Partitioning of Poly(amidoamine) Dendrimers between n-Octanol and Water

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Dendritic nanomaterials are emerging as key building blocks for a variety of nanoscale materials and technologies. Poly(amidoamine) (PAMAM) dendrimers were the first class of dendritic nanomaterials to be commercialized. Despite numerous investigations, the environmental fate, transport, and toxicity of PAMAM dendrimers is still not well understood. As a first step toward the characterization of the environmental behavior of dendrimers in aquatic systems, we measured the octanol–water partition coefficients (log$_{K_{ow}}$) of a homologous series of PAMAM dendrimers as a function of dendrimer generation (size), terminal group and core chemistry. We find that the log$_{K_{ow}}$ of PAMAM dendrimers depend primarily on their size and terminal group chemistry. For G1–G5 PAMAM dendrimers with terminal NH$_2$ groups, the negative values of their log$_{K_{ow}}$ indicate that they prefer to remain in the water phase. Conversely, the formation of stable emulsions at the octanol–water (O/W) interface in the presence of G6-NH$_2$ and G8-NH$_2$ PAMAM dendrimers suggest they prefer to partition at the O/W interface. In all cases, published studies of the cytotoxicity of Gx-NH$_2$ PAMAM dendrimers show they strongly interact with the lipid bilayers of cells. These results suggest that the log$_{K_{ow}}$ of a PAMAM dendrimer may not be a good predictor of its affinity with natural organic media such as the lipid bilayers of cell membranes.

Introduction

Nanotechnology has great potential to provide new and more effective functional materials to a broad range of industries including electronics, chemical, energy, water, food, and biomedical (1). Dendritic nanomaterials (NM), which include hyperbranched polymers, dendrigrafts, dendronized poly-
applicable to the assessment of the environmental fate and toxicity of NM such as carbon nanotubes, fullerenes, and metal oxide nanoparticles due to their tendency to aggregate in aqueous solutions; thus resulting in unpredictable bioavailability (17). Because dendrimer synthesis provides a variety of means for controlling molecular composition, size, shape, polydispersity, and solubility in aqueous solutions, dendrimers are ideal model systems for carrying out fundamental investigations of the environmental fate and toxicity of monodisperse and nonaggregated NM. As a first step toward the characterization of the environmental fate and behavior of dendrimers in aquatic systems, we initially focus on quantifying their partitioning between water and natural organic media using n-octanol as model system. We measured the log $K_{ow}$ of a homologous series of 18 PAMAM dendrimers as a function of dendrimer generation (size), terminal group, and core chemistry. We find that the log $K_{ow}$ of PAMAM dendrimers depend primarily on their size and terminal group chemistry. For G1-G5 PAMAM dendrimers with terminal NH$_2$ groups, the negative values of their log $K_{ow}$ indicate that they prefer to remain in the water phase. Conversely, the formation of stable emulsions at the octanol–water (O/W) interface in the presence of G6-NH$_2$ and G8-NH$_2$ PAMAM dendrimers suggest they prefer to partition at the O/W interface. In all cases, published studies of the cytotoxicity of Gx-NH$_2$ PAMAM dendrimers show they strongly interact with the lipid bilayers of cells (10–12). These results suggest that the log $K_{ow}$ of a PAMAM dendrimer may not be a good predictor of its affinity with natural organic media such as the lipid bilayers of cell membranes.

Materials and Methods

PAMAM dendrimers of different generations, core and terminal groups (Figure 1) were purchased (as methanol solutions or solids) from Dendritech, Sigma-Aldrich and Dendritic Nanotechnologies. Spectroscopic grade n-octanol and reagent grade trifluoroacetic acid were purchased from Sigma-Aldrich. OmniSolve acetonitrile was purchased from EMD.

Measurements of Octanol–Water Partition Coefficient (log $K_{ow}$). The octanol–water coefficients (log $K_{ow}$) of the PAMAM dendrimers were measured at room temperature using the standard “slow-stirring” method (16). Stock solutions of water-saturated octanol (WSO) and octanol-saturated water (OSW) were prepared by adding 450 mL of Milli-Q deionized water + 450 mL of octanol into a 1000 mL glass bottle with a tap at the bottom. The content of the bottle was mixed for 7 days by magnetic stirring using a Teflon-coated bar. The stirring speed was adjusted to create a vortex of ∼2 cm at the octanol–water interface. Following equilibration and phase separation, the OSW was collected by decantation; whereas the WSO was collected through the bottle tap. The log $K_{ow}$ measurements were carried out in 150 mL volumetric flasks. The concentration of each dendrimer was kept constant at 2.24 mM of equivalent terminal groups in all experiments unless otherwise specified. An aliquot of 25 mL of dendrimer + OSW was added to each flask. Following Diallo et al. (18), the pH of each dendrimer + OSW was adjusted to the desired value by addition of drops of concentrated HCl or NaOH. After pH adjustment, an aliquot of 90 mL of octanol into a 1000 mL glass bottle with a tap at the bottom. The content of the bottle was mixed for 7 days by magnetic stirring using a Teflon-coated bar. The stirring speed was adjusted to create a vortex of ∼2 cm at the octanol–water interface. Following equilibration and phase separation, the OSW was collected by decantation; whereas the WSO was collected through the bottle tap. The log $K_{ow}$ measurements were carried out in 150 mL volumetric flasks. The concentration of each dendrimer was kept constant at 2.24 mM of equivalent terminal groups in all experiments unless otherwise specified. An aliquot of 25 mL of dendrimer + OSW was added to each flask. Following Diallo et al. (18), the pH of each dendrimer + OSW was adjusted to the desired value by addition of drops of concentrated HCl or NaOH. After pH adjustment, an aliquot of 90 mL of WSO was poured slowly against the upper wall of the flasks, which were then sealed with a threaded cap to minimize evaporation. The sample solutions and reference solutions (OSW + dendrimers) were placed and mixed at 160–180 rpm on a Corning magnetic stirrer. A picture of the experimental setup is shown in Supporting Information (SI) Figure S1.
Following equilibration, the concentrations of dendrimers in the OSW phase of each sample solution (C_{d,OSW}) and reference solution C_{d,ref} were measured by high performance liquid chromatography (HPLC) (19). The HPLC system consisted of an Agilent 1100 vacuum degasser, a binary pump, an auto sampler and a column compartment with a UV diode array detector (G1315A). A Jupiter C5 silica-based reverse phase HPLC column (250 × 4.6 mm, 300 Å from Phenomenex, Torrance, CA) was used to separate the dendrimers. A Phenomenex Widepore C5 guard column (4 × 3 mm) was attached ahead of the analytical column. Milli-Q deionized water and acetonitrile (ACN) (50% v/v) were used as mobile phases. Trifluoracetic acid (0.14% v/v) was added to the mobile phases as ion pairing reagent and thus enabling dendrimer separation with a strictly reverse phase system. Typically, after a 5 min hold in 100% water, a linear gradient was employed from 0 to 50% ACN over 10 min followed by holding the system at 50% ACN water for 5 min. The injection volume was 25 μL with a solvent flow rate of 2 mL/minute. Dendrimer concentrations were determined by integration of the 210 nm chromatograms using Agilent’s Chemstation software. The dendrimer concentrations in the WSO phase and reference solution were measured three times and averaged to determine C_{d,WSO} and C_{d,ref}. The concentration of dendrimer in the octanol phase C_{d,OSW} = (C_{d,ref} - C_{d,WSO}) was determined by simple mass balance. logK_{ow} was expressed as follows:

\[
\log K_{ow} = \frac{C_{d,OSW}}{C_{d,ref}} 
\]

(1)

Results

To our knowledge, only a few measured values of logK_{ow} for dendrimers have been reported in the literature (20, 21). Thus, for the PAMAM dendrimers evaluated in this study, it was important to optimize the slow-stirring technique and HPLC assay to determine (1) the time required to reach equilibrium, (2) the optimum dendrimer concentration, and (3) the accuracy of the logK_{ow} measurements.

Measurements of Dendrimer Concentration by HPLC.

In a previous article, Diallo et al. (22) showed that UV–vis spectroscopy (λ = 201 nm) can be used to measure the concentration of PAMAM dendrimers in aqueous solutions. Because n-octanol absorbs UV light at λ = 210 nm, our initial attempts to measure dendrimer concentration with UV in OSW solutions were unsuccessful. Thus, in this study, we used HPLC to measure dendrimer concentrations in the reference aqueous and OSW solutions. This technique exploits the ability of protonated PAMAM dendrimers to form ion pairs with trifluoroacetic acid (TFA) that can be sorbed onto and eluted from a C5 HPLC column. Note that the hydrophobicity of a PAMAM-TFA ion pair will depend on the size of the dendrimer and its number/type of terminal groups. This suggests that the elution times of the sorbed PAMAM-TFA ion pairs will vary with generation and terminal group chemistry. SI Figures S2 and S3 show the effects of dendrimer generation and terminal group chemistry on elution time. For the Gx-NH₂ PAMAM dendrimers with ethylenediamine (EDA) core, we observed an increase in elution times with dendrimer generation (from 4.72 min for G1 to 7.3 min for G6). Similarly, the elution times of the nonamine terminated PAMAM dendrimers depend on terminal group chemistry: Tris- (t = 5.6 min), carboxylate (t = 6.3 min), amine (t = 6.3 min), succinic acid (t = 6.9 min), amidoethanolamine (t = 6.9 min), pyrrolidinone (t = 8.7 min). Our results are consistent with those reported by Islam et al. (19). In all cases, the chromatograms are well resolved thereby suggesting HPLC is effective at separating PAMAM dendrimers. SI Figure S4 shows (as a typical example) that HPLC with UV detection (λ ∼210 nm) can be used to quantify the concentration of PAMAM dendrimers in aqueous solutions. A series of standard solutions (10–60 μM of dendrimer) were used to generate the INSET calibration curve shown in SI Figure S4. Note that for all dendrimers evaluated in this study, we were able to detect and quantify very small differences (~1%) between the areas of their HPCL chromatograms in OSW solutions before and after equilibration with WSO solutions. SI Figure S5 shows a typical example of the integrated chromatographic areas of a G4 PAMAM dendrimer with succinic acid groups in OSW before (area = 6051 ± 6 counts) and after equilibration [area = 5985 ± 2 counts]. In all cases, we measured the HPLC chromatogram areas three times to ensure that they were consistent and reproducible. We also found that the HPLC chromatograms become less resolved as dendrimer concentration decreases (SI Figure S4). Below a dendrimer concentration of 10 μM, the chromatograms of the PAMAM dendrimers were not well resolved. This suggests that the HPLC method is only effective at quantifying dendrimer concentrations greater than 10 μM using our experimental setup and measurement procedures.

Optimum Reaction Time, Dendrimer Concentration and Accuracy of LogK_{ow} Measurements. Figure 2 illustrates the effect of reaction time and dendrimer concentration on the logK_{ow} of the G4-NH₂ PAMAM dendrimer. The logK_{ow} of this dendrimer reached constant value (~2.38) after 100 h of slow-stirring. De Bruin et al. (20) have reported that the transfer of highly hydrophobic compounds (with logK_{ow} > 5) from n-octanol to water in slow-stirring experiments reached equilibrium in 4–5 days. Interestingly, we also found that the transfer of highly hydrophilic PAMAM dendrimers from water to n-octanol also reached equilibrium in 4–5 days. Thus, we have allowed 120 h slow-stirring in all experiments. Figure 2 also shows that the logK_{ow} of the G4-NH₂ PAMAM dendrimer remains constant as the concentration of dendrimer was increased from 30 to 52 μM. Thus, dendrimer concentration was kept constant at 2.24 mM of equivalent terminal groups in all subsequent logK_{ow} measurements. For example, this corresponds to a concentration of 34.0 μM for all G4 PAMAM dendrimers with 64 terminal groups. Because the logK_{ow} measurements required significant amounts of dendrimers, we only carried out three replicate measurements in selected cases to estimate an upper limit for the accuracy of the logK_{ow} measurements. However, we used at least two replicate in all cases. Note that the logK_{ow} measurements were reproducible in all cases (Figures 3–6). We subsequently selected three generation (G4) with different terminal groups (amine, succinic acid and pyrrolidinone) and carried out three replicate logK_{ow} measurements for these dendrimers. These replicate measurements and average
The corresponding standard deviations are, respectively, equal to 0.02, 0.06, and 0.08 for the G4 PAMAM dendrimers with amine, succinamic acid, and pyrrolidinone terminal groups. Based on the results of these replicate experiments, we estimate the accuracy of the log\(K_{ow}\) of the PAMAM dendrimers evaluated in this study to be \(\sim 0.10\).

**Effects of pH on log\(K_{ow}\)**

We assessed the effect of solution pH on the log\(K_{ow}\) of PAMAM dendrimers using the G4-NH\(_2\) dendrimer as model system. Figure 3 shows the log\(K_{ow}\) values of the G4-NH\(_2\) dendrimer in acidic (pH 4.0), neutral (pH 7.4) and basic (pH 12.0) OSW solutions. Note that this dendrimer has higher (less negative) log\(K_{ow}\) (−2.15) at acidic pH (4) than at neutral (7.4) and basic pH (12). There is no significant change in the dendrimer log\(K_{ow}\) (−2.38) when pH changes from neutral to basic.

**Effects of Dendrimer Generation, Terminal Group and Core Chemistry on log\(K_{ow}\)**

Tables 1, 2, and 3 list the log\(K_{ow}\) values at physiological pH 7.4 of the 18 PAMAM dendrimers (Figure 1) evaluated in this study. Note that the log\(K_{ow}\) of the dendrimers are all negative thereby suggesting these dendrimers are hydrophilic. Figure 4 shows that the log\(K_{ow}\) of Gx-NH\(_2\) PAMAM dendrimers gradually decrease with generation/size (i.e., become more negative) from generation 1 (G1) to generation 4 (G4) followed by a slight increase at generation 5 (G5). To our knowledge, only a limited number of measured log\(K_{ow}\) values for PAMAM dendrimers have published. Najlah et al. (20) reported that G1-NH\(_2\) PAMAM dendrimers functionalized with two different terminal hydrophobic groups (terfenadine-succinyl and terfenadine-succinyl-diethylene glycol) have negative log\(K_{ow}\) values (−0.16). These measurements corroborate our finding of negative log\(K_{ow}\) values for the 18 PAMAM dendrimers evaluated in this study. Figure 5 and Table 2 illustrate the effect of dendrimer terminal group chemistry on the log\(K_{ow}\) of PAMAM dendrimers. We tested seven different dendrimer terminal groups: cationic (amine, amidoethylethanolamine), anionic (carboxylate, succinamic acid, and pyrrolidinone), and neutral (amidoethanol and tris(hydroxymethyl)amidomethane (Tris)). Note that the G4 PAMAM dendrimer with Tris terminal groups has the highest (less negative) log\(K_{ow}\) (−1.39).

Figure 6 and Table 3 show the variation of log\(K_{ow}\) values of G4-NH\(_2\) dendrimer with different core. There is a slight increase of the log\(K_{ow}\) values of the G4-NH\(_2\) PAMAM dendrimers as the number of their core carbon atoms increase from 2 (EDA) to 6 (DAH).
TABLE 2. Octanol–Water Partition Coefficients (log$K_{ow}$) of PAMAM Dendrimers with Different Terminal Groups at Room Temperature and pH 7.4

<table>
<thead>
<tr>
<th>generation</th>
<th>terminal group</th>
<th>log$K_{ow}$1</th>
<th>log$K_{ow}$2</th>
<th>log$K_{ow}$3</th>
<th>log$K_{ow}$ (reported)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>amine</td>
<td>−2.39</td>
<td>−2.36</td>
<td>−2.39</td>
<td>−2.38 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>amidoethanol</td>
<td>−2.47</td>
<td>−2.49</td>
<td>−2.48</td>
<td>−2.53 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>succinic acid</td>
<td>−2.53</td>
<td>−2.59</td>
<td>−2.48</td>
<td>−2.53 ± 0.06</td>
</tr>
<tr>
<td>3.5</td>
<td>sodium carboxylate</td>
<td>−2.30</td>
<td>−2.33</td>
<td>−2.33</td>
<td>−1.39</td>
</tr>
<tr>
<td>4</td>
<td>tris(hydroxymethyl) aminomethane</td>
<td>−1.39</td>
<td>−1.39</td>
<td>−2.39</td>
<td>−1.39</td>
</tr>
<tr>
<td>4</td>
<td>pyrrolidine</td>
<td>−2.65</td>
<td>−2.42</td>
<td>−2.39</td>
<td>−2.45 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td>amidoethylethanolamine</td>
<td>−2.54</td>
<td>−2.54</td>
<td>−2.45</td>
<td>−2.54</td>
</tr>
</tbody>
</table>

TABLE 3. Octanol–Water Partition Coefficients (log$K_{ow}$) of G4 PAMAM Dendrimers with Different Cores at Room Temperature and pH 7.4

<table>
<thead>
<tr>
<th>core* terminal group</th>
<th>log$K_{ow}$1</th>
<th>log$K_{ow}$2</th>
<th>log$K_{ow}$3</th>
<th>log$K_{ow}$ (reported)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDA amine</td>
<td>−2.39</td>
<td>−2.36</td>
<td>−2.39</td>
<td>−2.38 ± 0.02</td>
</tr>
<tr>
<td>DAB amine</td>
<td>−2.43</td>
<td>−2.35</td>
<td>−2.35</td>
<td>−2.35</td>
</tr>
<tr>
<td>DAH amine</td>
<td>−2.25</td>
<td>−2.25</td>
<td>−2.25</td>
<td>−2.25</td>
</tr>
<tr>
<td>DAD amine</td>
<td>−2.26</td>
<td>−2.33</td>
<td>−2.33</td>
<td>−2.33</td>
</tr>
<tr>
<td>Cyst amine</td>
<td>−2.62</td>
<td>−2.73</td>
<td>−2.62</td>
<td>−2.62</td>
</tr>
</tbody>
</table>

*EDA (ethylene diamine); DAB (diaminobutane); DAH (diaminoctane); DAD (diaminododecane), and Cyst (cystamine).

Discussion

Due to the wide utilization of log$K_{ow}$ in drug design, medicinal chemistry, and environmental assessment, a database of over 60,000 measured values of log$K_{ow}$ for organic molecules has been developed by the Biobyte Corporation (24). Several methods of estimations of log$K_{ow}$ from “structure” have also been developed during the last 20 years. Quantitative-structure activity relationships (QSAR) of estimation of log$K_{ow}$ include the fragment-based methods (CLOGP) of Hansch and Leo (25), the extended group contribution method (KLOGP) of Klopman et al. (26), and the atomic constant approach (ALOGP) of Goshe et al. (27). Although these QSAR approaches have been shown to reproduce very well the log$K_{ow}$ values of their training data sets, there are several classes of solutes such as peptides and nucleosides which often cause them “difficulties”. To quote Leo “when CLOGP fails badly, there are strong indications that it is conformational information that is lacking” (25).

The G4-NH$_2$ PAMAM dendrimer provides a good model system for explaining the effect of solution pH and macro-molecular conformation on the octanol–water partitioning behavior of PAMAM dendrimers. First, a G4-NH$_2$ PAMAM dendrimer exhibits three distinct charged states in water as a function of solution pH (18). At acidic pH (<5), all the primary and tertiary amine groups of a G4-NH$_2$ PAMAM dendrimer are protonated. At neutral pH (~7.0), only the primary amine groups of this dendrimer are protonated. Conversely, all the amine groups of the G4-NH$_2$ PAMAM become unprotonated and neutral at basic pH (>10.0). Figure 7 shows the 3D structures of a G4-NH$_2$ PAMAM in water with Cl$^-$ counterions at these three distinct protonation levels as determined from atomistic molecular dynamics (MD) simulations by Yi et al. (28). By using a modified Dreiding force field with interaction potentials that were fitted to high level density functional theory (DFT) calculations, Yi et al. (28) obtained calculated radii of gyration ($R_g$) (~2.11–2.20 nm) that were in excellent agreement with estimated $R_g$ from small angle neutron scattering (SANS) experiments (29). Note that the G4 PAMAM dendrimer undergoes drastic conformational changes as solution pH decreases (Figure 7). Although the $R_g$ of the G4-NH$_2$ PAMAM dendrimer does not vary with pH, Yi et al. (28) found that a decrease in solution pH causes a redistribution of mass within the G4 PAMAM dendrimer as its conformational changes from a “dense core” at high pH to a “dense shell” at low pH. They also found that the backfolding of the terminal NH$_2$ groups of the G4 PAMAM is mainly localized at the dendrimer periphery. Thus, the conformational flexibility of the G4 PAMAM dendrimer in aqueous solutions of different pH strongly suggest that QSAR based on group contributions (e.g., CLOGP, KLOGP, and ALOGP) cannot provide reliable estimates of log$K_{ow}$ for NM such as dendrimers.

At the present time, we do not have a quantitative atomistic model for explaining the effect of solution pH on the log$K_{ow}$ of the G4-NH$_2$ PAMAM dendrimer. However, this effect could be attributed to a competition between the electrostatic and cavity contributions to the free energy of transfer of a dendrimer from water to n-octanol. The log$K_{ow}$ (i) of a nonassociating solute “i” can be expressed as follows (30):

$$\log K_{ow} = \frac{-\Delta G_s^i}{2.30RT}$$

(2)

$$\Delta G_s^i = \Delta G_w^i - \Delta G_o^i$$

(3)

Where $\Delta G_s^i$ is the free energy of transfer of solute “i” from water to n-octanol; $\Delta G_w^i$ and $\Delta G_o^i$ are, respectively, the solvation free energies of “i” in n-octanol and water; $T$ is the temperature and $R$ is the ideal gas constant. Macroscopic solvent models can provide simple, fast, and reliable means of calculating the solvation energies of biological macro-molecules such as proteins (31). These models treat the solvent as a dielectric continuum and the solute as “low dielectric” molecular cavity with point charges placed on the atomic nuclei of its van der Waals surface. The basic premise of a macroscopic solvent model is that the electrostatic and nonpolar contributions to the solvation free energy of solute can be evaluated separately. Following Honig et al. (30), the solvation free energy of solute “i” in solvent “s” $\Delta G_s^i(\delta)$ can be expressed as follows:

$$\Delta G_s^i(\delta) = \Delta G_s^w(\delta) + \Delta G_s^o(\delta)$$

(4)

$$\Delta G_s^i(\delta) = \gamma_A A(\delta)$$

(5)

where $\Delta G_s^w(\delta)$ and $\Delta G_s^o(\delta)$ are, respectively, the electrostatic and cavity (i.e., nonpolar) contributions to the free energy of solvation of solute in solvent $s$; $\gamma_A$ and $A$ are, respectively, the microscopic surface tension and solvent-accessible surface area of solute “i” in solvent “s”. Substituting eqs 3, 4, and 5 into eq 2 yields

$$\log K_{ow} = \frac{[\Delta G_s^w(\delta) + \gamma_w A_w(\delta)] - [\Delta G_s^o(\delta) + \gamma_o A_o(\delta)]}{2.30RT}$$

(6)
Equation 6 provides a thermodynamic framework for explaining the effect of solution pH on log\(K_{ow}\) of the G4-NH\(_2\) PAMAM dendrimer. First, the negative values of the log\(K_{ow}\) of PAMAM dendrimers (Tables 1–3) indicate that dendrimer hydration free energy \(\Delta G^{h}_{w}(i) + \gamma_{w}A_{\gamma}(i)\) is very favorable (i.e., smaller than dendrimer solvation free energy in the octanol phase). Recall that at pH 4.0, all the primary and tertiary amine groups of a G4-NH\(_2\) PAMAM dendrimer are protonated (18). However, SANS experiments by Chen et al. (29) and atomistic MD simulations by Liu et al. (28) (Figure 7) have established that a significant fraction (>66%) of the protonated amine groups of a G4 PAMAM dendrimer become neutralized by bound Cl\(^-\) counterions at acid pH (<5). Moreover Liu et al. (28) have found that the solvent (water) accessible of the G4 PAMAM dendrimer is lower in magnitude (by ~15%) than at neutral pH. Thus, we hypothesize that the solvation free energy of the G4 PAMAM dendrimer in water \(\Delta G^{v}_{w}(i)\) to become less favorable as its cavity term \(\Delta G^{c}(i)\) decrease in magnitude and its electrostatic component \(\Delta G^{e}(i)\) becomes less negative due the neutralization of its protonated amine groups by bound Cl\(^-\) anions at pH 4.0.

Figure 3 and Table 1 show a slight increase of log\(K_{ow}\) for the G5 PAMAM dendrimer (~2.16) compared to that of the G4 PAMAM dendrimer (~2.38). SI Figure S6 shows that the G6-NH\(_2\) PAMAM dendrimer causes the formation of emulsions as illustrated by the cloudiness of the octanol–water (O/W) interface. We hypothesized this increased affinity for the O/W interface of the G5-NH\(_2\), G6-NH\(_2\), and G8-NH\(_2\) PAMAM dendrimers may be the result of changes in their hydrophilic–lipophilic balance due to global and local conformational events within the macromolecules. First, Gx-NH\(_2\) PAMAM dendrimers undergo a gradual transition in overall shape (from a more extended conformation for “earlier” generation dendrimers (G \(\leq 3.0\)) to a more compact/globular shape for “later” generation dendrimers (G \(\geq 5.0\)) (31). Second, atomistic MD simulations with explicit water molecules by Yi et al. (28) (Figure 7) have shown that the backfolding of the terminal NH\(_2\) groups of PAMAM dendrimers at neutral pH is mainly localized at the dendrimer periphery. Thus, it is reasonable to postulate that global and local conformational changes within the more compact and sterically crowded G6-NH\(_2\) and G8-NH\(_2\) PAMAM dendrimers could cause these macromolecules to act as amphiphiles at the O/W interface. This hypothesis is consistent with recent experiments showing that homologues of \(p\)-methyl benzyl alkylamines also undergo conformational changes and accumulate at the octanol–water interface (32). However, independent experimental measurements and atomistic MD simulations will be needed to corroborate this hypothesis.

Except for the PAMAM dendrimer with sodium carboxylate terminal group (G3.5), all dendrimers were fourth generation (G4) and thus have similar size and same number of tertiary amine and amide groups. Note that the highest (i.e., less negative) log\(K_{ow}\) value (~2.39) for the G4 PAMAM dendrimer with Tris terminal groups may be attributed due to its large number of terminal OH groups (64 \(\times\) 3 = 192). Thus, its increased affinity for the octanol phase could be attributed to increased H-bonding between the G4-Tris dendrimer macromolecules and \(n\)-octanol molecules. The log\(K_{ow}\) values of all the other G4 PAMAM dendrimers are comparable in magnitude ranging from ~2.54 to ~2.33. Not surprisingly, the log\(K_{ow}\) of the G4-NH\(_2\) PAMAM with EDA, DAB, DAH, and DAD cores are also comparable ranging from ~2.38 to ~2.25. However, the G4-NH\(_2\) PAMAM with cystamine core has the lowest log\(K_{ow}\) value (~2.62). At the present time, we do not have a quantitative explanation for this observation.

Environmental Implications

As previously stated, the logarithm of the partition coefficient of an organic compound between \(n\)-octanol and water (log\(K_{ow}\)) is one of the most widely used measures of its tendency to accumulate in natural biomedia such as the lipid membranes of animals, plants, and bacteria (15). Several investigators have shown that the lipid–water coefficients (log\(K_{ow}\)) of many polar and nonpolar organic pollutants are positively and often linearly correlated with their log\(K_{ow}\) (15). Because of these correlations, log\(K_{ow}\) is often employed as descriptor in QSAR used to evaluate the baseline toxicity of xenobiotic organic compounds as they accumulate in natural biomedia such as lipid bilayers (15). However, to our knowledge, correlations between the log\(K_{ow}\) of organic nanomaterials such as dendrimers and their accumulation in natural biomedia have not been established. Note that the G1-G5 PAMAM dendrimers with terminal NH\(_2\) groups evaluated in this study (Figure 1) have negative log\(K_{ow}\) values thereby suggesting they prefer to remain in water (Table 1). Conversely, the formation of stable emulsions at the octanol–water (O/W) interface in the presence of G6-NH\(_2\) and G8-NH\(_2\) PAMAM dendrimers suggest they prefer to

![FIGURE 7. Three-dimensional structures of a G4-NH\(_2\) PAMAM dendrimer in water (at low, neutral, and high pH) from atomistic molecular dynamics (MD) simulations (see ref 28). The green atoms are bound Cl\(^-\) counterions that neutralize the protonated primary and tertiary amine groups of the dendrimer.](http://pubs.acs.org)

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partition at the O/W interface (SI Figure S6). However, published atomic force microscopy (AFM) imaging and cytotoxicity studies show that Gx-NH₂ PAMAM dendrimers strongly interact with the lipid bilayers of cells (10–12). This suggests that logK₁₀₀ of a PAMAM dendrimer may not be a good predictor of its affinity with natural organic media such as the lipid bilayers of cell membranes.

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Supporting Information Available

Further details are shown in three tables and six figures. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

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