Effect of Protonation and PAMAM Dendrimer Size on the Complexation and Dynamic Mobility of 2-Naphthol

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The supramolecular dynamics of triplet excited states of the 2-naphthol@dendrimer (2-NpOH@n-SBD) noncovalently bound complex have been studied by fluorescence spectroscopy and laser flash photolysis. By varying the acidity and dendrimer size, polyamidoamine dendrimers are shown to possess both nonspecific and specific binding properties. For example, the studies show that 2-naphthol binds preferentially to the tertiary amine groups within the dendrimer interior and can be released by lowering the pH of the solution. On a per binding site basis, studies of the dynamics of 32-NpOH*@n-SBD reveal that the overall dynamics (entry and exit rate constants) are enhanced by increasing the acidity of the solution. Furthermore, the amount of protonation of tertiary amine groups within 2-SBD, 4-SBD, and 6-SBD was determined to be ~12%, ~10%, and <1%, respectively, at pH 6. The results presented here demonstrate that, in addition to being able to complex transition metals, organic molecules that are capable of hydrogen bonding to the internal amine moieties can also be bound to dendrimers.

Introduction

Complexation Dynamics in Supramolecular Dendritic Systems. Polyamidoamine (PAMAM) dendrimers are polymers composed of a central core (ethylenediamine in this study) with amidoamine branching units that extend outward in a symmetric fashion and consist of generations (n, layers) of these units. As such, the dendrimers contain both primary amines located in the outermost shell as well as amides and tertiary amines throughout the interior (Figure 1). The structural characteristics of these dendrimers have been well studied. In particular, it is worth noting that, as the generation of dendrimer increases, a substantial change in structure is typically observed around 4-SBD. Below 4-SBD, dendrimers are open and elongated, whereas above 4-SBD, they tend to be spherical and have densely packed outer shells. PAMAM dendrimers have been proposed for use in many areas of medical and health science (drug delivery, MRI contrast agents, and gene transfection). In addition, dendrimers have also served as models for histones, surfactants, catalysts, environmental cleanup agents, and nanomaterials. Of particular interest to this report are the recent publications that have highlighted the use of dendrimers as delivery systems for small hydrophobic molecules that are released as the pH is lowered.

Previous studies have compared larger dendrimers with micelles because both systems can act as supramolecular host molecules capable of binding small guest molecules (guest@host complexes). Photochemical and photophysical probing techniques have been widely used to explore the structural properties of the host systems. In the case of drug delivery or environmental cleanup agents, direct rate constants are required to evaluate not only the “tightness” of binding (K) but also the rate of movement between the complexation agent and the surrounding fluid environment.

PAMAM dendrimers have an outer shell of primary amines with a pKₐ in the range of 7–9 and internal, tertiary amine moieties with pKₐ values between 3 and 6. Since the pH of the solution can be varied, the number of unprotonated amines available for complexation by a proton donor can also be manipulated. Here, we use 2-naphthol (2-NpOH) as a probe that is capable of recognizing the dendrimer amines by forming hydrogen bonds.

Photophysics of 12-NpOH*. The ground-state acidity constant (pKₐ) of 2-NpOH is 9.3, whereas the pKₐ of the excited state (12-NpOH*) is 2.9 (Scheme 1). Previous studies of the photophysics of 12-NpOH* have shown that the neutral form deprotonates, yielding the anionic naphthoate, which emits at λ_{max} = 430 nm, while the neutral form fluoresces with λ_{max} = 360 nm. The pH of the local environment around 2-NpOH determines the relative emission quantum yields.

Using Excited States to Investigate Dynamic Processes. Evaluation of dynamic processes can be furnished by using excited states, provided that the rate of the diffusive process is fast relative to diffusion in and out of supramolecular systems, the dynamic rate constants for the reporter molecule cannot be made by directly studying the fluorescence. In contrast, the lifetimes of triplet excited states are much longer (microseconds). On this time scale, the probe molecule is expected to have enough time to diffuse in and out, as well as within, the macromolecular system.

Only a few studies that explore the properties of triplet excited 2-naphthol (12-NpOH*) have been carried out. The differ-
ence in acidity between the ground state ($pK_a = 9.3$) and the excited triplet state ($pK_a = 8.1$) is much smaller than the difference between the $pK_a$ of the ground state and the excited singlet state ($pK_a = 2.9$). In this study, we use features of molecular recognition to noncovalently bind 2-naphthol (2-NpOH) to small (2-SBD), medium (4-SBD), and large (6-SBD) dendrimers. Fluorescence spectroscopy is used to determine the binding site of the 2-naphthol@dendrimer interaction. In conjunction with these studies, we utilize the triplet excited state of 2-naphthol (3 2-NpOH*) to monitor the complexation dynamics and to evaluate the rate of exit ($k_-$) and entry ($k_+$) into the dendrimer host system as a function of pH.

Materials and Methods

2-Naphthol (Acros, 99.99%) was recrystallized from ethanol and water. Deionized water was obtained using a Millipore filtration system. Triethylamine (Fisher, peptide synthesis grade), ethylenediamine (Aldrich, 99.5%), triethanolamine (Aldrich, 98%), and N-methylacetamide (Aldrich, 99.99%) were used as received. Sodium nitrite (Aldrich, 99.99%), sodium hydrogen phosphate heptahydrate (99.99%), sodium dihydrogen phosphate monohydrate (Aldrich), and standard pH buffers (Fisher) were used without additional purification.

The synthesis of PAMAM dendrimers has been reported elsewhere. PAMAM dendrimers were obtained in methanolic stock solutions from Dendritech (Midland, MI). Solutions of PAMAM dendrimers were generated by taking a portion of the methanolic stock solution, drying the dendrimers to a film with a stream of argon, and then placing the dendrimers in a vacuum oven (~0.01 Torr) at room temperature overnight. The dendrimers were then diluted with an appropriate volume of pH-adjusted 0.1 M phosphate buffer in deionized water to reach the desired concentration. To produce a solution containing 2-NpOH@dendrimer, an aliquot of a filtered (Millex-PF, 0.8 μm), saturated solution of 2-NpOH in water was added into the dendrimer solution. The final absorbance at 308 nm was in the range of 0.3–0.5 (~50 μM). The solutions were then allowed to equilibrate overnight with magnetic stirring.

pH values were obtained on a Corning pH meter standardized at pH 4 and 7. UV–vis spectra were measured on a HP 8452A diode array spectrometer using quartz cells with path lengths of 1.0 cm. Emission spectra were recorded either on a Spex Fluorolog 1680 0.22 m double spectrometer or a Spex Fluoromax-2 spectrometer using quartz cells with path lengths of 1.0 cm. For the emission spectra, a wavelength interval of 1 nm and integration time of 1 s were used. Emission intensities taken for quenching plots were taken with an integration time of 2 s.

Laser flash photolysis experiments employed pulses (XeCl, 308 nm, ~30 mJ/pulse, pulse duration of 5–10 ns) from a Lambda Physik 50 excimer laser. The system and data acquisition process have been described elsewhere. Unless otherwise stated, in all transient absorption experiments the solutions were...
deoxygenated by bubbling argon through the solution for at least 30 min. For the quenching experiments where the rate of decay is measured at a single wavelength, 3.0 mL of the 2-NpOH/dendrimer solution was placed in a 1 cm × 1 cm Suprasil quartz cell. Fresh samples were prepared for each experiment. The deoxygenated quencher solution was added to the initial solution and shaken to ensure complete mixing. At each quencher concentration, six laser shots were obtained and the results were averaged. No photodecomposition over the course of each quenching experiment was evident for 2-NpOH in water or PAMAM dendrimers at pH >4 as judged from the lack of change in the ground-state UV–vis spectrum before and after photolysis. For transient absorption spectra, a total volume of 25 mL was used in a static 1 cm × 1 cm Suprasil quartz cell with additional holding capacity and the sample was shaken between every wavelength that was monitored. No difference in the ground-state UV–vis spectrum before and after photolysis was observed.

Results

Fluorescence Spectroscopy. In the case of 2-NpOH emission experiments, a quenching methodology is used whereby an extrinsic deactivator of the excited state is added to the 2-NpOH or 2-NpOH@dendrimer system. For steady-state fluorescence studies, the Stern–Volmer relationship (I/I = 1 + Ksv[Q]) can be applied where [Q] is the concentration of quencher, and I0 and I are the fluorescence intensities in the absence and presence of quencher, respectively. Ksv is the Stern–Volmer constant and is equal to kqτ, where kq is the quenching rate constant and τ is the intrinsic lifetime of 12-NpOH* fluorescence. In the absence of dendrimer, I/I monitored at 350 nm (emission from the neutral species) increases linearly with increasing [Q]. Addition of PAMAM dendrimers to a 2-NpOH solution resulted in a linear decrease in fluorescence intensities. All quenching plots had 8–15 points and had r² values greater than 0.97. The experiments were carried out with maximum dendrimer concentrations of 0.06, 0.25, and 1 mM for 6-SBD, 4-SBD, and 2-SBD, respectively. These concentrations correspond to 0.03 M in total dendrimer amines. The Stern–Volmer constants derived from the change of the fluorescence intensities at 350 nm (neutral 2-NpOH) are shown in Figure 2. In these experiments, the pH was held constant to within 0.05 pH units.

Figure 2a shows that, on a per dendrimer basis, the Stern–Volmer constant increases with generation. However, when normalized for the number of tertiary amines (Figure 2b), no significant change in the quenching efficiency is observed. Note that the singlet lifetime of the neutral 12-NpOH* remains relatively constant over the range of pH values studied here, so the changes reflect shifts in kq. These results indicate that a specific moiety of the dendrimer whose number is proportional to the generation is acting as the quencher of 2-NpOH fluorescence.

Since the primary and tertiary dendrimer amines have different pKa values, pH-dependent quenching provides insight into the identity of the excited-state deactivator. At pH 2.2, no quenching of the 12-NpOH* was observed for any generation. This indicates that no interaction between 2-NpOH and the portion of the dendrimer that can deactivate 12-NpOH* is occurring under the conditions employed. For the smaller generations (2-SBD and 4-SBD) above pH 4, little quenching with no significant pH dependence is seen. In an effort to determine which functional groups of the dendrimer induce the quenching of 12-NpOH* fluorescence, quenching studies of 12-NpOH* by triethylamine (pK1 = 10.7), ethylenediamine (pK1 = 6.8 and pK2 = 9.9), triethanolamine (pK1 = 7.8), and N-methylacetamide were carried out. Experiments conducted where the pH > pKa2–NpOH ~ 9.5 cannot be directly compared to the quenching studies involving the neutral species, because the emitting species is predominantly 2-naphthoate; thus, only experiments where pH < 9 were attempted. Results from quenching experiments below the pKa of triethylamine, triethanolamine, and ethylenediamine show that no significant quenching occurs when the amine group(s) are protonated. The Stern–Volmer constant for N-methylacetamide was less than 2 M⁻¹, indicating that minimal quenching by the amide moieties is occurring. As the pH increases, the efficiency of quenching of 12-NpOH* by the model primary and tertiary amines also increases. Furthermore, the amines have a Stern–Volmer constant approximately equal to that of the dendrimers on a per amine basis. These results suggest that 2-NpOH binds to the dendrimer amines that have a pKa in the range of 3–6. This is in the same pH range where the tertiary amines are expected to become protonated. In contrast, the primary amines generally protonate within a pH range of 6–9.

Transient Absorption Spectroscopy. Some of the characterization of 12-NpOH* has been covered in work by others, so here we concentrate on the properties of 12-NpOH* as required to understand the nature of experiments conducted in dendritic systems.
The efficient quenching of the broad signal and 10.5. The spectra were taken 1.3 and 1.1
2-NpOH* is 8.1, the processes monitored at lower pH values
Figure 3. Transient absorption spectra of 2-NpOH in water at pH 6 and 10.5. The spectra were taken 1.3 and 1.1 µs after the laser pulse, respectively. The broad spectral band >650 nm is attributed to the hydrated electron, the peak at 430 nm to 2-NpOH*, and the other bands to the naphthoxyl radical or a combination of 2-NpOH* and naphthoxyl radical. A representative decay for 2-naphthol in water at pH 6 monitored at 430 nm is shown in the inset.

Figure 3 shows the transient absorption spectrum taken 1.3 µs after a 308 nm laser pulse with a deoxygenated solution of 2-NpOH in water at pH 6 and 10.5.

Oxygen is a known deactivator of triplet excited states. By saturating the solution with oxygen, the spectral signal at 430 nm is eliminated, but the signals at 480 and 350 nm persist. On the basis of experiments by others and the oxygen quenching experiments, the peak at 430 nm is attributed to 2-NpOH* and the neighboring bands to 2-NpO* or a combination of 2-NpOH* and 2-NpO*. No growth kinetics for the formation of 2-NpO* are observed, because the biphotonic process of ionization that leads to radical formation probably occurs during the laser pulse. Bubbling of N2O, an efficient hydrated electron scavenger, into the sample eliminates the band at >650 nm, while leaving the other bands intact. The efficient quenching of the broad signal at >650 nm by N2O allows us to ascribe this band to the hydrated electron. In argon-saturated solutions, at pH 6, the lifetimes of 2-NpO*, 2-NpOH*, and the hydrated electron are 48, 25, and 1 µs, respectively. The lifetime of 2-NpOH* at pH 6 and at pH 8 was identical within experimental error and increased to 40 µs at even lower pH values. Since the pKa of 2-NpOH* is 8.1, the processes monitored at lower pH values are ascribed to neutral 2-NpOH*. At the same energy per pulse, the quantum yield of electron ejection from 2-NpO* is dramatically increased compared with that of 2-NpOH. This may cause the increased rate of photodecomposition observed in aqueous solutions where pH > pKa(2-NpOH).

In buffered solution, the lifetime of 2-NpOH*@[n-SBD] is 20 µs and does not change significantly with pH or dendrimer generation. Figure 4 shows the time-dependent transient absorption spectra of 2-NpOH*@[6-SBD]. No differences were seen for the smaller generations studied. Figure 4 shows that the lifetime of the spectral band at 430 nm (2-NpOH*) decays with a shorter lifetime than 2-NpO* (480 nm).

For the time-resolved transient absorption studies, where rate constants are determined, a modified Stern−Volmer equation in the form of \( k_{obs} = k_0 + k_q[Q] \) is used to determine the quenching rate constant, \( k_q \), where \( k_{obs} \) is the observed rate constant of decay of 2-NpOH*, and \( k_0 \) is the intrinsic rate of decay of 2-NpOH* in the absence of quencher. In homogeneous solution such as water, a plot of the dependence of \( k_{obs} \) on quencher concentration yields a straight line with a slope of \( k_q \).

Experiments where either protonated ethylenediamine or protonated triethylamine (pH < pKa) were added to aqueous solutions of 2-NpOH resulted in \( k_q \leq 10^8 \text{M}^{-1} \text{s}^{-1} \). Furthermore, experiments whereby concentrated dendrimers solutions (n = 2, 4, 6-SBD) were added into a 2-NpOH solution also yielded \( k_q \leq 10^8 \text{M}^{-1} \text{s}^{-1} \). These observations agree with other work, where protonated amines were found to be poor quenchers of 2-NpOH*.

In the case of microheterogeneous systems where a guest@host complex exists, the quencher may not have access to all regions of the host macromolecule. For example, in the case of a small, highly water soluble cationic quencher and cationic micelle, the quencher will tend to reside in the aqueous phase due to the electrostatic repulsion from the micelle surface. Studies on the dynamics of triplet excited states and quenchers have been employed to investigate both surfactant self-assemblies and direct host−guest complexes such as cyclodextrins. The model (eq 1) requires that different quenching efficiencies exist for the probe in water and for the probe within the macro molecular host (H). Key assumptions in this model are that (1) the concentration of free probe is small compared to the concentration of complexed probe (P), (2) the excited triplet state does not decay in the water phase by any process other than deactivation by the quencher, (3) the decay of the probe must follow pseudo-first-order kinetics for all quencher concentrations, and (4) the guest@host complex has a 1:1 stoichiometry. Under these conditions, eq 1 has been derived:

\[
k_{obs} = k_0 + k_p^- + k_{qH}[Q] - \frac{k_{qP}[H]}{k_{qP}[H] + k_o + k_{qQ}[Q]}(1)
\]

where \( k_0 \) and \( k_p^- \) are the decay rate constants for 2-NpOH*@[n-SBD] and free 2-NpOH*, respectively. \( k_p^- \) and \( k_{qP} \) are the exit and entry rate constants of 2-NpOH*@[n-SBD]. \( k_{qP} \) and \( k_{qQ} \) are the quenching rate constants in water and inside the supramolecule, respectively. If large enough quencher concentrations are used, the last term in eq 1 is rendered negligible and the \( k_{obs} \) becomes equal to \( k_{p^-} + k_{qP} - k_{qQ}[Q] \). In addition to quantitative analysis, by its shape, a quenching plot can qualitatively illustrate the relative amount of access of a quencher to the guest. For example, different curvatures are evident for quenching processes where the quencher has little or no access to the probe within the host \([k_{qQ}[Q] \ll (k_{p^-} + k_{qP})] \)
Using this solution, a quenching rate constant of 1 was prepared at pH 2 and used within an hour of being generated. In an effort to minimize quenching inside the dendrimer, one can employ a quencher with the same charge as the surface. However, amines are known to complex many transition metals, and in fact, recent work has shown that PAMAM dendrimers have a high affinity for metals such as copper and cobalt.11,37–39 For this reason, this approach could not be fully utilized, and nitrite, a highly water soluble, anionic quencher, was chosen. The main limitation in the use of an anionic quencher is that the quencher is attracted to the positively charged surface of the dendrimer and may deactivate the excited-state probe before complete exit from the macromolecule.

In buffered water solutions with pH values between 4 and 8, the quenching rate constant of 2-NpOH* by nitrite was found to be pH independent and equal to (3 ± 1) × 10^9 M^−1 s^−1 (Supporting Information). Nitrite decomposes in acidic solutions,40 so as a control, a fresh solution of the quencher was prepared at pH 2 and used within an hour of being generated. Using this solution, a quenching rate constant of 1 × 10^10 M^−1 s^−1 was obtained, indicating slightly smaller quenching efficiencies. In the absence of dendrimer, at all pH values studied, a strict linear relationship between kobs and [NO2 −] was observed until no measurable absorptive signal remained.

In the presence of dendrimers, NO2 − quenching of 2-NpOH* varied from linearity as shown in Figure 5.

The curvature of the plot increases at higher pH values, which is indicative that NO2 − has less access to the 2-NpOH@dendrimer complex at higher pH values. Furthermore, curvature is observed at pH 4 for only the largest generation studied (6-SBD). This suggests that the level of protonation inside the dendrimer is still low enough such that 2-NpOH can bind to the interior binding sites. It further suggests that the access of NO2 − to the 2-NpOH binding site is hindered. In a qualitative manner, the pH dependence of the curvatures in Figure 5 agrees with the fluorescence results (Figure 2), which show that the complexation properties of 2-NpOH change dramatically between pH 3 and 6.

Under conditions in which larger amounts of NO2 − are added to the 2-naphthol@dendrimer complex, kobs does not change significantly with the addition of more nitrite. However, the introduction of oxygen into the solution results in a strong reduction of the absorptive signal as well as a large increase in the rate of decay. This indicates that oxygen has access to areas of the dendrimer that nitrite does not and that the dendrimer is capable of protecting the complexed species.

The data were analyzed using eq 1, where the known values for the concentration of n-SBD, kobs, and kqo were obtained from different experiments. Independent experiments conducted under conditions where all of the 2-NpOH was assumed to complex to the dendrimer (large excess of dendrimer) were employed to evaluate kqo. At pH 6 and 8, kqo was invariant of dendrimer generation and found to be 3 × 10^9 and <10^10 M^−1 s^−1, respectively. The remaining parameters, kp+ and kp−, were the only parameters derived from the curve-fitting procedure. Table 1 lists the rate constants obtained from the curve-fitting process.

On a per dendrimer basis, the data (Table 1) reveal very large, bimolecular kp+ values, which depend on the concentration of dendrimer. This concentration takes the number of molecules of dendrimer into consideration, but not the number of amines, which are the actual binding sites for 2-NpOH (vide infra). For example, there are 254 tertiary amines in a single molecule of 6-SBD. The results demonstrate that larger dendrimers can complex a larger number of 2-NpOH molecules. Further, on the basis of the number of tertiary amines, an increase in pH increases the number of available binding sites, and thus, a decrease in the entry rate constant is observed. This occurs with a concomitant decrease in the exit rate constant of 2-NpOH* from the dendrimer macromolecule. At pH 8, where most of the surface amines are expected to be deprotonated, virtually no change in the exit rate constant is observed for all generations for the dendrimer studied. Equilibrium constants for the binding of the 2-NpOH* to dendrimers can be calculated on the basis of the dynamic rate constants (K = kp+/kqo). The equilibrium constants (per dendrimer) get larger as more amines become available for binding due to the dendrimer size and the level of protonation. For instance, for 6-SBD, K increases from 7900 to 9400 M^−1 as the pH of the solution increases from pH 4 to 8. It should also be noted that the values of K are relatively large for complexes based on noncovalent interactions.

It has been pointed out that data analyzed as per eq 1 (cf. Figure 5) is limited by the fact that, in many situations, the plateau at higher quencher concentrations is not well-defined and relies heavily on the data taken on the shortest time scales. As such, a double reciprocal plot stemming from eq 2, which is based on a similar model, has been offered as the preferred
method of analysis.\textsuperscript{33,34}

\[
\frac{1}{(k_{\text{obs}} - k_b)} = \frac{1}{k} \left[ \frac{k_{p+}[H]^+}{k_{pqo}[Q]} \right] \tag{2}
\]

In this analysis, in addition to the assumptions made in the derivation of eq 1, one assumes that the rate constant for decay of the excited guest molecule ($k_o$) is small compared to ($k_{p+}[H]^+$ + $k_{qo}[Q]$). In the $2$-NpOH@$n$-SBD system, this has been experimentally verified. Another assumption is that the NO$_2^-$ quencher has no access to the interior of the dendrimer and cannot quench $^\text{2}$-NpOH$^*$ complexed to the dendrimer. The validity of this latter assumption will be analyzed in the Discussion section below. All plots derived from the fitting of the data shown in Figure 5 by eq 2 were strictly linear. The values of $k_{p+}$ and $k_{p-}$ derived from this analysis showed the same trends and typically were within 15\% of the value shown in Table 1. This indicates that the binding of NO$_2^-$ to the interior of the dendrimer is minimal.

**Discussion**

The fundamental approach in this study is to use supramolecular recognition to help understand the complexation dynamics of $^\text{2}$-NpOH$^*$@$n$-SBD. In aqueous media, a competitive binding process for the amines occurs between protons and 2-NpOH. The key points (Scheme 2) are that 2-NpOH is found to bind to free, unprotonated amines that have a $pK_a$ in the range of 3–6; that on a per dendrimer basis the entry rate constant increases with dendrimer size; that the binding constants derived from the analysis of the dynamic behavior increase with increasing pH; and that the exit rate constant decreases with an increase in pH.

In terms of the fluorescence dendrimer quenching experiments, single photon counting fluorescence lifetime measurements reveal that the quenching appears to be static and the process can therefore be ascribed to complexation. The pH dependence of the quenching process in the fluorescence quenching experiments indicate that the dendrimer’s 3\(^\text{rd}\) functional groups ($pK_a = 3–6$) can quench the emission of

**TABLE 1: Exit and Entry Rate Constants Determined from the Curve-Fitting Process\textsuperscript{a}**

<table>
<thead>
<tr>
<th>dendrimer</th>
<th>$k_{p+}/10^8 \text{M}^{-1} \text{s}^{-1}$</th>
<th>$k_{p-}/10^6 \text{s}^{-1}$</th>
<th>$K (\text{M}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4</td>
<td>pH 6</td>
<td>pH 8</td>
</tr>
<tr>
<td>2-SBD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per molecule</td>
<td>19 ± 0.5</td>
<td>16 ± 5</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>per 3(^{\text{rd}}) amine</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>36</td>
</tr>
<tr>
<td>4-SBD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per molecule</td>
<td>250 ± 70</td>
<td>59 ± 8</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>per 3(^{\text{rd}}) amine</td>
<td>4 ± 1</td>
<td>0.95 ± 0.10</td>
<td>36</td>
</tr>
<tr>
<td>6-SBD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>per molecule</td>
<td>950 ± 100</td>
<td>1000 ± 300</td>
<td>150 ± 30</td>
</tr>
<tr>
<td>per 3(^{\text{rd}}) amine</td>
<td>3.7 ± 0.4</td>
<td>3.9 ± 1.1</td>
<td>0.59 ± 0.12</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The concentrations of $n$-SBD were $1 \times 10^{-3}$, $2.8 \times 10^{-4}$, and $7.0 \times 10^{-5}$ M for 2-SBD, 4-SBD, and 6-SBD, respectively. $k_o$, and $k_{qo}$ were set to the $2.7 \times 10^4 \text{s}^{-1}$ and $3 \times 10^6 \text{M}^{-1} \text{s}^{-1}$. For pH 4 and 6, $k_{qh}$ was fixed at the experimentally observed value of $2 \times 10^6$ and $3 \times 10^6 \text{M}^{-1} \text{s}^{-1}$, respectively, and assumed to be negligible at pH 8. The results are shown on a per dendrimer molecule and a per 3\(^{\text{rd}}\) amine basis. The magnitude of the rate constants is discussed in the text. Uncertainties in the rate constants were calculated from the average deviation of two experiments and are about 20\% for the equilibrium constants.
12-NpOH* upon binding. Moreover, little change is observed when pH \sim pK_a (1st amines), suggesting that the noncovalently bound 2-NpOH is preferentially bound to the tertiary amines in the dendrimer interior. The complexion of 2-NpOH probably involves the sharing of the 2-NpOH hydroxyl proton by the free amine (Scheme 2). The preference for exclusive complexion to the tertiary amines relative to the primary amines is quite striking but is in line with previous work that showed that the propensity for the formation of hydrogen bonds increases with the number of alkyl substitutions on the amine.41 In a manner analogous to micelles, in solutions where the tertiary amines remain unprotonated, additional favorable energetics could arise from the hydrophobic effect due to the relatively less polar dendrimer interior compared to that of the polar bulk water.

When studying host@guest systems, it is important to minimize possible interactions between two probe molecules within the same host. As such, the concentration of the 2-NpOH (~50 \mu M) was kept substantially lower than \([n\text{-SBD}]\). As has been done for micelles, a Poisson distribution was used to evaluate the probability of complexion of multiple probes in a single dendrimer.17 For 2-SBD, greater than 95% of all dendrimers had no 2-NpOH bound, and most of the remaining dendrimers contained a single probe. In the case of the 4-SBD, 85% contained no 2-NpOH, 14% had a single bound probe, and \(~1\%\) had two bound probes. The largest dendrimers studies (6-SBD) had 50% of all dendrimers free of 2-NpOH, 35% with one probe and 12% and 3% with two and three probes, respectively. Given the internal volume of 6-SBD, it is unlikely that interactions occur between 2-NpOH probes bound within the same dendrimer.

At this point, it is important to point out that the results from the fluorescence experiments are indicative of ground-state binding, while the laser flash photolysis experiments deals with the 2-NpOH excited triplet state. It has been noted that the dynamics of ground and excited states do differ32 and that this explains the results obtained by fluorescence, which show that 2-NpOH does not bind to dendrimers at pH 2 (K_q \approx 0 M^{-1}). We can then infer that at pH 2 the NO_2 quenching of 32-NpOH* is occurring exclusively in the aqueous phase.

One factor that also needs to be addressed is that of the level of protonation inside the dendritic polymer. A reduced number of 3° amines is available for complexion at pH < pK_a due to their protonation. As shown in Table 1, the k_p values corrected for the change in the number of 3° amines decreased with increasing generation at pH 8 (k_p = 1.1 \times 10^8 and 0.59 \times 10^8 M^{-1} s^{-1} for 2-SBD and 6-SBD, respectively). As the size of the dendrimer increases, there are more available binding sites between the core of the polymer and the layer of 1° amines at the surface. As such, it is less probable that 2-NpOH complexes to the binding sites closer to the core. This result is in line with other work, which showed that the area close to the surface of large, neutral dendrimers is quite congested.1 Furthermore, as the pH increases, on a per binding site basis, the entry rate constants decrease. This change may occur because of the electrostatic repulsion between cationic moieties, which may induce wider gaps between the branches, thereby allowing easier access to the unprotonated amine binding sites. Additional credence to this explanation lies in the increased overall dynamics at pH 6 compared with pH 8 (Table 1).

On a per dendrimer basis, the binding constant for the formation of 32-NpOH*@n-SBD decreases slightly with decreasing pH. Assuming that the interactions involved in the complexion process are similar to those observed in the fluorescence experiments, this result is in line with a competitive binding process between 2-NpOH and protons for the tertiary amines. Using these changes in K, the level of protonation can be estimated. The extent of dendrimer protonation depends highly on the experimental conditions (e.g., temperature and salt concentration) and so is not well established. Other work19,28 showed that a minimal number of tertiary amines in the dendrimer are protonated at pH 8. On the basis of the assumption that K is based exclusively on 3° unprotonated amines and that at pH 8 no 3° amines are in the protonated form, then an estimate of the number of internal amines that are unprotonated at other pH levels can be obtained. On the basis of the results in Table 1, at pH 6, we estimate that the interior tertiary amines are about 12%, 10%, and <1% protonated for 2-SBD, 4-SBD, and 6-SBD, respectively. Similar behavior has been predicted using simulations and is observed when Mn^{2+} and Cu^{2+} were used to probe the surface and interior of PAMAM dendrimers using EPR spectroscopy.28,39 In those studies, protonation of smaller sized dendrimers was found to be more probable than for larger dendrimers and the tertiary amines closer to the core were found to have a reduced pK_a relative to identical residues closer to the surface. Therefore, it is reasonable to expect that 2-SBD internal amines are more readily protonated than 6-SBD internal amines.

In summary, we have shown that dendrimers are unique in that they possess properties of micelle systems as well as those...
of well-defined receptor systems such as crown ethers. The results provide evidence that 2-NpOH preferentially binds to the tertiary amine groups within the dendrimer interior. Furthermore, as the dendrimer interior becomes increasingly charged, the spacing of the branches may expand, thereby leading to the increased dynamic behavior of the guest molecule. Since larger dendrimers have a greater number of amines that can bind the organic material, the overall binding constant on a per dendrimer basis increases with generation. This study also provides an estimate of the amount of protonation in the dendrimer interior and shows that, at a specific pH, the larger generations of n-SBD possess a less charged internal environment than the smaller generations. The results presented here also show that, in addition to being able to complex metal cations, organic material capable of hydrogen bonding to the amine moieties can also be bound.

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Supporting Information Available: Plots of $k_{\text{obs}}$ vs $[\text{NO}_3^-]$ in the absence of dendrimers for various pH values. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes