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Polymer 48 (2007) 2030–2034
polymer
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The unwinding of surfactant-induced helical structure of carboxymethyl amylose by single molecule force spectroscopy

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Received 12 December 2006; received in revised form 6 February 2007; accepted 13 February 2007
Available online 16 February 2007

Abstract

We have employed atomic force microscopy (AFM)-based single molecule force spectroscopy (SMFS) to investigate the unwinding process of the surfactant-induced helical structure of carboxymethyl amylose at the single-molecular level. In doing so, the nanomechanical fingerprint for the conformational transition of carboxymethyl amylose at about 270 pN is used as an indicator for identifying the single chain elongation. Upon the addition of NaCl and cetyltrimethylammonium bromide (CTAB), the force–extension curve of carboxymethyl amylose appears as a long flat plateau during the elongation of a single chain followed by the conformational transition. The flat plateau is attributed to the unwinding process of CTAB-induced helical structure of carboxymethyl amylose. By Gaussian fitting, the center value for plateau height histogram is 17 pN, which reflects the force needed to unwind the CTAB-induced helical structure of carboxymethyl amylose. We hope that this line of research provides an example of using characteristic shoulder plateau of α-linked glycan to identify single chain elongation, allowing for direct measurement of the unwinding force of supramolecular structure using SMFS.

Keywords: Single molecule force spectroscopy; Single polymer chain; Polysaccharide

1. Introduction

Single molecule force spectroscopy (SMFS), an atomic force microscopy (AFM)-based technique, has been widely used to investigate intermolecular and intramolecular interaction of macromolecules at the molecular level [1–19]. Force spectroscopy on polysaccharides has aroused an increasing interest because AFM-based SMFS is a powerful tool for investigating the force-induced conformational transition, the dynamics, and supramolecular structures of polysaccharides [20–29]. Force spectroscopy involves measuring the force required to extend a molecule by a certain length. The relationship between the force and extension gives a characteristic fingerprint for the conformational transition of the single molecule chain under study [1,2,20]. For example, force–extension relationships of α-linked glycan, such as amylose and dextran, display a characteristic force-induced transition from their chair conformation to the boat-like conformation [6,20–22]. It should be noted that the fingerprinting property of conformational transition can be used as an indicator for identifying whether a single polysaccharide molecule is stretched or not [28,29]. Polysaccharides can also form helical structures that are similar to the double helix of DNA. To unwind the helical structure of polysaccharide using SMFS can provide new physical insight into the formation of supramolecular structures generated by polysaccharides, such as xanthan [23], carrageenan [24], and curdlan [25]. Recently, Marszalek et al. have directly captured the formation of individual amylose helices in butanol or iodine [29].

Carboxymethyl amylose (CM-amylose), a linear polysaccharide of several thousand glucose residues linked by α-D-(1,4)-glucosidic bonds, is a well known host molecule forming helical inclusion complexes with organic compounds by hydrophobic interaction between guest molecules and the cavity

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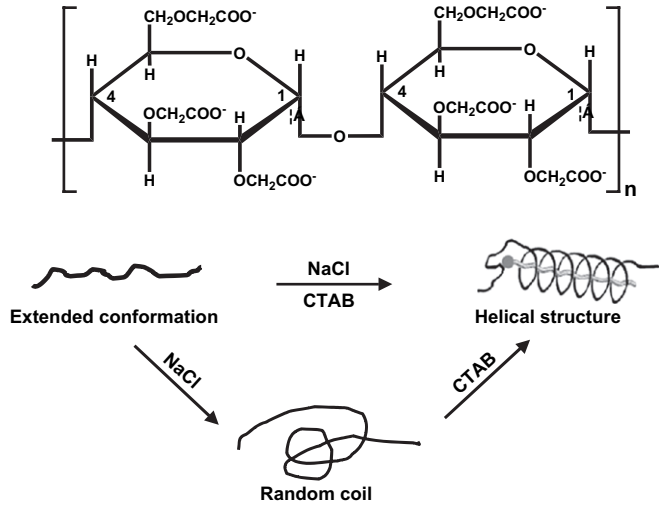
0032-3861/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2007.02.038



Scheme 1. The primary structure of carboxymethyl amylose (CM-amylose). The CM-amylose chain adopts an extended conformation in water and a random coil state upon the addition of salt. It exhibits a helical conformation and forms complex with guest molecules in a salt solution in the presence of CTAB.

of backbone [30–34]. As indicated in Scheme 1, the CM-amylose chain adopts an extended conformation because of the repulsion between charged repeating units in water. Upon the addition of salt, it exhibits as a random coil due to the ion screening effect. CM-amylose forms a helical complex with guest molecules in aqueous solution in the presence of salt and amphiphilic molecules with a long hydrophobic tail, such as cetyltrimethylammonium bromide (CTAB) [30,31,35]. These conformational transitions of CM-amylose in different solution conditions have been investigated by using NMR, viscometric and potentiometric titrations [31,34,36]. The formation of the helical structure is a cooperative process due to combination of hydrophobic and electrostatic interactions. However, all these researches focus on the formation process of surfactant-induced CM-amylose helix. Using SMFS allows investigating the unwinding of helical structure at the molecular level. In this article, we have comparatively studied the difference of force spectroscopy of single CM-amylose chain in different solution conditions by SMFS. The nanomechanical fingerprinting property of α-linked glycan is used as an indicator for identifying the single chain elongation. We hope that this work provides an example of using characteristic shoulder plateau of α-linked glycan to identify single chain elongation, allowing for direct measurement of the unwinding force of the surfactant-induced supramolecular structure using SMFS.

2. Experimental section

2.1. Materials and sample preparation

Commercially available carboxymethyl amylose (sodium salt, Sigma–Aldrich Corp.), cetyltrimethylammonium bromide (CTAB) and pyrene (Fluka, Corp.) were used without further purification. The 2% (w/w) NaCl solution was used as the media in all sample preparation. CM-amylose was dissolved in NaCl solution at a concentration of approximately 0.01 g/L. In force experiments, quartz slides were used as substrate. The slides were treated with a hot ''piranha'' solution (7/3 volume ratio, 98% H_2SO_4/30% H_2O_2) and followed by extensive rinsing with water before using. (Piranha solution reacts violently with organic materials, and should be handled with the extreme caution!) About 0.05 mL of sample solution was deposited onto a clean quartz slide and incubated for about 30 min. Then the slide was rinsed with water to remove the loosely adsorbed molecules.

2.2. Force measurements

All force–extension curves were measured by using Molecular Force Probe 3D (MFP-3D; Asylum Research, Santa Barbara, CA). Silicon nitride cantilevers from Veeco (Santa Barbara, CA) were used. The spring constants of cantilevers were in the range of 0.01–0.03 N/m, calibrated by measuring their thermal fluctuation [37]. The velocity of the tip retraction during the force measurement was 1.0 μm/s, if not specified. The experimental details of SMFS can be found in other references [38,39]. In brief, the polymer was immobilized onto the quartz substrate by physical adsorption. Prior to the measurements, a drop of liquid, acting as the buffer, was injected between the substrate and the cantilever holder, and then both the substrate and the cantilever were immersed in the buffer. By the movement of the piezo tube, the AFM tip was brought into contact with the sample for ca. 3 s under a contact force of several nanonewtons, allowing the polymer chain to physically adsorb onto the tip, and producing a connective bridge in-between. During the separation of the tip and the substrate, the polymer chain was stretched and the cantilever deflected. At the same time, a deflection–extension curve was recorded and then converted into a force–extension curve.

3. Results and discussion

The CM-amylose chain adopts a random coil state in aqueous salt solution. Before investigating the unwinding of the helical structure of CM-amylose, we have studied the single chain mechanical properties of CM-amylose in NaCl solution. Several typical force–extension curves of CM-amylose in 2% NaCl solution, obtained in different SMFS experiments with different cantilevers, are shown in Fig. 1. In spite of the different stretching lengths, all force–extension curves exhibit similar deformation characteristics: the force rises monotonically with the increasing extension, and then the deformation shows a shoulder plateau at about 270 pN. After the shoulder plateau, a stiffening of the polymer segment elasticity of the polymer is followed. And then the rupture point is reached when the force attains its maximum; after this the cantilever relaxes to its equilibrium position. This shoulder feature in the force–extension curves is very similar to the previous reports about CM-amylose [20,21]. The lengths of the stretched polymer segments are different, due to both the polydispersity of the polymer sample and the uncontrolled contact point of the

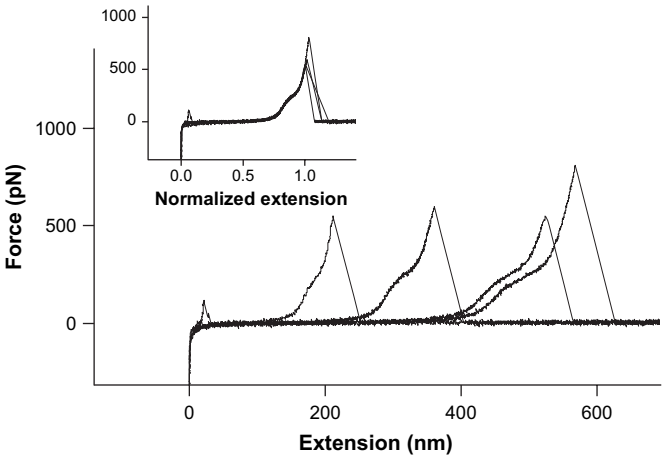


Fig. 1. Several typical force–extension curves of CM-amylose obtained in 2% NaCl solution. The inset shows the superimposition of the normalized force–extension curves.

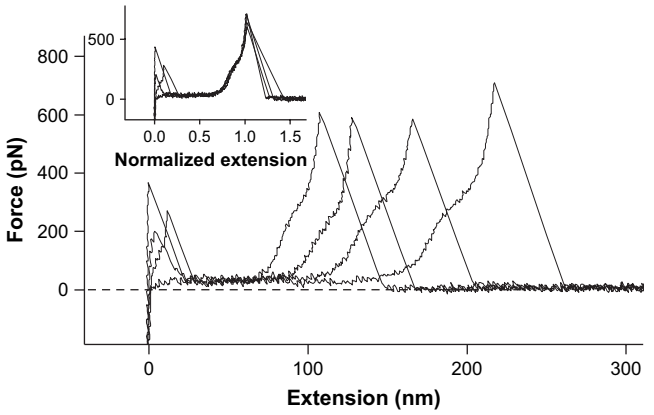


Fig. 2. Several typical force–extension curves of CM-amylose obtained in NaCl solution in the presence of 2×10^{-6} mol/L CTAB. The inset shows the superimposition of the normalized force–extension curves.

AFM tip with the polymer chain. To compare the force–extension relationship of the polymer chains with different stretch lengths, we normalized all the curves by dividing the corresponding lengths measured under the same force 500 pN [2,4]. Despite the different stretching lengths, the force–extension curves after the normalization can be well superimposed, as shown in the inset in Fig. 1. The good superimposition of these curves clearly shows that the elastic properties of polymer chains scale linearly with its length, and all filaments show a transition at the same force. In other words, these force–extension curves reflect single chain elongation. As reported, the characteristic transition at 270 pN in the force–extension curve of CM-amylose is caused by the force-induced chair-twist boat transition of glucopyranose rings, and this characteristic transition has been proved to be the nanomechanical fingerprinting property of α-(1,4)-linked glucosidic backbone [20–22].

In order to investigate the CTAB-induced helical structure of CM-amylose, we performed the force experiments with CM-amylose, which was treated with aqueous NaCl solution in the presence of 2×10^{-6} mol/L CTAB. Some typical force–extension curves obtained from this sample are shown in Fig. 2, which are different from those force–extension curves obtained with CM-amylose in NaCl solution. A long flat plateau appeared during each extension process. Although the force–extension curves have different stretching lengths, they have a similar characteristic: as the extension increases, each force–extension curve has a flat plateau, followed by the typical shoulder plateau at about 270 pN. The different lengths of flat plateau can be understood since the contact point between tip and sample varies, and the lengths of the surfactant-induced helices are probably different as well. For comparing the force–extension curves obtained from polymer chains with different stretching lengths, we have also normalized these force–extension curves under the force value of 500 pN [2,4]. As shown in the inset of Fig. 2, the force–extension curves can be well superimposed after being normalized, indicating a single chain elongation. Moreover, all of the filaments show similar long flat plateaus, indicating that the plateaus originate from the same conformational change.

We wondered if the flat plateau did result from unwinding of the CTAB-induced helical structure of CM-amylose. To clarify this issue, we have compared the normalized force–extension curves in NaCl solution and in NaCl solution plus CTAB, as shown in Fig. 3. In the low force region, the flat plateau of CM-amylose in the presence of CTAB becomes clear, comparing with the single chain stretching of CM-amylose random coils in NaCl solution. Although there exists clear difference between two force–extension curves in the low force region, we have found interestingly that the two normalized force–extension curves can be well superimposed in the high force region with the same shoulder plateau at force value of about 270 pN. This result indicates that the force–extension curves of CM-amylose in NaCl solution and in NaCl solution plus CTAB display the same elasticity in high force region, which infers that we have realized single chain elongation of CM-amylose in NaCl solution plus CTAB. The fact that the force–extension curves of CM-amylose exhibit flat plateau only in NaCl solution plus CTAB but not in NaCl solution, indicates that the flat plateau is corresponding to the unwinding of surfactant-induced helical structure of CM-amylose. It

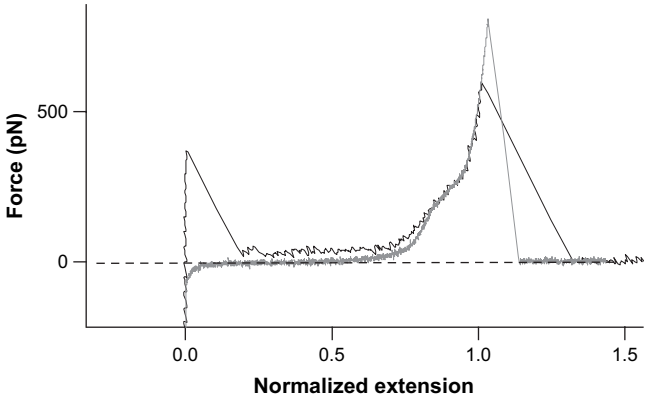


Fig. 3. Comparison of normalized force–extension curves of amylose in NaCl solution (gray trace) and in NaCl solution in the presence of 2×10^{-6} mol/L CTAB (black trace).

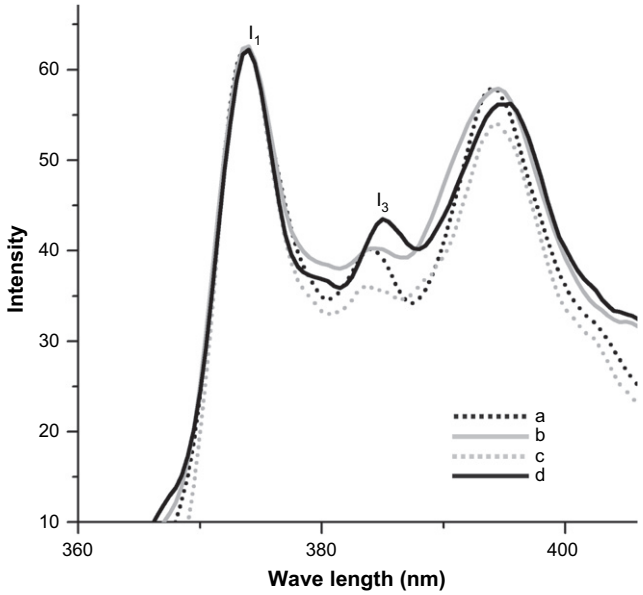


Fig. 4. Fluorescence spectra of pyrene in different solutions: (a) 2% NaCl, (b) 2.5 g/L CM-amylose, (c) 2×10^{-6} mol/L CTAB, and (d) 2.5 g/L CM-amylose plus 2×10^{-6} mol/L CTAB. The 2% NaCl solution is used as the media for the preparation of samples (b), (c) and (d).

should be noted that there are some force–extension curves without plateau, even though the sample was treated with CTAB. This result may indicate that CM-amylose is not transformed completely into the helical structure in aqueous solution of NaCl plus CTAB, which was also demonstrated elsewhere [31,32].

In order to confirm the formation of CTAB-induced helical structure of CM-amylose, we have used pyrene as a fluorescence probe to study the hydrophobic environment provided probably by the helical structure of CM-amylose in NaCl solution plus CTAB. Fluorescence emission spectra of pyrene in different solution conditions were recorded with a Perkin–Elmer LS 55 spectrofluorimeter. As shown in Fig. 4 and Table 1, the ratio I_1/I_3 in the fluorescence spectrum of pyrene decreases from 1.55 to 1.43 in NaCl solution of CM-amylose when CTAB is added. This result is rationalized by the formation of CM-amylose helical structure in NaCl solution plus CTAB and the cavities of helical structure provide a relative hydrophobic environment for guest molecules [30,31,40]. In other words, the decrease of the ratio I_1/I_3 supports the formation of surfactant-induced helical structure of CM-amylose in a way. It should be noted that the concentration of CTAB is lower than its critical micelle concentration (CMC) [41], so the decrease of the ratio I_1/I_3 in mixed solution of CM-amylose and CTAB was not caused by the formation of CTAB micelle.

Table 1
The vibronic band intensities of pyrene monomer fluorescence in different solution conditions in Fig. 4

Sample	I_1	I_3	I_1/I_3
(a) 2% NaCl	62.5	40.2	1.55
(b) 2.5 g/L CM-amylose	62.6	40.3	1.55
(c) 2×10^{-6} mol/L CTAB	62.4	36.0	1.73
(d) 2.5 g/L CM-amylose plus 2×10^{-6} mol/L CTAB	62.2	43.5	1.43

We have analyzed statistically the unwinding force of surfactant-induced helical structure of CM-amylose. The zero force baseline is determined as the section in the force–extension curve when the cantilever relaxes to its free condition. We chose 100 successive data points from the baseline region, and made the average value of these data points as the baseline value from each raw force–extension curve. We also chose 100 successive data points from the flat plateau region, and made the average value of these data points as the plateau value from this curve. The difference of two average values is regarded as the height of plateau. The histogram of the height of flat plateau is shown in Fig. 5. By Gaussian fitting, the center value for plateau height is 17 pN, which reflects the force needed to unwind the CTAB-induced helical structure of CM-amylose. In attempt to test whether the unwinding force is loading rate dependent or not, we have changed the velocity of AFM tip retraction during the force experiments. However, we have not found any distinct difference of the unwinding force for the tip retraction velocity in the range of 200 nm/s to 2.0 μm/s. We have also tried hard to provide information about the consecutive stretching–relaxing force–extension curves but did not succeed. For CM-amylose with existence of a large amount of CTAB, we have found that the CM-amylose can easily slip off from the tip or substrate, no matter how the rupture force is high or low. Considering the fact that the average unwinding force is 17 pN and the length of repeating unit of CM-amylose is approximately 0.45 nm [20], we can estimate that the average energy for the unwinding of CTAB-induced helical structure of CM-amylose is about 4.6 kJ/mol. In other words, the average energy for the unwinding of CTAB-induced helical structure of CM-amylose is about $1.86k_BT$ per repeating unit, as k_B is the Boltzmann constant and T is the temperature in Kelvin.

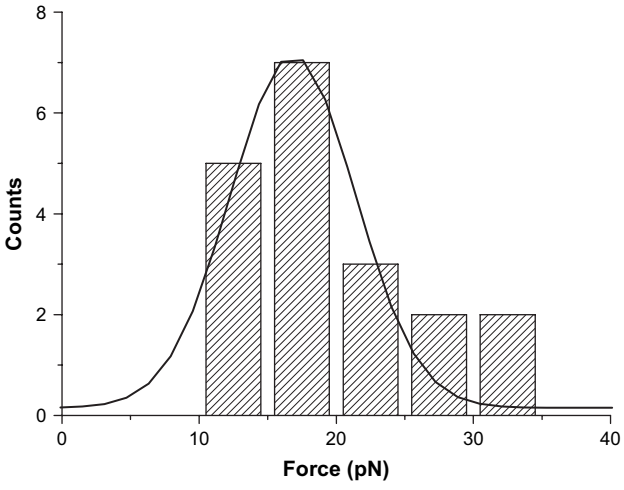


Fig. 5. Histogram for the unwinding force obtained from CM-amylose in NaCl solution plus CTAB. By Gaussian fitting, the center value for the flat plateau height is 17 pN, which reflects the force needed to unwind the CTAB-induced helical structure of CM-amylose. ($n = 19$, SD = 6.8 pN).

4. Conclusion

We have described the force profiles of CM-amylose in its random coil state and helical structure conformation. In the case of aqueous NaCl solution, we have obtained only monotonic force–extension curves, indicating the existence of random coils. However, the force–extension curves of CM-amylose in NaCl solution plus CTAB show a flat plateau, which is attributed to the unwinding of the helical structure of CM-amylose. This work presents an example of using characteristic shoulder plateau of α-linked glycan to identify single chain elongation, allowing for direct measurement of the unwinding force for the surfactant-induced supramolecular structure using SMFS.

Acknowledgment

The authors thank the Natural Science Foundation of China (20474035) and National Basic Research Program (2007CB808000) for financial support.

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