). Poly(allylamine) (PAA), a linear polyamine consisting of primary amines, and its derivatives were also used in the crosslinkage of chymotrypsin (13). PAA used as the macromolecular spacer has molecular weight of 100,000, containing about 1800 monomer residues (Scheme 2).

In the characterization of the biomimetic functional molecules constructed with PEI or PAA, knowledge of the degree of protonation of the amines present on the polymers is necessary. It is predicted that protonation of the amines is suppressed by cationic or hydrophobic groups present on the polymers. Quantitative measurement of the ionization of PEI or PAA, however, has not been previously performed. In this study, titration of PEI, lau₁₀PEI (a PEI derivative with 10% of the amino groups laurylated), and PAA was performed in order to obtain insights into the electrostatic and the hydrophobic effects exerted on the ionization behavior.

**PAA**

SCHEME 2

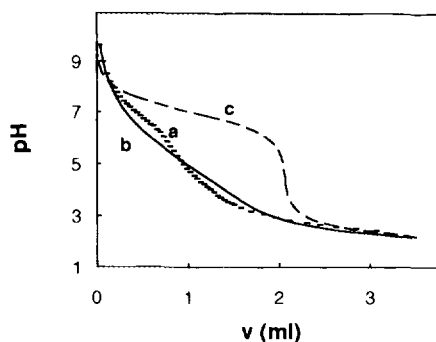


FIG. 1. The plot of pH against the volume (v) of 9.70×10^{-2} M HCl added to 0.01 resM PEI solution (20 ml). (a) Experimental data, (b) theoretical curve based on Eq. [11] and the parameter values summarized in Table I, (c) hypothetical curve obtained with Eq. [6] by assuming $pK_a = 7$.

EXPERIMENTAL PROCEDURES

PEI (MW 50,000–55,000) was purchased from Aldrich or Sigma and purified by dialysis or ultrafiltration (PM 30 membrane of Amicon) to remove fractions of small molecular weights. The molecular weight of the resulting PEI is estimated as ca. 60,000. Dry PEI was obtained by lyophilization.

The lauryl-containing PEI, lau₁₀PEI, was prepared according to the literature (14) and purified by dialysis against 25% ethanol (three times), 0.1 M NaCl (three times), and then water (five times). When the concentration of the polymer was adjusted to 0.0115 resM (concentrations of the polymers are expressed in terms of the monomer residue molar concentration) by dilution, pH of the solution was 5.92.

An aqueous solution of the HCl salt of PAA (0.02 resM, 50 ml), purchased from Aldrich, was dialyzed against water (2 liters, five times). After the volume of the resulting PAA solution was adjusted to 100 ml, the pH of the diluted solution was 9.66.

Titration of PEI, lau₁₀PEI, or PAA was performed with Dong-Woo pH meter DP 880 and with a microburet. Temperature was maintained at $25 \pm 0.1^\circ\text{C}$ with a Fisher scientific isotherm circulator Model 70.

RESULTS

The pH changes observed when 9.70×10^{-2} M HCl was added in small portions to the solutions of PEI, lau₁₀PEI, or PAA (20 ml, 0.01–0.05 resM) are illustrated in Figs. 1–4. The pH of partially titrated polymer solutions may be calculated by using equilibrium constants K_a (Eq. [1]) and K_w (Eq. [2]) as well as the equations for charge (Eq. [3]) or mass (Eq. [4] and [5]) balance. In these equations, B stands

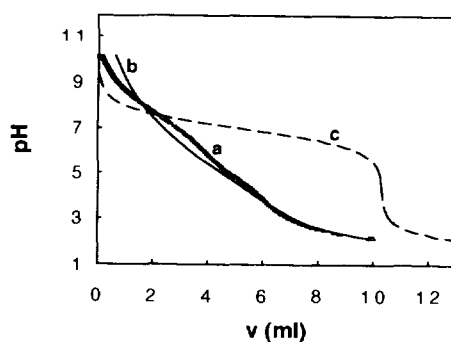


FIG. 2. The plot of pH against the volume (v) of 9.70×10^{-2} M HCl added to 0.05 resm PEI solution (20 ml). (a) Experimental data, (b) theoretical curve based on Eq. [11] and the parameter values summarized in Table I, (c) hypothetical curve obtained with Eq. [6] by assuming $pK_a = 7$.

for individual amino groups of the polymers whereas v_0 and v represent the initial volume of the polymer solutions and the volume of the HCl solution (concentration: $[HCl]_0$) added to the amine, respectively. In addition, it is assumed (Eq. [1]) that all of the amino groups have the same ionization constant for each polyamine. In the case of lau₁₀PEI or PAA, the polymer solution was partially neutralized with HCl in the preparation stage, and $[Cl^-]_0$ represents the concentration of Cl^- thus introduced initially to the solution of lau₁₀PEI or PAA.

$$K_a = [B][H^+]/[BH^+] \quad [1]$$

$$K_w = [H^+][OH^-] \quad [2]$$

$$[BH^+] + [H^+] = [Cl^-] + [OH^-] \quad [3]$$

$$[BH^+] + [B] = \{v_0/(v_0 + v)\}[B]_0 \quad [4]$$

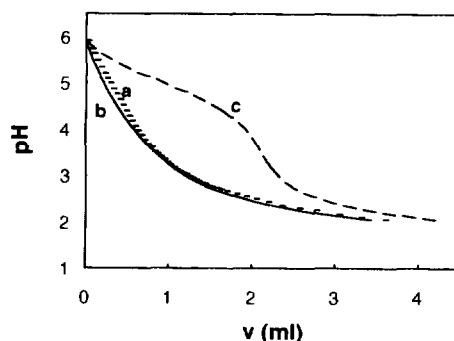


FIG. 3. The plot of pH against the volume (v) of 9.70×10^{-2} M HCl added to 1.15×10^{-2} resm lau₁₀PEI solution (20 ml). (a) Experimental data, (b) theoretical curve based on Eq. [11] and the parameter values summarized in Table I, (c) hypothetical curve obtained with Eq. [6] by assuming $pK_a = 5$.

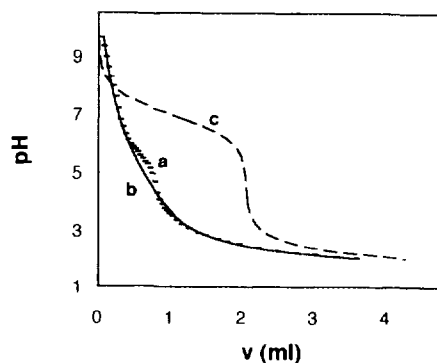


FIG. 4. The plot of pH against the volume (v) of 9.70×10^{-2} M HCl added to 0.01 resM PAA solution (20 ml). (a) Experimental data, (b) theoretical curve based on the parameter values summarized in Table 1, (c) hypothetical curve obtained by assuming $pK_a = 7$ (independent of pH).

$$[Cl^-] = \{v/(v_0 + v)\}[HCl]_0 + \{v_0/(v_0 + v)\}[Cl^-]_0. \quad [5]$$

Based on these equations, Eq. [6] is derived, which may be used in the analysis of the dependence of pH on v . The experimental data (curves **a** of Figs. 1–4), however, deviate considerably from the theoretical curves based on

$$v = v_0\{[H^+] + [B]_0/(1 + K_a/[H^+]) - [Cl^-]_0 - K_w/[H^+]\}/\{[HCl]_0 + K_w/[H^+] - [H^+]\}. \quad [6]$$

The observed data would be explained perfectly if ionization constants of all individual amino groups are considered. This is practically impossible because PEI and PAA contain ca. 1400 and 1800 amino groups, respectively. Instead, satisfactory fitting of curves **a** of Figs. 1–4 has been achieved by defining deprotonation quotient Q (Eq. [7]) as the ratio of the fraction of unprotonated amino groups and the fraction of protonated ones and by assuming that $\log Q$ is linearly related to pH (Eq. [8]). For the modified ionization scheme, the charge balance equation and the mass balance equation for the polymer become Eqs. [9] and [10], respectively, where $[P]_t$ and $[P]_0$ stand for the total concentration and the initially added total concentration, respectively, of the polymer expressed in terms of residue molar concentration. As the expression of the theoretical curves based on the modified scheme, Eq. [11] is derived from Eqs. [2], [5], and [7]–[10].

$$Q = f_B/f_{BH^+} = (1 - f_{BH^+})/f_{BH^+} \quad [7]$$

$$\log Q = \alpha pH + \beta \quad [8]$$

$$f_{BH^+}[P]_t + [H^+] = [Cl^-] + [OH^-] \quad [9]$$

$$[P]_t = v_0[P]_0/(v_0 + v) \quad [10]$$

$$v = v_0\{[H^+] + [P]_0/(1 + [H^+]^{-\alpha}10^\beta) - [Cl^-]_0 - K_w/[H^+]\}/\{[HCl]_0 + K_w/[H^+] - [H^+]\}. \quad [11]$$