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AFM Studies of High-Generation PAMAM Dendrimers at the Liquid/Solid Interface

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The structural distortion of high-generation poly(amidoamine) (PAMAM) dendrimers at the liquid/solid interface was investigated in situ using tapping mode atomic force microscopy (AFM). With a substantial compression along the surface normal but minor lateral expansion, isolated PAMAM dendrimers at the water/mica interface exhibit dimensions similar to those reported for the same species in dense, dried arrays, rather than those of isolated, dried units. On graphite substrates, adsorbed dendrimers exhibit weaker adhesion forces than on mica. AFM images of lower generation dendrimers at the water/graphite interface reveal additional features interpreted as large three-dimensional aggregates.

I. Introduction

Dendrimeric polymers recently have attracted increasing interest. Their defined size and structure along with flexible surface functionality makes them promising candidates for numerous applications ranging from chemical sensing to molecular transfer in biological environments.^{1–19} In particular, poly(amidoamine) (PAMAM) starburst dendrimers have shown promise for the transport of DNA material across cell boundaries.^{8,9} Such transfection capabilities require interaction with and

transmission through biological membranes while maintaining the association between transport vehicle and delivered agent. Clearly, a rational approach to such applications can benefit from a fundamental understanding of dendrimer interactions with surfaces.

Several studies have addressed the interaction of dendrimers with surfaces of varying hydrophobicity.^{20–36} Atomic force microscopy (AFM) measurements have quantified adhesion forces for single PAMAM dendrimers and have revealed substantial dendrimer distortion upon physisorption.^{21,22,25,30,34,35} Width to height aspect ratios ranging from 5 to 6 have been observed for the higher, structurally more rigid generations, and values in excess

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of 10 have been reported for smaller generation PAMAM dendrimers.^{22,30,34,35} Surface hydrophobicity, pH, and the presence of coadsorbates were found to be important in determining these aspect ratios.^{23,34,35} As expected, increasing charge on the dendrimers, which occurs at lower pH, leads to an effective volume expansion.^{34,35}

All quantitative reports of PAMAM dendrimer volume, size, and deformation on surfaces have so far been based on studies of dried films, often generated by spin-coating a solution onto the substrate. While yielding valuable information and providing exceptional accuracy and reproducibility, such efforts only yield indirect insight into fundamental questions of adsorption at the liquid/solid interface. The sensitivity of dendrimer shapes to the substrate type and to the presence of coadsorbate suggests that the aqueous solvent typically present in biological applications may influence dendrimer conformation. Shapes observed in dried films may be affected by the presence of residual water in dendrimers and its preferred contact angle with the substrate. The present study attempts to characterize high-generation PAMAM dendrimers in situ, at the interface between an aqueous solution and a hydrophobic or hydrophilic substrate.

II. Experimental Section

A. Sample and Substrate Preparation. Poly(amidoamine) (PAMAM) dendrimers, based on an ethylenediamine (EDA) core, were used in this study; their synthesis has been reported elsewhere.³⁷ Samples were obtained as methanol solutions from Dendritech (Midland, MI). To generate aqueous solutions, a portion of the methanolic stock solution was dried under a stream of argon and subsequently placed in a vacuum oven ($p \sim 0.01$ Torr) for several hours. The dried material was redissolved in deionized water to yield $0.2 \mu\text{M}$ aqueous stock solutions, which were stored at 4°C for a maximum of a few days. Immediately before use, a fraction of the stock solution was diluted further, typically to 20 nM concentration. Acidic solutions were generated by adding 0.1 M HCl , and pH values were measured using a Metrohm pH meter Model 744, calibrated at pH 4 and 7. To expose a fresh surface, mica and HOPG (ZYG grade; Advanced Ceramics) substrates were cleaved in air directly before use.

B. AFM Measurements. All measurements were performed on a Nanoscope III Multimode scanning probe microscope from Digital Instruments (Veeco Metrology Group; Santa Barbara, CA) using an "E" scanner. Oxide sharpened silicon nitride probes (Digital Instruments Model NP-S; manufacturer specification for nominal tip radius of curvature $5\text{--}40 \text{ nm}$) were used for imaging. Of the four cantilevers provided on each silicon nitride substrate, the small, narrow unit was found empirically to yield the most reliable results, allowing the oscillating probe mass to be minimized while maintaining a moderate spring constant (manufacturer specification 0.32 N/m). Setpoints were chosen close to the free oscillation amplitude to minimize forces exerted on the interfacial species. Effective resonance frequencies inside the fluid were approximately 8 kHz . To verify the absence of adsorbed contaminant features, the interface was first scanned in the presence of pure solvent. When no contamination was found, about $2\text{--}3 \text{ mL}$ of dendrimer solution was injected through a $0.2 \mu\text{m}$ particle filter into the O-ring sealed fluid cell.

III. Results and Discussion

Figure 1 shows a typical AFM image acquired upon exposing a mica surface to a 20 nM aqueous solution of generation 9 (G9) PAMAM dendrimers. In contrast to essentially featureless images of mica surfaces under pure water, a consistent set of small features can be seen which is attributed to adsorbed G9 PAMAM species. Detailed cross-sectional measurements on a large number of isolated features give an average height of $h = 4.7 \text{ nm}$ and

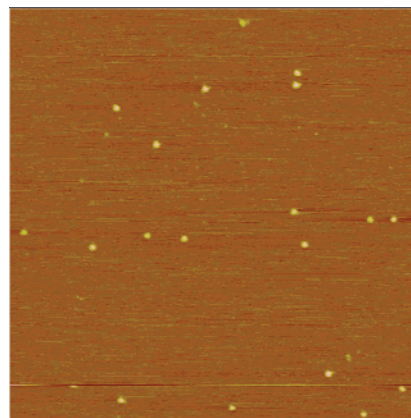


Figure 1. G9 PAMAM dendrimers on mica. Shown is a topographical tapping mode AFM image of individual G9 PAMAM dendrimers adsorbed at the water–mica interface. The scan area is $1 \mu\text{m} \times 1 \mu\text{m}$, and the height scale spans a range of 10 nm . The aqueous dendrimer solution in contact with the surface had a concentration of 20 nM and $\text{pH} \sim 7$.

a full width at half-maximum height of $w = 12.3 \text{ nm}$. Distributions of measured height and width values are characterized by standard deviations of about 1 nm . As this value may reflect both the standard error associated with each measurement and the standard deviation characteristic of the "true" dendrimer size distribution, repeated scans of the same particle would be required to quantify each contribution to the observed distribution. Essentially identical sizes of $h = 5 \text{ nm}$ and $w = 12.5 \text{ nm}$ are obtained when measuring all 19 particles displayed in Figure 1, except those two (i.e., the topmost and leftmost) with obvious signs of tip-induced distortions. The measured dendrimer height may be a function of interactions between adsorbed species and the AFM tip, especially in the presence of charges and a deformable adsorbate, although no noticeable dependence of feature height on tapping mode setpoint (i.e., on the normal force exerted while scanning) was observed. As always in AFM images, true surface features are dilated by a tip of finite size and imperfect shape, causing image distortions. The measured lateral dimensions of protruding objects such as dendrimers on a flat surface, therefore, should be regarded as an upper limit.

In the case of sufficiently rigid adsorbate species and known tip geometry, erosion algorithms can be applied to the recorded image to reconstruct the actual surface topology,^{38–48} subject to some limitations as an AFM image cannot carry information about surface regions inaccessible to the tip (i.e., where multiple contact points exist).^{38–48} For the present case, these limitations imply

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that typical AFM probes cannot determine whether the "contact angle" of the dendrimer with the surface is less than 90° as it appears in AFM images or more than 90° as expected for an idealized, oblate ellipsoidal dendrimer shape. Although the AFM tip used in the present case was not characterized using precalibrated samples, its shape can be assumed to be within the bounds given by manufacturer specifications. In addition, the AFM image itself can be used to estimate the tip shape (i.e., a so-called blind tip estimation). Both blind tip estimation and image erosion using estimated and model tips are offered by the software Deconvo1 (available from NT-MDT Co.), which is based on the algorithms published by Villarrubia.⁴⁸ This algorithm was utilized to erode the AFM images recorded in the present study.

The image erosion by any estimated or model tip must be self-consistent such that redilation of the deduced actual surface with the same tip recreates all aspects of the observed image topography that are not attributable to noise. In particular, the maximum height of an isolated feature on a flat substrate (e.g., a dendrimer on mica) observed in an AFM image is not affected by dilation and therefore must not be modified by the erosion procedure. In other words, the need for a self-consistent erosion process serves to place (upper) limits on tip size. When using cone-shaped model tips, it was found that any tip with a radius in excess of 5 nm (manufacturer specification 5–40 nm) could not have produced the observed features. As expected given the small feature height, this result is relatively independent of the chosen cone half-angle (manufacturer specification 35°). Although image erosion using $r_{\text{tip}} = 5$ nm significantly diminishes feature width near the top, the full width at half-height is merely reduced by 2 nm. This reduction may seem surprisingly small, given that the observed feature width is on the order of the nominal tip diameter; however, tips constructed by blind tip estimation (using the dendrimer image itself) gave similar results. Although the blind tip estimation process is affected by the chosen noise cutoff level, the need for self-consistency places an upper limit on tip size, leading to a reasonable upper limit on noise levels of approximately 10 Å. The resulting "worst-possible" tips change the feature shape significantly only near the very top, and the full width at half-height is reduced by no more than 2 nm. Within the assumptions implied in the image erosion process, in particular sufficient rigidity of the adsorbate features (i.e., dendrimers must not be moved or distorted by the scanning tip), it can be concluded that the unperturbed adsorbate geometry is likely characterized by a height of about 5 nm and a width at half-height of 10–12 nm.

Acidified dendrimer solutions (pH 3.3) produced a significantly higher surface coverage and adsorbate features of increased size. Typically, lateral as well as normal dimensions exceeded those of pH neutral dendrimers by a few nanometers. Exact quantification of these dimensions proved difficult due to an increased tendency for tip contamination at these lower pH values leading to imaging artifacts, an increased tendency to form dense arrays of adsorbate on the surface, and increased adsorbate dragging at small scan sizes. The volume expansion of PAMAM dendrimers upon acidification is plausible, given the high density of amine groups and resulting high charge density at low pH, and is in agreement with previous observations.^{34,35}

While dendrimer types based on rigid building blocks such as benzene moieties have been shown to display a high degree of shape persistence and rigidity,^{32,33} the flattening of PAMAM dendrimers upon adsorption

on a mica surface is in agreement with many studies of "dry" dendrimer adsorption on this and other substrates.^{21,22,25,30,34,35} A height reduction by about 55–70% from the solution diameter of 11.4 nm to values between 3.5 and 5 nm has been observed for isolated G9 PAMAM dendrimers in dried films on mica.^{30,34,35} However, isolated, dried dendrimers typically exhibit about twice the lateral diameter as compared with the in situ value seen here,^{30,34,35} which is approximately equal to the bulk solution diameter and is also very close to the measured lateral dimensions deduced for dendrimers packed into a well-defined array of dried adsorbate.³⁵ As the shell density of high-generation dendrimers is thought to be too great to permit significant interdigitation,^{49,50} the lateral dimensions measured for G9 units packed into a dense array should essentially represent true dendrimer sizes. Apparently, the close-packing of dried G9 PAMAM dendrimers into dense arrays reduces their lateral size to values similar to those assumed by isolated species in situ at the liquid/solid interface.

Clearly, the presence of a good solvent (i.e., water or other dendrimers) reduces the benefit gained from maximized surface interactions achieved by dendrimer flattening. The polar solvent water may also impose a penalty on dendrimer flattening, if such a shape change effectively reduces the polarity of the dendrimer surface. This could occur by making hydrophobic parts of the PAMAM dendrimer more solvent accessible (e.g., by turning branches "inside out" or creating openings in the shell). While such factors can explain the diminished lateral dendrimer expansion, the reason for the overall small dendrimer volume (as compared with bulk solution and isolated dried adsorbed dendrimers) is less obvious but no less important for the ability of dendrimers to function as transport vehicles.

Factors contributing to deviations between dendrimer dimensions determined in situ versus those measured for isolated species in dried films include effects of any residual water remaining after the drying procedure or of rehydration from the ambient laboratory atmosphere. The decrease in surface energy experienced by a water drop coming in contact with a (hydrophilic) mica surface and the resulting small contact angle causes any residual water on mica to exert large forces on an adsorbed species. Sufficiently flexible, hydrophilic dendrimers (e.g., PAMAM dendrimers) will be flattened and laterally expanded on such a surface. In the present case the lower distortion and looser binding on less hydrophilic substrates^{34,35} may be in part a consequence of an increased water–surface contact angle. In situ measurements at the liquid/solid interface may help to untangle the forces involved by avoiding the complications arising from molecularly thin water films on the surface of dendrimer molecules or on substrates of varying hydrophobicity. If looser binding and less shape perturbation, as reported for dried films on the more hydrophobic surfaces of graphite³⁵ and hydridosilsesquioxane resin,³⁴ are indeed consequences of dendrimer–surface interactions,^{34,35} then the same trends should hold in the presence of solvents.

Figure 2a shows an AFM image of an HOPG surface in contact with an aqueous solution of G9 PAMAM dendrimers. Comparison with Figure 1 reveals marked differences between the hydrophilic, charged mica and much more hydrophobic HOPG substrate. Instead of the more regular array of isolated features seen on a mica

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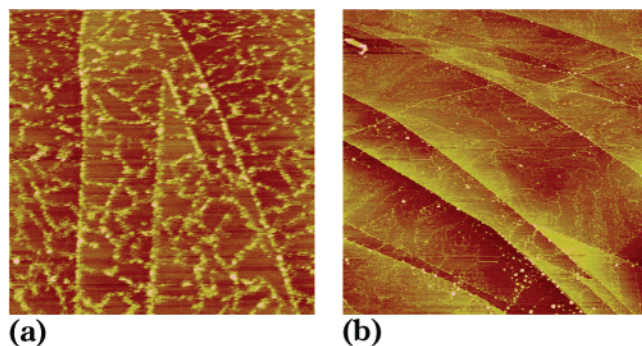


Figure 2. PAMAM dendrimers on HOPG. (a) Shown is a topographical tapping mode AFM image of G9 PAMAM dendrimers adsorbed at the water-graphite interface. The scan area is $2\ \mu\text{m} \times 2\ \mu\text{m}$, and the height scale spans a range of 10 nm. The aqueous dendrimer solution in contact with the surface had a concentration of 20 nM and pH ~ 7 . (b) Shown is a topographical tapping mode AFM image of G5 PAMAM dendrimers adsorbed at the water-mica interface. The scan area is $5\ \mu\text{m} \times 5\ \mu\text{m}$, and the height scale spans a range of 7.5 nm. The aqueous dendrimer solution in contact with the surface had a concentration of 0.3 μM and pH ~ 7 .

substrate, the graphite surface is covered by a web of curved lines composed of adsorbed dendrimers. This indicates an increased role of dendrimer-dendrimer over dendrimer-surface interactions in agreement with the study of Li et al.³⁵ However, the in situ images shown in Figure 2a do not exhibit the two-dimensional adsorbate assemblies seen in dried studies,³⁵ nor do all line segments follow exactly the visible pattern of detectable step edges. Although visible step edges have a propensity to be decorated with dendrimers and additional steps of single graphite layers may have gone undetected due to the much larger height of the dendrimers, the dense network of adsorbate lines clearly does not represent a pattern of step edges expected on this substrate. As apparent in Figure 2a, the majority of the adsorbate patterns on HOPG were not composed of easily identified smallest units. In addition, they were much more easily disturbed by the process of scanning the AFM tip, especially at smaller (submicrometer) scan sizes, effectively preventing the extraction of reliable and meaningful size parameters in this study. The dominance of one-dimensional patterns, although not changing significantly from scan to scan, may in part be a consequence of involuntary tip manipulation of the adsorbates. In any case, the results are in agreement with the concept of a weaker adsorbate-surface bond on graphite than on mica.

The formation of weblike adsorbate patterns at the water-graphite interface was also observed for dendrimers of lower generations. Figure 2b shows an in situ AFM image of G5 PAMAM dendrimers on an HOPG substrate. Aside from features assigned to isolated dendrimers and loosely connected strings of dendrimers, significantly larger objects are also present, which typically have oblate shapes with a well-defined circular footprint and diameters

reaching up to ~ 100 nm. In analogy to results reported for dried dendrimer films,³⁴ the formation of such large aggregate features occurs more readily on hydrophobic (graphite) substrates than on the more hydrophilic mica surfaces. The oblate aggregates at the interface are more prevalent when using aqueous solutions of lower generation dendrimers and were found to be essentially absent in alcoholic solvents (i.e., methanol, ethanol, and 1-butanol). Solution parameters such as pH and ionic strength were found to have at most a weak influence on dendrimer aggregation, with more acidic pH producing larger features. The aggregate formation may be caused by a change in dendrimer-dendrimer or dendrimer-solvent interactions induced by hydrophobic substrates. This could also account for the relatively high surface coverage seen on graphite as evident from a comparison of Figures 1 and 2, although reproducibility of coverages was limited. However, the participation of impurities present in the aqueous dendrimer solutions cannot be ruled out completely as a factor in the aggregation phenomenon.

IV. Summary and Conclusions

Poly(amidoamine) dendrimers adsorbed to mica and HOPG surfaces were probed in situ under aqueous solutions using tapping mode AFM. For G9 dendrimers on mica substrates, feature sizes in AFM images indicate that the surface adsorption is accompanied by a significant compression along the surface normal but only by very moderate lateral expansion. The resulting dimensions more closely resemble those found for species inside closely-packed, dried arrays than those of isolated, dried dendrimers. The more easily disturbed, weblike, interconnected features found at the water-graphite interface are interpreted as evidence for weaker adsorbate-surface binding than on mica. AFM images of G5 dendrimers provide evidence for the formation of large aggregates on the HOPG surface when water is used as solvent. Parameters such as the relative strength of surface binding, its consequence for dendrimer shape and size changes, and the possible tendency to form large aggregates may be important factors in understanding transfection capabilities of these interesting species. Future studies in our laboratory will further elucidate the role of solvent and surface polarity and the influence of cosolutes including species thought to modify water structure.

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