Reversible Collapse of Brushlike Macromolecules in Ethanol and Water Vapours as Revealed by Real-Time Scanning Force Microscopy

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Abstract: Environment-controlled scanning force microscopy allowed us to study adsorption and desorption of single poly(methacrylate)-*graft*-poly(*n*-butyl acrylate) brush molecules on mica in real time. The molecules transform reversibly from a two-dimensional, extended wormlike state to a compact globular state. The dynamics of the conformational transition was sufficiently slow in order to allow its observation by scanning force microscope in real time. The reversible transformation is effected by coadsorption of water or ethanol, the latter introduces the collapse. Adsorbing ethanol and water from the vapour atmosphere results in a change of the surface properties of mica, either favouring adsorption or desorption of the graft polymer. When the extended, tightly adsorbed poly(*n*-butyl acrylate) brush molecules are exposed to ethanol vapour, the macromolecules swell and contract to form compact globules. Exchanging the ethanol vapour to a humid atmosphere caused the molecules to extend again to a wormlike two-dimensional conformation. Coexistence of collapsed and extended strands within the same molecule indicates a single-molecule first-order transition in agreement with observations on Langmuir films previously reported.

Keywords: adsorption · conformational transitions · polymers · scanning probe microscopy · single-molecule studies

Introduction

Scanning force microscopy (SFM) is a powerful tool for visualization of single macromolecules at defined environmental conditions.[1] Thus, SFM has been successfully employed to study DNA,[2] RNA,[3] and their complexes with proteins,[4] surfactants[5] and other compounds. The ex situ visualization of conformational changes of DNA molecules has been carried out for the effect imposed by interaction with oppositely charged silanes,[6] with polymer nanoparticles,[7] surfactants,[8] Mg^{2+} ions (in ethanol),[9] spermidine,[10,11] polylysine,[12,13] poly(ethylene glycol)-poly(amidoamine) copolymer,[14] lipospermine and polyethylenimine.[15] Generally, the information obtained in these experiments was available before from electron microscopy studies.[16] Yet, a major advantage of SFM in comparison with electron microscopy is the possibility to investigate and visualize the dynamics of macromolecules in situ and in real time. Thus, condensation

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of DNA molecules has been observed directly in a water–alcohol mixture on mica[17,18] by an SFM equipped with a liquid cell. The molecules collapsed at increasing alcohol concentration and SFM enabled the observation of how the macromolecules unfolded partially as the alcohol concentration was decreased again.[18] Other examples concern in situ study of the DNA interaction with oppositely charged macromolecules[19] and with RNA polymerase.[20] Also the adsorption dynamics of DNA on mica have been studied at changing buffer conditions.[21]

The reason why most of these studies focus on DNA molecules is given by their size and rigidity in combination with the limited lateral resolution in SFM. Imaging of molecular details is determined by the tip–sample contact area,[22] which is typically a spot with a diameter of one nanometer and more.[23] As the contour of the rather stiff and thick DNA molecules is larger than the SFM lateral resolution they can be imaged rather easily by SFM.

In recent studies we had focused on brushlike macromolecules[1,24–26] that consist of a macromolecular backbone densely grafted by macromolecular side chains. The steric repulsion between the side chains imposes an effective rigidity of the brushlike macromolecule. At the same time the thickness facilitates easy visibility in SFM. Interaction, that is, adsorption of the side chains on a flat surface of mica or highly oriented pyrolytic graphite (HOPG) enforces an extended two-dimensional conformation of the backbone.[1,26] It has been shown,[1] that a conformational collapse of the brush molecules can be induced by increasing the surface pressure on a Langmuir monolayer of such macromolecules on a water subphase. The brush molecules underwent a transition from an extended two-dimensional wormlike to a compact globule conformation.[1] It was also shown that the transition could be reversed;[1] that is, when the surface pressure was reduced again, the compacted molecules unfolded and resumed an extended conformation. At a critical pressure the collapsed and the extended state coexisted even within one molecule. This observation has been regarded as an indication that the transition is of the first-order type. However, in order to image the molecular structure at the different states of compaction the monolayer film had to be transferred onto a substrate. This approach did not allow the observation of the dynamics of the conformational transition of single molecules.

Here we report for the first time a real-time study on the reversible collapse of single brush molecules on mica as a flat substrate. As analysed earlier, also theoretically, the conformation of the brush molecules is determined by the balance of the interfacial interaction forces and entropic forces.[1,26,27] Controlled variation of the environment, for example, introduction of solvent vapour or change in temperature, can shift this balance and effect conformational changes. Kumaki et al.[28,29] have revealed that poly(methyl methacrylate) blocks of PS-*b*-PMMA molecules (PS = polystyrene) deposited onto mica can transform into an extended conformation if the sample is exposed to water vapour saturated atmosphere. Apparently, the presence of a thin-layer of absorbed water can enhance the mobility of the chains and affect their conformational rearrangement. Balnois and Wilkinson[30] and Morozov et al.[31] have also reported that the morphology of macromolecules deposited onto mica can change significantly if the sample is exposed to humid air.

This study concerns in situ SFM imaging of poly(methacrylate)-*graft*-poly(*n*-butyl acrylate) (PMA-*g*-P*n*BuA) brushes on mica that were exposed to water and ethanol vapours. It is demonstrated that the dynamics of the collapse to a globule and its reversion can be controlled and followed in real time.

Results

Deposited by the spin-coating procedure, the polymer brushes attained a highly extended wormlike conformation (see Figures 1, 2a, 3a, 5a vide infra). Generally, we did not observe any significant difference for the molecular conformation on mica or on HOPG, in spite of the very different polarity of these substrates. Most likely the side chains adsorb on mica by the interaction of the ester groups, while the adhesion to HOPG is facilitated by the interaction of the *n*-butyl groups with the graphite surface structure. In both cases side chains are pinned down and the brush is extended in the two-dimensional surface plane. The substrates evidently differ in the strength by which they bind the molecules during the spin-coating process; that is, at equal conditions, solution concentration and so forth, more molecules were adsorbed on mica than on HOPG.

When brush molecules deposited on HOPG were exposed to ethanol vapour, both the long and short brush molecules thickened slightly. At the same time the molecules gained some mobility and aligned along the HOPG terraces or clustered to disclike islands. Thus, mobility can be introduced without desorption, that is, collapse. Exposure of the brushes on HOPG to water vapour did not result in any swelling, as observed by SFM, and the mobility was also not increased significantly. Evidently, the limited swelling and the stability of the extended conformation of the brush molecules on HOPG is an indication that the ethanol effects the interaction of the side chains with the HOPG surface only slightly. Only in the case of more polar substrates are ethanol and H_2O expected to form a well-defined ad-layer that can moderate the interaction of the brush molecules with the substrate strongly. This is clearly the case for mica (muscovite).

The conformation of PMA-*g*-P*n*BuA brushes on mica is essentially that of a flat two-dimensional brush:[1,32] the extended backbone is surrounded by a two-dimensional aureole of the side chains (see Figure 1). The fact that the brushlike macromolecules adsorbed mostly as extended separated polymer chains with only few intersection points indicates that the deposition of the molecules takes place by adsorption from solution, that is, the molecules attach to the surface before capillary and dewetting forces cause agglomeration.[33–35] The repulsion of the side chains in a good solvent (chloroform) favours an extended conformation of the macromolecules.[36,37]

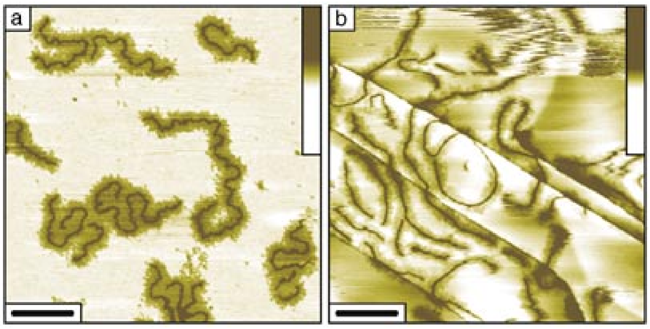

Figure 1. High-resolution SFM-image of PMA-*g*-P*n*BuA(L) brush molecules on a) mica and on b) HOPG. One can see the two-dimensional corona of side-chains surrounding the backbone of the graft polymers. Bar size: 150 nm, height scale: 5 nm.

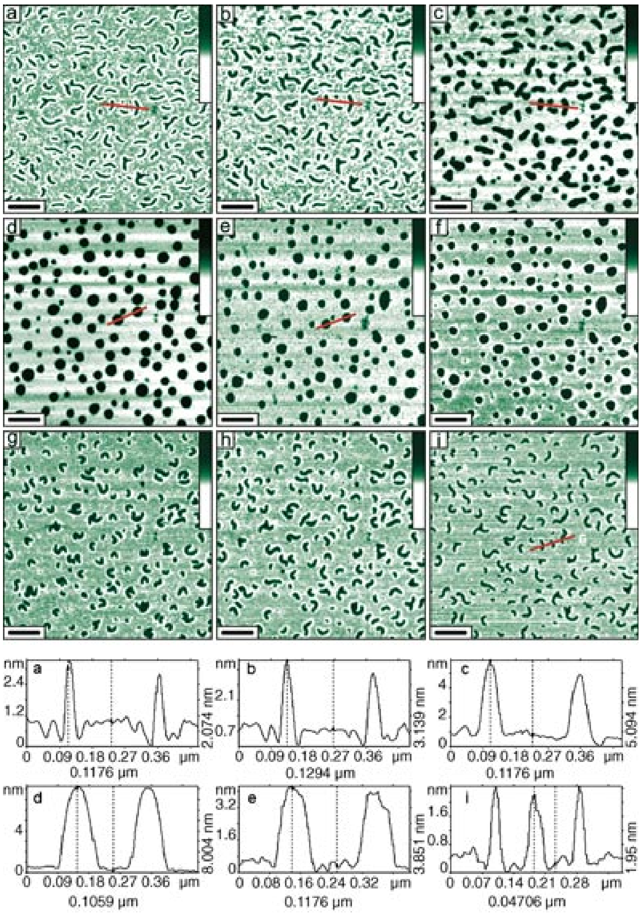

Figure 2. Sequence of SFM images that demonstrate the collapse/decollapse dynamics for PMA-*g*-P*n*BuA(s) molecules on mica in an atmosphere with varying partial pressure of ethanol and water. a) Initial image of sample in dry N_2 atmosphere; b) image obtained during injection of absolute ethanol to the sample chamber; c,d) images obtained 14 min (c) and 23 min (d) after ethanol injection; e) image after the sample space was purged with dry N_2 again, molecules remain in the collapsed state; f) image obtained during injection of water to the sample space, g–i) images obtained 15 min (g), 24 min (h), and 1.5 h (i) after water injection. The temperature was kept constant at 24 ± 1 °C corresponding to a partial vapour pressure of 7.4 kPa for ethanol and 3 kPa for H_2O. Bar size: 300 nm, height scale: 10 nm.

The extended adsorbed state is stable in dry air and in a nitrogen atmosphere. All our attempts to effect the collapse of the molecules by raising the temperature failed. Even at temperatures as high as 200 °C, the molecules did not desorb, but started to degrade. However, when we saturated the atmosphere above the molecules on mica with ethanol vapour, we observed a transformation of the molecular conformation already at room temperature. A collapse of the single, isolated molecules was monitored by a series of SFM images as shown in Figure 2a–e. In this series, we studied short PMa-*g*-P*n*BuA molecules. The transformation was observed in real time and the images in Figure 2 represent the most important stages. Figure 2a depicts the brush molecules deposited and observed in a dry atmosphere. Recording of Figure 2b was started when ethanol was injected into the sample chamber and the atmosphere became saturated by alcohol vapour (scanning from bottom to top). At the bottom of the image the molecules are unchanged when compared with Figure 2a; however, at the top of the image, we recognise a considerable increase in thickness; this can be explained by uptake of ethanol, that is, swelling. The height in the SFM profile increased from about 1.5–2.5 nm up to 3–5 nm.

After about ten minutes, the swollen brushes started to contract to form hemispherical particles with a height of 4–8 nm (Figure 2c–d). Complete transformation to globules was observed after 20–30 minutes exposure to ethanol vapour. The molecules also gained mobility and started to diffuse along the surface. Because of the surface/line tension the molecules tended to aggregate to clusters of several molecules when the sample was exposed to ethanol for even longer times.

The transformation could be arrested at any state when the ethanol-saturated atmosphere was replaced by dry nitrogen (the sample chamber was purged with N_2). Deswelling of the globules resulted in a decrease in height by 30–60 %, but not in significant change in the molecular conformation.

The images in Figure 2f–i depict the effect that was observed when water was introduced into the sample chamber subsequent to the drying procedure. Once the atmosphere got saturated by water vapour, the aggregates started to separate and the individual molecules adopted again an extended conformation; that is, the molecules “crawled” apart from each other.

After several hours the motion of the molecules ceased and it may be assumed that the extended worm-like slightly curved conformation approached the equilibrium state (Figure 2i). The observed distance between the worm-like molecules (actually, only the backbone is depicted in Figures 2 and 3) is larger than 25–30 nm, which corresponds well to the width of the corona of side chains as depicted in Figure 1. The height of the molecules exposed to a water-saturated atmosphere was measured to be the same as at dry conditions. This indicates that the uptake of water or swelling of the brush molecules is small and negligible compared to the situation found for ethanol (Figure 2b–d).

The observed curvature of the molecular conformation agrees with a model recently proposed by us.[26] Based on a scaling approach, the model demonstrates that a two-dimensional brush tightly adsorbed on a flat substrate will preferentially adopt a curved state with an uneven distribution of the side chains between the two sides of the backbone, provided that the side chains are long enough. The driving force is a gain in configurational entropy. For selected particular molecules we could compare the lengths before and after the "collapse–decollapse" cycle. A decrease by not more than 5–10% was measured; this can be explained by the redistribution of side chains from an even left/right distribution (as cast) to an uneven left/right distribution after equilibration by the ethanol–water vapour treatment.[26]

The high-molecular-weight PMA-*g*-P*n*BuA(L) brushes behaved very similar to the shorter molecules discussed above. However, due to the increased length, the transformation was more impressive and also the observation gave further details. Figure 3 depicts a series of images taken at different times after the molecules, which were deposited in an extended conformation, were exposed to ethanol vapour. Firstly, swelling of the individual molecules resulted in an increased width and height. Typically, the height of PMA-*g*-P*n*BuA(L) brushes above the substrate was equal to 0.2–0.8 nm before exposure to ethanol vapour, see Figure 3a. As a result of the exposure to ethanol vapour the height increased to 1.5–4 nm, see Figure 3b–d. Collapse of the extended swollen chain could, however, only be achieved if besides ethanol (absolute) also a small amount of water was introduced into the sample atmosphere ($P|_{H_2O,\ 24^\circ C}=3$ kPa, $P|_{\text{ethanol},\ 24^\circ C}=7.4$ kPa). When exposed to the ethanol–water vapour, first the smaller molecules started to collapse preferentially at the chain end. This is seen in Figure 3c–e by the occurrence of small nodules at the ends of the chains. These nodules increase in size and move toward the centre of the molecule as they "eat up" the inner segments (see Figure 3e–h). The collapse stops with coagulation of two nodules to an individual globule.

If segments of different molecules were in direct contact with each other, the collapse proceeded in a more complicated manner. The contact line of the strands stuck together, grew further and thus intermolecular aggregates were formed and finally adopted a hemispherical shape. Further exposure to ethanol vapour resulted in further aggregation to multimolecular globules. Again the transformation could be arrested at any stage by venting the chamber with nitrogen. This allowed it to "freeze" the molecules in a partially compacted conformation, see, for example, the dumbbell conformations in Figure 3f–i.

In ethanol vapour the height of globules formed from individual molecules was in the range of 6–12 nm. Drying with nitrogen resulted in 20–50% decrease of the globule size.

As in the case of the shorter brushlike molecules, the longer ones also "crawled" back on the surface and extended again when the atmosphere was saturated with water. Figure 4 presents a series of images taken at different stages of the decollapse process in real time. Before water was in-

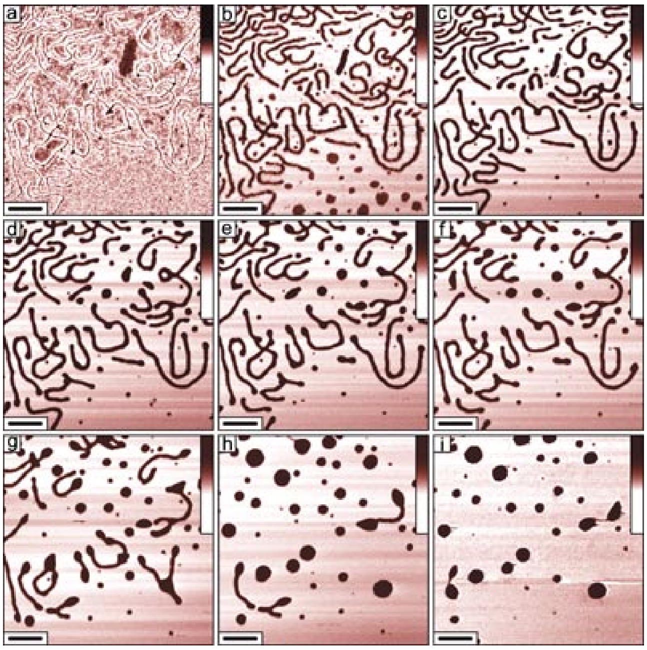

Figure 3. Scanning force microscopy of the different stages of the collapse for long PMA-*g*-P*n*BuA(L) brushes on mica in ethanol vapour. The temperature was kept constant at 24±1°C. a) Initial image of the spin-coated sample thoroughly dried under nitrogen; b) image obtained in absolute ethanol vapour 18 min after injection of absolute ethanol. In the atmosphere saturated by pure ethanol, the molecules swelled but did not collapse. When after 1 h 35 min, a droplet of water was added into the chamber, the collapse of the individual molecules proceeded. c–e) Images recorded at 1 h 47 min (c), 2 h 04 min (d), and 2 h 57 min (e) after the first ethanol injection. At the small water concentration the structural transformation stopped again at the state shown in e, and only when at 3 h 05 min after ethanol injection another droplet of water was added into the chamber total collapse could be observed. f–h) Images obtained 3 h 14 min (f), 3 h 23 min (g), and 3 h 32 min (h) after ethanol injection; i) Final image of the collapsed molecules as arrested after purging the sample chamber with dry N_2. Bar size: 300 nm, height scale: 10 nm.

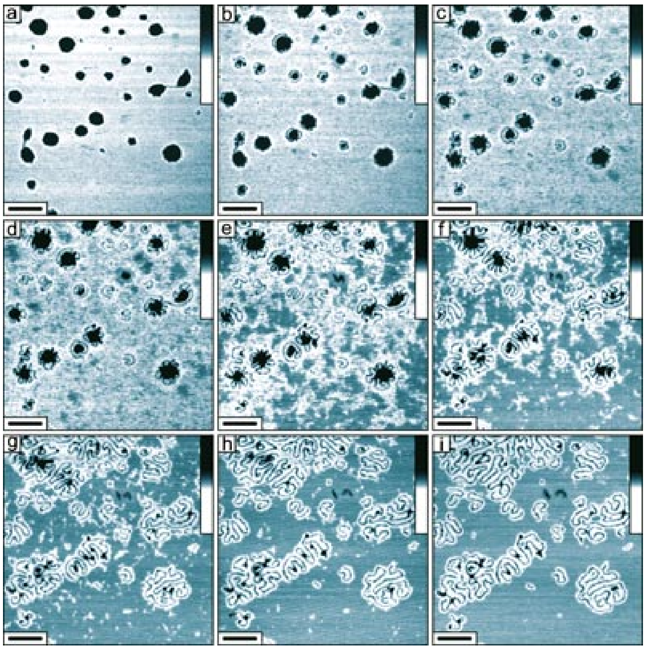

Figure 4. Scanning force microscopy of the different stages of the decollapse of the long PMA-*g*-P*n*BuA molecules on mica in a water saturated atmosphere. The figure depicts the same section as shown in Figure 3 starting with the sample after drying with nitrogen flow. The temperature was kept constant at 24±1°C. a–i) images obtained 13 min (a), 22 min (b), 30 min (c), 39 min (d), 1 h (e), 1.5 h (f), 2 h (g), 4 h (h) and 15 h (i) after water injection. Bar size: 300 nm, height scale: 10 nm.

troduced, the sample had been thoroughly dried in a nitrogen flow to remove any traces of ethanol. One can see how individual strands of the molecules "crawl out" of the globules and untwine. The molecular motion stopped when the molecular strands were separated by a distance of 20–35 nm and had adopted a distinct curvature.[26]

Again, the height values measured in dry atmosphere (in air or in nitrogen) and in water saturated atmosphere did not differ. This indicates little uptake of water, that is, swelling. In contrast to the shorter brushlike molecules, we did not observe a noticeable change in the contour length after the collapse decollapse cycle humidified molecules. This is consistent with the model described in reference [26], which predicts an uneven side chain distribution only for longer side chains as in the case of the first polymer (Figure 2). Thus, longitudinal shrinkage is expected to be less pronounced here.

The critical role of water for the molecular mobility is demonstrated by the following observation: when the sample with the brush molecules deposited on mica was dried extensively after spin-coating (under N_2 flow), we observed thickening by the uptake of absolute ethanol, but the brush molecules did not start to collapse over a period of at least 12 h. This is similar as described above for HOPG. Some mobility and swelling can be introduced without effecting the collapse. When we added, however, only a small amount of water (see also the discussion of the result shown in Figure 3) into the sample space the collapse occurred within few minutes for those brushes previously swollen in ethanol vapour. Thus, it is concluded that swelling of the brush molecules is not sufficient to introduce the rod–globule transition, but that in addition an ad-layer of water must be formed at the surface of mica.

"Lubrication" or competitive desorption by water is also supported by the following observation. When we attempted to flush the brush molecules from the mica substrate by putting a droplet of a solvent onto the rotating sample in the spin-coater, we found that water was much more efficient in comparison to ethanol or even the good solvent chloroform.

So far we described that wet ethanol vapour favours the collapse, while pure water vapour promotes extension of brushlike molecules. We also wanted went to determine the critical ethanol/water ratio for the transition from the extended to the globular state. Injection of an 80:20% v/v ethanol/water mixture into the environmental chamber was most efficient to effect a fast collapse of the long brush molecules. In this case complete collapse took place over a period of 30 minutes, see Figure 5. Injection of an ethanol/water mixture with 20% vol or less of ethanol into the environmental chamber did not induce collapse, while injection of an ethanol/water mixture with 30% vol of ethanol (or more) induced collapse in half an hour. When the ethanol/water composition was 25:75% v/v, the molecules collapsed but did not formed well-shaped globules. Moreover, over a period of several hours in the same atmosphere, the molecules started decollapsing (very slowly). Such a reversibility effect should be most pronounced near the critical composition. The delayed decollapse can be explained by the different vapour pressures. Ethanol is more volatile than water and depending on the relative amounts of ethanol/water in the vapour, water adsorption at the mica surface can be delayed. When we inject a liquid mixture into the chamber, this will initially result in a relatively higher concentration of ethanol in the vapour phase than in equilibrium. But after the equilibration, the ethanol concentration in the film adsorbed at the mica surface will reflect the composition of the liquid introduced to the sample space. So, we may conclude, that the critical composition for the adsorbed film is also about 30% vol of ethanol. Figure 6 shows equilibrated structures for the long brush molecules adsorbed on mica after they had been exposed to ethanol/water vapour of different composition.

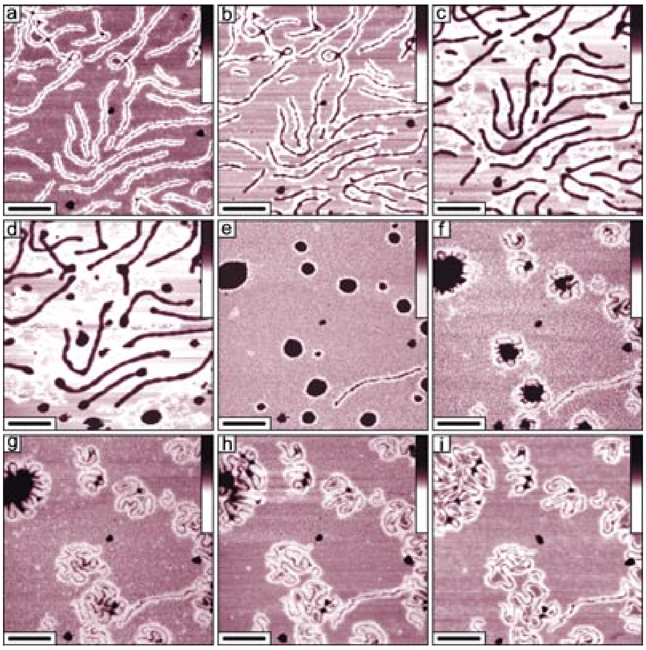

Figure 5. SFM visualization of collapse/decollapse dynamics for long PMA-*g*-P*n*BuA(L) brushes on mica in ethanol/water vapour. a) Initial image of the dry sample as spin cast; b–d) images obtained 9 (b), 18 min (c) and 26 min (d) after injection of 80:20% ethanol/water mixture; e) image of compacted molecules after purging with dry nitrogen; f–i) images recorded 17 min (f), 35 min (g), 1 h (h) and 16 h (i) after water injection. The temperature was kept constant at 24 ± 1 °C Bar size: 250 nm, height scale: 10 nm.

Discussion

The observation of a critical ethanol/water composition and the fact that the collapse was only observed on mica, but not on the less strongly interacting HOPG, is considered a key point for the explanation of the experimental observation. Water is a very poor solvent and ethanol is still a nonsolvent for the PMA-*g*-P*n*BuA brush molecules[38] that can only swell molecules. The comparison of the results on mica and on graphite indicates that the difference in solubility is not the key factor for the extension and collapse, but the interaction of the solvent with the substrate.

As both water and ethanol adsorb strongly at the surface of mica, they also moderate the surface properties of mica

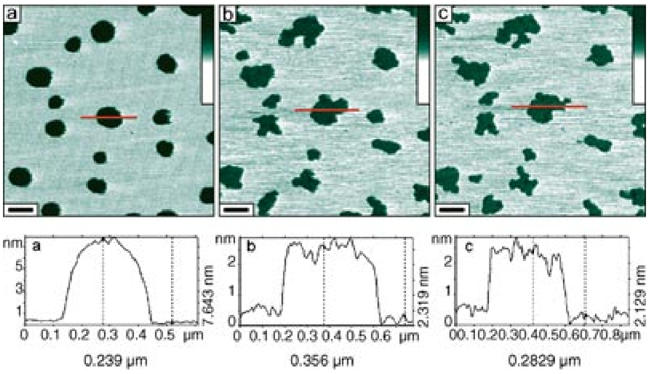

Figure 6. SFM images of collapsed and partially collapsed long PMA-*g*-P*n*BuA brush molecules after equilibration under ethanol/water vapour of different composition. a) Image at 30 % vol of ethanol in injected liquid mixture (60 h after the injection), b) after 24 h at 25 % vol of ethanol (the same place) and c) after 8 h at 20 % vol of ethanol (the same place). The temperature was kept constant at 24° ± 1 °C Bar size: 300 nm, height scale: 10 nm.

strongly. This leads us to the following explanation. The *n*-butyl acrylate units of the side chains compete in adsorption with the water and ethanol molecules. As long as neither water nor ethanol is present, they stick strongly to the surface of mica. Correspondingly, no desorption or collapse of the brushlike macromolecules was observed even at elevated temperature. With water or ethanol introduced into the atmosphere, an ad-layer is formed on top of the mica surface and in between the mica and the adsorbed brush molecules. This ad-layer moderates the interaction of the brush molecules with the substrate.

In a rather simplistic picture we can describe the adhesion/extension versus desorption/collapse balance by spreading of the molecules at surfaces of different surface energy.

Surface tension of pure water (72 $\mathrm{mN\,m^{-1}}$) is much higher than that of ethanol (22 $\mathrm{mN\,m^{-1}}$). The surface tension of water/alcohol mixture decreases monotonically with an increase of ethanol content and is equal to about 35 $\mathrm{mN\,m^{-1}}$ at 30 % vol of ethanol concentration.[39] The surface tension of poly(*n*-butyl acrylate) is found in the range of 31–34 $\mathrm{mN\,m^{-1}}$.[38] Thus, for the water/ethanol mixture at the point of transition from collapse to decollapse the surface tension of the ethanol/water mixture is similar to that of the brush polymers. If the liquid layer at the surface has a higher water content, surface tension forces will promote flat coverage by the less polar polymer, that is, a monolayer of the side chains. The increase of the ethanol fraction, and, consequently, the decrease of the surface tension in the adsorbed layer reduces the interaction with the substrate and favours the collapse to a swollen globule. These surface tension forces may be regarded as an extra contribution to the total balance of the macromolecule–solvent interaction that becomes dominant in the monomolecular layer. Thus, in contrast to the bulk situation, water is a good two-dimensional solvent for the brush molecules effecting their expansion, while ethanol favours their collapse. Because of the poor interaction of water and ethanol with HOPG these solvents cannot effect the collapse/decollapse at this substrate. It must be noted that this simplistic picture does not take into account other factors that distinguish mica and HOPG. Thus, mica has a polyionic surface densely covered by potassium cations. Furthermore layers of adsorbed water[40,41] and ethanol[42] molecules on mica demonstrate properties that differ significantly from those of the bulk liquids.

Conclusion

We demonstrated, that single-molecule collapse/decollapse processes on a flat substrate can be visualized in real time by SFM operated in a controlled vapour atmosphere. Adsorption of an ultrathin film of liquid adsorbed from the vapour at the surface of mica can significantly influence the structure of an adhering macromolecule and induce a conformational transition. The macromolecule–solvent interaction in ultrathin films fundamentally differs from the interaction in a bulk, because the contribution of surface tension forces is of a great importance in the two-dimensional case. The studied brush molecules collapse in a “two-dimensional” solvent with low surface tension (ethanol), and extend in a “two-dimensional” solvent with high surface tension (water). The controlled collapse and “crawling” back of the isolated brush molecules demonstrates an efficient mechanism for molecular mobility to be developed further in future studies.

The obtained results shed some light on the fact, that macromolecules deposited onto mica are strongly affected by the environmental humidity. With the most used ambient-atmosphere sample-preparation techniques it is impossible to avoid the formation of an ultrathin solvent film.

Experimental Section

The PMA-*g*-P*n*BuA brush molecules were prepared by grafting *n*-butyl acrylate from a poly[2-(2-bromo propionyloxy)ethyl methacrylate] (pBPEM) macroinitiator by using an atom-transfer radical polymerization reaction as described before.[43] The two samples employed in this study differed in the lengths of the macromolecular backbone and of the grafts as well. Table 1 summarized the corresponding degrees of polymerization.

Milli-Q water (Milli-Q Plus 185), ethanol and chloroform of an analytical grade (Merck, > 99.8 %) were used throughout all experiments. Mica (muscovite) and highly oriented pyrolytic graphite (HOPG) were chosen as substrates on which the polymer molecules were deposited by spin-coating from a solution in chloroform ($c = 0.002$–$0.02\ \mathrm{mg\,mL^{-1}}$; 2000 rpm; 25 s) using a WS400 spin processor (Laurell Technologies, USA).

Table 1. Molecular characterization of poly(methacrylate)-*graft*-poly(*n*-butyl acrylate) brush molecules (BPEM: 2-(2-bromo propionyloxy)ethyl methacrylate, *n*BuA: *n*-butyl acrylate)

Name	Macroinitiator, DP	Side chains, DP	Numbers of grafts per monomer in the backbone
PMA-*g*-P*n*BuA(L)	pBPEM, ~3700	*n*-BuA, ~30	> 0.9
PMA-*g*-P*n*BuA(s)	pBPEM, ~400	*n*-BuA, ~60	> 0.9

SFM images were recorded by means of the acoustic mode with a "PicoSPM" scanning probe microscope (Molecular Imaging, USA). In order to introduce water or ethanol vapour to the atmosphere around the sample, we injected 0.3–0.7 mL of water, ethanol or a mixture of both into the microscope environmental chamber (volume of ~1.2 L). The injection was performed without interruption of the scanning procedure through an inlet in the device panel. In order to replace the vapour by a dry atmosphere and to remove also traces of ethanol or water from the sample surface, dry nitrogen (99.999 % purity, MTI IndustrieGase AG, Germany) was blown through the chamber. Topographic SFM-images were collected with an information density of 512 × 512 points at 1 Hz scanning frequency. We used silicon FM-W cantilevers (NanoWorld, Switzerland) with a resonance frequency of 68–87 kHz. Editing of the SFM-images and image analysis was done by means of the PicoScan (Molecular Imaging, USA) and the FemtoScan software (Advanced Technology Center, Russia).

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