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Investigation of a stimuli-responsive copolymer by atomic force microscopy

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Abstract

The aim of this study was to investigate the stimuli-responsive behaviour of a pH- and temperature sensitive polymer system, i.e. a random copolymer of *N*-isopropylacrylamide and acrylic acid by atomic force microscopy (AFM). AFM allowed high resolution images of the physical states of the polymer chains at different conditions. Different collapsed and extended chain conformations of a random copolymer were seen over a wide range of pH and temperature. The shapes of both the individual copolymeric chains and globules can be clearly identified. Here we demonstrate the potential of AFM for characterising the stimuli responsive behaviour of such "smart" polymers. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: AFM; Stimuli-responsive copolymer

1. Introduction

The atomic force microscope and its application to the investigation of polymer structure and surface properties has continued to grow in recent years. This technique can address many surface-related problems in polymer science [1–4]. The atomic force microscope has been used in the investigation of polymer morphology, polymer degradation, polymer–biomolecule interactions, surface manipulation and the mechanical properties of polymers [5–7]. Frazier et al. employed AFM for the first time to measure the topographical changes due to the hydration–dehydration states of individual dextran molecules using a monolayer film [8]. The distinct benefit that AFM offers is the ability to differentiate three-dimensional structures of polymers by direct observation.

Intelligent polymer systems have recently been of increasing interest in biomedical and biotechnology fields including drug delivery systems [9,10], artificial organs [11], control of protein-ligand recognition [12], sensors [13], affinity separations, and immunoassays [14,15]. These polymers respond to changes in external conditions by undergoing large and sharp reversible phase transitions [16–19]. During phase transition, a polymer solution turns from transparent to cloudy, which indicates the changes in polymer chain conformation. It would be very advantageous for potential applications of these polymer systems to be able to observe directly their conformational changes caused by the changes in environmental conditions. In this paper, we show that AFM is capable of imaging the actual physical states of polymer chains at nanometer- or subnanometer scale resolution as a function of temperature and pH by employing a pH- and temperature-sensitive polymer which is a random copolymer of *N*-isopropylacrylamide (NIPAAm) and acrylic acid (AAc).

2. Experimental

2.1. Materials

NIPAAm (Eastman Kodak, Rochester, USA) was purified by recrystallisation from *n*-hexane. AAc (Aldrich, Milwaukee, USA) was redistilled in vacuum. Dimethylformamide (J.T. Baker, Phillisburg, USA) was used without further purification. 2-Mercaptoethanol (ME, 98%, Aldrich, Milwaukee, USA) was redistilled before used. 2-2′ Azoisobutyronitrile (AIBN, J.T. Baker, Phillisburg, USA) was recrystallised from methanol. All other reagents used were of analytical grade.

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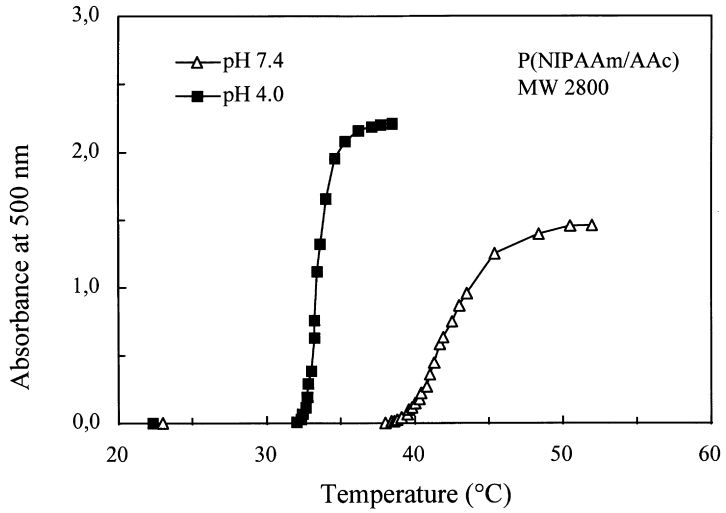

Fig. 1. Absorbance of 0.2 wt% solutions of poly(NIPAAm/AAc) in citric acid-phosphate buffers (CPB, 50 mM) at pH 7.4 and pH 4.0 vs. temperature. The temperature at 10% of maximum absorbance was defined as the LCST.

2.2. Copolymer synthesis

Hydroxyl terminated random copolymer of NIPAAm and AAc was prepared by free radical chain transfer copolymerisation of NIPAAm and AAc in dimethylformamide at 60°C for 1 h using AIBN and ME as free radical initiator and chain transfer agent, respectively. The molar ratio of NIPAAm/AAc/ME/AIBN in the polymerisation recipe was 100/4/4/0.5. At the end of the copolymerisation time, the copolymer was precipitated in diethylether, filtered through a sintered glass filter, washed several times with diethylether, and dried in vacuum at 40°C for 24 h. The copolymer composition was analysed by dissolving it in an excess of 5 mM NaOH to neutralise the carboxyl groups and then back-titrating the excess of NaOH with 5 mM HCl. Molecular weight (MW) was determined by vapour pressure osmometry (VPO, model OSV111 from Knauer, Germany). The temperature- and pH-sensitive behaviour of the copolymer was studied by spectrophotometrically determining the absorbance of the polymer solutions at pH 4.0 and 7.4 as a function of temperatures at a fixed wavelength (500 nm). A UV-vis spectrophotometer (Bausch & Lomb Spectronic 1001, USA) with a jacketed cuvette holder was used to determine the phase separation temperature, also called the lower critical solution temperature (LCST). The heating rate was 0.2°C/min. 0.2 wt% solutions of copolymer in citric acid-phosphate buffers (CPB, 50 mM) at pH 4.0 and pH 7.4 were employed and the ionic strength of the solutions was maintained by adding appropriate amounts of NaCl. The temperature at 10% of maximum absorbance was defined as the LCST.

2.3. Atomic force microscopy

The atomic force microscope used in this study was an East Coast Scientific instrument (ECS Ltd., Cambridge, UK) and was operated in contact (dc) mode. The tips used were the short narrow variety of Nanoprobe™ cantilevers (Digital Instruments, Santa Barbara, CA) with a nominal force constant of 0.38 nm^{-1}. Images were manipulated using ECS Image™ software and colour has been added to the images using Photoshop 3.0 (Adobe Systems) to enhance discrimination of fine detail. For AFM experiments, the sample solutions at pH 7.4 and 4.0 were prepared by dissolving poly(NIPAAm/AAc) in pure distilled water and adjusting the pH of the solutions to the appropriate level using either HCl or ammonia, both of which are sublimible and so would not be present on the mica after drying of the solutions. The final concentration of the samples was 0.1 mg/ml. 2 μl drops of the sample were deposited onto freshly cleaved mica, which was heated to the same temperature as the sample solution. The temperatures studied were as follows; 40°C and 33°C for sample solution at pH 7.4 and 4.0, respectively, and 20°C (room temperature) for both of two solutions. The samples were then imaged under butanol with no further treatment after drying process ($\approx$ 5 min) in air at the same temperature with the sample solution. The reason why the polymers were imaged under butanol was to prevent damage or displacement by eliminating adhesive forces between the AFM tip and the mica. This also eliminates the humidity effect which occurs in ambient conditions.

3. Results and discussion

In this study, we synthesised a random copolymer of NIPAAm and AAc containing 5.5 mol% AAc with the number average molecular weight of 2800. The AAc content of the copolymer is of major importance regarding the temperature sensitivity at pHs above the pK_a of AAc. Copolymers of NIPAAm with AAc should have

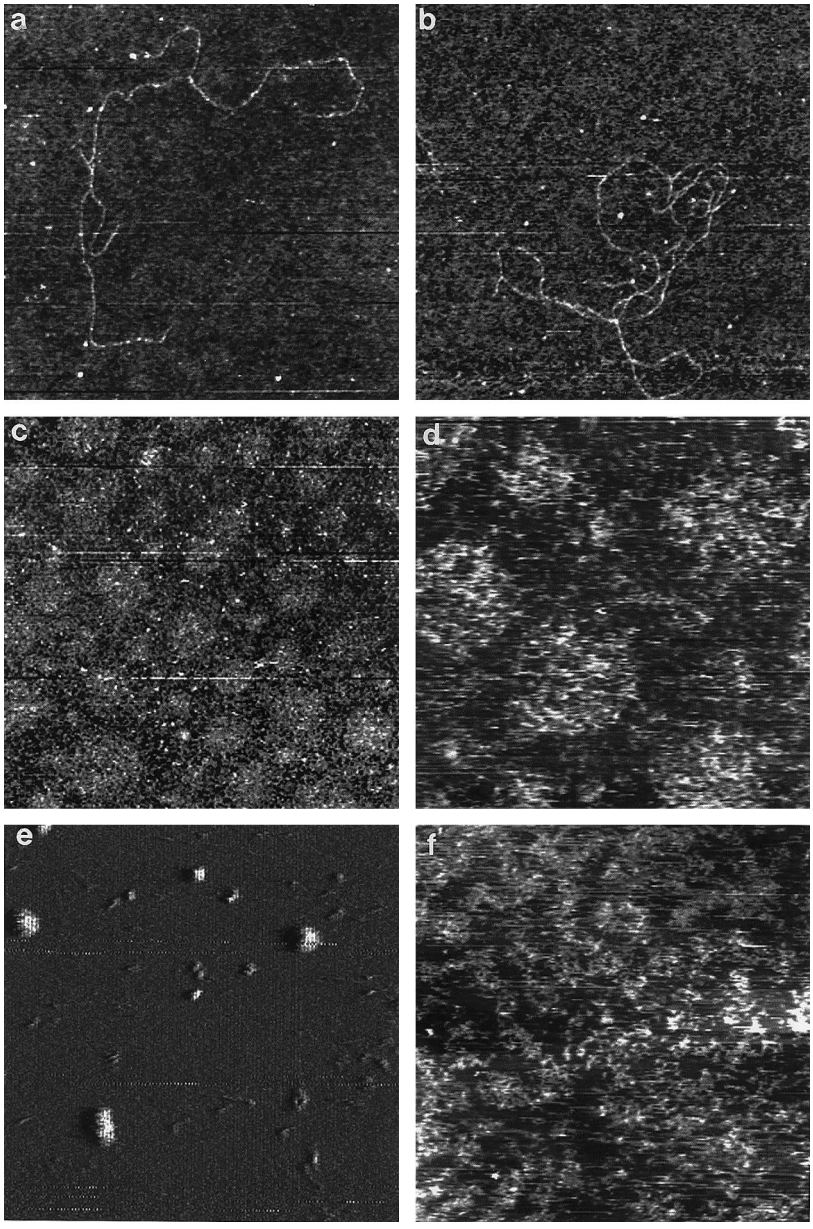

Fig. 2. (a) AFM image of poly(NIPAAm/AAc) copolymer chains at pH 7.4 and 20°C, image size 1.6 × 1.6 μm; *z*-scale of 46.6 nm. (b) Another area of the same sample, image size 1.6 × 1.6 μm; *z*-scale of 42 nm. (c) AFM image of poly(NIPAAm/AAc) copolymer chains at pH 4.0 and 20°C, image size 1.6 × 1.6 μm; *z*-scale of 46 nm. (d) AFM image of poly(NIPAAm/AAc) copolymer chains at pH 4.0 and 20°C, image size 0.6 × 0.6 μm; *z*-scale of 23 nm. (e) Error signal mode AFM image of poly(NIPAAm/AAc) chains at pH 7.4 and 40°C, image size 1 × 1 μm; *z*-scale of 35 nm. (f) AFM image of poly(NIPAAm/AAc) chains at pH 4.0 and 33°C, image size 1.2 × 1.2 μm; *z*-scale of 68 nm.

an LCST above that of homo-poly(NIPAAm) (32°C) at all pHs, because both the acidic and ionic states of the AAc monomer units are more hydrophilic than NIPAAm units [20,21]. For high AAc content, as pH is raised above the pK_a of AAc, the LCST is not observed simply due to the ionisation of the carboxylic acid groups to carboxylate groups. Random copolymer of NIPAAm and AAc containing 5.5 mol% AAc retained its reversible phase transition over useful temperature and pH ranges, especially at physiological conditions (such as pH 4.0–7.4 and 37°C) suggesting possible applicability of the polymer in biomedical systems and biotechnology.

Fig. 1 shows the effect of temperature on the phase transition behaviour of poly(NIPAAm/AAc) chains in solutions at pH 4.0 and pH 7.4. Raising the temperature causes release of hydrophobically bound water and increases both the intra- and inter-chain hydrophobic interaction, which leads to collapse and aggregation of polymer chains. Thus, phase separation of the polymer solution occurs, causing a sharp increase in the absorbance. At constant ionic strength, increasing pH leads to an enhanced hydrophilicity of the

copolymer chains due to an increase in the [COO^-/COOH] ratio in the copolymer. This produces a higher phase transition temperature of the copolymer solution. LCSTs of poly (NIPAAm/AAc) were found to be ca. 33°C and 40°C at pH 4.0 and pH 7.4, respectively. At pH 7.4 and 20°C, the copolymer chains are expected to be completely in their hydrated state and have an expanded coil conformation, because this temperature is less than the LCST of the copolymer at pH 7.4 (i.e. 40°C), and also the effect of ionic repulsion between chains due to the ionic state of AAc units in the copolymer is significant [17,18,22]. Fig. 2a and b is representative AFM images of NIPAAm/AAc copolymer chains which clearly mirror the highly extended nature of the chains. However, the molecular dimensions observed by AFM reveals that the process of association between extended chains occurs. Although the solutions initially prepared for this study were diluted, drying the solutions during sample preparation does lead to an increase in the polymer concentration and also an increase in the inter-chain interactions. It is possible that this process will induce the high degree of association observed by AFM. At this point, it is worth to note that drying process does not cause any preferential orientation of polymer chains on the mica substrate as seen in Fig. 2a and b which are the images taken from different areas of the same sample. Line profiles taken across the image suggest a polymer height of ~1–2 nm with an appropriate doubling of height (2–4 nm) at cross-over points in the polymer chains. The width of a single polymer chain is ca. 9.3 nm. This value is much larger than would be expected for an individual polymer chain and is probably the result of the finite size of the AFM tip with respect to the molecular width. The radius of curvature of the AFM tip is typically 10–30 nm, i.e. some 10–20 times larger than the molecular width [23]. The result of this size mismatch is that the profile, which the tip traces out as it passes over the molecules is correspondingly broadened. Side-to-side association between overlapping chains may also cause overestimation of molecular thickness. The individual chains have different length, which reflects the molecular size distribution of the copolymer. From AFM data the average end to end distance, r, of a single copolymer chain is measured to be ca. 300 nm which is ~2 orders of magnitude longer than any reasonable estimate of r by using conventional models for molecular size calculations [24,25]. This result suggests that there are interactions between chain ends or between overlapping chains leading to relatively long strands. In addition, interpretation of the predicted value of r using conventional models, such as the equivalent freely jointed chain model, appears at odds with the highly extended chains as those seen in Fig. 2a and b. Because the interaction between the chain segments and solvent molecules is strongest for the copolymer molecule in its fully extended state, i.e. the number of polymer segment-solvent contacts is the highest, the actual chain dimension of the copolymer molecule is expected to be much higher than its unperturbed dimension calculated from the equivalent freely jointed chain model.

At pH 4.0 and 20°C, the copolymer chains had a globular appearance in the AFM images (Fig. 2c and d), although the solution was found to be completely transparent under these conditions (Fig. 1). At low magnification (Fig. 2c) globular aggregates with a range of dimensions were observed. The higher magnification image shown in Fig. 2d confirms this. The sizes of globules are calculated to be approximately 90–250 nm. At room temperature the transparency of copolymer solution at pH 4.0 suggests that the globules formed under these conditions are highly hydrated. Extensive hydration may be simply due to the high content of the thermosensitive component, NIPAAm, in the copolymer. This will not allow the polymer chains to collapse at room temperature, which is lower than LCST of homopoly(NIPAAm), even though the value of pH is below the pK_a value of the pH sensitive component, AAc [26]. From the AFM image (Fig. 2d) the aggregate structures do not appear to be densely packed, which may explain the transparency of copolymer solutions at pH 4.0 and room temperature.

Fig. 2e shows an AFM image of poly(NIPAAm/AAc) chains at pH 7.4 and 40°C. Since the copolymer at pH 7.4 exhibits a LCST at 40°C, it is expected that the chains will start to collapse and tend to aggregate. This is due to the more hydrophobic character of NIPAAm units at 40°C. The globular structures seen in Fig. 2e are more densely packed (having sizes ranging between 42–108 nm) than those observed in Fig. 2c and d. However these copolymeric chain globules do not exhibit any further aggregation behaviour. This may result primarily from the ionic interactions between copolymer chains. The COOH groups of AAc units are strongly ionised at pH 7.4, which leads to ionic repulsion between copolymer chains and prevents them from aggregating. It should be noted that the polymer solution at these conditions just begins to become turbid, but does not exhibit any significant phase transition yet. At pH 4.0 and 33°C, both the pH- and temperature-sensitive components of the copolymer favour phase-separation. Release of hydrophobically bound water from NIPAAm units and the presence of the COOH form of the AAc units give a more hydrophobic character to the entire copolymer chain. It may be expected that inter- or intra-chain hydrogen bonds form between COOH groups of AAc units. This may result in bonding of the chains, and lead to clouding of the solution. The physical state of the copolymer chains exhibiting this behaviour was also obtained by AFM imaging and is shown in Fig. 2f. The image appears to show a random distribution of small chains of dimensions consistent with individual copolymers.

4. Conclusions

AFM has proved to be a unique and useful microscopic technique to gain direct information about the stimuli-responsive behaviour of an intelligent polymer system.

The images obtained in the present study provide a clear demonstration of how the technique may be used to study physical states and conformational changes of a stimuli-responsive polymer on the nanometer scale. Intelligent polymer systems may have widespread utility, particularly in medicine and biotechnology. An increased understanding of the behaviour of such polymers at the molecular level and their conjugates with other molecules, i.e. proteins, enzymes, oligopeptides, may lead to additional or enhanced applications.

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