Single Macromolecule Nanomechanical Design: Poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) Graft Copolymers of Varying Architecture

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Introduction

With the advent of nanotechnology, the behavior of individual polymer chains is increasingly being studied due to their potential to act as molecular springs, switches, sensors, shock absorbers, and motors. Single molecule force spectroscopy (SMFS) or "tensile testing" is a powerful technique that is fast enabling rational nanomechanical design of synthetic polymers for such applications.[1−3] Recently, we reported the synthesis and characterization of a series of poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol) or poly(HEMA-*g*-EG) neutral graft copolymers of varying macromolecular architecture with such functions in mind.[3,4] For accurate SMFS experiments, a number of experimental factors were addressed,[3,4] including (1) end-functionalization for strong and specific end-tethering to a planar substrate, (2) high enough molecular weight (preferably ∼100 kg/mol) to enable long enough extensions beyond the nonspecific surface forces regime and clear observation of nanomechanical profiles, and (3) verification of low enough grafting densities after chemisorption to ensure probing of single molecules. The graft copolymers studied here were also designed to be water-soluble since aqueous solutions are typically much easier to work with than organic solvents in current nanomechanical devices and also, in the longer term, to enable the use of such polymers for biomedical applications.

SMFS on poly(HEMA-*g*-EG)$_{120K}$ in aqueous solution was reported by us previously (Figure 1).[3] The numerical subscript in the abbreviated polymer name label (referred to in the previous sentence) is the number-average molecular weight, M_n, of the graft copolymer in g/mol (as determined by ^{1}H nuclear magnetic resonance (NMR)) "K" is an abbreviation for 1000. This particular graft copolymer had a poly(ethylene glycol) or PEG side-chain molecular weight, MW_{PEG}, of 2080K, an average number of PEG chains per PHEMA (poly(2-hydroxyethyl methacrylate)) chain, N_{PEG}, of ∼8, corresponding to a 1% molar ratio of PEG to HEMA (graft density), and a number-average degree of polymerization ratio of EG (ethylene glycol) to HEMA (2-hydroxyethyl methacrylate), $DP_{n,EG}/DP_{n,HEMA}$, of 0.4. Poly(HEMA-*g*-EG)$_{120K}$ behaved similar to an extensible freely jointed chain[5] with a statistical segment length, a, between 0.5 and 1.0 nm and segment elasticity, $k_{segment}$, between 5 and 15 N/m, both of which were largely insensitive to solution ionic strength. This relatively low value of the resisting force to extension suggested minimal noncovalent intramolecular interactions and that the PEG side chains were quite effective in causing local expansion of the PHEMA backbone and overcoming hydrophobic collapsing forces between the HEMA methyl groups. In this paper, we report the single molecule nanomechanical properties of a number of poly(HEMA-*g*-EG) graft copolymers with varying macromolecular architecture, in particular with $DP_{n,EG}/DP_{n,HEMA}$ between 0 (PHEMA) and 1.8. Characterization data and corresponding schematics of the architecture of these polymers are given in Figure 2 of ref 4.

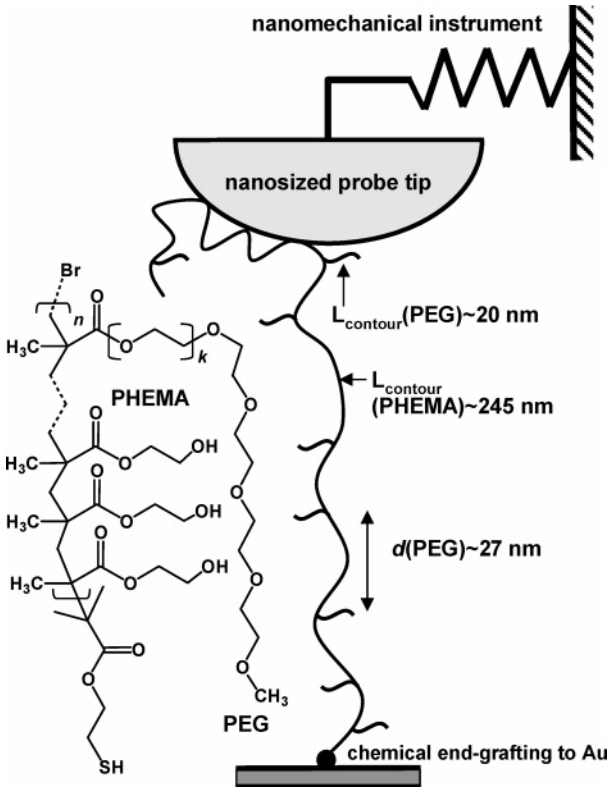

Figure 1. Schematic of single molecule force spectroscopy experiment on poly(HEMA-*g*-EG)$_{120K}$ (poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol)) graft copolymer reported previously (not drawn to scale).[3] The numerical subscript in the abbreviated polymer name label (referred to in the previous sentence) is the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000, as determined by ^{1}H nuclear magnetic resonance (NMR). PHEMA is the poly(2-hydroxyethyl methacrylate) component, and PEG is the poly(ethylene glycol) component of the graft copolymer. This polymer was chemically end-grafted to a Au substrate at low densities via a thiol end group, and a nanomechanical device[6] was employed to tether individual polymer chains to a nanosized Si_3N_4 probe tip and extend it away from the substrate at a (relatively) constant displacement rate of 1 μm/s. The polymeric dimensions labeled above were calculated from ^{1}H NMR data where $L_{contour}$ is the average contour length and d(PEG) is the average extended separation distance between PEG chains.

Experimental Section

Polymer Architectures. The polymers employed in this study were as follows: $PHEMA_{61K}$, poly(HEMA-*g*-EG)$_{122K}$ (MW_{PEG} = 475K, N_{PEG} = 87, molar ratio of PEG to HEMA (graft density) = 14%, $DP_{n,EG}/DP_{n,HEMA}$ = 1.1), poly(HEMA-*g*-EG)$_{106K}$ (MW_{PEG} = 2080K, N_{PEG} = 20, molar ratio of PEG to HEMA = 4%, $DP_{n,EG}/DP_{n,HEMA}$ = 1.8).[4] These data were compared with that of poly(HEMA-*g*-EG)$_{120K}$ (MW_{PEG} = 2080K,

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$N_{PEG} = 8$, molar ratio of PEG to HEMA = 1%, $DP_{n,EG}/DP_{n,HEMA} = 0.4$).[3]

Preparation of End-Grafted Polymer Mushrooms on Gold-Coated Silicon Wafers. 1 cm × 1 cm pieces of a gold-coated silicon wafer[3] were cleaned by first placing them in a piranha solution of H_2SO_4/H_2O_2 (3:1) bath at room temperature for 10 min (*Warning! Piranha solution is extremely oxidizing, reacts violently with organics, and should only be stored in loosely tightened containers to avoid buildup of pressure*). The samples were then extensively rinsed with deionized (DI) water and ethanol, followed by drying in a stream of N_2. All DI water (18 MΩ·cm resistivity, Purelab Plus UV/UF, US Filter, Lowell, MA) used for cleaning, storage, and SMFS experiments was filtered through Millipore syringe filters (pore size = 0.22 μm) prior to use. Each sample was incubated in a polymer solution of concentration 0.05 mg/mL (which is much lower than the polymer critical overlap concentration) for 1 h at room temperature. These chemisorption conditions were found to produce low enough grafting densities (measured by atomic force microscopy imaging) for SMFS experiments. After incubation, the samples were rinsed copiously in methanol, acetone, DMF, methanol, and DI water followed by storage in DI water to equilibrate for 12 h.

Single Molecule Force Spectroscopy (SMFS). SMFS experiments were conducted using a cantilever-based instrument, the 1-D Molecular Force Probe (1DMFP[6]) set on a Halcyonics MOD-1 active vibration isolation system, to measure force, F (nN), vs probe tip−sample separation distance, D (nm) (henceforth referred to as "distance") on "approach" (i.e., probe tip advancing toward the surface) and "retract" (i.e., probe tip moving away from surface). Force vs distance curves were measured at room temperature using Thermomicroscopes microfabricated V-shaped Si_3N_4 cantilevers (cantilever length of 320 μm, cantilever width of 22 μm, cantilever thickness of 0.6 μm, spring constant of 0.01 N/m, and resonant frequency of 7 kHz) with the square-pyramidal probe tip. The experiments were performed at a constant z-piezo displacement rate = 1 μm/s reversing immediately between approach and retract cycles with a z-piezo range = 1 μm (slow enough to minimize hydrodynamic effects), and the rate of data acquisition = 4000 points/s, in DI water (pH = 5.6). Approximately 1000 force spectroscopy experiments were performed at ∼20 different sites on each polymer-functionalized Au substrate. The probe tip end radius was found to be equal to ∼25 nm for all of the experiments reported in this paper, as measured via scanning electron microscopy (JEOL 6320 SEM with an operating voltage of 5 kV and magnitude of 64000×). The distances reported were normalized by the experimentally observed polymer contour length, $L_{contour}$, to obtain relative extension for direct comparison of multiple SMFS experiments, as described previously.[7]

Results and Discussion

Figure 2a shows typical nanomechanical data for end-grafted $PHEMA_{61K}$ ($DP_{n,EG}/DP_{n,HEMA} = 0$) homopolymer in deionized water which was observed in ∼10% of the total force spectroscopy experiments performed while the remaining ∼90% predominantly exhibiting a single surface adhesion pull-off on retract at $D = 0$ and no subsequent long-range adhesive events (Table 1). Here, we see that an initial nonspecific surface adhesion exists between $D/L_{contour} = 0-0.2$ with adhesion force magnitudes up to ∼1 nN (off the y-scale of Figure 2a), typical for hydrophobic surfaces such as $PHEMA_{61K}$, which is insoluble in DI water at room temperature.[4] For $D/L_{contour} > 0.2$, the force profile on retract exhibits plateaus of constant force with increasing extension. This phenomenon has been predicted[8,9] and observed experimentally for synthetic polymers in poor solvents[10,11] and has been interpreted in two ways. The first is termed a *Rayleigh instability* which consists of a first-order transition into a "ball−string" configuration where the applied tension draws out one monomer at a time into a thin filament from a collapsed, surface-bound globule due to high polymer−solvent interfacial energy.[8,10] The second involves an assumption that the polymer chain is adsorbed on the surface as a series of loops and at slow enough extension rates the monomers have enough time to detach and re-form multiple times so that the tension is distributed over all the monomers in the chain.[9] As described previously,[8−11] both of these explanations involve pulling of a chain one monomer at a time from an attractive potential into a zero mean potential that arises from the poor solvency of the chain in the solution. Figure 2b is a histogram plot of the probability distribution for the experimentally observed $PHEMA_{61K}$ plateau forces and shows a bimodal distribution with a total mean and standard deviation for the

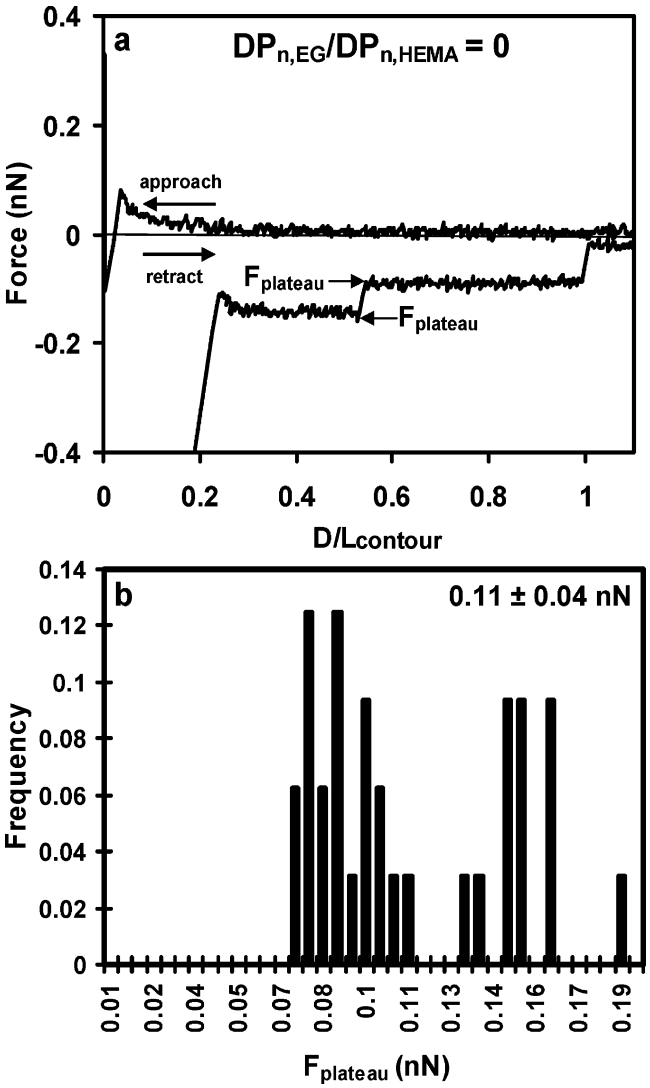

Figure 2. (a) Typical nanomechanical data for end-grafted $PHEMA_{61K}$ homopolymer (poly(2-hydroxyethyl methacrylate)) on approach (upper data set labeled with arrow pointing toward the left) and retract (lower data set labeled with arrow pointing toward the right) in deionized water. Positive values of force are repulsive and negative values are attractive. Two plateaus are shown with the two plateau forces ($F_{plateau}$) for this particular experiment indicated by the two arrows at $D/L_{contour} = 0.58$ where $L_{contour}$ is the extended contour length of the bridging polymer chain segment. (b) Probability distribution of plateau forces observed on retract in the nanomechanical experiment on $PHEMA_{61K}$ in deionized water; inset values are the mean and standard deviation for the data set.

Table 1. Parameters Describing Macromolecular Architecture of the Polymers Studied (As Calculated from ^{1}H Nuclear Magnetic Resonance (NMR))[4] and Percentages of Adhesion Events Observed in Various Force Spectroscopy Datasets for Chemically End-Grafted PHEMA and Poly(HEMA-*g*-EG) Graft Copolymers[a]

	PHEMA	poly(HEMA-*g*-EG)		
M_n (K)	61	120	122	106
MW(PEG)	0	2080	475	2080
$DP_{n,EG}/DP_{n,HEMA}$	0	0.4	1.1	1.8
mol PEG/mol HEMA	0	1	14	4
surface adhesion only or nonadhesion	87.9	91.3	84.6	68.7
plateaus	10.6	0.0	0.6	4.3
nonlinear peak	1.5	8.7	14.8	27.0

[a] M_n is the number-average molecular weight of the graft copolymer in g/mol, and "K" is an abbreviation for 1000. MW_{PEG} is the PEG (poly(ethylene glycol)) side-chain molecular weight, and $DP_{n,EG}/DP_{n,HEMA}$ is the a number-average degree of polymerization ratio of EG (ethylene glycol) to HEMA (2-hydroxyethyl methacrylate). PHEMA is poly(2-hydroxyethyl methacrylate). Approximately 1000 measurements were employed for each data set presented.

data set equal to 0.11 ± 0.04 nN. Depending on which interpretation is employed to explain the plateau events, the quantized distribution of plateau forces could represent multiple filaments being drawn out of the same collapsed globule and bridging the surface and probe tip in parallel or different regions of the same polymer chain adsorbed in parallel to the probe tip and simultaneously detaching from the surface.

Figure 3 shows representative SMFS data on retract for the end-grafted poly(HEMA-*g*-EG) copolymers in DI water at three different values of $DP_{n,EG}/DP_{n,HEMA}$, which was observed to occur in 9−30% of the total force spectroscopy experiments (Table 1), while the majority of the remaining experiments exhibited a single surface adhesion pull-off on retract at $D = 0$ and no subsequent long-range adhesive events (Table 1). The single, nonlinear attractive peaks observed are due to the stretching of individual polymer chains bridging the substrate and the probe tip, which had nonspecifically adsorbed a number of tethering polymer chain segments during the course of the force spectroscopy experiment.[3] The linear data recorded for $D/L_{contour} > 1.0$ on retract represents a mechanical instability of the cantilever that takes place after detachment of the polymer chain from the probe tip and, hence, does not represent the true desorption interaction profile. It should be noted that since the magnitude of the polymer−probe tip detachment forces was observed to always be less than that needed to cleave the weakest covalent bond (Au−S[7]), it is assured that the polymer chain always detaches from the probe tip after completion of each force spectroscopy experiment.

A noticeable change in the shape and magnitude of the single macromolecule elasticity curves was observed depending on the macromolecular architecture, in particular an increasing resistance to extension with increasing $DP_{n,EG}/DP_{n,HEMA}$. This phenomenon was quantified in Figure 4, which shows an increase in the force necessary for 90% relative extension, $F(0.9D/L_{contour})$, of a single polymer chain with increasing $DP_{n,EG}/DP_{n,HEMA}$; an increase in magnitude of ∼3.5× was observed from $DP_{n,EG}/DP_{n,HEMA} = 0.4-1.8$. Unpaired, two-tailed *t*-tests were performed on these data showing all combinations to be statistically different ($p < 0.05$) except for $DP_{n,EG}/DP_{n,HEMA} = 0$ ($F_{plateau}$) compared to $DP_{n,EG}/DP_{n,HEMA} = 0.4$.

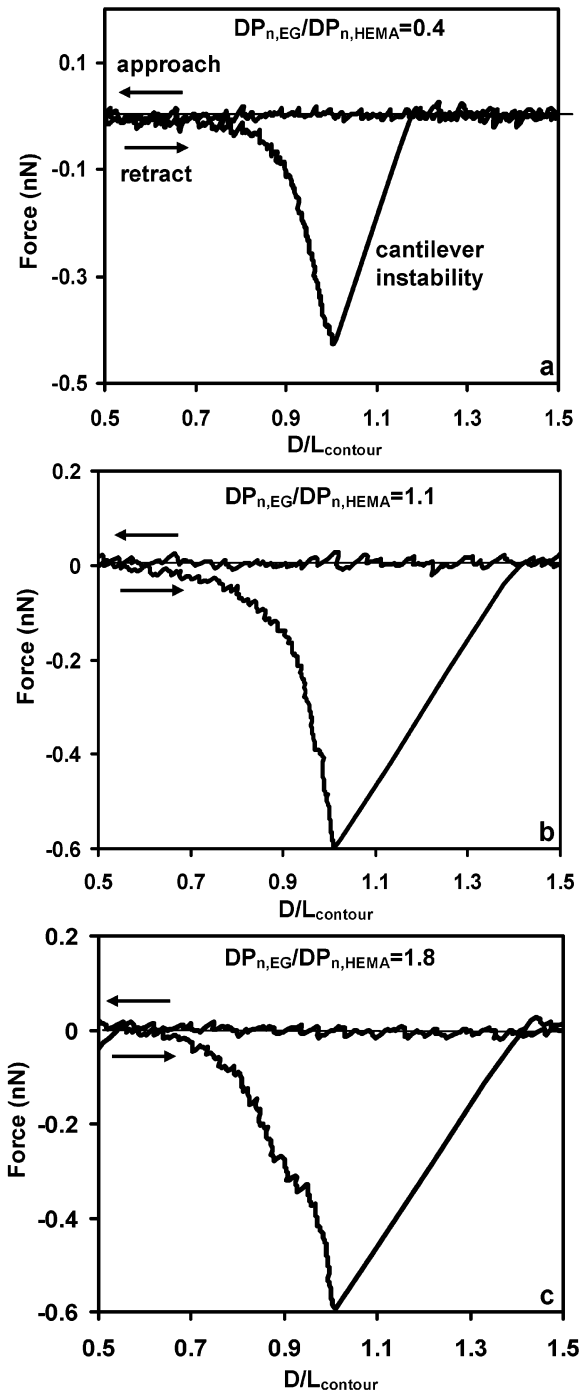

Figure 3. Typical single molecule force spectroscopy data for end-grafted copolymers in deionized water: (a) poly(HEMA-*g*-EG)$_{120K}$, MW_{PEG} = 2080K, $DP_{n,EG}/DP_{n,HEMA}$ = 0.4, (b) poly(HEMA-*g*-EG)$_{122K}$, MW_{PEG} = 475K, $DP_{n,EG}/DP_{n,HEMA}$ = 1.1, and (c) poly(HEMA-*g*-EG)$_{106K}$, MW_{PEG} = 2080K, $DP_{n,EG}/DP_{n,HEMA}$ = 1.8 on approach (upper data set labeled with arrow pointing toward the left, zero baseline) and retract (lower data set labeled with arrow pointing toward the right). Positive values of force are repulsive and negative values are attractive. Poly(HEMA-*g*-EG) is (poly(2-hydroxyethyl methacrylate-*g*-ethylene glycol)) graft copolymer. The numerical subscript in the abbreviated polymer name label is the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000, as determined by ^{1}H nuclear magnetic resonance (NMR). MW_{PEG} is the PEG (poly(ethylene glycol)) side chain molecular weight, and $DP_{n,EG}/DP_{n,HEMA}$ is the number-average degree of polymerization ratio of EG (ethylene glycol) to HEMA (2-hydroxyethyl methacrylate). $L_{contour}$ is the extended contour length of the bridging polymer chain segment.

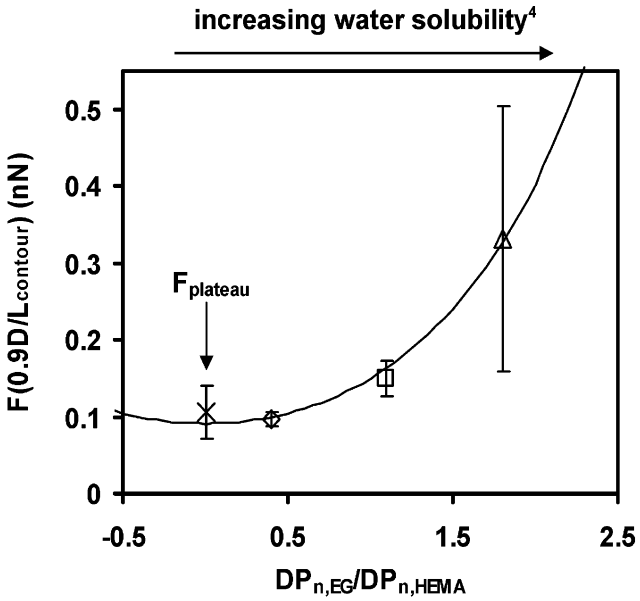

Figure 4. Force necessary for 90% relative extension ($D/L_{contour}$) for individual end-grafted polymer chains in deionized water as a function of $DP_{n,EG}/DP_{n,HEMA}$ except for $DP_{n,EG}/DP_{n,HEMA} = 0$ ($PHEMA_{61K}$) where the force plotted is $F_{plateau}$. D is the extensional distance, and $L_{contour}$ is the extended contour length of the bridging chain segment during a single molecule force spectroscopy experiment. $DP_{n,EG}/DP_{n,HEMA}$ is the number-average degree of polymerization ratio of EG (ethylene glycol) to (2-hydroxyethyl methacrylate). PHEMA is poly(2-hydroxyethyl methacrylate). The symbols indicate experimental means, hi−lo bars represent experimental standard deviations for each data set, and the solid red line is an empirical, two-parameter curve fit to $y = A\cosh(Bx)$ ($A = 0.0909$, $B = 1.0853$, $R^2 = 0.98779$) shown in order to emphasize trend in the data.

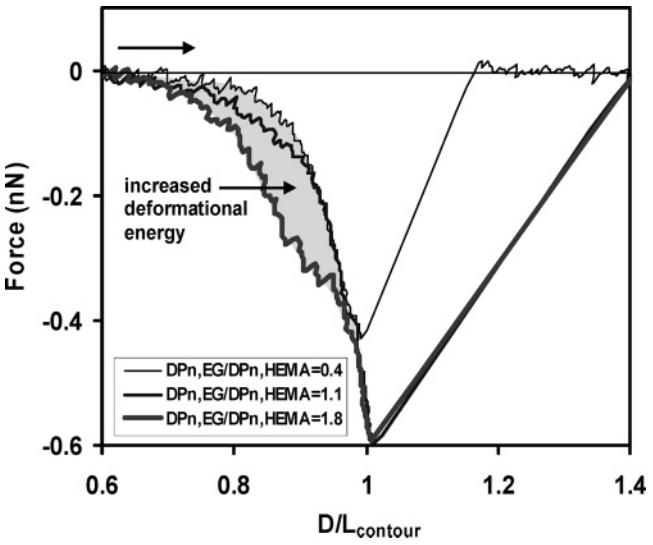

Figure 5. Overlay of typical single molecule force spectroscopy data on retract for end-grafted copolymers in deionized water; poly(HEMA-*g*-EG)$_{120K}$, MW_{PEG} = 2080K, $DP_{n,EG}/DP_{n,HEMA} = 0.4$ which has the lowest force during much of the extension range, poly(HEMA-*g*-EG)$_{122K}$, MW_{PEG} = 475K, $DP_{n,EG}/DP_{n,HEMA} = 1.1$ which has an intermediate force, and poly(HEMA-*g*-EG)$_{106K}$, $MW_{PEG} = 2080$K, $DP_{n,EG}/DP_{n,HEMA} = 1.8$ which has the highest force during the majority of the extension range. The shaded area corresponds to the increase in single molecule deformational energy from $DP_{n,EG}/DP_{n,HEMA} = 0.4-1.8$. D is the extensional distance, and $L_{contour}$ is the extended contour length of the bridging chain segment during a single molecule force spectroscopy experiment. The numerical subscript in the abbreviated polymer name label is the number-average molecular weight, M_n, of the graft copolymer in g/mol and "K" is an abbreviation for 1000, as determined by ^{1}H nuclear magnetic resonance (NMR). MW_{PEG} is the PEG (poly(ethylene glycol)) side chain molecular weight and $DP_{n,EG}/DP_{n,HEMA}$, is the a number-average degree of polymerization ratio of EG (ethylene glycol) to HEMA (2-hydroxyethyl methacrylate). The arrow pointing to right indicates the retraction direction.

The extensional force observed for the highest graft density poly(HEMA-*g*-EG) copolymers ($DP_{n,EG}/DP_{n,HEMA} = 1.8$) was found to be up to 2× larger in magnitude than that known for PEG homopolymer in aqueous solution,[12] which exhibits a reversible, strain-induced conformational transition from the water-bound, contracted, helical trans−trans−gauche (*ttg*) state to the more extended trans−trans−trans (*ttt*) state. An increase in the standard deviation of the extensional force values for the poly(HEMA-*g*-EG) copolymers was also observed with increasing $DP_{n,EG}/DP_{n,HEMA}$, suggesting a larger degree of heterogeneity in the polymeric supramolecular structure.

Correspondingly, Figure 5 shows an overlay of typical SMFS data on retract for end-grafted poly(HEMA-*g*-EG) copolymers of varying $DP_{n,EG}/DP_{n,HEMA}$ in DI water (corresponding to the data given in Figure 3), which shows a significant increase in the deformational energy or work of extension (i.e., the area under the force vs relative extension curve).

An increase in the single molecule resistance to extension and deformational energy may have two possible origins: entropic (i.e., a decrease in a, the local statistical segment length[5]) or enthalpic (i.e., an increase in the macromolecular noncovalent interactions[12]). Previously, the graft copolymers studied here were found to have an inverse temperature solubility in aqueous solution with a cloud point that increased with $DP_{n,EG}/DP_{n,HEMA}$ and insolubility observed for $DP_{n,EG}/DP_{n,HEMA} \leq 0.4$.[4] These data are not consistent with a decrease in a, and additionally, fits of the $DP_{n,EG}/DP_{n,HEMA} = 1.8$ block copolymer SMFS data to the extensible freely jointed chain model[5] yielded unrealistically low values of a, both suggesting an enthalpic origin to this trend. Direct H-bonding interactions between the −OH groups of the HEMA backbone and the −O− groups of the PEG side chains or of HEMA with itself via the −OH and −O−C=O groups is not likely, since this would be expected to decrease solubility.[13] Hence, one likely possibility for origin of the noncovalent macromolecular interactions could be via a water bridging mechanism between the HEMA main chain and PEG side chains.

Conclusions

In this paper, we have shown a variation in the extensional single molecule nanomechanical properties depending on macromolecular architecture of graft copolymers of poly(HEMA-*g*-EG). In particular, a statistically significant increase in the force necessary for relative extension (and hence, deformational energy) was observed with increasing $DP_{n,EG}/DP_{n,HEMA}$; for example, up to 3.5× at 90% relative extension for $DP_{n,EG}/DP_{n,HEMA} = 0.4-1.8$. This trend in the data was suggested to arise from noncovalent macromolecular interactions, possibly via a water bridging mechanism between the HEMA main chain and PEG side chains. Such synthetic graft copolymer systems hold potential to establish single-molecule structure−property rela-

tionships as well as design single macromolecule nanomechanical properties.

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