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Conformation of single polyelectrolyte chains vs. salt concentration: Effects of sample history and solid substrate

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Abstract

Using AFM single polymer molecule experiments we compare conformations of adsorbed on the mica substrate (in the isoelectric point) polyelectrolyte molecules (PE) of poly(methacryloyloxyethyl dimethylbenzylammonium chloride) at different concentrations of Na_3PO_4 salt. We compare the conformations at solid–liquid interface (1) with the conformations of dry samples obtained if the samples of the adsorbed PE were slowly dried (2) and if the PE chains were rapidly deposited and dried on the surface (3). The experiments demonstrate very similar conformations for the cases (1) and (2), while the case (3) differs substantially. The results suggest that the conformations (1) and (2) are formed under strong influence of the substrate. In all cases we observed very pronounced transitions from the extended coil conformation to the pearl necklace-like globule with an increase of salt concentration. The necklace-globules in the cases (1) and (2) appear at a higher salt concentration as compared to the case (3). The strong van der Waals interactions with the solid substrate stabilize the PE globule.

Keywords: Single molecules; AFM; Polyelectrolyte

1. Introduction

The study of polyelectrolyte (PE) behavior on the level of single molecules opens new possibilities to collect important information for polymer and colloidal science. Behavior of PE chains in solutions and in adsorbed state is crucial for adsorption processes, chromatography and other separation methods, design and applications of new advanced materials and technologies such as drug delivery systems, sensors, responsive materials, catalysts, miniaturized devices, lab-on-chip and microfluidic technologies [1,2]. Scanning probe microscopy (SPM) from the very beginning has attracted great interests as a powerful tool for the study of matter on the level of molecules and atoms [3]. Atomic force microscopy (AFM), as one of major constitutive part of SPM, allows for the visualization of fine conformational details of polymer molecules under air atmosphere [4–9] and under liquid medium [10–14]. In a series of works AFM was used to study conformational transitions of PE molecules [15]. Most of the AFM studies were performed with dry samples of polymer chains deposited from a solution on mica substrates and dried for the AFM experiments on air. There are doubts about these kinds of experiments. The major concern is about the effect of solid substrates on conformation. There are two limiting cases: (1) polymer chains adsorb, and equilibrate on the surface. The dry sample represents an equilibrium conformation of the adsorbed polymer chain. (2) Polymer chains are trapped by the substrate. In the latter case the dry sample reflects the conformation of 3D–2D projected polymer coil. We may speculate that the first case will be observed at strong polymer–substrate interactions, while the second case is the most probable scenario for a weak polymer–substrate interaction.

The second important problem is the effect of surface/-capillary forces on the conformation when solvent evaporates. We may speculate that the effect of solvent evaporation will be much larger for the chains weakly interacting with the substrate. The combination of both the substrate and solvent effects can be formulated as an effect of the sample history on the result of AFM investigations. The same conclusions may be done for SEM and optical microscopy studies of single polymer molecules. Thus, results of single polymer molecule experiments may be less useful and inconclusive because of the sample history effect. The statistical analysis of the polymer chain conformation was used to solve the problem. The idea of

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this approach is to compare dimensions of the chains (radius of gyration, end-to-end distance, and persistence length) obtained from the images with those predicted by theoretical models. Two models have been used: the model of ideal worm-like chain and the model of random walk chain with excluded volume. However, neither of these models can describe the conformation of a real polymer chain at solid–liquid interface exactly. The models neglect the interaction with the solid substrate and can be used in the case of a weak polymer–substrate interaction. The latter case is not interesting for the visualization of dry samples, because the conformation is strongly damaged by solvent evaporation. Thus, an analysis of the experimental chain statistics with the models may be interesting, but the obtained result is not a convincing argument.

Only few examples in literature report on the AFM study of polymer chain conformations in a liquid cell. Recently, we have succeeded in a high resolution visualization of (poly(2-vinylpyridine)) chains on mica surface in situ under aqueous solutions [16,17]. This experience allowed us to compare the conformations of PE chains on the mica substrate using samples prepared in different conditions. Although, comparing the polymer chain conformations will require a quantitative analysis of chain dimensions, in this paper we present the first qualitative glance on the PE chain conformations affected by the sample history. We used three different protocols for the sample preparation. (1) The samples (H1 series) were prepared and visualized in situ in liquid cell of an atomic force microscope when the PE solution was injected in the cell and PE chains adsorbed on the mica substrate. (2) The PE molecules were adsorbed from the solution for 30 min. Afterwards, the solvent (water) was slowly evaporated (H2 series). (3) The deposited drop of the PE solution in 1 min was rapidly removed from the mica substrate with a N_2 gas gun discharge (H3 series). In our experiments we used solutions of poly(methacryloyloxyethyl dimethylbenzylammonium chloride) (PMB) in water (pH3) with added Na_3PO_4 salt. This system is an example of a strong van der Waals polymer–substrate interaction. The earlier publication reported on a very pronounced effect of the salt on the conformation of PMB molecules [8]. The mica has the isoelectric point at pH 3. Thus the substrate charge has no influence on the chain conformation. An increase of the ionic strength of the solution induces the cascade of abrupt conformational transitions due to the intrachain segregation from elongated coil to compact globule conformations through intermediate pearl necklace-globule conformations with different amounts of beads per chain. In this paper we study the phase transition for all three H1, H2, and H3 series of samples.

2. Experimental section

2.1. Materials

Poly(methacryloyloxyethyl dimethylbenzylammonium chloride) (PMB, $M_w = 690{,}000$ g/mol, characterized by NMR, GPC, Viscometry) was synthesized as described elsewhere [18]. Hydrochloric acid (36.5–38%, EM Science, NJ) and Na_3PO_4, 12-hydrate, crystal (99.8%, J. T. Baker, NJ) were used for the preparation of PMB solutions in Millipore water. Muscovite mica of V-1 grade (Structure Probe, PA) was used as received (discs of 15 mm diameter).

2.2. Solutions

A stock PMB solution (0.1 g/L) was prepared in Millipore water at pH 3 (HCl). A series of stock Na_3PO_4 solutions were prepared and adjusted to pH 3 with HCl. All solutions were filtered using a Millex-LCR 0.45 μm (Millipore, MA) filter. The working solutions were prepared by mixing the stock PMB solution (0.5 mL) with the stock Na_3PO_4 solutions (99.5 mL) to obtain the PMB concentration of 5×10^{-4} g/L. pH values of the working solutions were measured after the AFM experiments to avoid contact of the solutions with the surface of the glass electrode of a pH-meter (PE adsorbs on the glass surface, that results in a change of the concentration of the working solution). We excluded glass instruments and containers (mainly polyethylene materials were used) from all preparation steps to avoid adsorption of the polymer molecules on the walls.

2.3. AFM experiments

AFM images were recorded using a MultiMode Scanning Probe Microscope (Veeco Instruments, NY) operated in tapping mode. The Muscovite mica discs were glued to metal 15 mm in diameter supporting disks (Structure Probe, PA) with an epoxy composition. The freshly-cleaved mica discs were used for the experiments. A tapping force of about 98% of the amplitude set point was used to minimize possible effects of the tip on the chain conformation. Conformations of all the molecules visualized retained unchanged for hours of multiple scans in the AFM experiments. Thickness of single polymer chains was measured and averaged over more than 300 molecular fragments for every sample. The fragments were selected from different locations on the images.

2.4. Under aqueous media

AFM experiments were performed using a silicon nitride probes NP (Veeco Instruments, NY) with the radius of 20 nm, the spring constant of 0.32 N/m, and the resonance frequency of ~ 9 kHz under aqueous media. A thermal drift of the scanner was minimized by the incubation of the assembled cell with the mounted mica disc for 0.5–2.5 h to equilibrate heat flows in the cell induced by the laser beam. A mechanical drift induced by the O-ring was decreased by precise placing the O-ring to minimize lateral stresses. The polymer solutions were injected directly into the fluid cell and technically (because of the sequence of the steps: adjustment of laser beam reflection; first manual search of resonance frequency; first ‘rough’ approach of the tip to the surface; tip withdrawal; second adjustment of the resonance frequency near the surface; second ‘fine’ approach to the surface) it was possible to start the

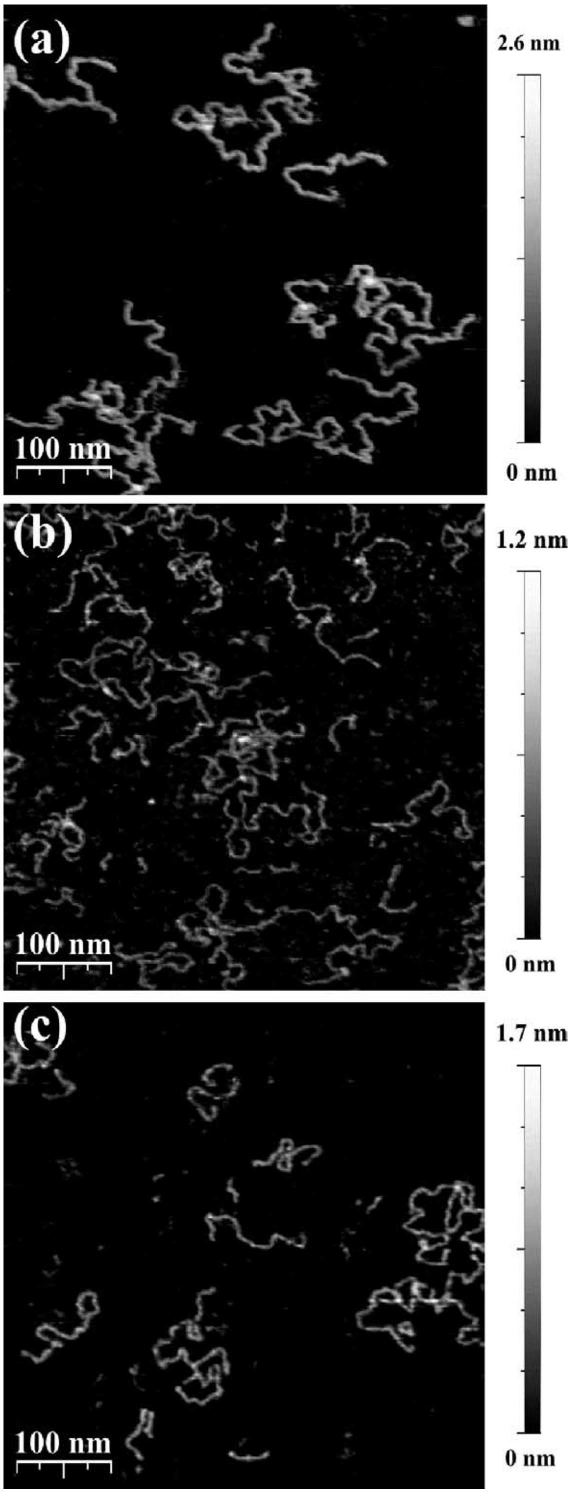

Fig. 1. AFM images of single PMB molecules adsorbed from their 5×10^{-4} g/L solution (pH 3) (no salt added): (a) under aqueous medium (under the solution, from which the molecules were adsorbed); (b) after 30 min equilibration and slow overnight drying; (c) after rapid deposition.

measurements in about 7 min after the injection of the polymer solution. The measurements were performed at amplitude set points ranging from 0.6 to 2.2 V. The tapping force of about 98% of the set points was used to minimize the effects of the tip on the chain conformation.

2.5. Under air atmosphere

AFM experiments were performed using a silicon probes BudgetSensors BS-Tap300 (Innovative Solutions Bulgaria, Bulgaria) with a radius of <10 nm, a spring constant of 40 N/m, and a resonance frequency of 250–300 kHz under air atmosphere. In a one series of the experiments 0.2 mL of the working PMB solution was deposited onto the freshly cleaved mica for 30 min in a humid atmosphere (99% humidity) in a sealed container (in order to minimize evaporation of the drop). Afterward, the sample was rinsed with water and slowly dried overnight in the container (40% humidity). In another series of experiments a drop of the working PE solution was deposited on the mica substrate. The drop was rapidly removed from the substrate in ca 1 min with a N_2 gas gun discharge. This sample (H3 series) preparation is similar to that published elsewhere [8]. The samples were rinsed with water and dried with nitrogen gas. The prepared samples were tested under air atmosphere of $\sim$40% relative humidity at the 1.7 V amplitude set point.

3. Results and discussion

For *non-saline* solutions (Fig. 1) PMB chains were observed in flat extended conformations for all three kinds of samples. The surface concentration of polymer chains decreases in the range H2, H1 and H3 samples, respectively. The same sequence was observed for all experiments reported in this article. This sequence was expected, since the H2 sample had the longest contact with the polymer solution and water. All chains from the solution were deposited on the substrate. The H3 sample had the shortest time of the contact with the polymer solution. We found no significant differences in the chain conformations for all three histories of the samples. All molecules appear as self-avoided extended coils with a few points of a chain superposition. However, the apparent thickness of non-superimposed fragments was different for different samples (Table 1). Such a discrepancy between polymer chain thicknesses obtained in different conditions is known in literature [19]. One of the most probable explanations is the effect of adsorbed water molecules. Water adsorbed on dry samples may change the interaction of the AFM tip with the sample. That may result in the underestimation of the thickness of the molecules. However, the noticeable difference between H2 and H3 sample cannot be explained using that

Table 1
Thickness of single PMB chains of different history as function of salt concentration

Concentration of salt Na_3PO_4 (mM)	Single chain thickness (nm)		
	H1	H2	H3
0	0.69 ± 0.24	0.24 ± 0.10	0.45 ± 0.17
4	0.70 ± 0.20	0.21 ± 0.10	0.38 ± 0.15
8	0.62 ± 0.26	0.31 ± 0.15	0.38 ± 0.18
24	0.49 ± 0.18	0.40 ± 0.13	–

argument, because both the dry samples were scanned in the same conditions. We will discuss this issue below.

Adding the Na_3PO_4 salt (4 mM) results in a contraction of the coils affected by the electrostatic screening and counterion condensation effects (Fig. 2). We observed well-pronounced difference between H3 and H1, H2 samples. The conformation of the PE chains in the H3 samples is much more compact. Moreover, a few beads were observed on the PMB chains exhibiting the initial stages of the coil-to-globule transition studied earlier by Kiriy et al. [8].

Increasing salt concentration (8 mM Na_3PO_4) led to a further condensation of the coils (Fig. 3). The difference between the samples demonstrates the same tendency as mentioned above. The rapidly dried sample H3 shows very compact conformation with well pronounced pearl necklace-globule morphology. Here, we see also an appearance of beads on the H2 sample.

Finally, for the highest Na_3PO_4 concentration 24 mM (Fig. 4) we observed the pearl necklace-globule morphology for the H1 and H2 samples. The sample H3 was found in the shape of compact globules (single beads) with a non-resolved fine morphology. We were not able to measure thickness of the H3 chains in this conformation. The H2 and H3 chains possessed a conformation of a swollen strongly compressed globule. The fine morphology of these globules is well resolved on the AFM images.

Consequently, the series of experiments demonstrates very pronounced difference between, on the one hand, the rapidly dried samples H3 and, on the other hand, the H1 samples measured in situ under solution or the slowly dried H2 samples. The H3 samples are more sensitive to ionic strength of the solutions. At high salt concentrations the H3 samples produce compact globules, while the H2 and H1 samples show some kind of a ‘delay’ in their response to increased ionic strength. We explain this difference by the different states of the polyelectrolyte molecules in these samples. The H3 samples were obtained by a rapid deposition of polymer molecules. We may speculate that they retain in a collapsed in *Z*-direction solution conformation of the chains, because they are trapped on the surface by the rapid deposition (due to the rapid evaporation of water). The samples H1 and H2 represent the adsorbed conformations formed under a strong effect of the interactions with the substrate. From the comparing of the H1 and H2 samples we may conclude that solvent evaporation has no noticeable damaging effect on the conformations. The results demonstrate that coil-to-globule transitions in solution and at interface are different. This difference is introduced by a strong van der Waals interaction of polymer chains with the substrate. The solid–polymer interactions results in the compression of the polymer globules. The formation of the beads was observed at a higher salt concentration when the hydrophobic interactions are stronger than the intrachain electrostatic repulsion and the interaction with the mica substrate.

Table 1 presents the collection of values of chain thickness for all experiments. As it was mentioned above the very pronounced difference between, on the one hand, H1 samples and on the other hand, H2 and H3 samples could be explain by a principally different interaction of the AFM tip with the substrate in aqueous environment and on air. However, the difference between H2 and H3 samples rather involves some

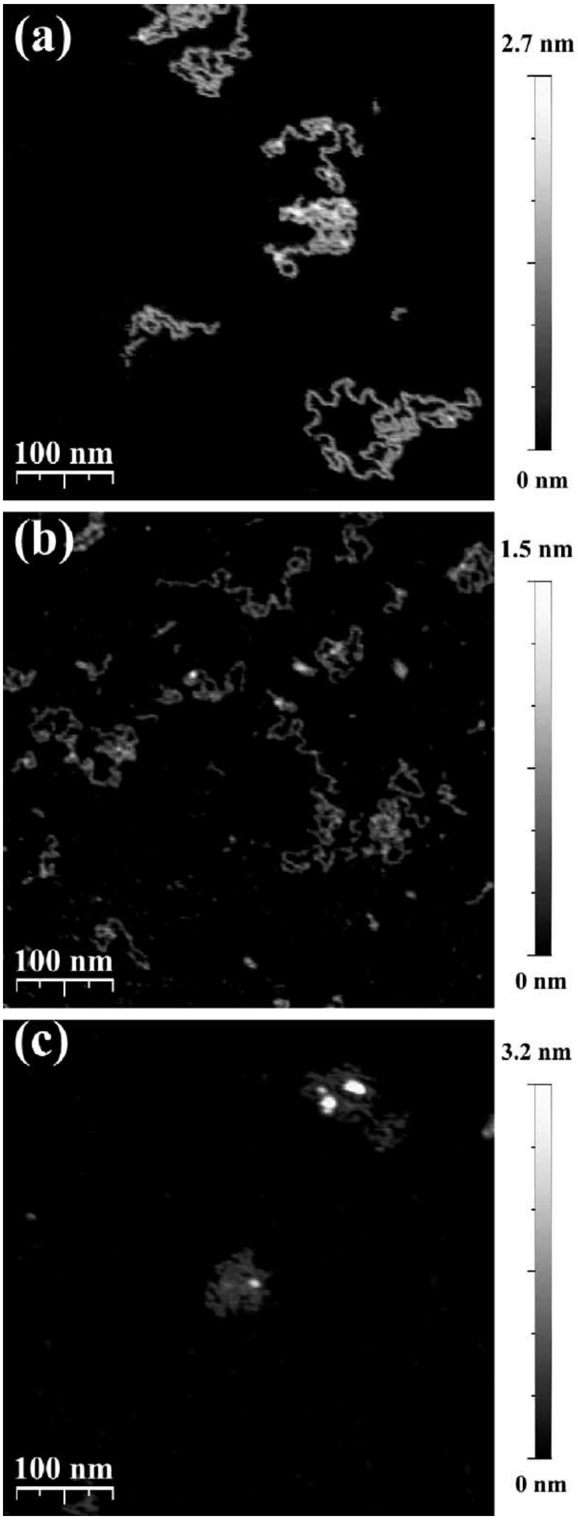

Fig. 2. AFM images of single PMB molecules adsorbed from their 5×10^{-4} g/L solution (pH 3) containing 4 mM of Na_3PO_4: (a) under aqueous medium (under the solution, from which the molecules were adsorbed); (b) after 30 min equilibration and slow overnight drying; (c) after rapid deposition.

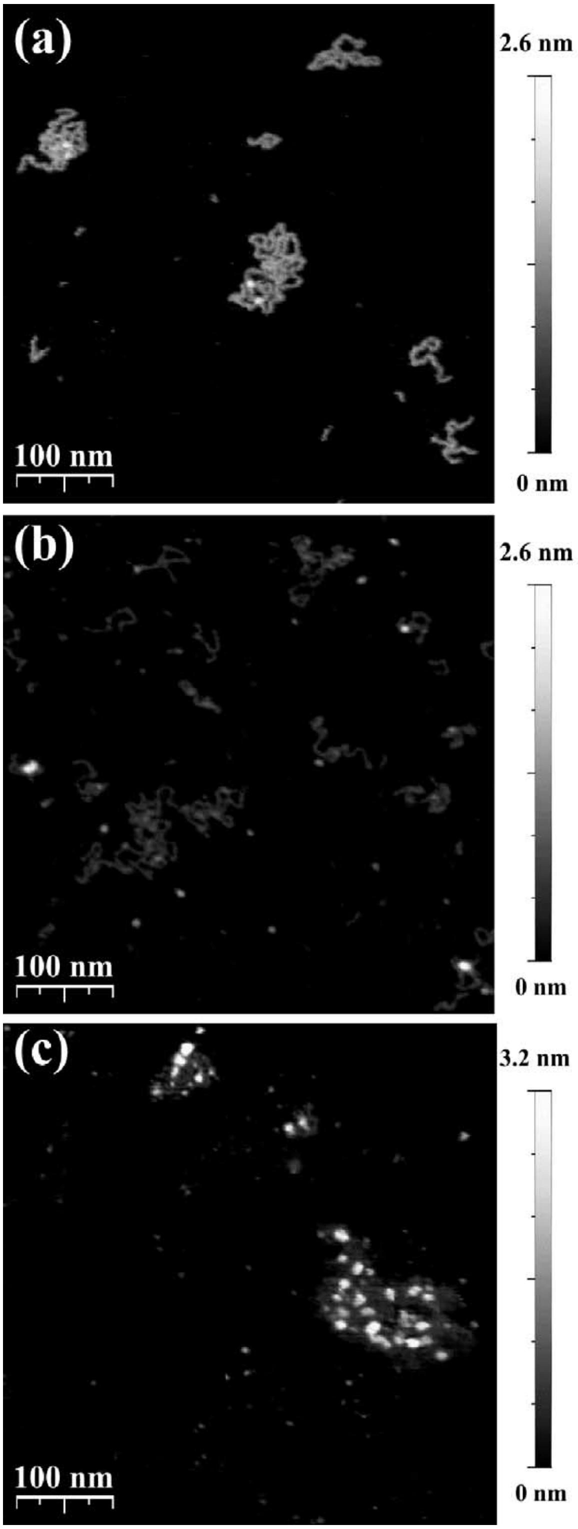

Fig. 3. AFM images of single PMB molecules adsorbed from their 5×10^{-4} g/L solution (pH 3) containing 8 mM of Na_3PO_4: (a) under aqueous medium (under the solution, from which the molecules were adsorbed); (b) after 30 min equilibration and slow overnight drying; (c) after rapid deposition.

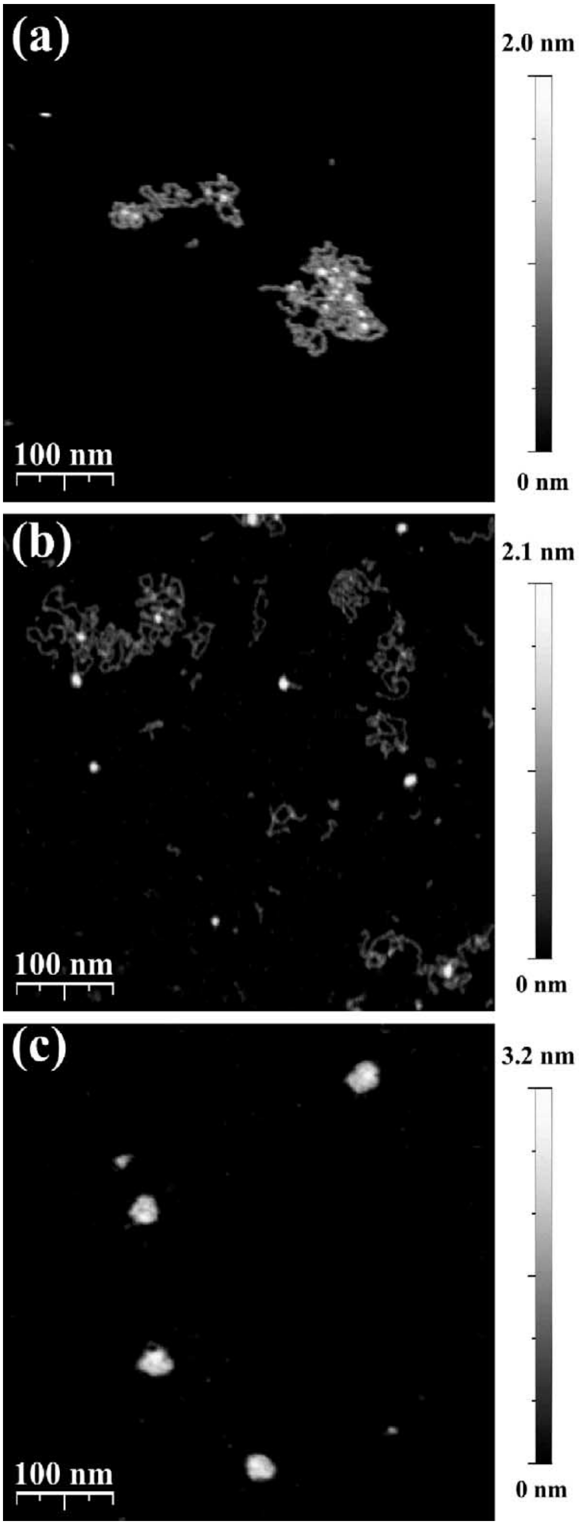

Fig. 4. AFM images of single PMB molecules adsorbed from their 5×10^{-4} g/L solution (pH 3) containing 24 mM of Na_3PO_4: (a) under aqueous medium (under the solution, from which the molecules were adsorbed); (b) after 30 min equilibration and slow overnight drying; (c) after rapid deposition.

other arguments. We may speculate that for H2 samples the interaction with the substrate affects a ‘flattening’ of the backbone when the side groups are rearranged to approach an optimal interaction with the substrate. The rapidly deposited molecules in H3 samples experience glass transition. They cannot approach an equilibrium conformation on the surface. Thus, they are deposited in the backbone conformation which is similar to the solution conformation.

All three samples demonstrate tendency to change the chain thickness, if salt concentration increases. An increase of salt concentration results in a screening of electrostatic interactions, and condensation of counterions. Both these effects will change the interaction of the AFM tip with the polymer chain in aqueous environment. Consequently, the balance of the interactions for H1 samples results in a decrease of an apparent chain thickness. Both H2 and H3 samples are interacting with the tip in the same conditions; however, they show opposite tendencies for the thickness change vs. salt concentration. We may speculate that the thickness change is affected by the sample history. For H3 samples the interaction with the substrate is less important and the thickness decrease may be explained by a decrease of swelling degree of the backbone at high salt concentrations. We may expect that charged backbone will have higher salvation number (‘swelling’) as compared to less charged polymer chains. Thus, the increase of salt concentration may course a ‘deswelling’ of the backbone in that moment when the chain was deposited and frozen by solvent evaporation. For H2 samples the interaction with the substrate is very important. The counterion condensation inhibits the ‘flattening’ of the backbone in the adsorbed state in contrast to the non-saline solution. This difference in sample history for H2 and H3 samples can be a reason for the inverse relationships of chain thickness vs. salt concentration observed in the experiments.

4. Conclusions

The sample history demonstrates very pronounced effect on the single polymer chain conformations visualized in the AFM experiments. Rapidly deposited polyelectrolyte chains are likely in *Z*-direction collapsed chain conformations which are less deformed by the interaction with the substrate. Slow adsorption of the polymer on the mica substrate results in the conformations formed under the strong effect of the interfacial interactions. The difference between the samples is very pronounced in terms of the chain shape, size, and backbone thickness. Slow water evaporation has no damaging effect on the chain conformation in the *X*–*Y* plain for the investigated cases.

An increase of salt concentration induces the chain-to-globule phase transition. The intermediate states can be described as pearl necklace-like globules deposited from saline solutions. A rapid deposition brings very dense globules on the surface, while adsorbed chains are rather in a swollen globule conformation. The beads in the necklace-like globules have a height which corresponds to the double polymer chain thickness and originate from the chain superposition in the globules. The adsorbed polymer undergoes the coil-to-globule transition at higher salt concentrations as compared to the chains in the solution.

The experimentally measured thickness of the polymer chains is also affected by polymer history and salt concentration. The equilibrated on the surface chains are thinner than the chains rapidly deposited from solutions.

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