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Conformational Flexibility of Poly(ethylenimine) and Its Derivatives

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Degree of protonation of the amino groups and the equilibrium constant for binding of Ni(II) ion by the amino groups were measured for poly(ethylenimine) (PEI), the PEI derivative containing lauryl groups (Lau-PEI), the PEI derivative containing β-cyclodextrin (CD-PEI), and the PEI derivative containing *o,o′*-dihydroxyazobenzene (DHAB-PEI). The amino groups of Lau-PEI, CD-PEI, and DHAB-PEI resisted protonation much more strongly compared with those of PEI. In addition, Ni(II) binding by the amino groups of Lau-PEI, CD-PEI, and DHAB-PEI was much weaker than that of PEI. These are taken to indicate that Lau-PEI, CD-PEI, and DHAB-PEI possess compact conformations in order to minimize the hydrocarbon–water interfacial area. The high conformational flexibility of the polymer backbone results in the formation of hydrophobic microdomains by aggregation of hydrophobic moieties. Implications of the conformational flexibility on the design of artificial enzymes are also discussed.

INTRODUCTION

Branchy poly(ethylenimine) (PEI) contains ethylamine as the repeating unit. A typical PEI of MW 60 000 contains ca. 350 primary amines, ca. 700 secondary amines, and ca. 350 tertiary amines. The tertiary amino nitrogens are the branching points. PEI has been used as the backbone of many artificial enzymes in view of branchy structure, high solubility in water, and ready modification of its amines by acylation, alkylation, or imine formation (*1–3*).

The major obstacle faced in the design of artificial enzymes using synthetic polymers as the molecular backbone has been the lack of specific binding sites. We have utilized β-cyclodextrin (CD) as the binding site for aromatic substrates and macrocyclic metal centers as that for anionic substrates in designing artificial enzymes based on PEI (*4*, *5*). Additional catalytic elements have been randomly introduced to PEI derivatives containing CD or macrocyclic metal centers (*6*, *7*). Cooperativity has been observed by the multiple catalytic elements introduced into the same PEI backbone.

Each PEI derivative is expected to possess unique conformation in solution as enzymes do. As the origins for folding of enzyme backbones are important for enzymology, it is desirable for the design of artificial enzymes to obtain insights

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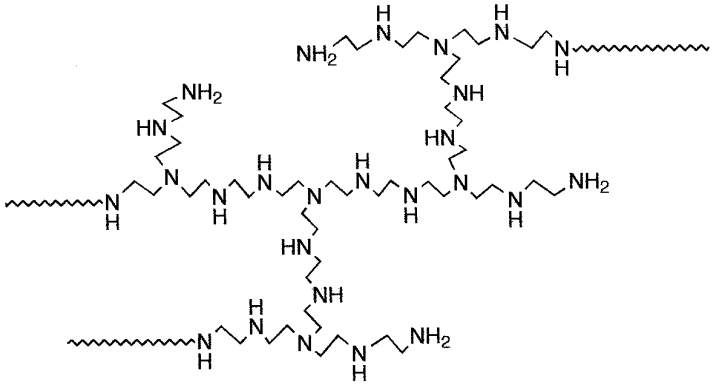


PEI

into the conformational flexibility of the macromolecular backbone. In this study, how the conformation of PEI is affected by attachment of hydrophobic residues such as lauryl, CD, or *o*,*o*′-dihydroxyazobenzene (DHAB) groups is examined. For this purpose, protonation and/or Ni(II) binding of the amino groups of PEI, the PEI derivative containing lauryl groups (Lau-PEI), the PEI derivative containing CD rings (CD-PEI), and the PEI derivative containing DHAB moieties (DHAB-PEI) are measured.

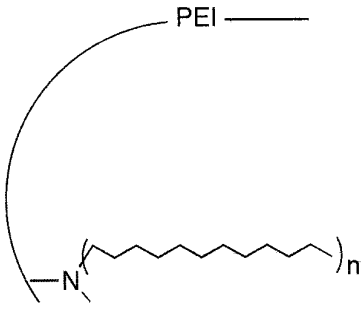
EXPERIMENTAL PROCEDURES

PEI and Its Derivatives

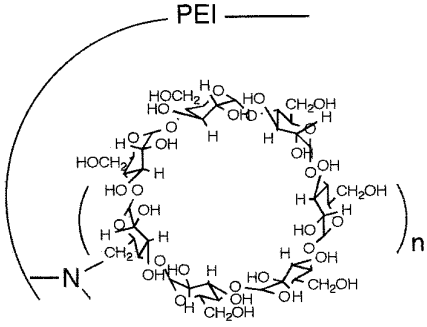
PEI was purchased from Aldrich and purified by dialysis to remove fractions of small molecular weights. Laurylation of PEI was carried out as reported in the literature to obtain Lau-PEI (*8*), in which the content of the lauryl group was estimated as 12 residue mol%. CD-PEI was prepared as reported previously (*4*), and the content of CD in CD-PEI was 1.2 residue mol%. Preparation of DHAB-PEI was reported previously (*9*), and the content of DHAB moieties in DHAB-PEI was 1.8 residue mol%.

Measurements

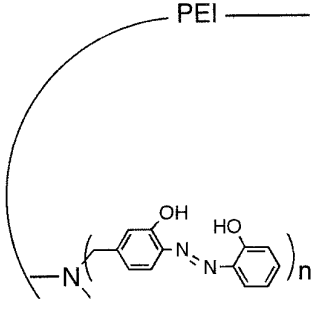
pH measurements were carried out with a Dong-Woo DP 880 pH meter. Buffer used at pH 7.00 was *N*-(2-hydroxyethyl)piperazinyl-*N*′-2-ethanesulfonate (0.05 M). ICP analysis of Ni(II) ion was performed with a Laptam S410 plasmascan.



Lau-PEI



CD-PEI



DHAB-PEI

RESULTS

To obtain information on microenvironments of amino groups of CD-PEI and DHAB-PEI relative to those of PEI, basicity of the amino groups was examined by titration with HCl. To a solution of the PEI derivative, a standard HCl solution was added dropwise and the resulting pH readings were recorded. From the total concentration ($[HCl]_0$) of added HCl and the corresponding pH reading, the fraction of protonated amine (f) of the PEI derivatives was calculated according to Eqs. [1] and [2] where $[NH^+]$ and $[N]_t$ stand for the residue molar concentrations of protonated amines and the total residue molar concentration of amines, respectively. Fractions of amino groups protonated at various pH values are illustrated in Fig. 1. For CD-PEI and DHAB-PEI, some of the amino groups of the PEI backbone are protonated during the synthetic stage. The fraction of protonated amino groups of CD-PEI or DHAB-PEI should have been considerably lowered by repetitive dialysis in the purification stage and would not affect the results of Fig. 1 appreciably. Ionization of phenolic hydroxy groups of DHAB-PEI does not affect the results considerably due to the low content of the DHAB group. The data for Lau-PEI were obtained from the results reported previously (*10*) and those for triethylenetet-

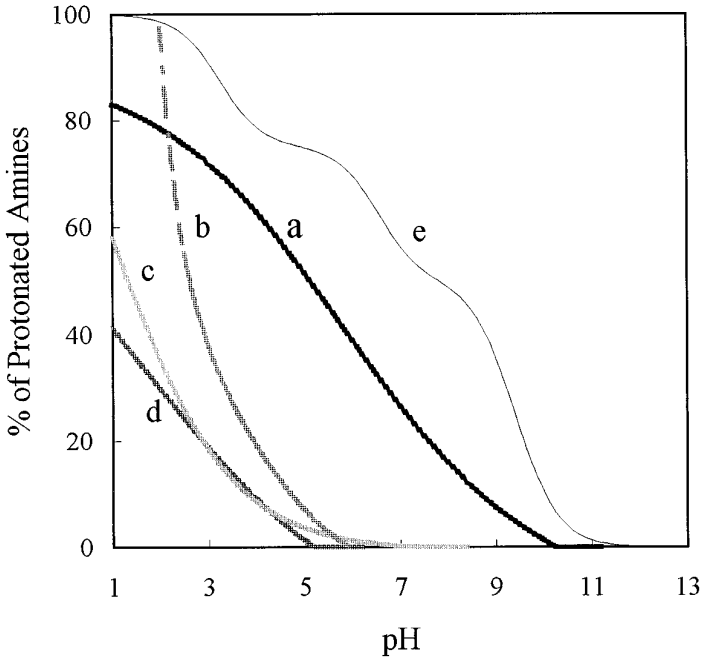


FIG. 1. Fractions of protonated amines for PEI (a), Lau-PEI (b), CD-PEI (c), DHAB-PEI (d), and triethylenetetramine (e) at various pH values and 25°C.

ramine ($NH_2(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$) calculated from p$K$ values reported in the literature (*11*).

$$[HCl]_0 = [H^+] + [NH^+] \quad [1]$$

$$f = [NH^+]/[N]_t \quad [2]$$

To examine suitability of backbones of the PEI derivatives for metal complexation, formation constants (K_f) for the Ni(II) complexes of PEI, Lau-PEI, and CD-PEI were measured at pH 7.00 and 25°C in this study. Various types of small molecules including metal ions and organic molecules are complexed to PEI derivatives, and sometimes a large number of the small molecules are bound to each molecule of a PEI derivative. The binding of small molecules can be simplified, however, as independent complexation to individual binding sites. This approximation is valid as long as the complexation to a binding site does not affect the succeeding bindings appreciably and has been found to be valid for binding of several types of small molecules to PEI derivatives (*4–7*, *12*, *13*).

When K_f for metal complex formed on a PEI derivative is very large, the concentration of unbound metal ion cannot be measured directly. Instead, K_f can be estimated from the results of exchange of the metal ion between the PEI derivative

$$\text{Ni(II)-BS-PEI} + n\,\text{CL} \overset{K_{ex}}{\rightleftharpoons} \text{Ni(II)(CL)}_n + \text{BS-PEI}$$

$$\text{Ni(II)} + \text{BS-PEI} \overset{K_f}{\rightleftharpoons} \text{Ni(II)-BS-PEI}$$

$$\text{Ni(II)} + n\,\text{CL} \overset{K^{CL}}{\rightleftharpoons} \text{Ni(II)(CL)}_n$$

SCHEME 1

and a competing ligand (*12*, *13*). As indicated by Scheme 1, K_f ($= K^{CL}/K_{ex}$) is calculated from K_{ex} and K^{CL}. Values of K_{ex} are measured experimentally and those of K^{CL} are obtained from the literature (*14*). In Scheme 1, BS-PEI indicates individual binding sites for Ni(II) ion on a PEI derivative.

To measure K_{ex}, a solution containing Ni(II) ion, a PEI derivative, and a competing ligand was dialyzed in a narrow and tall dialysis casing against the corresponding buffer solution. After the mixture was shaken at 25°C for a week, the total concentration of Ni(II) ion contained in the buffer solution outside the dialysis casing was measured by ICP analysis. Since K^{CL} is sufficiently large, the concentration of uncomplexed Ni(II) ion is negligible and the Ni(II) concentration measured by ICP analysis corresponds to the equilibrium concentration of Ni(II)$(CL)_n$. By performing a set of dialysis experiments employing various total concentrations ($[CL]_0$) of the competing ligand, the dependence of $[Ni(II)(CL)_n]$ on $[CL]_0$ is obtained. An example of the plot of $[Ni(II)(CL)_n]$ against $[CL]_0$ is illustrated in Fig. 2 for competition of PEI with picolinic acid (PA) for Ni(II) ion.

When nitrilodiacetic-3-propionic acid (NDPA) is used as the competing ligand (log $K^{CL} = 8.80$ at pH 7.00 and 25°C) for Ni(II) ion to form Ni(II)(NDPA), a 1:1-type complex, Eq. [3] was used to estimate K_{ex} from the dependence of $[Ni(II)(CL)_n]$ on $[CL]_0$. When iminodiacetic acid (IDA) was used as the competing ligand (log $K^{CL} = 9.42$ at pH 7.00 and 25°C) for Ni(II) ion to form $Ni(II)(IDA)_2$, a 1:2-type complex, Eq. [4] was used to estimate K_{ex} from the dependence of $[Ni(II)(CL)_n]$ on $[CL]_0$. When PA was used as the competing ligand (log $K^{CL} = 17.20$ at pH 7.00 and 25°C) for Ni(II) ion to form $Ni(II)(PA)_3$, a 1:3-type complex, Eq. [5] was used to estimate K_{ex} from the dependence of $[Ni(II)(CL)_n]$ on $[CL]_0$:

$$[Ni(II)(CL)] = [-\alpha + (\alpha^2 + 4K_{ex}[Ni(II)]_0[CL]_0)^{1/2}]/2K_{ex}, \quad [3]$$

where $\alpha = K_{ex}([BS\text{-}PEI]_0 - [Ni(II)]_0) + [CL]_0$;

$$[Ni(II)(CL)_2] = [-\alpha + (\alpha^2 + 4K_{ex}[Ni(II)]_0[CL]_0^2)^{1/2}]/2K_{ex}, \quad [4]$$

where $\alpha = K_{ex}([BS\text{-}PEI]_0 - [Ni(II)]_0) + [CL]_0^2$; and

$$[Ni(II)(CL)_3] = [-\alpha + (\alpha^2 + 4K_{ex}[Ni(II)]_0[CL]_0^3)^{1/2}]/2K_{ex}, \quad [5]$$

where $\alpha = K_{ex}([BS\text{-}PEI]_0 - [Ni(II)]_0) + [CL]_0^3$.

In Eqs. [3]–[5], $[Ni(II)]_0$ and $[BS\text{-}PEI]_0$ represent the total concentration of Ni(II)