Study on Polymer Micelles of Hydrophobically Modified Ethyl Hydroxyethyl Cellulose Using Single-Molecule Force Spectroscopy

4 AUTHORS, INCLUDING:

Shan Zou
National Research Council Canada
59 PUBLICATIONS   1,341 CITATIONS

SEE PROFILE

Wenke Zhang
Jilin University
41 PUBLICATIONS   925 CITATIONS

SEE PROFILE

Study on Polymer Micelles of Hydrophobically Modified Ethyl Hydroxyethyl Cellulose Using Single-Molecule Force Spectroscopy

Shan Zou,[†] Wenke Zhang,[†] Xi Zhang,*,[†] and Bingzheng Jiang[‡]

Key Lab of Supramolecular Structure and Spectroscopy, Department of Chemistry, Jilin University, Changchun 130023, People's Republic of China, and Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, People's Republic of China

Individual hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC) molecules under different conditions were elongated using a new atomic force microscope (AFM) based technique−single-molecule force spectroscopy (SMFS). The critical concentration of HM-EHEC for micelle-like clusters at a solid/liquid interface was around 0.8 wt %, which is lower than that in solution. The different mechanical properties of HM-EHEC below and above the critical concentration were displayed on force−extension curves. Through a comparison with unmodified hydroxyethyl cellulose, substituent-induced effects on nanomechanical features of HM-EHEC were investigated. Because of hydrophobic interactions and cooperative binding with the polymer, surfactants such as sodium dodecyl sulfate (SDS) dramatically influence the elastic properties of HM-EHEC below the critical concentration, and further addition of SDS reduces the interactions between the hydrophobic groups and the surfactant.

Introduction

The great advancement of nanoscale techniques has enabled the manipulation of single molecules and provided detailed insight into intermolecular and intramolecular forces of individual molecules, as well as molecular mechanisms without any averaging process.[1−8] With its high lateral resolution and extreme force sensitivity, atomic force microscopy (AFM) has become one of the most widely used near-field microscopic techniques.[9] In addition to topographic imaging, AFM can measure and map many types of surface forces and lateral properties. A new technique based on AFM−single-molecule force spectroscopy (SMFS), first introduced by H. E. Gaub−has been implemented and used to measure the elastic properties and deformation of natural[8,10−18] and synthetic polymers.[19−24] In these studies, single-molecule force−exten-

sion curves have revealed a wealth of fingerprint-like characteristics, such as the stretching of normal random-coil polymer chains,[19−21] the chair-to-boat conformational transition of individual glucopyronose rings,[8,10,16] the unfolding process of Ig titin domain,[11,12] and some other supramolecular rearrangements of biomacromolecules[13] and synthetic polymers.[22−24] Recently, M. Grandbois et al. measured the rupture force of single covalent bonds under an external load,[25] which cannot be obtained using conventional methods. Even more recently, H. Fuchs et al. reviewed studies on the properties of individual macromolecules and inter- and intramolecular forces using force spectroscopy.[26]

Hydrophobically modified polymers (HM-polymers) are attracting increasing attention mainly because of their unique rheological properties and potential industrial

* Author to whom correspondence should be addressed. E-mail: xi@jlu.edu.cn. Fax: 0086-431-8923907.

† Jilin University.

‡ Chinese Academy of Sciences.

(1) Florin, E.-L.; Moy, V. T.; Gaub, H. E. *Science* **1994**, *264*, 415.

(2) Florin, E.-L.; Gaub, H. E. *Science* **1994**, *266*, 257.

(3) Radmacher, M.; Fritz, M.; Hansma, H. G.; Hansma, P. K. *Science* **1994**, *265*, 1577.

(4) Bustamante, C.; Marko, J.; Siggia, E.; Smith, S. *Science* **1994**, *265*, 1599.

(5) Lee, G. U.; Chrisey, L. A.; Colton, R. J. *Science* **1994**, *266*, 771.

(6) Cluzel, P.; Lebrun, A.; Heller, C.; Lavery, R.; Viovy, J.; Chatenay, D.; Caron, F. *Science* **1996**, *271*, 792.

(7) Smith, S. B.; Cui, Y.; Bustmmante, B. *Science* **1996**, *271*, 795.

(8) Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J.; Gaub, H. E. *Science* **1997**, *275*, 1295.

(9) Binning, G.; Quate, C. F.; Gerber, C. *Phys. Rev. Lett.* **1986**, *56*, 930.

(10) Marszalek, P. E.; Oberhauser, A. F.; Pang, Y. P.; Fernandez, J. M. *Nature* **1998**, *396*, 661.

(11) Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. *Science* **1997**, *276*, 1109.

(12) Marszalek, P. E.; Lu, H.; Li, H. B.; Carrion-Vazquez, M.; Oberhauser, A. F.; Schulten, K.; Fernandez, J. M. *Nature* **1999**, *402*, 100.

(13) Li, H. B.; Rief, M.; Oesterhelt, F.; Gaub, H. E. *Adv. Mater.* **1998**, *10*, 316.

(14) Rief, M.; Gautel, M.; Schemmel, A. *Biophys. J.* **1998**, *75*, 3008.

(15) Carrion-Vazquez, M.; Oberhauser, A. F.; Fowler, S. B.; Marszalek, P. E.; Broedel, S. E.; Clarke, J.; Fernandez, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3694.

(16) Li, H. B.; Rief, M.; Oesterhelt, F.; Gaub, H. E.; Zhang, X.; Shen, J. C. *Chem. Phys. Lett.* **1999**, *305*, 196.

(17) Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Nat. Struct. Biol.* **1999**, *6*, 346.

(18) Zhang, W. K.; Xu, Q. B.; Zou, S.; Li, H. B.; Xu, W. Q.; Zhang, X. *Langmuir* **2000**, *16*, 4305.

(19) Li, H. B.; Liu, B. B.; Zhang, X.; Gao, C. X.; Shen J. C.; Zou, G. T. *Langmuir* **1999**, *15*, 2120.

(20) Ortiz, C.; Hadziioannou, G. *Macromolecules* **1999**, *32*, 780.

(21) Bemis, J. E.; Akhremitchev, B. B.; Walker, G. C. *Langmuir* **1999**, *15*, 2799.

(22) Li, H. B.; Zhang, W. K.; Zhang, X.; Shen, J. C.; Liu, B. B.; Gao, C. X.; Zou, G. T. *Macromol. Rapid Commun.* **1998**, *19*, 609.

(23) Li, H. B.; Zhang, W. K.; Xu, W. Q.; Zhang, X. *Macromolecules* **2000**, *33*, 465.

(24) Oesterhelt, F.; Rief, M.; Gaub, H. E. *New J. Phys.* **1999**, *1*, 1.

(25) Grandbois, M.; Beyer, M.; Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Science* **1999**, *283*, 1727.

(26) Janshoff, A.; Neitaert, M.; Oberdoerfer, Y.; Fuchs, H. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 3212.

Scheme 1.   Chemical Structures of Hydrophobically Modified Ethyl Hydroxyethyl Cellulose (HM-EHEC) and Unmodified Cellulose (HEC)

HM-EHEC: R = $—CH_2—CH_2O—C_6H_4—C_9H_{19}$

HEC: R = H
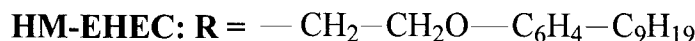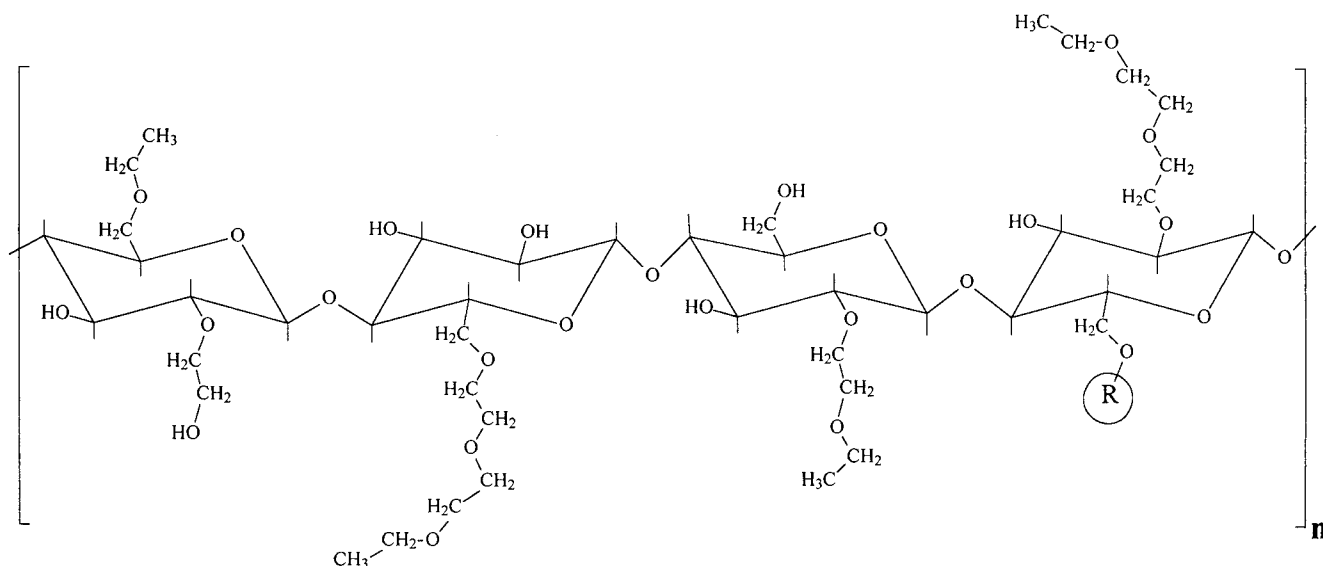
applications. These water-soluble materials can be used as rheology modifiers in, for example, enhanced oil recovery and latex paints. In aqueous solution, the hydrophobic moieties are able to form micelle-like clusters through hydrophobic interactions when above a certain polymer concentration.[27,28] These polymers thus exhibit particular rheological properties in solution as a result of the reversible dissociation of physical links that occurs under shear. The ability to form hydrophobic associations should also increase their field of use, with emulsification and surface modification as potential applications.[29] In the process of self-association of HM-polymers,[30] surfactants are known to bind and self-assemble at the polymers' hydrophobic moieties and to dramatically affect the rheological properties of the polymer, depending the concentration of surfactant.[31–33]

The properties of associative polymers have been extensively studied, but very little is known about their behavior at solid/liquid interfaces.[28] We believe that the polymer micelles of hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC) should provide a good system to study experimentally intermolecular interactions in macromolecular systems, which might reflect some extent of the concept of cohesion entanglement.[34] In this paper, by using single-molecule force spectroscopy (SMFS), we investigate the mechanical properties of HM-EHEC under different concentration conditions and identify the

critical polymer concentration for the formation of micelle-like clusters at solid/liquid interfaces. The different elastic properties of HM-EHEC below and above the critical concentration are compared. To facilitate an understanding of the deviation of the force curves, unmodified hydroxyethyl cellulose (HEC) is studied comparatively to differentiate the substituent-group-induced effects on the nanomechanical properties of the polymer chains. Because the proper concentration of the surfactant can dramatically influence the properties of HM-polymer, we study the elastic features of the HM-EHEC polymer chain complex with different concentrations of the surfactant sodium dodecyl sulfate (SDS).

Experimental Part

Single-Molecule Force Spectroscopy (SMFS). The experimental details of SMFS by AFM have been described elsewhere.[8,13,21] Briefly, when an AFM tip is brought into contact with a polymer sample that has been immobilized on a substrate, binding with a single chain can be achieved because of the molecular dimensions of the tip apex. Upon retraction of the tip, the polymer is stretched, and the restoring force is measured as a function of the separation distance, resulting in a force–extension curve.

Calibration. Silicon nitride cantilevers from Digital Instruments (DI, Santa Barbara, CA) and PARK Scientific Instruments (PARK, Sunnyvale, CA) were used for all force measurements. Each cantilever was calibrated after a given experiment using the equipartition method, measuring the thermal excitation of the tip to compute its spring constant.[35,36] The measured spring constants of the cantilevers varied between 42 and 65 mN m^{-1}.

Materials and Sample Preparation. Hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC) and unmodified hydroxyethyl cellulose (HEC) were kindly provided by Prof. B. A. Wolf (Institut fuer Physikalische Chemie, Johannes Gutenberg-Universität, Mainz, Germany). The HM-EHEC prod-

(27) Badiger, M. V.; Lutz, A.; Wolf, B. A. *Polymer* **2000**, *41*, 1377.
(28) Volpert, E.; Selb, J.; Candau, F.; Green, N.; Argillier, J. F.; Audibert, A. *Langmuir* **1998**, *14*, 1870.
(29) Thuresson, K.; Karlström, G.; Lindman, B. *J. Phys. Chem.* **1995**, *99*, 3823.
(30) Thuresson, K.; Nilsson, S.; Lindman, B. *Langmuir* **1996**, *12*, 2412.
(31) Thuresson, K.; Lindman, B. *J. Phys. Chem. B* **1997**, *101*, 6460.
(32) Joabsson, F.; Rosén, O.; Thuresson, K.; Piculell, L.; Lindman, B. *J. Phys. Chem. B* **1998**, *102*, 2954.
(33) Thuresson, K.; Söderman, O.; Hansson, P. Wang, G. *J. Phys. Chem. B* **1996**, *100*, 4909.
(34) Qian, R. Y. *Macromol. Symp.* **1997**, *124*, 15.

(35) Florin, E. L.; Rief, M.; Lehmann, H.; Ludwig, M.; Dornmair, C.; Moy, V. T.; Gaub, H. E. *Biosens. Bioelectron.* **1995**, *10*, 895.
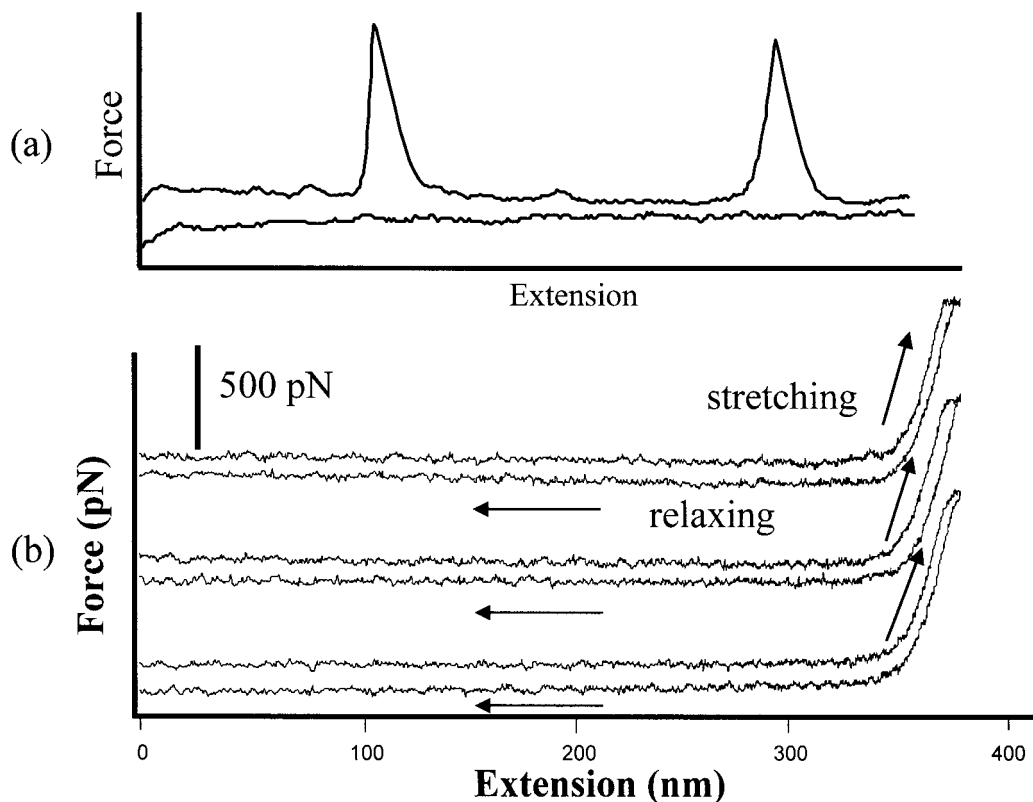(36) Butt, H. J.; Jaschke, M. *Nanotechnology* **1995**, *6*, 1.

Figure 1. Reversible extension of HM-EHEC. (a) Multiple molecules had attached to the tip. During the extension, the shorter one detached first. (b) Stretching and relaxing traces of HM-EHEC (0.56 wt %) binding with 2.5 mM SDS. For clarity, the two traces in one cycle are offset.

uct is commercially known as Bermocoll EHM-100. The chemical structure of the polymer is given in Scheme 1. The hydrophobic modification of HM-EHEC consists of a nonylphenol substitution of ca. 1.7 mol %, and the molecular weight of the polymer is reported to be 100 kg/mol. Before being used, the polymer was purified as described in the literature.[27,29] HEC was used without further purification.

HM-EHEC and HEC were dissolved in Millipore water at concentrations of 0.02, 0.046, 0.21, 0.57, 0.8, 1.0, 1.4, and 2.5 wt % and 0.05, 0.18, 0.23, 0.97, 1.87, and 1.92 wt %, respectively. These polymer solutions with different compositions were homogenized in the refrigerator for several days before being conveyed to the SMFS instrument. Each mixture system of HM-EHEC or HEC and SDS was formed by using ca. 0.55% polymer solution miscible with 2.5 or 20 mM SDS, respectively. The appropriate amount of polymer solution was deposited onto a clean glass substrate and stored for 6 h isolated from the air. Then, most of the solution on the glass was removed, and the sample was immediately used for experiments. The coupling of the cellulose to the tip in this study was based on a nonspecific interaction; it has been shown in various studies that this nonspecific attachment can hold forces of up to nano-newtons.[8,11−16,19]

Results and Discussion

Evidence on Single-Chain Elongation. In all SMFS experiments reported here, the data listed or treated represent the mechanical properties of a single chain. To begin, we verify that the stretched chain in the experiments was a single chain with the following procedures.

First, we can confirm the single-chain attachment at the tip on one side and at the substrate on the other side through a repeated stretching−relaxing experiment. In cases where the tip adsorbs more than one chain, the shorter chain would bear a higher load and would detach first from the tip, resulting in a dramatic drop in load, as shown in Figure 1a. If there is no symptom of the detachment of the stretched chain end during stretching,

we assume that it is the single-chain elongation experiment. Second, the attached molecule could be stretched and relaxed repeatedly. If there is no change in the force−extension curves of a stretching and relaxing cycle, as shown in Figure 1b, it is proposed to be the stretching of a single chain. Third, in case of the very small chance that the tip stretches the ends of two chains, e.g., with contour lengths of 200 and 400 nm, simultaneously, we could demonstrate their behavior with a model calculation. An extended Langevin function

$$x(F) = \left[\coth\left(\frac{Fl_k}{k_B T}\right) - \frac{k_B T}{Fl_k}\right]\left(L_{contour} + \frac{nF}{K_{segment}}\right) \quad (1)$$
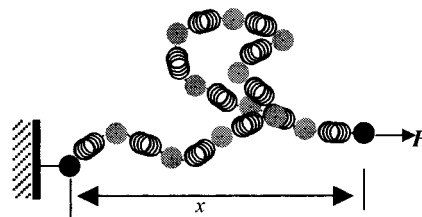
derived from the modified freely jointed chain (FJC) model, can treat the polymer as a chain of statistically independent segments of lengths l_k (Kuhn length) that can be deformed under stress.[1,11] Here F reflects the applied force; n is the number of segments, which equals $L_{contour}/l_k$; k_B is the Boltzmann constant; and T is the temperature. The elasticity of a modified FJC chain is dominated by the entropic contribution in the low-force regime; at high forces, bond angle torsion and bend give rise to a molecule-specific segment elasticity that is governed by enthalpy as well as entropy. In previous studies,[1,8,11−16,19−23] we and others have shown that the stretching of some single
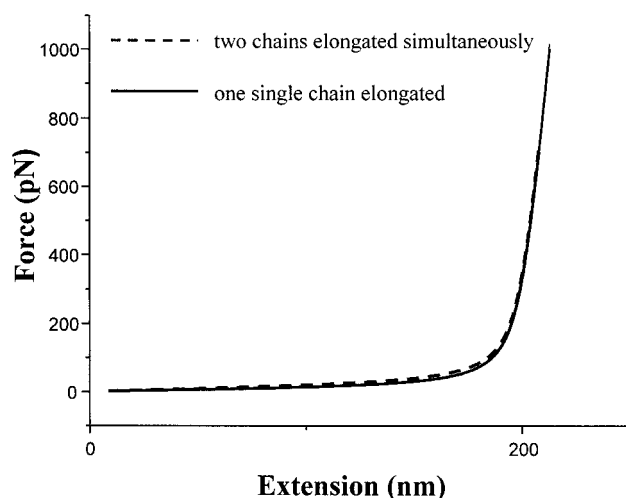
Figure 2. Calculated force−extension curves assuming one single chain with a shorter contour length stretched and two chains with different lengths simultaneously stretched.
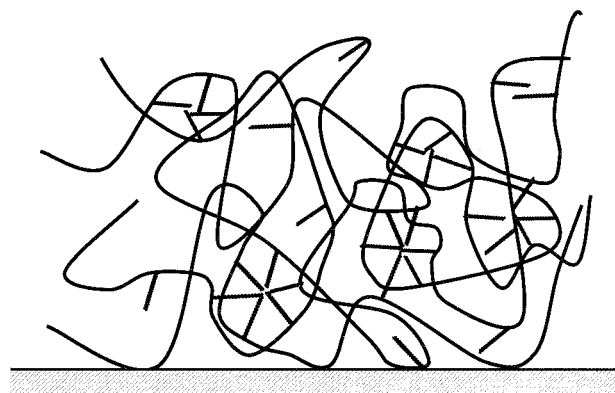

Figure 3. Possible model of a micelle-like cluster for HM-EHEC.

polymer chains is well-described by the modified FJC model. According to a modified FJC model, the extension−force relationship can be expressed by eq 1. The force, $F(x)$, is the inverse function of the extension, $x(F)$, in this situation. If $F_1(x)$ and $F_2(x)$ are the forces for chains with contour lengths of 200 and 400 nm, respectively, then the force−extension curves could be illustrated as in Figure 2. The solid curve in Figure 2 corresponds to the stretched chain with the shorter contour length of 200 nm, and the dashed line corresponds to that of the two chains stretched simultaneously. It is obvious that the force difference between the two curves is comparable to experimental error. If the second chain were longer than 400 nm, the difference would be even smaller. This implies that the effect of the other chain, which is longer than 400 nm, on the force−extension curve could be neglected. Finally, we could identify single-chain stretching by the unique shape of the master force−extension curves. If the recorded force−extension curves in experiments could be normalized (the normalization process will appear later) and superimposed very well, then a conclusion could be drawn that they all reflect single-chain elongations. Therefore, we could conclude that the force−extension behavior shown above represents single-chain characteristics from the procedures mentioned above.

Identification of the Critical Concentration of HM-EHEC for Micelle-like Clusters at Solid/Liquid Interface. Figure 3 shows the probable structure of micelle-like clusters of HM-EHEC. The results of a series

Table 1. Statistical Data of the Slow Elasticity Traces of Experimental Curves

concentration (wt%)	0.02	0.05	0.211	0.57	0.8	1.0	1.4	1.9	2.5
ratio of the slow elasticity traces (%)	0	0	0	0	2	37.5	47.4	57.5	60
sum of experimental curves	264	61	136	198	94	118	82	208	49

of typical force−extension curves of HM-EHEC chains measured on samples of different concentrations by using different cantilevers are shown in Figure 4a. Because the tip and surface attachments can occur anywhere along the HM-EHEC chains, the lengths of the HM-EHEC chains stretched between the tip and the sample vary from 90 nm to more than 1 μm. All of the force curves show a sharp increase when the extension approaches the detachment point, and then the force drops suddenly to zero.

To compare the force−extension relationship and the segment elasticity of polymers with different contour lengths, the traces were normalized by their lengths at the same given force value. The normalization process has been described elsewhere.[37] Briefly, the length value can be obtained for a selected force value, e.g., 400 pN; then, the extensions of the force curves are divided by this length. Finally, the force−extension traces are normalized, as shown in Figure 4b. All of the force curves can be superimposed well in the high-force regime, where the deformation is thought to be dominated by the stiffness of the bond-angle potentials of the polymer chains. In the low-force regime, however, there mainly exist two kinds of force curves, "slow" curves and "steep" curves. The superposition of each kind of normalization force curve indicates single-polymer chain elongation.

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. To identify the critical concentration for the micelle-like clusters, we carefully summarized the numbers of these slow curves in samples with different concentrations and calculated the probability of slow curves in all experiments. The results are shown in Table 1. Using these data, the probability of slow curves was plotted versus concentration, as shown in Figure 5.

From Figure 5, we observe that almost no slow curves appeared below the concentration of 0.8 wt %. However, when the concentration was above 0.8 wt %, there were more and more slow curves. Because the slowness is due to the hydrophobic interactions of the hydrophobically modified groups, we identified the critical concentration for micelle-like clusters as around 0.8 wt %. This value is lower than the reference reported datum, 1.87%[27] in solution, because the experiments that we performed were at the interface between the polymer solution and the solid substrate.

Comparison of the Elastic Features of HM-EHEC Below and Above the Critical Concentration. From the normalized force curves at different concentrations (shown in Figure 6), one can observe that below and above the critical concentration, the force curves are obviously different in terms of the elasticity of the chains at the

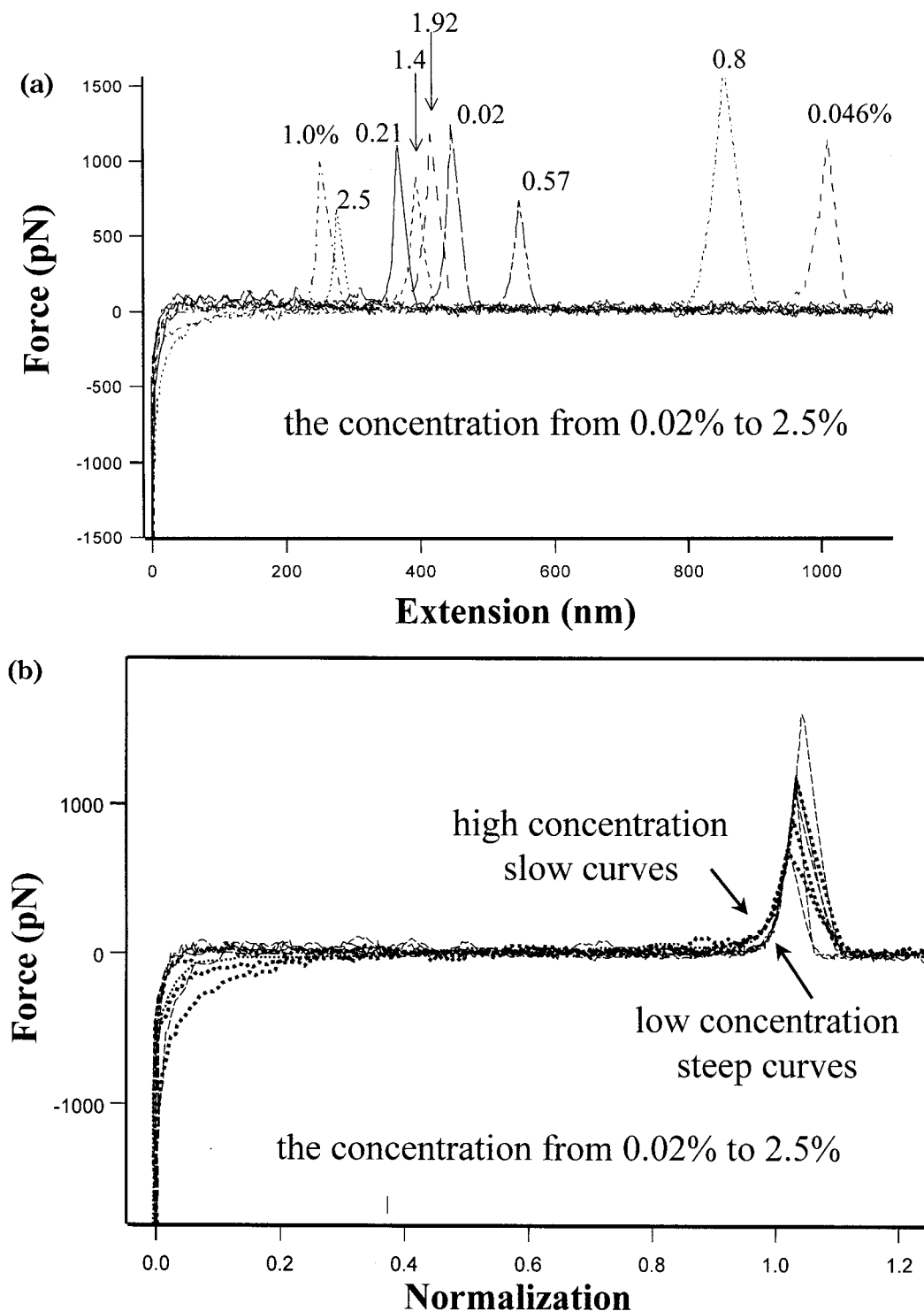(37) Zhang, W. K.; Zou, S.; Wang, C.; Zhang, X. *J. Phys. Chem. B* **2000**, *104*, 10258.

Figure 4. (a) Some typical force extension curves for HM-EHEC samples of different concentrations using different cantilevers. (b) Normalized force curves of HM-EHEC shown in Figure 4a.

beginning of the elongation. The deviation of the two kinds of force curves in the low-force regime is about 110 pN. The force law of polymer chains under tension can be derived from the FJC model. To know more about the mechanical characteristics of the HM-EHEC chains, the measured force−extension curves were fitted by the FJC model to obtain the parameters of the mechanical properties. The FJC model treats a polymer as a chain of statistically independent segments of length l_k (Kuhn length). The force law of the FJC model is given by the Langevin function. The elasticity of a freely jointed chain comes purely from entropic contributions. The FJC model

can only describe the elastic behaviors of the polymer chain at low forces, but a great discrepancy between the predictions of the FJC model and the experimental data could be found at high forces.[19,37] Following Bustamante[7] and Gaub,[8] a modified FJC model was used to fit the data. This modified FJC model is constructed from a fixed number of segments whose lengths increase upon stress (eq 1). The fit curves were superimposed very well with the experimental force curves, and all are plotted in Figure 7. Figure 7a shows the results for experiments performed below the critical concentration, which gave a Kuhn length of 1.60 ± 0.10 nm and a segment elasticity of 26 500 ±
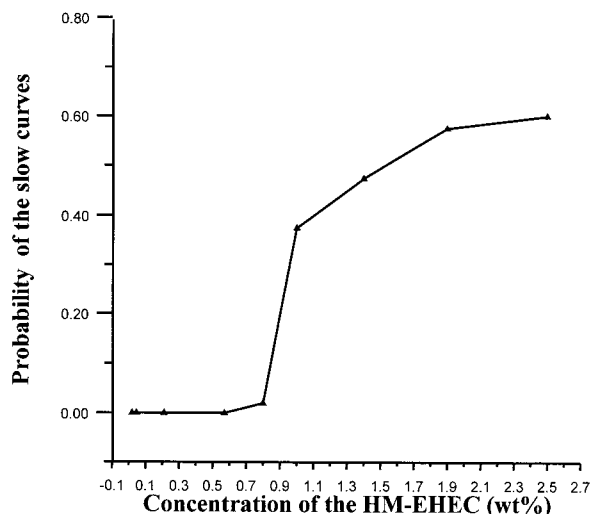
Figure 5. Determination of the critical concentration for micelle-like clusters of HM-EHEC at a solid/liquid interface.

1600 pN/nm. Figure 7b presents the results for experiments performed above the critical concentration, which gave a Kuhn length of 1.10 ± 0.15 nm and a segment elasticity of $25\,500 \pm 1500$ pN/nm. The parameters in Figure 7 represent the fit results of the force−extension curves, and the inset figure displays the fully normalized force traces about each kind of curve, which also indicate single-molecule stretching. Also, the use of various scan rates from 500 to 16 000 scan/s did not show any changes in the elasticity of HM-EHEC.

In the high-force regime, which reflects the mechanical features of the main chains, all of the force curves exhibited the same elastic characteristics. Because the FJC model is not able to consider the long-range interactions in the polymer coils, the differences presented in the low-force range might be due to the measured elasticity reflecting the mechanical properties of the segment part along with the hydrophobic groups, thus leading to shorter Kuhn lengths for the polymer chains.

Substituent-Induced Effects on Elastic Properties of the HM-EHEC Chains. To confirm the true origin of the differences in the force curves in the low-force regime and to investigate the effects of side chains, we measured unmodified hydroxyethyl cellulose (HEC) using SMFS. The HEC/water system is known to have a very good miscibility, so that it does not form micelles or clusters below a certain concentration.[38]

As before, the representative force−extension curves of HEC at different concentrations were measured using different samples and cantilevers, and the results are shown in Figure 8a. An interesting finding is that, despite the various of contour lengths, these force curves were able to be normalized and superimposed very well, as shown in Figure 8b, which comprehensively records the single-chain stretching process.

In comparing the normalized force curves of HM-EHEC with those of HEC, we found that the elasticity of HEC was different from that of HM-EHEC above the critical concentration in the low-force regime, as shown in Figure 9. The inset shows an expanded view of the region around a normalization of 1.0. In addition, the force curves of HM-EHEC obtained below the critical concentration were almost as the same as the force curves of HEC. As a consequence of its different mechanical features, the force−extension curves of HEC were fitted by the modified FJC model to obtain information on its elastic characteristics, and the fit trace superimposed well with the measured force curve. The results are presented in Figure 10.

From the fit data, one can clearly detect a difference in the elasticities of the two kinds of polymers, and the only difference in their structures is their hydrophobic sub-
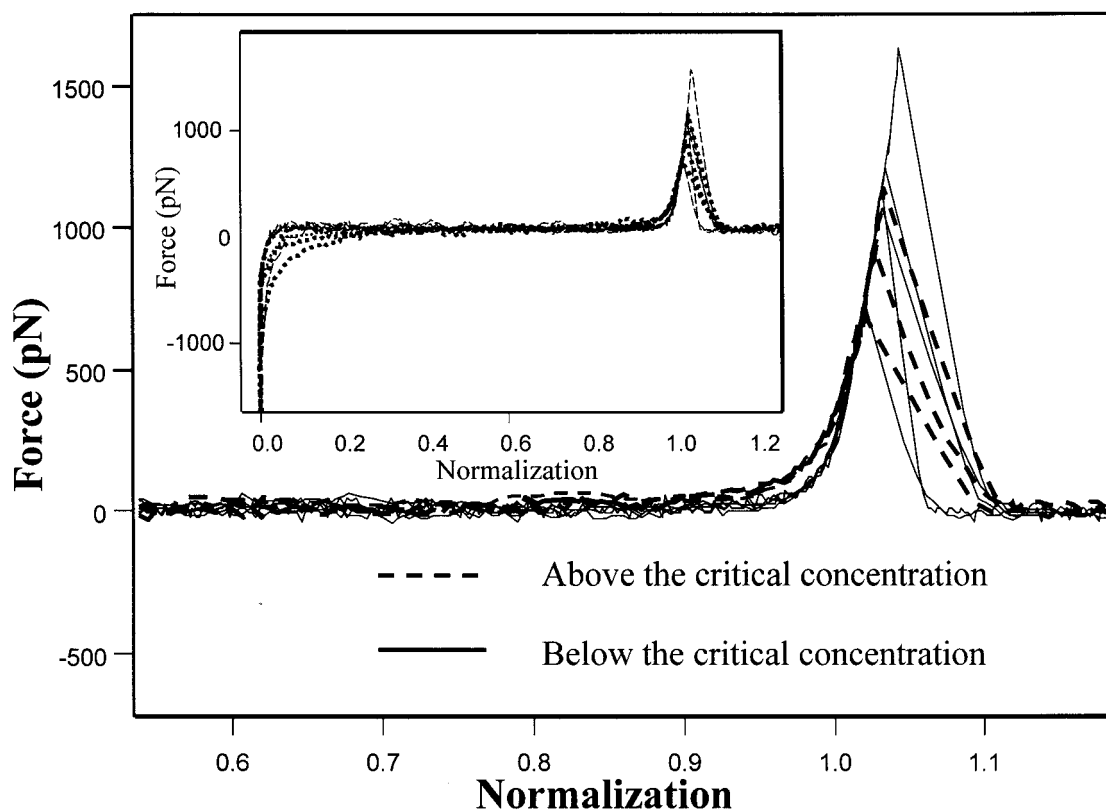

Figure 6. Comparison of normalized force curves in aqueous solution below and above the critical concentration.
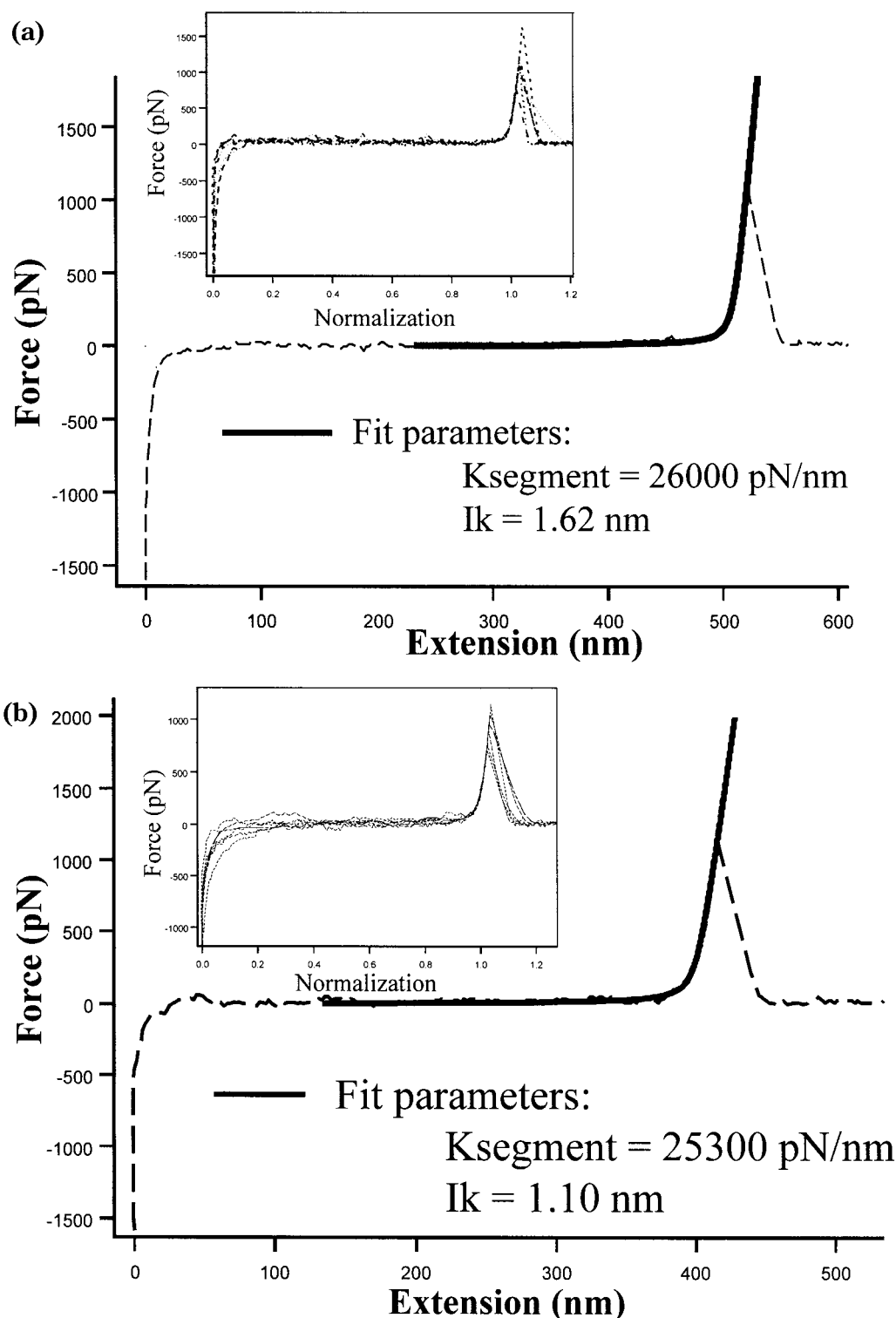
(a)

(b)

Figure 7. (a) Measured force—extension curves of a HM-EHEC chain below the critical concentration superimposed by a fit with a modified FJC model. (b) Measured force—extension curves of a HM-EHEC chain above the critical concentration superimposed by a fit with a modified FJC model.

stituent groups. As for $K_{segment}$, HEC and HM-EHEC have the same kind of glucopyranose ring backbone, and it is reasonable to assume that they have almost the same $K_{segment}$ value. For HM-EHEC below the critical concentration, the polymer chains cannot form any kind of micelle-like structures, so HM-EHEC exhibits almost the same elastic properties as HEC at all concentrations.

Hence, we can conclude that HM-EHEC can form the micelle-like clusters through the hydrophobic interactions of the long-chain substituent groups, which induces the differences in elasticity between the HEC and HM-EHEC chains.

Influence on Surfactant Binding with HM-EHEC. During the past decade, hydrophobically modified polymers have found increasing use as stabilizers in emulsions and dispatchers of active substances in drug formulations,

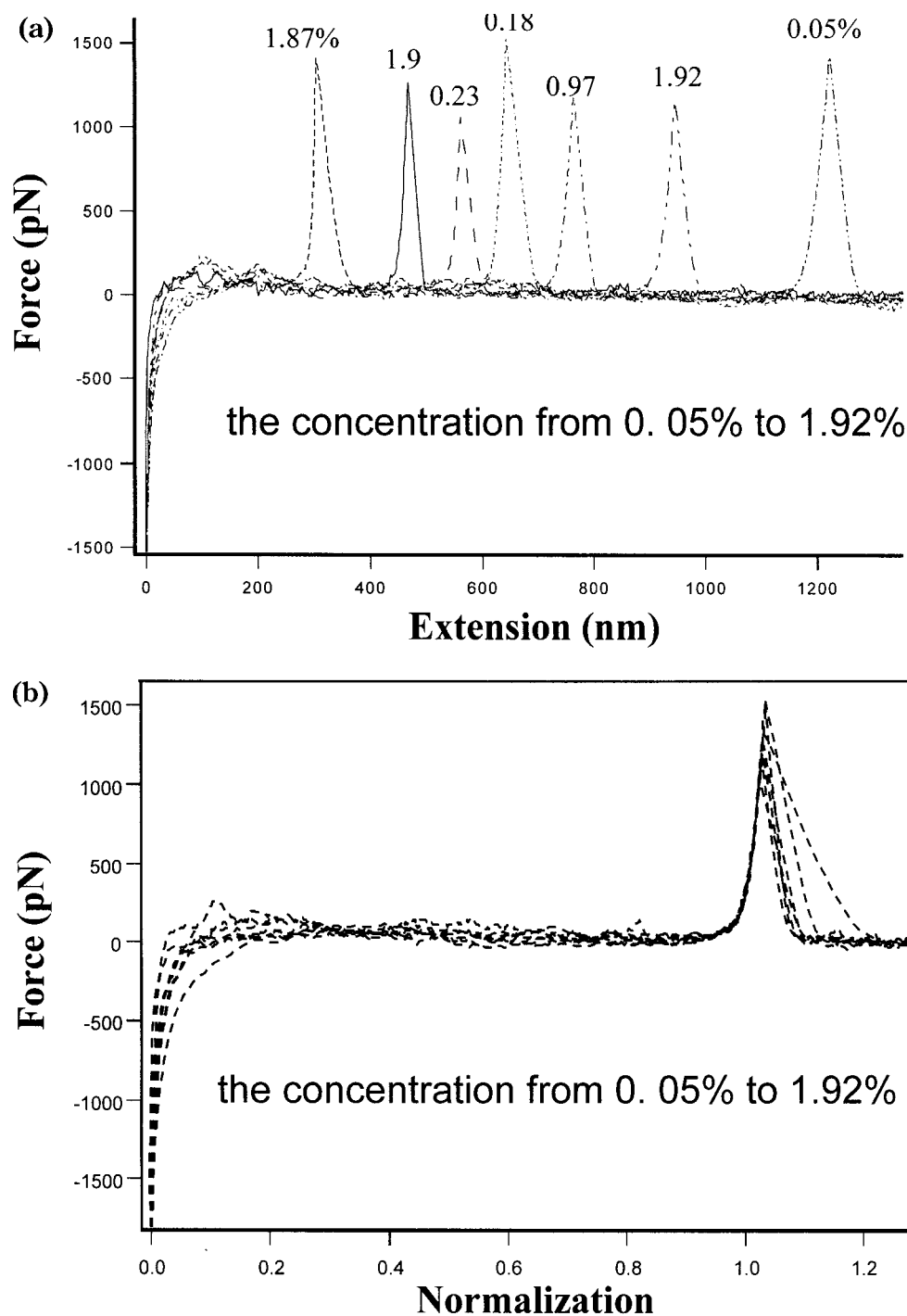(38) Gerharz, B.; Horst, R.; Wolf, B. A. *Colloid Polym. Sci.* **1996**, *274*, 439.

Figure 8. (a) Superposition of some typical force—extension traces of HEC at different concentrations. (b) Normalized force curves of HEC shown in Figure 8a.

as well as in the modification of surfaces from hydrophobic to hydrophilic or vice versa.[29] In recent years, the number of investigations has increased with a natural focus on the particular behaviors of the aqueous solutions and the changes of some properties after the addition of cosolutes such as surfactants. Many researchers have reported that the small surfactant sodium dodecyl sulfate (SDS) can bind with hydrophobically modified polymers and that addition of the proper concentration of SDS (~2−4 mM) can greatly enhance the interactions between the hydrophobic groups.[29−33,40] With further addition of SDS to the

solution, up to a surfactant concentration of 20 mM, the hydrophobic interactions of the side chains can be reduced dramatically.[29−32] Using SMFS to study HM-EHEC binding with different concentrations of SDS can provide further information about the mechanism of the mixture as well as assisting in molecular identification.

For the sake of comparison, the normalized force traces of a pure polymer sample are plotted with the normalization force curves of samples to which SDS had been added in Figure 11. For small amounts of SDS (2.5 mM), the normalized force curves of HM-EHEC under the critical concentration in the low-force regime deviate from those of the pure polymer system and from those of systems with more added SDS system. Along with the previous

(39) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122.
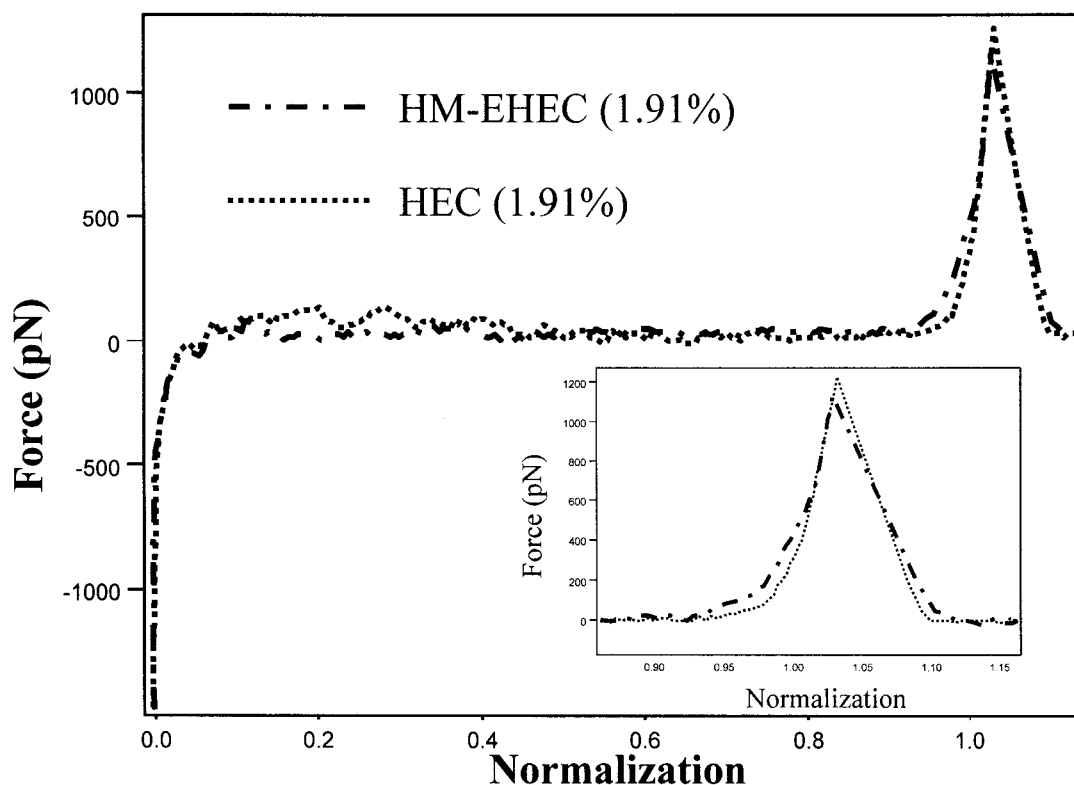(40) Aubry, T.; Moan, M. *Macromolecules* **1998**, *31*, 9072.

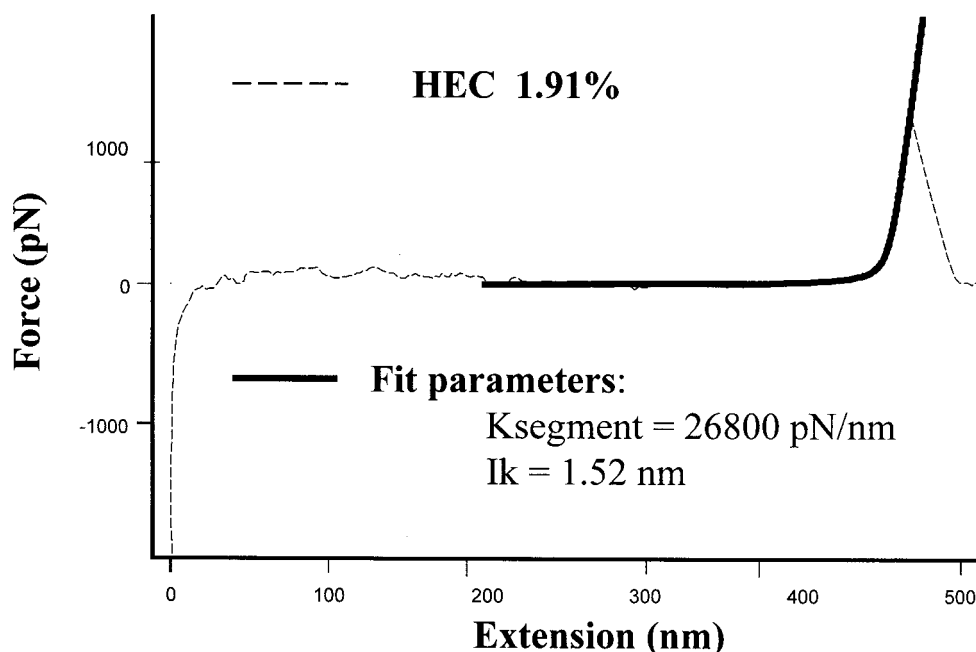**Figure 9.** Comparison of normalized force curves between HM-EHEC and HEC.

Figure 10. Force curve of a single HEC chain superimposed by a fit with a modified FJC model.

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.[31] Upon the addition of more SDS (20 mM) to the polymer/ surfactant system,[33] the micelles that have been formed by the association of the polymer and surfactant might be destroyed. Consequently, the deviations of the force curves in the low-force regime disappear, and the force traces

show characteristics similar to those of systems of polymer below the critical concentration that do not contain surfactant.

In summary, the stretching−relaxing experiments using single-molecule force spectroscopy provide ample evidence that a single polymer chain is deformed. SMFS on HM-EHEC has been carried out, and the critical concentration of HM-EHEC for micelle-like clusters at a solid/liquid interface has been identified. The force−extension curves clearly reflect the differences in the mechanical properties for systems below and above the critical concentration.
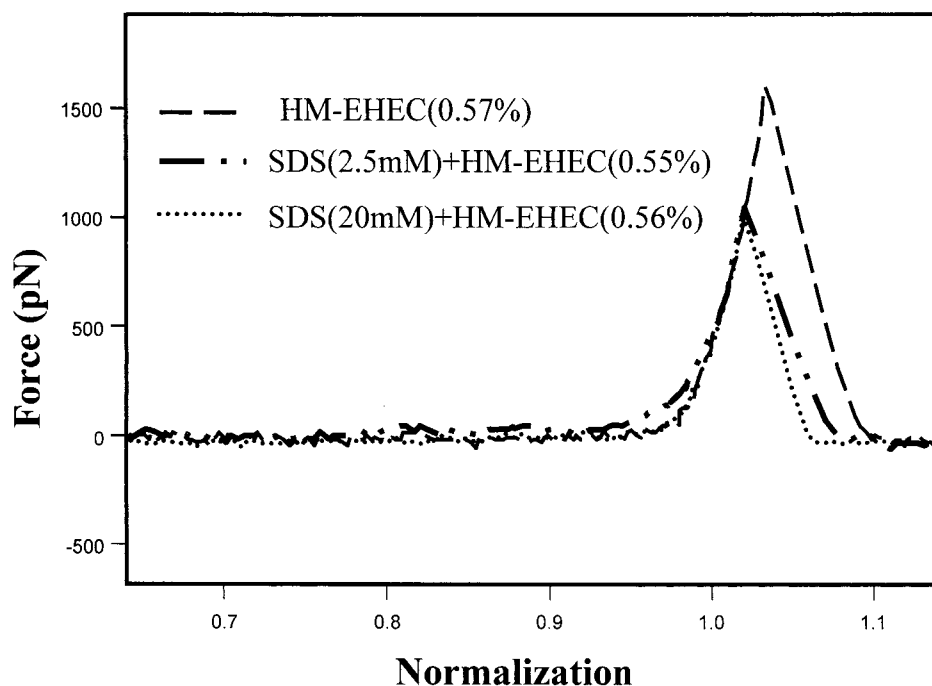
Figure 11. Comparison of normalization force curves for pure HM-EHEC, HM-EHEC with 2.5 mM SDS, and EHEC with 20 mM SDS.

When the experimental conditions were fixed above the critical concentration, the polymer chains can present strong hydrophobic interactions through the formation of micelle-like clusters. Compared with unmodified HEC, the substituent groups could induce effects on the formation of the micelle-like clusters through hydrophobic interactions among the polymer chains. The proper concentration of the surfactant SDS greatly influences surfactant binding with HM-EHEC below the critical concentration. A relatively high concentration of about 20 mM of the surfactant SDS reduces the influence of the SDS on the polymer.

LA010329D