Communication

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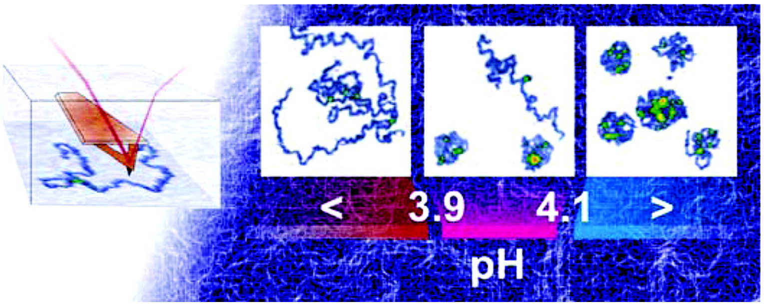

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AFM Single Molecule Experiments at the Solid−Liquid Interface: In Situ Conformation of Adsorbed Flexible Polyelectrolyte Chains

Yuri Roiter and Sergiy Minko*

Department of Chemistry, Clarkson University, 8 Clarkson Avenue, Potsdam, New York 13699-5810

Received August 24, 2005; E-mail: sminko@clarkson.edu

The study of conformation details of polymer chains adsorbed at the solid−liquid interface addresses a range of important questions: interactions with solid surface, dynamics of adsorbed chains, and changes affected by environmental conditions, such as pH, ionic strength, and solvent quality, all of which are of vital importance for material science.[1] Due to resolution constraints, the optical microscopy is limited to the study of giant biological molecules.[2] Atomic force microscopy (AFM) is the only existing method for direct 3D visualization of polymer chains.[3,4] In this work, we extend AFM methods to monitor conformations of flexible synthetic polymer molecules in situ at the solid−liquid interface. We study single poly(2-vinylpyridine) (P2VP) protonated cationic polyelectrolyte chains adsorbed on an oppositely charged mica surface under aqueous medium at different pH (Figures 1and 2).

Protonated P2VP is a weak hydrophobic polyelectrolyte. Water is a poor solvent for P2VP. The protonated polymer is soluble in water due to release of counterions. Conformation of the P2VP coil is balanced by Coulomb repulsion and short-range van der Waals attraction. As the degree of protonation decreases or the solution ionic strength increases, extended polymer coils undergo the sharp coil-to-globule phase transition. Studying P2VP solution properties, Puterman et al.[5] found that the phase transition took place in a very narrow pH range around pH 4. AFM single molecule experiments revealed abrupt conformational changes at 0.02 M NaCl solutions at pH 3.5.[6] The transition passes through an intermediate state when polymer globules split into necklace-like conformations (dumbbell, trimbell, etc. structures).[7] For adsorption of hydrophobic polyelectrolytes, theory predicts a quite complex phase diagram because of the additional effect of the surface charge density of the adsorbent.[8] Both the regime of a 2D extended coil and the regime of a 3D compressed globule, depending on the surface charge density, contributions of nonelectrostatic interactions with the substrate and solvent quality were predicted theoretically. However, direct study of the conformations of the adsorbed polyelectrolyte chains was done only for giant rigid DNA molecules.

The experiment was conducted as follows. A freshly cleaved disk of the V-1 grade muscovite mica (Structure Probe, PA) was mounted in a liquid cell of a MultiMode SPM (Veeco Instruments, NY). The cell was filled with 10^{-3} g/L P2VP ($M_n = 152\,000$ g/mol, $M_w/M_n = 1.05$, secondary standard, Aldrich) aqueous solution at the given pH value. The first scan was made 7 min after the contact of the solution with the mica substrate. The sample was scanned using NP (Veeco Instruments, NY) silicon nitride probes with a resonance frequency in aqueous media of ~9 kHz at an amplitude set point in the range from 0.6 to 2.2 V and the tapping force of about 98% of the set point. We have demonstrated that under these conditions the sample was not affected by the probe (see Supporting Information for more experimental details).

The self-developed software[9] was used for processing the images to quantify the characteristics of chain conformations at the solid−liquid interface (see Supporting Information).

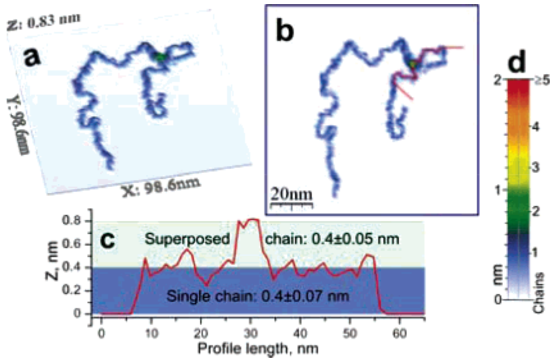

Figure 1. Single P2VP molecule adsorbed on mica under pH 3 aqueous solution: (a) 3D AFM image, green zone corresponds to the chain self-superposition; (b) 2D AFM image, red curve denotes the profile shown in (c). (d) Z-scale bar shows a number of superposed chains with an assumption of 0.4 nm height increment.

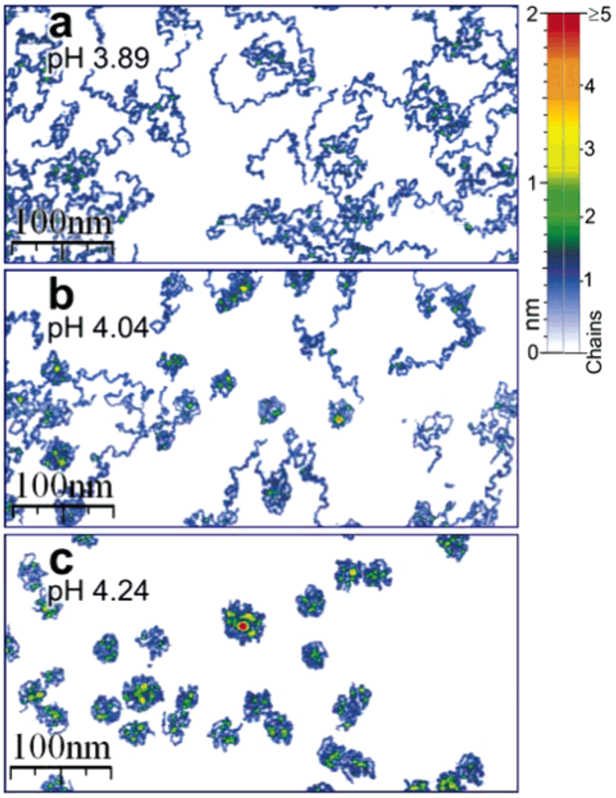

Figure 2. AFM-visualized conformations of adsorbed P2VP molecules: (a) pH 3.89, extended coils; (b) pH 4.04, intermediate state; (c) pH 4.24, compact coils. Z-scale bar shows a number of superposed chains assuming the height increment of 0.4 nm.

The P2VP molecules appear in the AFM images as ~0.4 nm thick coiled chains (Figure 1). The images reveal loops, which were

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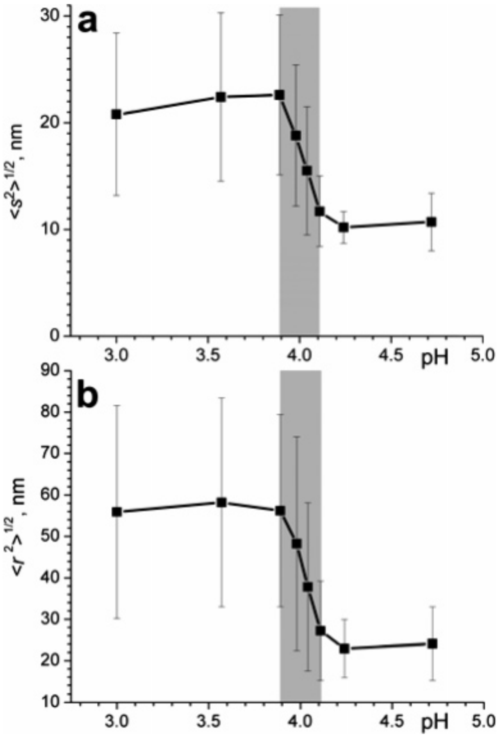

Figure 3. Directly measured values of rms end-to-end distance (a) and rms radius of gyration (b) of P2VP single molecules adsorbed on mica surface versus pH. The gray zone is a pH range of the conformation transition. All lines are given to guide the reader. Standard deviation bars are the estimations of characteristic distributions.

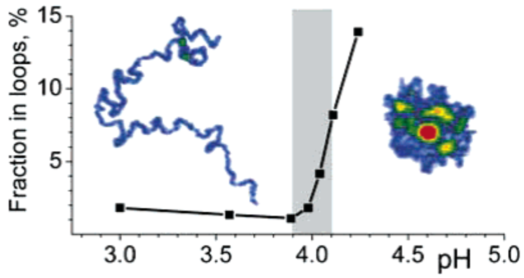

Figure 4. Fraction of monomer units in loops versus pH. The gray zone is a pH range of the conformation transition. Line is given to guide the reader.

identified as points of chain self-superposition. In these points, the cross-section (Figure 1c) is 2 times thicker (~0.8 nm). Subsequent scans within 3 h showed no change of the conformation. Very rarely, we observed small changes in the conformation of some fragments; however, the number of these events was negligibly small.

At pH 3.0−3.9, ionized P2VP in less than 10 min approaches a flat conformation (Figures 1 and 2a) with root-mean-square (rms) radius of gyration ($\langle s^2\rangle^{1/2}$) $\approx$ 22 nm, rms end-to-end distance ($\langle r^2\rangle^{1/2}$) $\approx$ 55 nm (Figure 3). The conformation stays unchanged for hours. No diffusion, exchange, or desorption takes place. Variation of the surface charge of mica[10] from the isoelectric point (pH 3) to strongly charged state (pH 3.9) has no effect on the conformation. That suggests a strong dominating van der Waals interaction with the substrate surface. Less protonated P2VP (pH > 4.1) possesses a conformation of a strongly compressed, in *Z*-direction, 3D compact coil (globule) (Figure 2c) with a small fraction (up to 15%) of segments in loops (Figure 4). The adsorbed globules are immobile. Obviously, strong van der Waals intramolecular and polymer−substrate interactions stabilize the conformation. In the intermediate regime ($3.9 < \mathrm{pH} < 4.1$), both the flat extended and compact coils coexist (Figure 2b). Some molecules are in conformations that resemble "ball with a tail" or dumbbell-like conformations reported earlier for the dried samples.[6] The values of $\langle s^2\rangle^{1/2}$ and $\langle r^2\rangle^{1/2}$ decrease by factor of 2 (Figure 3). At the same time, we obtained a sharp increase of the fraction of segments in the loops (Figure 4 inset). In some points, the thickness of the globule approaches 2 nm.

Presented here are results that are in good qualitative agreement with theory, which predicts the transformation of adsorbed PE chains from an extended coil conformation through transition state to a compressed globule as the ionization degree decreases.[8] However, there are several unexpected experimental facts. *First*, the adsorbed chains are "frozen" by a strong interaction with the mica. *Second*, number-averaged (over 400 molecules) contour length of the adsorbed molecules, $L_C = 204$ nm, is about 30% shorter than the value of 310 nm estimated from P2VP molecular weight and the size of monomer unit (0.219 nm for P2VP 3_1-helix[11]). Thus, we may speculate about a "wavy" substructure that is not resolved by AFM (similar to that observed for thicker molecules by Rabe et al.[3]). Such a substructure may be formed via the rotation around the backbone, adapting a P2VP spatial helix to optimal position on an oppositely charged mica surface (see Supporting Information). The conformation of a charged adsorbed chain supposedly reveals two levels of organization. The upper level is a flat polymer coil, and the hypothetical lower level formed by the "wavy" substructure constituted several monomer units.

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Supporting Information Available: Experimental details, pH versus HCl in P2VP solution, effects of the AFM tip and experiment run time on chain conformations, data processing, scheme for the "wavy" structure, screenshots of molecules processed, and complete ref 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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