Anal. Chem. **1999,** *71,* 4418−4422

Spin-Stretching of DNA and Protein Molecules for Detection by Fluorescence and Atomic Force Microscopy

Hiroki Yokota,*,† James Sunwoo,† Mehmet Sarikaya,‡ Ger van den Engh,† and Ruedi Aebersold†

Departments of Molecular Biotechnology and Material Sciences & Engineering, University of Washington, Box 357730, Seattle, Washington 98195

We have developed a rapid and efficient way of stretching DNA and denatured protein molecules for detection by fluorescence microscopy and atomic force microscopy (AFM). In the described method, a viscous drag created by a transient rotational flow stretches randomly coiled DNA molecules or denatured proteins. Stretching is achieved by dispensing a droplet of sample solution containing DNA or denatured protein on a $MgCl_2$-soaked mica surface. We present fluorescent images of straightened λDNA molecules and AFM images of stress-sheared, reduced von Willebrand factor as well as straightened λDNA. The described quick and reliable spin-stretching technique will find wide applications in the analysis of single biopolymer molecules.

In any cell or molecular biological investigation, whether studying protein, DNA, or RNA, it is necessary to extract material from a large number of cells, and thus to accept the interpretational limitations due to inherent heterogeneity of such preparations. Since differences in structural features such as biochemical modifications or subunit composition and stoichiometry of any one protein, protein/DNA, or protein/RNA complex frequently reflect a difference of activity, it is easy to see that, due to the sample heterogeneity, one is limited to measuring an average or at least dominant structural state. To overcome such limitations and to detect the full distribution of structural states present in the sample, it is necessary to be able to study single molecules and/or complexes individually. Stretching and immobilizing biopolymers on a flat surface provides an excellent sample preparation for single-molecule detection by fluorescence microscopy and atomic force microscopy (AFM). Fluorescent images of straightened chromatin or DNA, for instance, have been found informative in fiber-FISH mapping[1−4] and optical restriction mapping.[5−8]

Stretching DNA has been achieved using various forces such as magnetic force,[9] laser-optical force,[10−13] dielectric force,[14−16] surface tension,[17,18] and viscous drag.[19] These stretching methods require either a delicate polymer end-labeling or a careful substrate surface modification. None of the methods has been applied to stretching and immobilizing protein molecules. In this report, we have developed an easy and reliable method for stretching DNA and protein molecules using a transient rotating flow. Samples prepared in this manner can be studied by fluorescence microscopy as well as by AFM.[20−24] Stretching was achieved by spinning an atomically flat mica plate with a dc motor at 4000−7000 rpm and dispensing a droplet of DNA or denatured protein solution on a spinning center. We demonstrate that this spin-stretcher effectively stretches 48.5-kbp λDNA and denatured,

* Corresponding author (current address): Departments of Mechanical Engineering and Anatomy, Indiana University Purdue University at Indianapolis, 635 Barnhill Drive, MS-504, Indianapolis, IN 46202. (phone) 317-274-2448; (fax) 317-274-9744; (e-mail) hiroki@anatomy.iupui.edu.

† Department of Molecular Biotechnology.

‡ Department of Material Sciences & Engineering.

(1) Parra, I.; Windle, B. *Nature Genet.* **1993**, *5*, 17−21.
(2) Haaf, T.; Ward, D. C. *Hum. Mol. Genet.* **1994**, *3*, 629−633.
(3) Weier, H.-U. G.; Wang, M.; Mullikin, J. C.; Zhu, Y.; Cheng, J.-F.; Greulich, K. M.; Bensimon, A.; Gray, J. W. *Hum. Mol. Genet.* **1995**, *4*, 1903−1910.
(4) Michalet, X.; Ekong, R.; Fougerousse, F.; Rousseaux, S.; Schurra, C.; Hornigold, N.; van Slegtenhorst, M.; Wolfe, J.; Povey, S.; Beckmann, J. S.; Bensimon, A *Science* **1997**, *277*, 1518−1523.
(5) Schwartz, D. C.; Li, X.; Hernandez, L. I.; Ramnarain, S. P.; Huff, E. J.; Wang, Y.-K. *Science* **1993**, *262*, 110−114.
(6) Wang, Y.-K.; Huff, E. J.; Schwartz, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 165−169.
(7) Meng, X.; Benson, K.; Chada, K.; Huff, E. J.; Schwartz, D. C. *Nature Genet.* **1995**, *9*, 432−438.
(8) Yokota, H.; Johnson, F.; Lu, H.; Robinson, R. M.; Belu, A. M.; Garrison, M. D.; Ratner, B. D.; Trask, B. J.; Miller, D. L. *Nucleic Acids Res.* **1997**, *25*, 1064−1070.
(9) Smith, S. B.; Finzi, L.; Bustamante, C. *Science* **1992**, *258*, 1122−1126.
(10) Smith, S. B.; Cui, Y.; Bustamante, C. *Science* **1996**, *271*, 795−799.
(11) Quake, S. R.; Babcock, H.; Chu, S. *Nature* **1997**, *388*, 151−154.
(12) Wang, M. D.; Yin, H.; Landick, R.; Gelles, J.; Block, S. M. *Biophys. J.* **1997**, *72*, 1335−1346.
(13) Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. *Science* **1997**, *276*, 1109−1112.
(14) Smith, S. B.; Aldridge, P. K.; Callis, J. B. *Science* **1989**, *243*, 203−206.
(15) Washizu, M.; Kurosawa, O. *IEEE Trans. Ind. Appl.* **1990**, *26*, 1165−1172.
(16) Asbury, C. L.; van den Engh, G. *Biophys. J.* **1998**, *74*, 1024−1030.
(17) Bensimon, A.; Simon, A.; Chiffaudel, A.; Croquette, V.; Heslot, F.; Bensimon, D. *Science* **1994**, *265*, 2096−2098.
(18) Bensimon, D.; Simon, A. J.; Croquette, V.; Bensimon, A. *Phys. Rev. Lett.* **1995**, *74*, 4754−4757.
(19) Zimmermann, R. M.; Cox, E. C. *Nucleic Acids Res.* **1994**, *22*, 492−497.
(20) Binnig, G.; Quate, C. F.; Gerber C. *Phys. Rev. Lett.* **1986**, *56*, 930−933.
(21) Bustamante, C.; Vesenka, J.; Tang, C. L.; Rees, W.; Guthold, M.; Keller, R. *Biochemistry* **1992**, *31*, 22−26.
(22) Siedlecki, C. A.; Lestini, B. J.; Kottke-Marchant, K.; Eppell, S. J.; Wilson, D. L.; Marchant, R. E. *Blood* **1996**, *88*, 2939−2950.
(23) Allison, D. P.; Kerper, P. S.; Doktycz, M. J.; Spain, J. A.; Modrich, P.; Larimer, F. W.; Thundat, T.; Warmack, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8826−8829.
(24) Hansma, H. G.; Kim, K. J.; Laney, D. E.; Garcia, R. A.; Argaman, M.; Allen, M. J.; Parsons, S. M. *J. Struct. Biol.* **1997**, *119*, 99−108.

10.1021/ac9902695 CCC: $18.00
Published on Web 08/20/1999

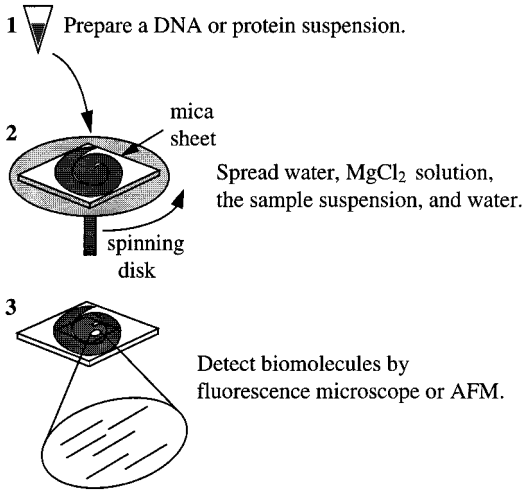

Figure 1. Schematic diagram illustrating the procedure for spin-stretching DNA or denatured protein molecules. For details, see Materials and Methods.

reduced human von Willebrand factor.

MATERIALS AND METHODS

DNA and Protein Preparation. For spin-stretching DNA, we used 48.5-kbp λ-phage DNA (GibcoBRL). A 20-ng sample of λDNA was suspended in a 50-μL solution containing 4 mM $MgCl_2$ and 0.5 μM YOYO-1 fluorescent dye (Molecular Probes Inc.). In contrast to ethidium bromide, which increases DNA contour length up to 40%, YOYO-1 has been observed to associate with double-stranded DNA without significant effect on the intrinsic contour length of the molecule in the time scale (<30 min) used in this report.[25]

For protein stretching, we used human von Willebrand factor, (vWF, a generous gift from L. H. Ericsson and K. A. Walsh), a large multimeric plasma glycoprotein. A monomer subunit of vWF is composed of 2050 amino acids (260 kDa), of which 8.3% are cysteine residues and up to 22 carbohydrate side chains are linked to 12 Asn and 10 Thr/Ser residues, with carbohydrates constituting 18.7% of the total mass.[26,27] Approximately 1 μg of vWF was suspended in a 100 μL of PBS solution containing 6 M guanidine hydrochloride (pH 5.4) and 200 mM DTT and incubated at 24 °C for 5 h before stretching.

Spin-Stretching DNA and Denatured Protein Molecules. Figure 1 illustrates a schematic diagram for spin-stretching DNA or denatured protein. A freshly cleaved mica sheet (25 mm × 25 mm, Ted Pella Inc.) was mounted horizontally on the spin-stretcher using a double-sided tape, and the mica plate was spun by a dc motor (Edmund Scientific) at 6−18 V. A spin speed in rpm without any load is approximated by the formula, $10v^2 + 42v + 3900$, where v is a voltage (in V). The operational speed with a mounted mica (50−100 mg) is slightly lower than the rpm value indicated by the formula. A series of 10-μL droplets was gently dispensed on the spinning center by a pipet, in the following order and with ∼30-s intervals: H_2O for prerinsing, 10 mM $MgCl_2$ solution for precoating the surface, DNA or protein sample solution, and H_2O for postrinsing the surface. It has been reported that a small amount of $MgCl_2$ added to the DNA suspension increases the DNA−surface affinity.[8] The mica surface dried out before the addition of the next solution. The entire spin-stretching operation from prerinsing to postrinsing the surface by water requires only a few minutes.

DNA Imaging by Fluorescence Microscopy. We applied ∼5 μL of antifade solution containing 90% glycerol and 0.1% phenyldiamine (pH 7.5) to the sample immobilized on the mica surface and subsequently placed a 25-mm square cover slip atop the surface. We imaged fluorescently labeled DNA molecules by a CCD camera (model TEA/CCD-1517-K/1, Princeton Instruments, Inc.) mounted on a Zeiss Axiophot fluorescence microscope with 1000× magnification. The YOYO-1 DNA dye has an excitation maximum at 491 nm and an emission maximum at 509 nm. A 100-W mercury arc lamp (USH-102DH, Ushio) was used with a combination of an excitation filter (BP450-490), a dichroic mirror (FT510), and an emission filter (BP515−565, Chroma Technology). The exposure time was 5−10 s, and the field of view was 67.3 μm × 85.5 μm (517 pixels × 658 pixels).

The CCD images were analyzed using IP Lab software (Signal Analysis Corp.). The end-to-end length of DNA molecules in the peripheral region (r = 10 mm) was measured, where r is the radial distance from the spinning center. The DNA lengths were measured in a pixel unit (one pixel = 0.13 μm) and converted into micrometers. We included only straightened DNA that was enclosed in a rectangle of aspect ratio ∼20.

DNA and Protein Imaging by Atomic Force Microscopy. We used a Nanoscope III AFM (Digital Instruments, Inc., Santa Barbara, CA) to image the protein molecules immobilized on the mica. The AFM was operated in the ambient air condition in the tapping mode at ∼200 kHz, a frequency near resonance. The scan rate was 1 Hz with a scan field of view of 1 μm × 1 μm to 5 μm × 5 μm. The Si_3N_4 tips used had an estimated curvature of ∼20 nm. Images were flattened to remove the background curvature of the mica surface and analyzed using NIH Image 1.60 image analysis software.

RESULTS

Spin-Stretching of DNA. To determine the proper conditions for stretching DNA using the spin-stretcher (Figure 1), we spin-stretched fluorescently labeled 48.5-kbp λDNA on a mica surface and examined the distribution and morphology of the immobilized molecules by fluorescence microscopy. Control DNA, deposited gently on the surface without using the spin-stretcher, displayed the shape of a random coil (Figure 2A). DNA molecules spin-stretched at ∼5800 rpm (12 V) exhibited a straight configuration uniformly aligned in one direction (Figure 2B). We examined the morphology of DNA molecules prepared at various spin rates with and without $MgCl_2$ precoating of the mica sheet. The following observations were made (data not shown): spin-coating the mica surface with a 100 mM $MgCl_2$ solution prior to spin-stretching DNA enhanced retention of DNA; DNA was aligned and straightened better at a region slightly away from the spinning center (r = 10 mm) than at the region near the center (r < 5 mm); and spinning at approximately 4500−5800 rpm (6−

(25) Gurrieri, S.; Wells, K. S.; Johnson, I. D.; Bustamante, C. *Anal. Biochem.* **1997**, *249*, 44−53.
(26) Titani, K.; Kumar, S.; Takio, K.; Ericsson, L. H.; Wade, R. D.; Ashida, K.; Walsh, K. A.; Chopek, M. W.; Sadler, J. E.; Fujikawa, K. *Biochemistry* **1986**, *25*, 3171−3184.
(27) Jenkins, P. V.; Pasi, K. J.; Perkins, S. J. *Blood* **1998**, *91*, 2032−2044.

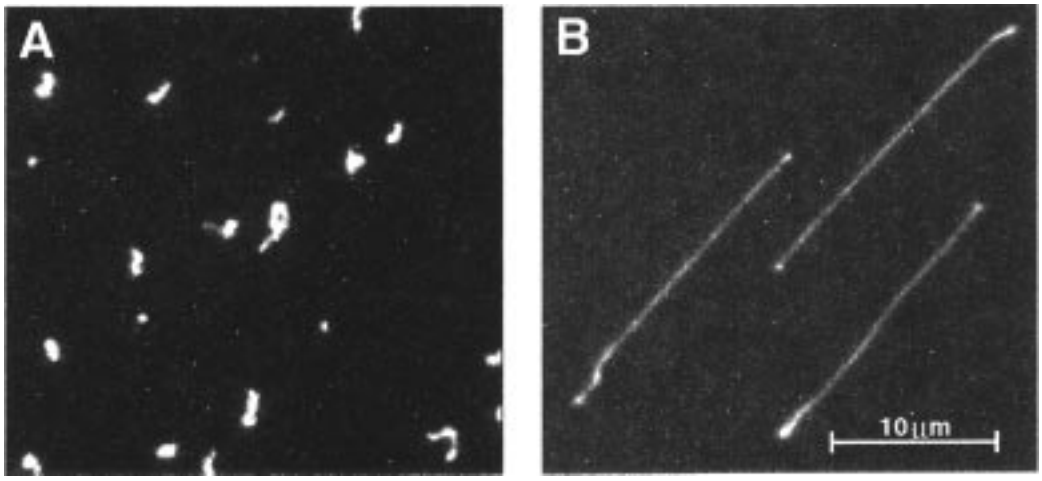

Figure 2. CCD images of fluorescently labeled λDNA molecules. The bar is 10 μm. (A) Control λDNA gently deposited on mica surface without spin-stretching. (B) λDNA spin-stretched at ~5800 rpm (12 V) and immobilized at 10 mm from the spinning center on mica surface.

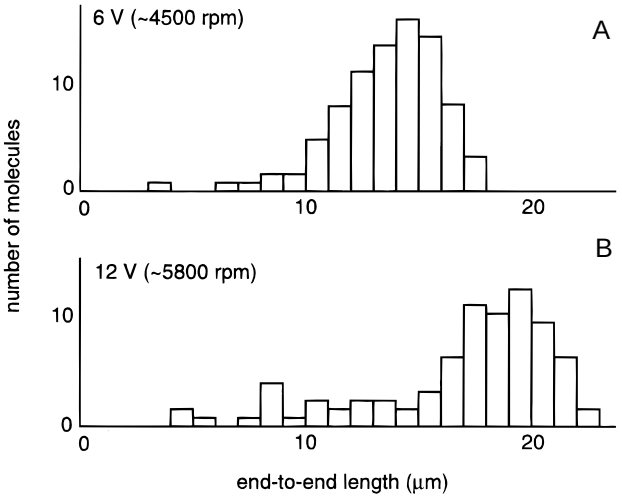

Figure 3. Histogram showing the distribution of end-to-end λDNA length. The length of straight DNA, which is enclosed in a rectangle of aspect ratio ~20, was measured. (A) Histogram corresponding to the spin rate of ~4500 rpm (6 V) at $r = 10$ mm. The mean length was 14.6 ± 2.5 mm ($N = 106$). (B) Histogram corresponding to the spin rate of ~5800 rpm (12 V) at $r = 10$ mm. The mean length was 16.9 ± 4.3 mm ($N = 100$).

12 V) effectively straightened λDNA on a mica surface. At a significantly higher spin rate (>7900 rpm, 18 V), DNA molecules started to break.

End-to-End λDNA Length. To investigate the effects of spin rate and radial distance from the spinning center on spin-stretched DNA molecules, we measured the end-to-end DNA lengths from fluorescent images and determined the length distribution (Figure 3). When the DNA was spin-stretched at ~4500 rpm (6 V) and the sample was applied to the peripheral region ($r = 10$ mm), the mean end-to-end DNA length was 14.6 ± 2.5 μm (mean ± SD, $N = 106$) with a median of 14.9 μm. When the DNA was straightened at ~5800 rpm (12 V), the mean end-to-end DNA length was comparatively longer at 16.9 ± 4.3 μm ($N = 100$) with a mean length of 18.1 μm. Since the natural contour length of λDNA is 16.5 μm (0.34 μm/kbp × 48.5 kbp), the median length was 10% shorter at ~4500 rpm and 10% longer at ~5800 rpm than the natural length. When the DNA was spun at a higher spin rate (~7900 rpm, i.e., 18 V), some DNA molecules were apparently fragmented. DNA fragments were also detected by AFM (data not shown). The mean length was 11.9 ± 7.2 μm ($N = 106$, $r = 10$ mm). The observed shorter mean value with a longer standard deviation than DNA straightened at 4500−5800 rpm is consistent with DNA fragmentation. DNA molecules, straightened at ~5800 rpm, were imaged by AFM (Figure 4).

Spin-Stretching von Willebrand Factor. To investigate whether the spin-stretcher can be used to stretch protein molecules, we examined vWF reduced by DTT and denatured by guanidine hydrochloride. Human vWF proteins were spin-stretched on a $MgCl_2$-soaked mica surface at ~5300 rpm, and immobilized vWF was imaged by AFM. As a control, we suspended vWF in PBS without DTT or guanidine hydrochloride and gently deposited the sample on the surface. The AFM image of control vWF shows a globular shape in a variable size, suggesting that vWF forms multimers of variable composition (Figure 5A). Spin-stretched counterparts were uncoiled or straightened, although their morphology varied from one surface region to another (Figure 5B and C). The end-to-end length of straightened vWF in Figure 5C is 1.6 μm. A linearized vWF monomer is roughly 780 nm (0.38 nm/amino acid (aa) × 2050 aa) in length. Since the stretched vWF in Figure 5C is ~210% of the linearized monomer, it obviously contains two or more monomers. The inset in Figure 5C illustrates a cross section of vWF. The relatively wide and nonuniform width observed along extended molecules may result from the multiple oligosaccharide side chains as well as the convolution of the finite size of the AFM scanning tip with the molecule.

DISCUSSION

We describe a rapid and efficient method of straightening DNA or denatured protein molecules in a form in which they can be observed by fluorescence and atomic force microscopy. In the described "spin-stretching" method, a flat mica surface is simply spun at ~5000 rpm, and a droplet containing biomolecules is dispensed on the rotating surface. The entire process of stretching and immobilizing biomolecules takes only a few minutes. We present the CCD images of fluorescently labeled λDNA molecules and the AFM images of unreduced forms and denatured and reduced forms of vWF protein molecules.

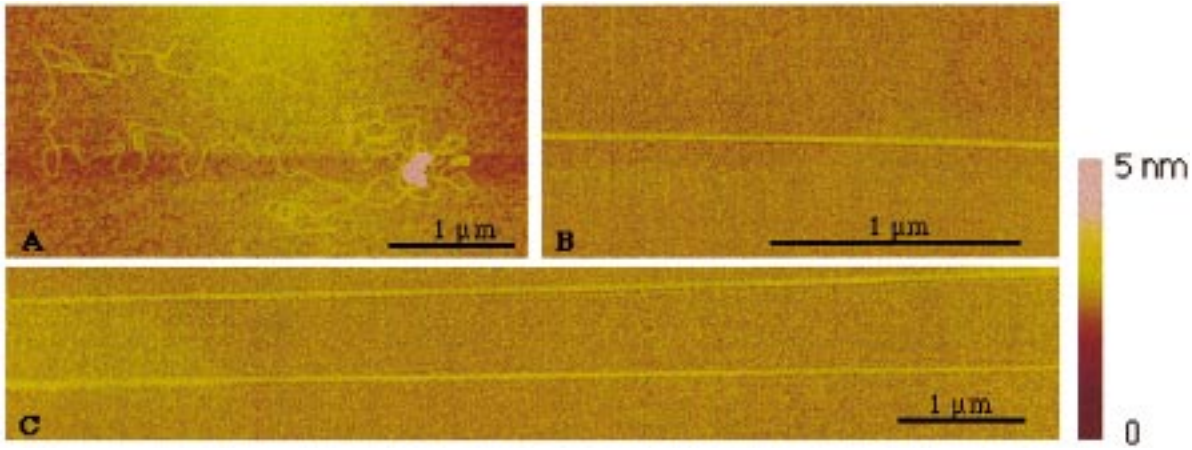

Figure 4. AFM height image of λDNA (48.5 kb). Height is indicated by a color code with dark (0 nm) and light (5 nm). The bar is 1 μm. (A) Control DNA without spin-stretching. (B, C) Straightened λDNA.

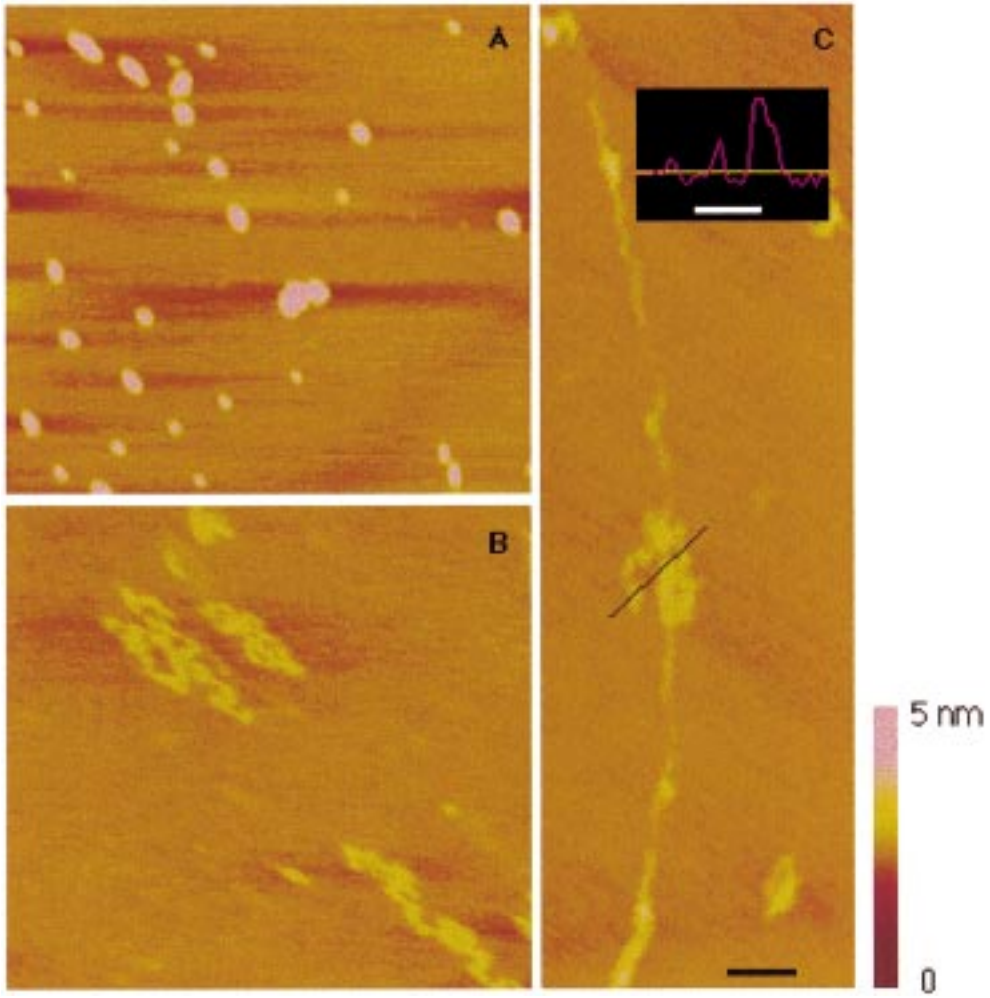

Figure 5. AFM height images of vWF. The black bar in (C) is 100 nm for (A−C). (A) Control vWF gently deposited on mica without denaturation. (B) Decondensed vWF by denaturation and spin-stretching at 10 V (~5300 rpm) on mica. (C) Linearized vWF multimer by denaturation and spin-stretching at 10 V. The contour length is 1.6 μm. The inset displays the height across the straightened vWF indicated by the black line. The maximum height is 0.6 nm. The white bar in the inset is 100 nm.

The proposed method for stretching DNA and denatured protein molecules has a number of advantages and unique features. First, the spin-stretching operation is simple and easy to apply to both DNA and protein. The spin-stretcher can spin-coat or spin-rinse the surface, and the entire operation takes only a few minutes. Second, no end-labeling or specific molecular modification is necessary for spin-stretching. Third, by changing the spin rate or sample solvents, retention and straightness can be controlled easily. For instance, DNA or proteins can be fragmented on purpose at a high spin rate. Fourth, the same

sample can be used for detection by fluorescence microscopy as well as AFM and a simultaneous detection of topographical and fluorescent images should be possible.

A viscous force from the substrate surface acting on biomolecules during spin-stretching mediates the gentle stretching of DNA and protein molecules. In laminar flow, a shear stress on a rotating disk surface is estimated as $\tau = 0.8\mu r(\omega^3/\upsilon)^{0.5}$ (dyn/cm^2), where μ is the absolute viscosity of the medium (poise), r is the radial distance from the center of the disk (cm), ω is the angular velocity (rad/s), and υ is the kinematic viscosity (Stokes).[28,29] The shear force to λDNA, spun at ~5000 rpm, is roughly estimated by the formula as ~10 pN using $\mu = 0.01$ P and $\nu = 0.01$ Stokes for water. Because a small droplet dispensed on the rotating surface does not form a steady shear flow and the DNA solution used in this study is more viscous than water, the actual shear stress can be higher than this estimation. Based on the chemical energy in a P−O bond (600 kJ/mol) or a C−N bond (750 kJ/mol), the force necessary to break these covalent linkages is approximately a few nanonewtons for a few nanometer bond length.[30] In our experiments, a gentle stretching was attained with a relative surface speed of 5−6 m/s (5000−6000 rpm, τ = ~10 pN) and DNA fragmentation was observed at ~8 m/s (~8000 rpm, τ = ~20 pN). Since DNA fragments receive varying shear force depending on their length, it would be interesting to study whether the optimum speed is a function of fragment length.

In our spin-stretcher experiment, the peak frequency in the histograms of the λDNA lengths was 14−15 (at ~4500 rpm) and 19−20 μm (at ~5800 rpm). Under a critical stress of ~65 pN, λDNA is reported to undergo a highly cooperative transition from a linear spring to an overstretched form, and this transition occurs at ~17 μm end-to-end length.[10,31] On the basis of our length measurement, the total tensile force acting on biomolecules was estimated below the critical stress at ~4500 rpm and above at ~5800 rpm. A relatively broad distribution of end-to-end length by the spin-stretching method may result from a transient flow pattern on the rotating disk. Recently Smith et al. studied the conformational dynamics of λDNA in a steady shear flow and observed large, aperiodic temporal fluctuations.[32] The shear flow in this report was unsteady since the small fluid volume we used was unable to uniformly coat the surface and a solution dried out in a few seconds. Further analysis is needed to understand the underlying mechanism of molecular stretching in the described method.

Unlike DNA stretching, the proper condition for protein stretching may differ from one protein to another since each protein has its own stable conformation, solubility, and hydrophobicity. Also, the denatured conformations are clearly affected by external conditions such as solvent composition, temperature, pH, and ionic strength as well as the amino acid sequence.[33] We chose human vWF for testing our spin-stretcher, since vWF is a large multimeric protein with a monomer of 260 kDa in mass and it is reported that vWF undergoes a shear stress-induced conformational transition from a globular state to an extended chain conformation.[22,26] The extended length of 1.6 μm in our experiment is about twice as long as a straightened monomer, suggesting that the extended length is attributed to a dimer.

(28) Levich, V. G. *Physicochemical Hydrodynamics*; Prentice-Hall: Englewood Cliffs, NJ, 1952; p 60.
(29) Hulbert, S. F.; Klawitter, J. J. *J. Biomed. Mater. Res.* **1974**, *8*, 137−153.
(30) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 79th ed.; CRC Press: Boca Raton, FL, 1998; Chapter 9, Table 1.
(31) Cluzel, P.; Lebrun, A.; Heller, C.; Lavery, R.; Viovy, J.-L.; Chatenay, D.; Caron, F. *Science* **1996**, *271*, 792−794.
(32) Smith, D. E.; Babcock, H. P.; Chu, S. *Science* **1999**, *283*, 1724−1727.
(33) Dill, K. A.; Shortle, D. *Annu. Rev. Biochem.* **1991**, *60*, 795−825.

ACKNOWLEDGMENT

We thank L. H. Ericsson and K. A. Walsh (Department of Biochemistry, the University of Washington) for the kind gift of von Willebrand factor. We appreciate D. A. Nickerson, and B. J. Trask (Department of Molecular Biotechnology, the University of Washington) for providing valuable comments. This work was supported in part by the National Science Foundation, Science and Technology Center for Molecular Biotechnology (NSFBIR-9214821AM), and Sumitomo Electric Industries. AFM facilities were provided, partly, by a DURIP grant through ARO.

Received for review March 10, 1999. Accepted July 19, 1999.

AC9902695