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Supramolecular Research by Single Molecule Force Spectroscopy

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Summary: Single molecule force spectroscopy (SMFS) is a new kind of technique based on atomic force microscope, which allows detecting force as low as pico-newtons directly. Herein based on our recent work, we want to demonstrate the investigation of supramolecular structures and interactions in polymer systems by SMFS, such as desorption force between polymer and substrate, identifiability of polymer micelle and its interaction with surfactant, splitting force of ion-induced helical structure in polysaccharide. It shows well that SMFS is a powerful tool in the study of supramolecular science.

Keywords: intermolecular interaction; single chain force spectroscopy; supramolecular structure;

Introduction

Single molecule force spectroscopy (SMFS), a new technique based on atomic force microscopy, has allowed studying intermolecular and intramolecular interactions in macromolecular systems with extremely high force sensitivity.[1] It has been proven that this method can provide much new information, which is not available by conventional methods, such as elastic property of single polymer chain,[2-3] force induced conformational transition in polysaccharide,[4] unfolding forces of protein domains,[5] unbinding forces of receptor-ligand complexes [6] and so on. These experiments have given insights into the forces stabilizing the complicated supramolecular structures of biomacromolecules as well as synthetic polymers.

The force measurements are carried out on a home-made SMFS setup.[7] The sample is immobilized onto a suitable substrate by physical adsorption and then immersed into a liquid cell. An AFM tip is brought into contact with the sample so that some molecules could adsorb onto the tip, due to non-specific interaction between the tip and the polymer and a connective molecular bridge is built between the tip and polymer. During the separation of the tip and the sample, the molecule is stretched. At this time, the deflection-piezo path curve was recorded and converted into a force-extension curve (force curve, in brief). Since the measurement is carried out at a solid/ liquid interface, the buffer can be easily changed.

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Interfacial Interaction between Polymer and Substrate

How the polymer chain attaches and adheres to a solid surface is not only of fundamental basic interest, but related to many practical applications in materials science. By means of SMFS, we attempt to measure the desorption force of the polymer from the modified substrate directly at molecular scale, and moreover, to correlate the force pattern and the conformation of the polymers at the interface.

The driving force of the fabrication of poly(4-vinylpyridine) (PVP) on either hydroxyl or amino groups modified quartz is thought to be mainly hydrogen-bonding.[8] The typical force profile of PVP from the amino groups modified substrate in methanol is shown in Figure 1a. The "saw-tooth" like force curve can be obtained when the stretching velocity varied from 2.02 μm/s to 0.202 μm/s, and the rupture force is around 180 pN independent of the stretching velocity. This may indicate that the PVP chain exists as loops at the interface. If we change the substrate from amino modified quartz to hydroxyl modified quartz, the similar force curve profiles with different rupture force (260 pN) could be observed. This result infers that the polymer chain is desorbed from the substrate, not from the tip. Therefore, each force peak would correspond to the desorption of a loop structure from the substrate.

If the interaction between the substrate and the polymer is based on hydrogen-bonding, this interaction can be influenced upon adding a little bit acetic acid into the buffer. An interesting finding is that the number of force peaks in one curve reduced remarkably in this case, though the rupture force is still around 180 pN. One possible reason is that carboxyl groups can interact with both pyridine groups of PVP and amino groups at the modified substrate. As a result, the number of hydrogen bonds formed between PVP and amino groups decreased remarkably, so is the anchor points of PVP at the interface. The desorption force value (180 or 260 pN) is much larger than a single hydrogen-bonding, and this may be corresponding to a segment desorption, which would result in several hydrogen bonds broken at one time, or the combination of hydrogen-bonding and van der Waals interactions that caused the final desorption force.

The adsorption of poly (diallyldimethylammonium chloride) (PDDA) on negatively charged substrate is based on electro-static interaction.[9-10] Typical force curve of PDDA desorbed from sulfonic groups modified substrate is shown in Figure 1b. Different from the force pattern of multi-peaks in PVP system described above, there exists a long plateau at about 120 pN for the desorption of PDDA from the negatively charged substrate. The smooth desorption reveals that the polymer chain would adopt a "train-like" conformation on the substrates. The

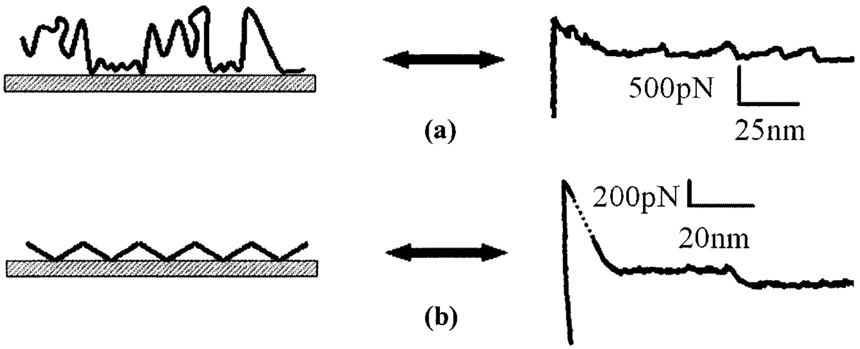

Figure 1. From adsorption conformation to force curve profile. (a) Typical force profile of PVP on amino modified substrate. (b) Typical force profile of PDDA on sulfonic modified substrate.

similar experiments are carried on different substrates, e. g. mica and hydroxyl modified quartz. The typical force profiles of PDDA from the two negatively charged substrates also have a long plateau but with different force value. The force value of the plateau increased gradually from hydroxyl groups modified quartz (~60 pN), mica (~90 pN) to sulfonic groups modified quartz (~120 pN), indicating that the different surface charge density of the three substrates results in different desorption force.

In each of the above two systems, no obvious difference of the desorption force is found when different stretching velocity is applied. Therefore, the desorption forces are obtained in an equilibrium condition,[1] and the force values are valuable in the estimation of the driving force in layer-by-layer assembly.

Interaction between Polymer and Surfactant

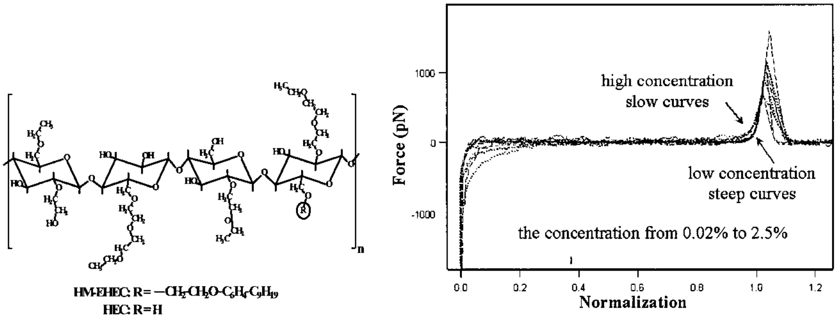

Figure 2. (a) The molecular structure of HM-EHEC and HEC. (b) Normalized force curves of HM-EHEC obtained under different concentration.

Hydrophobically modified polymers (HM-polymers) are able to form micelle-like clusters through hydrophobic interactions above a certain polymer concentration.[11] These polymers thus exhibit particular rheological properties in solution as a result of the reversible dissociation of physical links that occurs under shear. In the process of self-association of HM-polymers, surfactants are known to bind and self-assemble at the polymers' hydrophobic moieties and to dramatically affect the rheological properties of the polymer. In our system, the HM-polymer we used is hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC), the structure of the polymer is given in Figure 2a. The hydrophobic modification of HM-EHEC consists of a nonylphenol substitution of ca. 1.7 mol%. All of the force curves of single HM-EHEC chain at different concentrations show the elasticity of the chain, and these curves can be fitted well by the modified freely-joint-chain (FJC) model. However, these curves show a little difference at the low force region and can be classified into two types: slow and steep (see Figure 2b). Furthermore, all of the slow force curves only appear in the case of high concentration. We believe that this is related to polymer micelle-like clusters through interchain hydrophobic interactions under high-concentration conditions, which contributes to the elastic properties of HM-EHEC. Interestingly, we find that the probability of the steep curves is dependent on the polymer concentration. When the concentration is above 0.8 wt%, there are more and more slow curves. Because the slowness is due to the hydrophobic interactions of the hydrophobically modified groups, we would identify the critical concentration for micelle-like clusters as around 0.8 wt%. This value is lower than the reference reported datum, 1.87% in solution, because the experiments here are performed at the interface between the polymer solution and the solid substrate.

The interaction between hydrophobically modified polymers and small surfactant, sodium dodecyl sulfate (SDS), is an interesting problem and this has been revealed by SMFS too. For the addition of small amounts of SDS (2.5 mM), the normalized force curves of HM-EHEC under the critical concentration in the low-force region deviate from those of the pure polymer system and from those of systems with more SDS added. Along with the previous study, these results indicate the deformation of some special structures, which might be caused by enhanced interactions of the hydrophobic moieties binding with the surfactants. The polymer hydrophobic tails could serve as nucleation sites and provide an attraction to drive the association between the polymer and the surfactant. Upon the addition of more SDS (20 mM) to the polymer/surfactant system, the micelles that have been formed by the association of the polymer and surfactant might be destroyed due to the self-organization of SDS. Consequently, the deviations of the force curves in the low-force region disappear, and the force traces show characteristics similar to those of systems of polymer below the critical concentration without surfactant.

Interaction between Polymer and Specific Ion

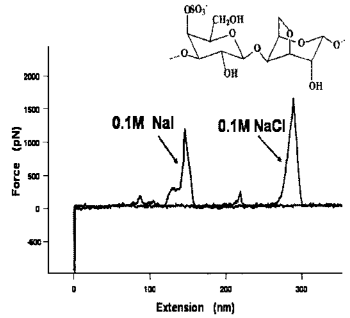

Figure 3. Stretching and relaxation force curves indicating the irreversible process of helix unfolding.

Carrageenan is an important polysaccharide extracted from different species of marine red algae. One of its useful properties is to induce gelation in aqueous solution depending on the counterions and temperature, corresponding to a coil-helix-gel transition. The single chain force curve of native ι–carrageenan, fitted well by the modified FJC model, shows clearly that it exists as coil structures and no suprastructure in the native state.[12] Interestingly, we found that if we treat the polymer with iodide sodium (NaI), the resulting force curve would show a long plateau at about 300 pN (see Figure 3). But the similar treatment with NaCl didn't give any effects. As the detailed structure of such a NaI-induced helix is not known yet, it was suggested that iodide, among others, can bind to ι–carrageenan. Such binding is proposed to promote helix formation but to hinder helix-to-gel transition. Since the long plateaus in the force curves could not be observed for the coil state, but only for the helix state induced by 0.1 M NaI, their occurrence must be ascribed to the splitting or unfolding of such helix structure of ι–carrageenan.

By keeping the stretching force below than the rupture force, we can stretch and relax the same ι–carrageenan chain repeatedly. When the force is raised above 300 pN, the molecule yields and the relaxation trace is no longer identical to the deformation trace, indicating that the transition from helix to coil is a completely irreversible process.

In summary, single molecule force spectroscopy has proven to be a powerful and sensitive tool in probing the delicate structures of macromolecules at molecular level, leading to open up a new field of single molecule mechanochemistry.

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