Complex Formation by Electrostatic Interaction between Carboxyl-Terminated Dendrimers and Oppositely Charged Polyelectrolytes

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Complex formation between a carboxyl-terminated cascade polymer (generation 3) and several cationic polyelectrolytes of varying linear charge density was studied as a function of ionic strength, by turbidimetric titration and dynamic light scattering. Tetramethylammonium chloride was used to adjust the ionic strength in order to avoid sodium counterion binding to dendrimer carboxyl groups. Complex formation occurred abruptly at a critical pH, as signaled by a sudden change in either the turbidity or the apparent Stokes radius from dynamic light scattering. The pH, of incipient complex formation was converted to the critical surface charge density \( \sigma_c \). Under conditions of low or moderate ionic strength \( I_0 \), it was confirmed that \( \sigma_c \) is roughly proportional to \( \kappa \xi \), where \( \kappa \sim 10^2 \) is the Debye–Hückel parameter and \( \xi \) is the linear charge density of the polyelectrolyte.

Introduction

Intermacroionic complex formation is a basic phenomenon in biological systems as well as in a number of technological processes; however, in the case of biological systems such as protein–DNA and enzyme–substrate complexes, the mechanism of intermolecular interaction is difficult to fully elucidate in physicochemical terms. For example, ligand binding constants, related to biological activity, cannot be quantitatively explained solely on the basis of fundamental variables such as macromolecular charge density or the electrostatic screening effect of the counterion. For such reasons, and also because of the charge density or the electrostatic screening effect of the counterion, we focus on complex formation arising from electrostatic interactions in synthetic systems of polyelectrolytes and oppositely charged colloid particles. The colloidal particle in this study is a symmetric, uniform, and spherical dendrimer which provides an ideal model for small charged colloids.

The binding of polyelectrolytes and oppositely charged particles depends predominately on three variables: the ionic strength \( I_0 \), the polymer linear charge density \( \xi \), and the colloid surface charge density \( \sigma^c \). Phase-transition-like behavior for the interaction of polyelectrolytes with oppositely charged particles has been predicted by several theoretical investigations. Previous experimental studies on micelle–9–12 dendrimer–13,14 and protein–polyelectrolyte15–17 systems have revealed the existence of such critical conditions for complexation. Studies with polyelectrolytes and oppositely charged micelles have suggested the empirical relationship

\[
\sigma^c \xi \sim \kappa
\]

where \( \kappa \) is the Debye–Hückel parameter \( (\kappa \sim 10^2) \) and \( \sigma^c \) is the critical surface charge density of the micelle at the point of incipient complex formation. In the case of a dendrimer–polyelectrolyte system, it was observed that \( \sigma^c \) depends on the ionic strength \( I_0 \), but the relationship among \( \sigma^c, \xi \), and \( \kappa \) was not confirmed.

In this report, we examine the dependence of \( \sigma^c \) on \( \xi \) and \( \kappa \). The quantity \( \xi \) is varied by using copolymers of (methacrylamido)propyltrimethylammonium chloride (MAPTAC) and acrylamide (AAM). Sodium counterion binding to carboxyl-terminated dendrimer has been demonstrated by potentiometric titration, so it may be expected to affect complexation; therefore, both TMACl and NaCl were used to adjust the ionic strength \( I_0 \). The mechanism of phase separation is also briefly discussed in this study, since observations of phase separation by complexation may help us to understand the mechanism.

of precipitation and complex coacervation (liquid–liquid polyelectrolyte phase separation) in general.

**Experimental Section**

**Materials.** The third-generation cascade methan[4]-[3-oxo-6-oxa-2-azahexylidene]-[3-oxo-2-azapentylidene]-3-propanoic acid (G3) has 108 terminal COOH groups and a theoretical molecular weight of 12 345.20

Poly(diallyl dimethyl ammonium chloride) (PDADMAC), molecular weight 458 000, was synthesized by free-radical polymerization of diallyl dimethyl ammonium chloride, purified, and characterized in the laboratory of Dr. W. J. aege (Fraunhofer-Institut, Teltow, Germany). Poly(methacrylamido)propyl tri-methylammonium chloride (PMAPTAC) was a gift from Cairol corporation, Stamford, CT. Copolymers of MAPTAC and acrylamide (AAm), 60, 40, and 30% MAPTAC, were prepared by Dr. Takeshi Sato at Osaka University. A 15% MAPTAC and AAm copolymer was from j efferson chemical Company, Inc., Bellema, Texas. Studies with micelle polyelectrolyte or protein–polyelectrolyte systems have shown that polymer MW has no effect on

corresponding to 100 was about 1 min. After mixing, solutions with transmittances magnetic stirring, and the time interval between measurements before turbidimetric titration. All titrations were carried out with

The solutions (0.10 g/L) were filtered with 0.2

photodiode detector. All solutions were filtered with 0.2

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where \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, and \( \eta \) is the solvent viscosity. Our interest is only the determination of the pH at which \( R_{\text{app}} \) changes abruptly, so its absolute value is not of primary concern.

## Results and Discussion

### Potentiometric Titration

The relationship between pH and \( \alpha \) for dendrimer G3 in 0.1 M TMACl and NaCl is shown in Figure 2. The results in TMACl and NaCl converge below pH 5, but the slope in NaCl is steeper than that in TMACl in the region between pH 5 and pH 7. This result suggests that the binding of Na\(^+\) to dendrimer increases the acidity of the carboxylic acid groups, an effect not seen for the nonbinding TMA\(^+\). The tendency of Na\(^+\) to bind to carboxylate has been well-documented.\(^{21}\) To avoid counterion binding, TMACl was therefore used to control ionic strength in addition to NaCl.

Dendrimer solutions of 0.5 g/L at various ionic strengths of TMACl were titrated with 0.50 \pm 0.002 M HCl and NaOH. Titration curves of G3 in various TMACl concentrations are shown in Figure 3 as the \( \alpha \)-dependence of the apparent pK\(_a\), pK\(_a\) = pH + log(1 - \( \alpha \)/\( \alpha \)).

### Phase Separation and Complexation between Dendrimer G3 and Polyelectrolyte

Turbidimetric titrations for dendrimer G3 in the presence of MAPTAC--AAm (40%/60%) copolymer in TMACl at various ionic strengths are shown in Figure 4A. The sudden increase in turbidity at a well-defined pH\(_c\) arises because phase separation is a result of complexation. The increase in pH\(_c\) with ionic strength is due to screening. The behaviour of PDADMAC--G3 in NaCl is characterized by a steep increase in turbidity at a well-defined pH\(_c\) as shown in Figure 4B, which is followed by their redissolution at high pH due to charge reversal. Evidence of soluble complex formation may be discerned also for the other homopolymer, PMAPTAC, as seen in Figure 7. The "steps" between pH\(_c\) and pH\(_c\) in Figure 7 result from the instrumental limit of \( \pm 0.1\%\)T; however, the instrumental drift of the system is less than 0.1 per 1 h, so the subtle increase in turbidity from pH 3.5 to pH 4.1 is real.

The pH dependence of \( R_{\text{app}} \) for PDADMAC in Figure 6 indicates the presence of soluble complexes before phase separation. Such soluble complexes were also observed in protein--polyelectrolyte and micelle--polyelectrolyte systems. Since phase separation is likely to occur by charge neutralization,\(^{22}\) soluble complexes presumably bear some excess charge. The situation is represented schematically in Figure 8. When complex formation occurs at low \( \alpha \), the bound dendrimer charge cannot cancel the highly charged polyelectrolyte and excess charge arises. Thus, soluble complex is observed in systems showing low pH\(_c\).

Although the linear charge density \( \zeta \) of PDADMAC is nearly the same as that of the 40% MAPTAC copolymer, its pH\(_c\) is lower. This shift of pH\(_c\) is too large to explain by the difference between Na\(^+\) and TMA\(^+\), since the effect of counterion binding is small at low ionic strength. Similar anomalous behavior has been observed in protein--polyelectrolyte systems (e.g., bovine serum albumin with PDADMAC versus a 50/50 copolymer of trimethyammoniopropylacrylate (CMA) with acrylamide)\(^{23}\) and in micelle--

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polyelectrolyte systems (Triton X-100/sodium dodecyl sulfate with PDADMAC versus PMAPTAC). In both cases, binding of anionic colloids to polycations was stronger for PDADMAC. One explanation may be to consider the polyelectrolyte as a cylinder, whose surface charge density then depends on the side chain length. Since the distances between the backbone and the side chain charge are about 6 Å for PMAPTAC and about 2 Å for PDADMAC, the value 0.008 (electronic charges per Å$^2$) for the cylinder geometric surface charge density obtained for PDADMAC is considerably larger than the value 0.005 obtained for the CMA-AAA copolymer, whose linear charge density is nearly the same as that of the 50% MAPTAC-AAA copolymer. Consequently, G3 binding is stronger for PDADMAC than for MAPTAC-AAA copolymers.

Critical Surface Charge Density. The proton dissociation constant $K$ is defined as

$$K_{app} = [COO^-][H^+]/[COOH] = \alpha[H^+]/(1 - \alpha)$$  

Table 1. pH$_c$, $\alpha_c$, $\alpha_g$, $\alpha_{GC}$, and $\xi_{GC}$ for PMAPTAC and Copolymers of MAPTAC and Acrylamide Obtained from Turbidimetric Titration at Various Ionic Strengths

<table>
<thead>
<tr>
<th>MAPTAC (%)</th>
<th>$\xi$ (x10$^8$ cm$^{-1}$)</th>
<th>I</th>
<th>pH$_c$</th>
<th>$\alpha_c$</th>
<th>$\alpha_g$ (x10$^{-4}$ esu/cm$^2$)</th>
<th>$\alpha_{GC}$ (x10$^{-4}$ esu/cm$^2$)</th>
<th>$\alpha_{GC}^*$ (x10$^3$ esu/cm$^3$)</th>
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<tr>
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<td>0.30</td>
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<td>0.08</td>
<td>1.1</td>
<td>0.7</td>
<td>3.0</td>
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<td>0.07</td>
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<td>1.7</td>
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<td>0.17</td>
<td>2.3</td>
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<td>15</td>
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<tr>
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<td>0.10</td>
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</tr>
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<td>2.2</td>
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</tr>
<tr>
<td>0.100</td>
<td>5.25</td>
<td>0.10</td>
<td>5.25</td>
<td>0.30</td>
<td>4.1</td>
<td>2.6</td>
<td>1.5</td>
</tr>
</tbody>
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Figure 5. pH$_c$ versus ionic strength in TMACl for PMAPTAC (O), MAPTAC-AAA copolymer (60%/40%) (X), MAPTAC-AAA copolymer (40%/60%) (x), MAPTAC-AAA copolymer (30%/70%) (v), MAPTAC-AAA copolymer (15%/85%) (C), MAPTAC-AAA copolymer (60%/40%) in NaCl (A), and PDADMAC in NaCl (O).

Table 2. pH$_c$, $\alpha_c$, $\alpha_g$, $\alpha_{GC}$, and $\xi_{GC}$ for PMAPTAC ($\xi = 0.16 \times 10^8$ cm$^{-1}$) Obtained from Turbidimetric Titration at Various Ionic Strengths

<table>
<thead>
<tr>
<th>I</th>
<th>pH$_c$</th>
<th>$\alpha_c$</th>
<th>$\alpha_g$ (x10$^{-3}$ esu/cm$^2$)</th>
<th>$\alpha_{GC}$ (x10$^{-4}$ esu/cm$^2$)</th>
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<td>0.5</td>
</tr>
<tr>
<td>0.50</td>
<td>3.7</td>
<td>0.1</td>
<td>1.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 6. pH dependence of the apparent Stokes–Einstein radius for G3 + PDADMAC in 0.5 M NaCl (O) and G3-PMAPTAC-AAA (15%/85%) in 0.1 M NaCl (O).

Figure 7. Plots of turbidimetric titration for G3 + PMAPTAC at 0.3 M NaCl (O) and TMACl (O).


If the electrostatic free energy required to dissociate one mole of protons at a given degree of dissociation is $\Delta G_a(\alpha)$, then

$$pK_{app} = pK_0 + 0.434\Delta G_a(\alpha)/RT \quad (8)$$

where $R$ is the gas constant and $K_0$ is the characteristic dissociation constant when electrostatic interactions with other dissociated groups are absent. $pK_0$ is generally obtained as $pK_{app}$ in the limit of $\alpha = 0$. Since $\Delta G_a(\alpha)$ is the work of transferring a proton from the bulk solution to the dendrimer surface, then if $e\psi_0 = \Delta G_a(\alpha)$,

$$\psi_0 = 2.303(pK_a - pK_0)kT/e \quad (9)$$

where $e$ is the elementary charge, $4.8 \times 10^{-10}$ esu, $N$ is Avogadro’s number, and $\psi_0$ is the surface potential (statvolt).

The relationship between $\psi_0$ and $\alpha$ for spheres is given by the Gouy–Chapman relation:

$$\sigma_{GC} = (\epsilon \psi_0/4\pi)(\kappa + 1/a) \quad (10)$$

where $\kappa$ is the Debye–Hückel parameter, $\epsilon$ is the dielectric constant of the solvent (taken as 78), and $a$ is the dendrimer radius: 1.73 nm at acidic pH. The geometric critical surface charge density was also estimated as $\sigma_0 = nea/4\pi a^2$, where $n = 108$.

Results listed in Tables 1 and 2 are also plotted as the absolute value of $\sigma_{GC}$ against $\kappa$ in Figure 9. Also included are results for the reverse-charged micelle–polyelectrolyte system, comprised of DMDAO (a cationic micelle with radius 2.6 nm) and PAMPS (an anionic polyelectrolyte with charge spacing nearly the same as that of PMAPTAC).

As seen in Figure 9, the dependence of $\sigma_{GC}$ on $\kappa$ is linear within the rather wide limits of experimental error, and the slope is an inverse function of polyelectrolyte charge density, as expected from eq 1. In conflict with eq 1, the straight lines determined by the least-squares method do not extrapolate to $\sigma_{GC} = 0$ at $\kappa = 0$. However, since the number of COO− ions per dendrimer is less than 10 when $\sigma_{GC} < 0.5$, it is difficult to justify a model of a uniformly charged sphere for G3.

The results obtained for six different systems, over a range of ionic strengths, are summarized in Figure 10 as the dependence of $\sigma_{GC}$ on $\kappa$. Aside from two aberrant data points, the results for PMAPTAC and MAPTAC copolymers roughly conform to a single curve, with the results for the polyelectrolyte–micelle system forming a parallel curve, shifted toward smaller $\sigma_{GC}$. The large radius of DMDAO micelles relative to that of G3 may be expected to favor polyelectrolyte binding, much as the binding of PDADMAC to G3 is stronger than its binding to G1 at equal $\sigma_{GC}$. This leads to complex formation at lower $\sigma_{GC}$. The values of $\sigma_{GC}$ for PDADMAC are much smaller, possibly reflecting the large geometric charge density of DMDAO.


PDADMAC (see above). However, most of the data obtained for G3 in the range \( k = 0.5 \times 10^7 \) to \( 1.7 \times 10^7 \) \( (0.025 < I < 0.25) \) when presented on a log-log plot approximate a straight line with slope 1.1 ± 0.1, corresponding to \( \alpha_{GC} = (\text{constant})k^{-1} \), in close agreement with eq 1. This is also quite close to the \( x^{1.2} \) dependence of \( \alpha_{GC} \) arising from the model of Muthukumar\(^5\) in the range of low \( k \).\(^17\)

The considerable scatter among the combined data in Figure 10 may result from several sources. First, we assume that the copolymers have homogeneous charge distributions. The copolymer sequence distribution could, in principle, lead to MAPTAC-rich domains of the polycation which preferentially bind to G3. Second, in parametrizing the polymers solely according to average charge spacing, we neglect the effects of polymer composition on chain stiffness, which has been shown to have a large influence on \( \alpha_{GC} \).\(^17\) This effect should become particularly important for small colloid particles such as G3, where complexation may involve substantial local deformation of the polymer chain.\(^27\)

**Conclusions**

Electrostatic interactions between dendrimer G3 and polyelectrolytes of varying charge density (\( \xi \)) confirm the occurrence of complex formation as a phase-transition-like phenomenon. The critical condition \( \alpha_{GC} \) depends on \( \xi \) and \( k \) and, in TMACl, is expressed by \( \alpha_{GC} \approx k^{1.1} \) in the range \( 0.025 < I < 0.25 \). Na\(^+\) impedes the complexation by counterion binding. The difference of \( \alpha_{GC} \) between PDADMAC and the MAPTAC–AAm copolymer suggests consideration of polyelectrolyte charge density according to a cylindrical model. When highly charged polyelectrolytes such as PDADMAC bind to G3 at small \( \alpha_{GC} \), soluble complexes may appear because charge asymmetry precludes stoichiometric charge neutralization.

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