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Imaging Structured Water and Bound Polysaccharide on Mica Surface at Ambient Temperature

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Abstract: The presence of a water layer on the surface of muscovite mica under ambient conditions is well established. The water molecules are well ordered and seem to be oriented, leading to an icelike monolayer (probably ferroelectric) in epitaxial relation with the mica surface. We have imaged and characterized the height and contact angle of ordered water layer(s) formed by wetting and de-wetting processes on mica surfaces at different states of hydration by tapping mode atomic force microscopy. Implications that the presence of such an ordered water layer may have for imaging of biological samples are also discussed, with consideration of data for the polysaccharide hyaluronan.

Introduction

The existence of an ordered or partially ordered water layer on mica surfaces has been established at ambient temperature and humidity by measurements of repulsive hydration force,[1] scanning polarization force microscopy,[2-4] conductivity measurements combined with ellipsometry,[5] sum force generation vibrational spectroscopy,[6] surface potential measurements,[7] infrared spectroscopy,[8] and X-ray reflectivity,[9] but it has only been topographically imaged by standard atomic force microscopic methods at low temperatures.[10,11] The structured water layer closest to the mica surface is considered to be based on an icelike layer of hexagonally arranged water molecules. (The first layer is variably referred to as a monolayer or bilayer, because the oxygens in the buckled rings of an icelike layer lie in two planes, which may be separated by as much as 0.1 nm.) The structure of the first water layer on mica is ferroelectric,[7] with oxygen atoms facing the air interface and O−H bonds either pointing toward the mica surface or linking adjacent water molecules.[6] Simulations by Odelius et al.[12] suggest additional distortion driven by hydration of K^+ ions at the mica surface, but Park and Sposito[13] found K^+ ions to fit within the first layer of water.

A structured water layer may have significant effects on the interactions among mica particles and other molecular species in colloidal suspensions or at hydrated surfaces. Thus commercial applications of mica in products such as cosmetics may be affected by the extent of mica surface hydration and the difference in interaction of hydrated vs unhydrated mica with other components of the suspensions. Additional interest in this phenomenon derives from the use of mica as a common surface for imaging of biological macromolecules by atomic force microscopy. The macromolecular interaction with the surface may be anticipated to depend strongly on the structure of water at the surface, and the affinity of the macromolecule for structured water vs the mica surface itself.

Here we report that we have been able to achieve high-resolution topographic imaging of the structured water layer on mica surfaces at room temperature and humidity, using tapping mode atomic force microscopy (AFM). The formation, destruction, and characteristics of the icelike layer are examined as a function of several environmental and instrumental factors. The significance of the water layer in binding a polysaccharide is examined.

Experimental Section

Materials. Mica disks, 1 cm diameter, were obtained from Digital Instruments. Hyaluronan was a gift from Genzyme Biosurgery. A stock solution was prepared by dissolving the hyaluronan in 0.15 M NaCl to a final concentration of 0.5 mg/mL. Shortly prior to use, it was diluted 5-fold with water.

AFM Measurements. Imaging was performed using a Digital Instruments Nanoscope IIIa Multimode Scanning Probe Microscope, equipped with a type EV scanner, operating in tapping mode. The instrument was calibrated using a standard having a step height of 24.5 ± 1.5 nm, from Ultrasharp silicon grating set TGS 02 (Digital Instruments). Nanoprobe SPM etched silicon cantilever probes of 125 μm nominal length, driven at a resonance frequency of about 300 kHz, were used for scanning. Signal amplitude from the free resonance of the tip was set at 3.000 V; amplitude set point during scanning was varied in the range of 1.500−2.300 V. In general, the set point was kept as high as possible ("light tapping") without losing connection

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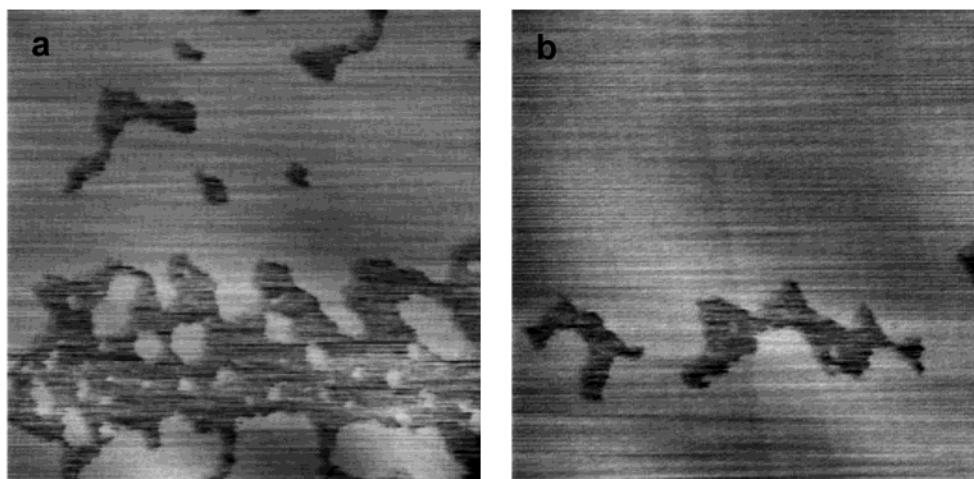(13) Park, S.-H.; Sposito, G. *Phys. Rev. Lett.* **2002**, *89*, 085501.

Figure 1. Wetting of a freshly cleaved mica surface. Tapping mode AFM images showing appearance of ordered water islands on a freshly cleaved mica surface, after (a) 2 h and (b) 2 h 2 min. Image size 2 μm $\times$ 2 μm $\times$ 2.5 nm.

with the surface, to minimize the force of interaction between tip and surface. The scan rate was 2−3 Hz, and data were stored in 256 $\times$ 256 pixel format. Images were processed using the Nanoscope version 4.43r8 software. For images to be used in measuring heights, the only image processing was zero-order flattening.

For imaging under controlled conditions, an atmospheric hood for environmental control (Digital Instruments) was used. Humidity was varied in the range 30−60% by controlling the ratio of N_2 gas flowing into the chamber from a dry line and a line passed through a water flask. Temperature and humidity in the hood were measured with an Omega RH-30-2 humidity sensor; temperature and humidity in the room were measured with a Fisher Thermo Humidity Jumbo display hygrometer.

For the experiments designed to follow the formation of a water layer on mica, the freshly cleaved mica surface was immediately mounted on the sample holder and scanned, either continuously or intermittently, at different amplitude set points, until the formation of the water layer was complete. In some cases formation of a water layer was studied as a function of humidity under the atmospheric hood.

For the experiments designed to follow the partial destruction of a preformed water layer, 4 μL of H_2O was deposited either on a freshly cleaved mica or on a mica cleaved up to 16 h earlier, and stored protected from dust (the latter being called "prehydrated" mica). After 1 min, the mica was rinsed once with 100 μL of H_2O and gently blow dried with N_2. This procedure mimics the normal procedure we employ in sample deposition and surface washing for analysis of polysaccharides. Another method for surface preparation was cleavage of the mica under flowing water. In some cases, the process of water layer destruction was accelerated by placing a beaker containing a desiccant material (Drierite) under the atmospheric hood, or by flowing dry nitrogen in the hood, and imaging at controlled low relative humidity. Our results showed no dependence of the de-wetting process on different experimental approaches.

The laser light and optical microscope fiber optic source remained on continuously unless noted otherwise in the text.

Analysis of hyaluronan was carried out as previously described.[14] In short, 4 μL of sample solution was deposited on a prehydrated mica surface and allowed to stay for 2 min. The surface was rinsed twice with 100 μL of H_2O and blow dried with a gentle N_2 flow.

Contact Angle Measurements. Contact angle determinations were performed using a Rami-Hert, Inc., Model 100.06 contact angle measurement system with tilting base. The programs used for analyzing the images were Apple Video Player version 1.3.2 and NIH Image version 1.57. For contact angle measurements the mica surface was

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cleaved 3 days before the measurements, and left dust-protected at room conditions (relative humidity about 60%).

Results and Discussion

Figure 1 shows two images of a mica surface, obtained by tapping mode AFM under ambient conditions of temperature and relative humidity, in which formation of a structured water layer can be observed. The mica surface had been freshly cleaved and then imaged repeatedly. Initial images showed only a flat surface. Scanning was then interrupted, and laser and optical microscope lights were temporarily turned off. The scan shown in Figure 1a was subsequently obtained approximately 2 h after cleavage, and that shown in Figure 1b approximately 2 min later. Islands of water appeared on the surface and progressively extended. After surface coverage was complete, the images again showed an apparently flat surface.

In repeat studies, the induction time for formation of the structured water layer on freshly cleaved mica was highly variable and not well correlated with humidity. Instead, surface contamination with dust, occasionally observed, seemed to decrease the nucleation induction time. The atmospheric hood, primarily used to control humidity, was also used to minimize dust contamination.

The use of tapping mode at low applied force in the AFM is key to imaging the structured water layer on mica at room temperature. Ogawa and Majumdar[10] and Bluhm and Salmeron,[11] using contact mode AFM, were able to observe formation of a structured water layer only at temperatures below 0 °C. This is because the contact mode applies strong lateral force at the surface, disrupting the water layer. Ogawa and Majumdar[10] also employed tapping mode AFM, but only at low temperature.

Imaging when the instrument had just been turned on was also preferred for observing the formation of a structured water layer. In the multimode AFM used in this study, the sample disk sits on the piezo crystal-containing tube. After scanning for several hours, the tube heats up and transmits this heat to the sample. This heating effect on the surface makes the formation of an ordered layer impossible.

After the completion of a structured water layer, it is possible to increase the amplitude set point of the instrument (i.e., the applied force can be reduced) to values that are impossible to
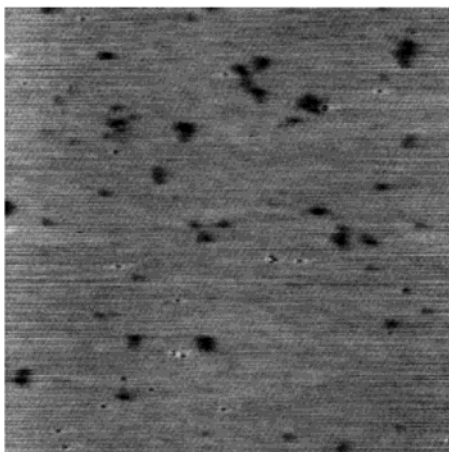
Figure 2. Partial removal of structured water layer on a prehydrated mica surface. Tapping mode AFM image showing holes formed in a prehydrated mica surface after repeated imaging. Image size 2 μm × 2 μm × 2.5 nm.

Figure 3. Distribution of island height (or, equivalently, hole depth) of the ordered water layer on mica.

Figure 4. Tapping mode AFM image of mica surface containing a thick layer of ordered water, incompletely formed in regions presumed to contain impurities. Image taken at $t = 6$ min after mica cleavage. Image size 2 μm × 2 μm × 2.5 nm.

reach at the beginning of the experiment. It is likely that additional, less structured water layers build up on the surface. The capillary effect due to the presence of this water can keep the tip from losing the surface.

We routinely prehydrate mica surfaces by storing freshly cleaved mica overnight, protected from dust. Imaging such surfaces shows complete or nearly complete layers of structured water, similar in appearance to Figure 1b. Since mica surfaces are commonly considered to become hydrophobic upon exposure to the atmosphere, a hydrated mica surface was subjected to contact angle measurements. While it was not possible to measure the contact angle on freshly cleaved mica because of the static charge, after hydration time of approximately 3 days in a dust-protected environment the contact angle was 22.7 ± 0.7°. The surface was hydrophilic, but apparently less hydrophilic than ice.

The expected water contact angle on ice is 0°, and hence, a contact angle of 22.7 ± 0.7° suggests that water molecules cannot form strong H-bonds with the structured water on mica. A similar phenomenon was observed in water contact angles on self-assembled monolayers (SAMs) of 11-hydroxyundecane-1-thiol (HUT) on gold and silver surfaces. Thus, while the water contact angle of highly ordered HUT/Au SAM surfaces was ≤5°, it was ~20° on HUT/Ag SAM surfaces.[15] It was suggested that because of the S···S distance on gold, water molecules fit well at the hexagonal cavities in the OH surface, lying flat and forming three H-bonds. On the other hand, in HUT/Ag SAMs, with S···S distance of ~4.6 Å, water molecules can interact only with the OH protons and can form H-bonds only through their oxygen atoms.

Miranda and co-workers[6] used sum-frequency generation vibrational spectroscopy of D_2O on mica and concluded that at full coverage the film is icelike with no dangling O−D groups, in agreement with molecular dynamics simulations.[12] This was further confirmed by Bluhm and co-workers,[7] who carried out surface potential measurements of ice on mica and concluded a growth of surface induced ferroelectric ice. Therefore, we propose that the observed water contact angle is due to the inability of water to form multiple H-bonds with the water molecules in the spontaneously formed icelike water layer.
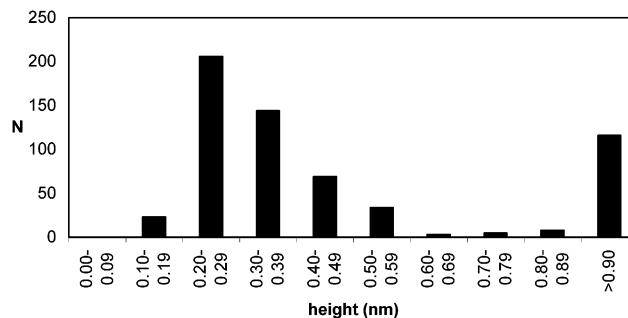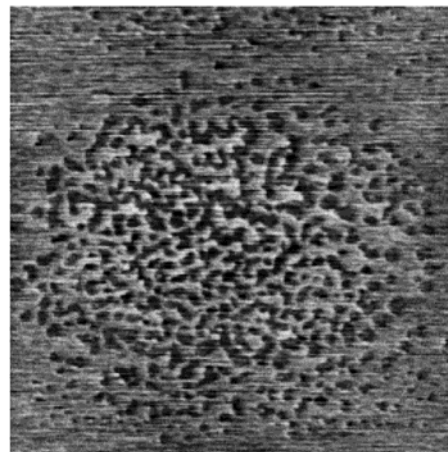
It is also possible to observe the reverse process of destruction of a structured water layer on mica. A fully hydrated surface appears flat, but repeated imaging with continuous exposure of the surface to stray laser light and the fiber optic light of an optical microscope accessory, both localized within an area of approximately 1 mm², as well as the heat generated by the piezo crystal holding the sample and heat transferred from the tip, causes changes in the structured water layer (Figure 2). The images characteristically show holes in an otherwise flat icelike layer.

Destruction of the water layer has also been imaged by other groups,[10,11] in studies performed at low temperatures. It was induced either by reducing humidity or by increasing the temperature at constant humidity. However, in these reports, the process was started when the first layer was not yet complete; a reduction in size of the water islands and the formation of holes on their surface is observed. The depth of holes, in these cases, refers to a single layer. In our case, the prehydrated mica surface, upon initial scanning, appears flat, being completely covered by structured water; destruction of such a complete preformed water structure frequently involves formation of holes greater than one layer in depth.

The distribution of measured heights of the forming structured water layer or, equivalently, the depth of the holes in an otherwise uniform structured water layer is presented in Figure 3. These data were obtained by 608 height measurements performed on seven different mica surfaces. Considering only data corresponding to the main peak between 0.1 and 0.6 nm,

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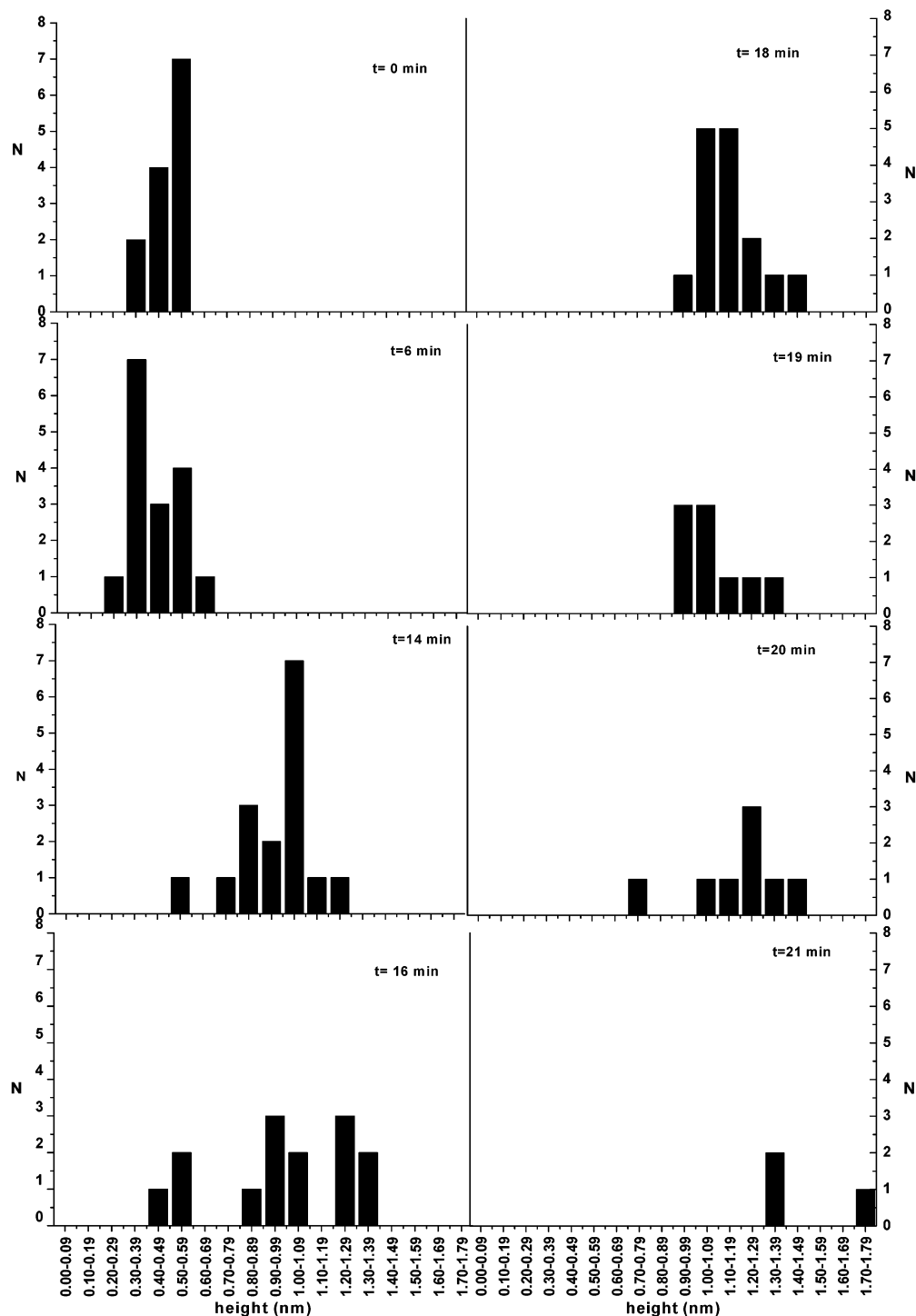
Figure 5. Distribution of structured water layer height measurements made by AFM, for mica sample shown in Figure 4, as a function of time.

the average height is 0.33 ± 0.04 nm, consistent with the concept that we are imaging primarily a single buckled layer of water molecules above the background. Less frequently observed are heights corresponding to multiple ice layers. It is important to emphasize that the actual depth of the whole ice structure could be higher than that measured here, because we are only measuring the height difference between two visible layers. The experimental setup does not allow us to follow the buildup of the layers from the mica surface with complete certainty: we can only see the height of the steps relative to each other. If the bottom layer is in fact the mica surface, then

the measured height is the ice layer depth. If the bottom layer is a continuous layer of ice, then we are only measuring the thickness of the next monolayer(s).

Height data clearly corresponding to multiple structured water layers have been obtained for surfaces on which holes remained for an extended period of time, probably because of the presence of impurities. Such impurities seem to be hydrophobic, but their exact composition is not known. They are occasionally present on the surface, and could derive from natural contaminations of the mica layer or from the cleavage and/or sample handling procedure. Figure 4 shows an image of such a sample, and the

distribution of heights for this surface is reported in the histogram in Figure 5 as a function of time. It is possible to observe how the peaks move toward higher values, showing the increase of the layer thickness. But again, it is difficult to state whether these values represent the total thickness of the structured water layer, measured from the bare mica surface, or whether the apparent surface is merely a lower layer of water.

Although most of our images were taken applying a low force (high amplitude set point, usually about 1 V less than the 3 V free amplitude signal), we have tested the effect of the force applied on the aspect of the water layer. Lowering the amplitude set point (increasing force) during formation of the water layer resulted in imaging a completely flat surface, probably because the higher force applied on the tip allowed it to penetrate the forming layer. Similarly, increasing the applied force during imaging of ice layer destruction gave images where the holes were still visible, but their number and depth were reduced. Our hypothesis is that, using tapping mode under high force, the tip can penetrate one or more layers of structured water but still reveal the lower lying layers. Scanning by contact mode, which results in the greatest force applied on the surface, invariably results in a smooth surface image (as also observed by other groups[7,10,11]).

In our AFM studies of the anionic polysaccharide hyaluronan, deposited on mica and imaged using tapping mode in air at ambient humidity and temperature, we have observed that the polysaccharide chain sometimes appears to lie on top of a rugged surface. Furthermore, we noted that storing a freshly cleaved mica surface overnight in a dust-protected environment prior to sample deposition is advantageous for obtaining good images of molecularly combed polysaccharide. The present work shows that stored mica develops a structured water surface. This prehydrated surface has strong attraction for the polysaccharide hyaluronan. Figure 6 shows an image of hyaluronan deposited on a prehydrated mica surface. The hyaluronan chain lies on top of the structured water layer, since the measured height of the molecule (ca. 0.5 nm) relative to the higher aspects of the rugged surface is consistent with the diameter of the polysaccharide structure. The "unnatural" aspect of the molecule (highly extended, as if the persistence length were very long relative to the 4−5 nm persistence length found in aqueous salt solution) probably reflects favorable interactions such as hydrogen bonding, which keep the chain fully extended. When hyaluronan is deposited on freshly cleaved mica, before the structured water layer is formed, it acts as a defect in the formation of the water layer, allowing the polysaccharide to adopt a more relaxed conformation, with a characteristic appearance more appropriate for solvated hyaluronan.

Our tapping mode AFM topographic measurements are consistent with previous estimates of the thickness of the icelike layer on mica surfaces at ambient conditions of temperature and relative humidity. Depending on the method used, the
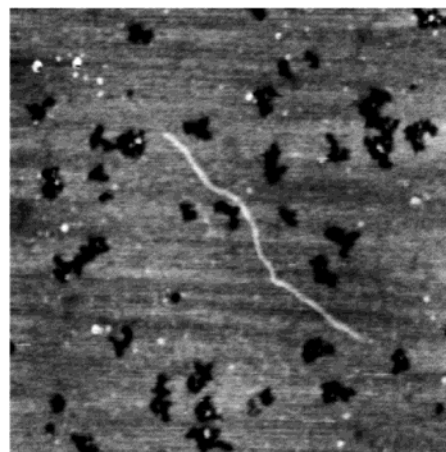

Figure 6. Effect of hydration on polysaccharide imaging. Tapping mode AFM image of hyaluronan polysaccharide deposited on a prehydrated mica surface. Image size 1 μm × 1 μm × 2.5 nm.

structured water layer thickness has been estimated[1,2,5] to be 0.25 ± 3 nm, 0.2 nm, and 0.35 nm. Distance from the mica surface to the center positions of oxygens in the water molecules has been estimated to be 0.25 nm.[9,13] This compares favorably with our average height of 0.33 nm. The average height may correspond to a buckled monolayer of ice, but our results do not exclude partial ionization of water, according to the model presented[16] for structured water on the Ru(0001) surface.

At low temperature, thickness has been measured[10,11] by AFM as 0.44−0.8 nm (contact mode) and 0.70−0.71 nm (tapping mode). This suggests increased probability for multiple layers as the temperature is reduced.

Conclusions

Our data show that tapping mode AFM can easily image the structured water layer formed on mica under ambient conditions. This technique may be able to penetrate nonstructured water on top of the structured layer(s) at low-tapping force, but can penetrate the icelike layers only at higher force. We observe evidence for a continuous icelike layer of structured water at ambient temperature and humidity, the thickness of which may correspond to multiple layers under some conditions. The ordered water has a strong affinity for hydrophilic macromolecules such as hyaluronan polysaccharide.

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