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Direct Force Measurements on Double-Stranded RNA in Solid-State Nanopores

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ABSTRACT Solid-state nanopores can be employed to detect and study local structure along single molecules by voltage driven translocation through the nanopore. Their sensitivity and versatility can be augmented by combining them with a direct force probe, for example, optical tweezers. Such a tool could potentially be used to directly probe RNA secondary structure through the sequential unfolding of duplex regions. Here, we demonstrate the first application of such a system to the study of RNA by directly measuring the net force on individual double-stranded RNA (dsRNA) molecules. We have probed the force on dsRNA over a large range of nanopore sizes from 35 nm down to 3.5 nm and find that it decreases as the pore size is increased, in accordance with numerical calculations. Furthermore, we find that the force is independent of the distance between the optical trap and the nanopore surface, permitting force measurement on quite short molecules. By comparison with dsDNA molecules trapped in the same nanopores, we find that the force on dsRNA is on the order of, but slightly lower than, that on dsDNA. With these measurements, we expand the possibilities of the nanopore-optical tweezers to the study of RNA molecules with potential applications to the detection of RNA-bound proteins, the determination of RNA secondary structure, and the processing of RNA by molecular motors.

KEYWORDS Solid-state nanopores, RNA, optical tweezers, single-molecule biophysics

Recently, a new tool for single-molecule studies has emerged in the form of nanopores. Here, charged molecules of interest immersed in an electrolyte are translocated through a narrow pore of several nanometers in cross-section by the application of a strong electrical field over the pore. When the molecule is inside the nanopore, it blocks a certain portion of the ionic current. Using this approach, nanopores can not only be employed for molecular counting, but also for the analysis of molecular structure or nucleic acid–protein interactions. For example, they can be used to detect single proteins in solution¹ or to monitor protein binding on dsDNA.^{2,3} More recently, it was shown that solid-state nanopores can also be employed for the study of RNA; homopolymeric ssRNA was shown to be easily distinguishable from dsDNA and dsRNA as it traverses 8–10 nm pores, which was attributed to stretching of the flexible ssRNA on the length scale of the pore.⁴ Both biological and solid-state nanopores have also been proposed as candidate systems for genomic sequencing of dsDNA,^{5,6} and indeed, it has recently been shown in the biological α -Hemolysin nanopore that different bases could be distinguished, albeit under the condition that they are translocated through the nanopore as isolated nucleotides.⁷

The versatility of solid-state nanopores in particular can be enhanced through their integration with optical tweezers,

permitting the controlled positioning of molecules with respect to the pore and the direct measurement of the forces acting on them.^{8,9} In such an experiment to date demonstrated for dsDNA,⁸ molecules are attached to an optically trapped bead and moved close to the nanopore where the electrical field can “capture” the molecule in the pore. In contrast to free translocation experiments, here the optically trapped bead prevents the molecule’s subsequent translocation to the other side of the pore. Furthermore, the trapped bead’s position directly monitors the force applied to the molecule in the nanopore. Such a force measurement is performed in parallel with the ionic current recording, providing a simultaneous readout of the molecule’s spatial configuration in the nanopore and the force to which it is subjected.

Since both the force and the nanopore current report on the local charge density and size of the molecules inside the nanopore,^{10,11} their combined readout can serve as an indicator for the presence of proteins or other local structure along nucleic acids. In addition, forces could be selectively applied to proteins bound to nucleic acids simply by choosing a nanopore size smaller than the nucleic acid–protein complex. The use of a local force probe holds particular interest for the study of RNA as it provides a method to sequentially unravel and probe its secondary structure.^{12,13} However, for this to be accomplished, several challenges must be overcome. First, while translocations of RNA molecules through biological nanopores^{14,15} and, more recently, solid-state nanopores⁴ have been demonstrated, the use of

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Received for review: 11/24/2009

Published on Web: 00/00/0000

the combined nanopore-optical tweezers system has not yet been applied to RNA. One major difficulty here is that trapping a single molecule inside the nanopore requires the optically trapped bead to be moved into close proximity to the nanopore, so that the molecule can enter it with reasonable probability. Upon capture, however, the bead must be prevented from being pulled against the nanopore membrane to avoid aspecific attachment. In practice, this imposes a criterion on the minimum length of the molecule under study; its radius of gyration (R_g) should be at least on the order of hundreds of nanometers, as this is the typical distance the bead is displaced from its equilibrium position in the optical trap to balance the force on the molecule. Specifically for RNA this is a stringent requirement, as many RNA molecules are single-stranded and thus very flexible, resulting in $R_g \sim 10\text{--}100$ nm even when they comprise many kilobases (kb). Even for dsRNA,¹⁶ which is less flexible,^{17,18} this criterion is challenging, as the biochemical synthesis of such molecules rapidly decreases in efficiency for transcript lengths greater than 5 kb. In addition, one must consider the potential influence of the resulting short contour lengths on the measured experimental signals, that is, the force and nanopore current.

Here, we demonstrate the first study of RNA in the combined nanopore-optical tweezers apparatus. We have successfully synthesized a long (12.7 kb) dsRNA molecule that can readily be captured inside our nanopores, and we present the forces measured on these dsRNA molecules, comparing these to the forces on dsDNA molecules of the same contour length. We show that the ionic current blockades occasioned by single dsRNA and dsDNA molecules upon capture in the nanopore are in excellent agreement with results from translocation studies.⁴ Furthermore, we demonstrate that the measured forces are independent of the distance of the bead to the nanopore, down to distances as close as 250 nm from the nanopore, which would allow such measurements to be performed with molecules of even shorter contour lengths. Finally, we show that our results agree qualitatively with numerical calculations of the forces on these molecules over a large range of nanopore sizes from 35 nm down to 3.5 nm in diameter. In particular, the exploration of this small nanopore size range opens the way for the study of local structure of smaller molecules such as single-stranded RNA, for which secondary structure determination using established techniques such as NMR and X-ray scattering remains challenging.¹⁹

METHODS

Experimental Setup. Our combined nanopore-optical tweezers setup has been previously described^{9,20} with several minor differences. The trapping laser used is a 4W Coherent Compass 1064–4000 M (Coherent, Santa Clara, US), and the piezo stage employed is a P561.3CD (Physik Instrumente, Karlsruhe, Germany).

Sample Preparation. Nanopores were fabricated in 20 nm SiN membranes by a highly focused TEM beam as previously described²¹ and stored in a solution containing 50 % ethanol and 50 % ddH₂O directly after drilling. Prior to use, the SiN membrane was thermally oxidized at 200 °C for 10–30 min before use in order to reduce aspecific attachment of beads or nucleic acids to the surface. Subsequently, the sample was treated with O₂ plasma at room temperature for 30 s, mounted into the flow cell, and immediately flushed with electrolyte. All experiments were performed in 1 M KCl, 10 mM Tris-HCl (pH = 8.0), 1 mM EDTA, and 0.01 % triton-X100 (Roche, Switzerland). These high ionic strength conditions were chosen for a number of reasons such as good signal-to-noise in the nanopore current, reduced sticking of the molecules to the nanopore, optimal capturing efficiency over the entire range of nanopore sizes studied, as well as straightforward comparison with published data.^{4,8,9} Conversely, the influence of low ionic conditions on the forces experienced in nanopores is not yet well understood.¹¹ The addition of triton-X100 was found to further reduce sticking of the beads to the membrane.

All experiments were performed with streptavidin-coated polystyrene microbeads of 1.87 ± 0.03 μm in diameter (Spherotech, IL). The 12.7 kb dsDNA fragment was synthesized via PCR amplification as previously described.²² A single biotin molecule was incorporated at the 5' end of the forward PCR primer, allowing for the specific attachment of one end of the molecule to our beads. The 12.7 kb dsRNA was synthesized via in vitro transcription of the two complementary strands of a different dsDNA construct, which was obtained by reverse transcription of the equine arteritis virus (EAV) genome and subsequent PCR amplification thereof.²³ The strand corresponding to the original EAV genomic RNA was fully transcribed, whereas the complementary strand was transcribed into two adjoining segments of 530 bases and 12161 bases. Transcription of the 500-nt segment was performed in the presence of a 1:1 mixture of rUTP and bio-rUTP, causing the segment to have a number (up to 60, estimated) biotin-labeled bases, which allows for the specific attachment of the molecules to our beads. The three segments were then hybridized in a formamide-based buffer previously described by Liphardt and co-workers.²⁴ The hybridization mixture was incubated at 55 °C for 3 h, and subsequently cooled down to room temperature with a cooling rate of 1.5 °C/5 min. The hybridization mixture was immediately dialyzed against 2 L of 0.5 \times SCC at 4 °C.²⁵ Dialysis was ended after 2 h, and the resulting RNA hybrid was purified by RNeasy (Promega), eluted with 1 \times TE and stored at –20 °C.

Prior to each experiment, the nucleic acid molecules were incubated with the beads for at least 30 min in a solution containing 200 mM KCl and TE (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0). For the experiments involving RNA, the beads were additionally rinsed, first twice with 0.1 M NaOH and then twice with TE prior to the incubation with the RNA

molecules, to reduce potential RNase contamination. Concentrations of the molecules on the beads were chosen such that during the experiments single molecules are easily captured in the nanopore (within tens of seconds), but capture of multiple molecules is avoided as much as possible. We found that for nanopores of 5 nm and smaller this required molecule concentrations about 100× higher than those for large nanopores (>20 nm).

Trap Calibration. Prior to each experiment, the trap stiffness of each individual bead was determined by a Lorentzian fit to the power spectrum of the bead position recorded over tens of seconds²⁶ at a distance of more than 20 μm from the nanopore surface. For beads that were incubated with very high molecule concentrations the apparent trap stiffness was found to be significantly (10–30%) lower than for uncoated beads of the same size. In these cases, we have used the average trap stiffness determined from the uncoated beads and taken the difference between the two values as the error in the trap stiffness.

Modeling. Numerical calculation of the forces on dsRNA and dsDNA was done using code from van Dorp and co-workers.¹¹ The dsDNA and dsRNA are approximated as cylinders with uniformly distributed surface charges. The surface charge density of dsDNA, which has a linear charge density λ_{bare} of -0.96 nC/m and a diameter D of 2.0 nm, equals -0.15 C/m². The surface charge density of dsRNA, which has linear charge density $\lambda_{\text{bare}} = -1.23$ nC/m and $D = 2.6$ nm diameter, likewise equals -0.15 C/m². Values for the linear charge densities and diameters are obtained from the published crystal structures of these molecules (pdb ID 2BNA²⁷ for dsDNA and ID 3CIY for the dsRNA, after the bound protein, which does not distort the double-stranded helix of the A-RNA, was deleted²⁸).

RESULTS

We have measured forces on dsRNA and dsDNA molecules in nanopores integrated with optical tweezers. At the start of an experiment, an optically trapped bead with one or more molecules attached to its surface is held in the cis side in close vicinity of the nanopore (Figure 1a, left). A voltage bias is then applied over the nanopore (using two electrodes on both sides of the nanopore), leading to a strong electrical field in the vicinity of the nanopore, and an ionic current passing through it. Together with the ionic current, we track the position of the bead by monitoring the optical reflection signal of a separate detection laser incident on the bead. When the bead is held in close proximity to the nanopore, the electrical field (which typically extends only tens to hundreds of nanometers into the solution) will pull the negatively charged nucleic acid molecule through the nanopore toward the trans side until the force is balanced by the optical force on the bead (Figure 1a, right). The details of this force balance were described in detail recently^{11,29} and will be discussed below in the context of our measurements. Upon capture into the nanopore, the ionic current

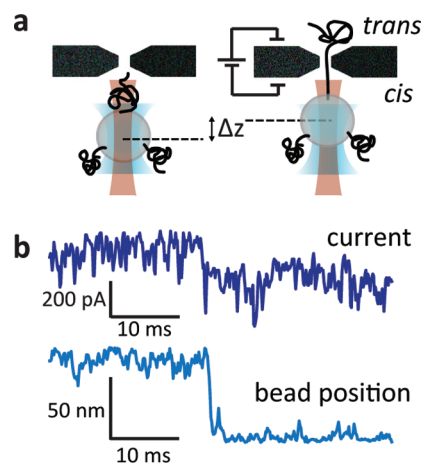


FIGURE 1. Principle of the experiments. (a) Schematic of the experiment. A dsRNA molecule, attached to an optically trapped bead, is held close to the nanopore. The bead position is monitored by a separate detection laser (red). Application of a voltage bias across the nanopore results in an electrical field that pulls the charged molecule into the pore. The optical restoring force on the bead, however, prevents complete translocation of the molecule, and consequently the molecule is held taut inside the nanopore. (b) Example of such a capture event. When the molecule enters the pore, it blocks a portion of the ionic current through the nanopore (dark blue curve). This current drop is accompanied by a simultaneous change in the position of the trapped bead (light blue curve). The bead position stabilizes to a new equilibrium position at which the optical force balances the total electro-osmotic force on the molecule.

through the nanopore is partially blocked by the presence of the molecule, which together with the change in bead position serves as a clear indication that a molecule is captured.

A typical capture event for a single dsRNA molecule in a 6 nm pore is shown in Figure 1b. A short while after the application of a bias voltage across the pore (+100 mV in this experiment), the current signal is observed to suddenly decrease in a stepwise fashion (Figure 1b, dark blue line). Simultaneously the position signal changes, commensurate with movement of the bead toward the nanopore (Figure 1b, light blue line). From the change in the current signal, we compute the change in nanopore conductance $\Delta G (= \Delta I / V_{\text{bias}})$ that for this event equals 2.07 ± 0.35 nS. This value is in good agreement with the ΔG observed for untethered dsRNA molecules translocating through similarly sized nanopores,⁴ for which an average value of 2.0 ± 0.4 nS was found at this voltage. The change in the position signal of the bead (in volts) can be related to an actual displacement Δz (Supporting Information) of approximately 64 ± 5 nm, which corresponds to an effective force of 8.6 ± 2.3 pN given the typical trap stiffness of 135 ± 25 pN/μm (Supporting Information, Methods).

By sequentially trapping dsRNA and dsDNA in the same nanopore, we can directly compare both the conductance changes caused by the presence of these molecules in the nanopore and the forces exerted on them under the influence of the electric field. In Figure 2a, we show the changes

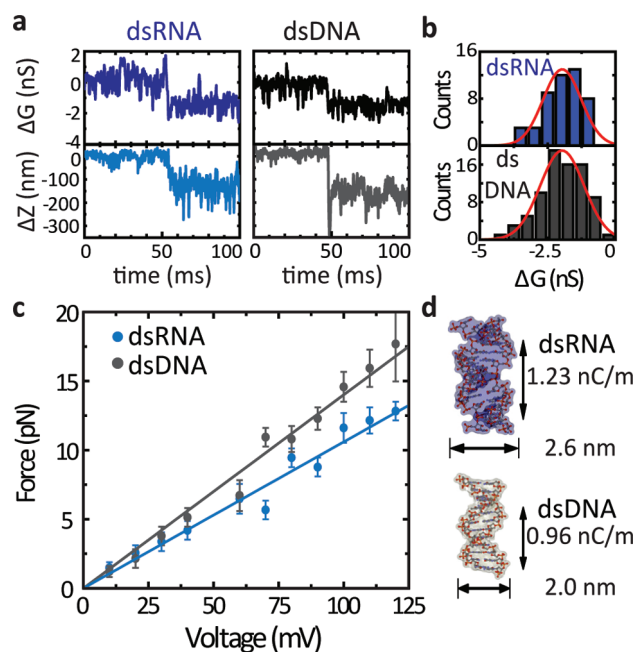


FIGURE 2. Capturing dsRNA and dsDNA molecules. (a) Comparison of the raw data in a 22 nm pore at 100 mV bias voltage illustrating captures of a dsRNA (dark and light blue) and dsDNA molecule (black and gray). The current signal (top, dark traces) and bead position signal (bottom, light traces) are shown for both types of molecules. (b) Histograms of the conductance change $\Delta G (= \Delta I/V_{\text{bias}})$ for dsRNA and dsDNA, extracted from the current signal on capture or extraction. The values for ΔG compare well to earlier results in which molecules were freely translocated through similarly sized pores.⁴ (c) Force versus voltage relationship for dsRNA (light blue) and dsDNA (gray) molecules successively captured in the same 22 nm pore. The solid lines correspond to linear fits to the data. As the bias voltage is varied, the corresponding change in bead position Δz is measured, from which the force can be calculated. Here, the initial bead position is $\sim 0.75 \mu\text{m}$ from the pore surface, and error bars stem from the uncertainties in the determination of the trapping strength and the bead position (Supporting Information). (d) Images of the dsRNA and dsDNA helices as determined via X-ray crystallography, indicating the molecular diameters and charge densities.^{27,28}

in conductance and bead position occasioned by dsRNA (blue trace) compared to that of dsDNA (black trace), sequentially trapped in a 22 nm nanopore. Focusing first on the conductance changes, it is clear from Figure 2a that the change in conductance is very similar for the two molecules, which is not unexpected given their similar dimensions (Figure 2d). Histograms summarizing all our measurements of the conductance change occasioned by trapped molecules of dsRNA and dsDNA, where these changes were determined either upon capture into or extraction out of the pore, are shown in Figure 2b. For the dsRNA we find a mean value of $\Delta G_{\text{dsRNA}} = -1.96 \pm 0.70$ nS (mean and standard deviation determined from a maximum likelihood Gaussian fit to the data). This value is in excellent agreement with translocation results of dsRNA of similar length through nanopores of the same diameter and thickness, where a mean value of $\Delta G_{\text{dsRNA}} = -2.0 \pm 0.4$ nS was found at 100 mV bias voltage.⁴ Similarly, for the conductance blockade from dsDNA we find an average value of $\Delta G_{\text{dsDNA}} = -1.97 \pm 0.81$ nS, which is

again in good agreement with results obtained from translocation studies involving the same dsDNA fragment. There, a mean value of $\Delta G_{\text{DNA}} = -1.6 \pm 0.5$ nS was found at 100 mV bias voltage.⁴ The value for ΔG_{dsDNA} is somewhat higher than the value previously reported in nanopore-optical tweezers trapping experiments,⁸ which is readily explained by the fact that these previous experiments were performed with nanopores formed in thicker membranes, resulting in a smaller change in conductance.

We now compare the forces experienced by dsRNA and dsDNA molecules as they are tethered in the nanopore. The electrical field in the pore vicinity causes a force on the molecules toward the trans side, as the phosphodiester backbone of nucleic acids is negatively charged in aqueous solution. At the same time, positively charged counterions screening the nucleic acid are forced by the electrical field to flow in the opposite direction, leading to an electro-osmotic drag force from the fluid on the molecule in the direction of the cis side. The resulting net force (always directed toward the trans side) is balanced by the optical restoring force on the bead, which we determine directly by measuring the bead position. Comparing the structures of the molecules (Figure 2d), it is not a priori clear how the force on dsRNA should compare to that on dsDNA. Certainly, the purely electrical force on dsRNA toward the trans side should be higher, reflecting the 30 % increase in its linear charge density compared to dsDNA. However, this increase is partially canceled by a larger electro-osmotic drag force in the opposite direction, as the surface area that experiences fluid drag from the counterions is also increased (the dsRNA helical diameter being ~ 30 % bigger than that of dsDNA). Since the magnitude of this latter effect also depends on the pore size as discussed below, the difference between the net force on dsRNA and dsDNA is not readily estimated.

By varying the bias voltage across the nanopore we can directly determine force curves for dsRNA (Figure 2c, blue) and for our control molecule, dsDNA (Figure 2c, gray). We find that the measured force on the bead depends linearly on the voltage for all molecules probed, allowing us to determine the force per voltage f on the molecule from the slope of a linear fit to the forces at different bias voltages. For the measurements shown in Figure 2c, conducted in the same 22 nm pore as used in Figure 2a,b, the linear fit through the points yields a force per voltage of $f_{\text{dsRNA}} = 0.11 \pm 0.02$ pN/mV and $f_{\text{DNA}} = 0.14 \pm 0.03$ pN/mV, where the errors reflect the uncertainties in the determination of both the trap stiffness and the position of the beads. Therefore, within error, the forces dsRNA and dsDNA are roughly equal in this nanopore.

Prior to investigating this in greater detail, we will first address an important question related to performing these force measurements with short molecules and at very close distances to the nanopore: are the measured forces strongly dependent on the molecule length and the distance separating the bead from the nanopore membrane? The maximum

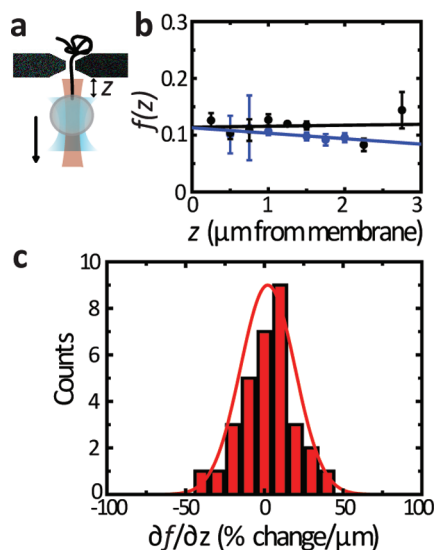


FIGURE 3. Measured forces are independent of the separation between optical trap and nanopore. (a) Schematic. The bead position z is defined as the distance between the surface of the bead and that of the nanopore membrane. After capture the distance z is varied (signified by the black arrow) and at every position z the force per voltage $f(z)$ is calculated from force curves (Figure 2c). (b) Average force per voltage determined for dsRNA (blue) and dsDNA (black) as a function of bead position z in a 32 ± 4 nm pore. The dependence of the force per voltage on the bead position is determined by the slope of a linear fit to the data. (c) Histogram of the slopes $\partial f(z)/\partial z$ expressed in percent change/micrometer for all dsRNA and dsDNA molecules for which f was determined at more than two positions. The mean of a maximum likelihood Gaussian fit to the histogram is $2 \pm 17\%$, where the error is the standard deviation.

distance achievable while maintaining a molecule inside the pore is limited to the contour length of the molecules (a condition achieved at high voltages, where the molecule is fully stretched). For our 12.7 kb dsRNA and dsDNA molecules this distance amounts to about 3.2 and 4.2 μm , respectively, at least 4-fold shorter than in the case of the 48 kb ($\sim 16 \mu\text{m}$ contour length) bacteriophage- λ dsDNA molecules utilized in previous measurements using this experimental setup.^{8,11,30} To check first if the force depends on the length of the molecule, we have compared the forces on our shorter dsDNA molecules to those on λ -DNA in the same nanopores (at equal distances to the nanopore membrane) and found no noticeable difference (data not shown). Next, to see if the proximity of the nanopore membrane influences the optical trap stiffness and hence the values of the measured forces, we have investigated the forces over distances ranging from 250 nm to 3 μm from the nanopore membrane. Here, a molecule was initially captured in the pore, and subsequently the distance between the bead and the nanopore was varied while maintaining the molecule in the pore through the application of a small voltage bias (30 mV). Force curves such as in Figure 2c were then recorded at different positions z , where z is the distance between the bead surface and the nanopore membrane (Figure 3a). The resulting dependence of the force per voltage versus z for

dsRNA molecules (blue) and dsDNA molecules (black) in a nanopore of 32 ± 4 nm diameter is plotted in Figure 3b. Each data point corresponds to the average force per voltage at a given position z , $\langle f(z) \rangle$, determined from measurements in this nanopore repeated on different single molecules. For both dsRNA and dsDNA, we fit the data using a linear relation (solid lines) and determine accompanying slopes $\partial f(z)/\partial z$ of -12.7% (μm dsRNA) and 1.2% (μm dsDNA). Thus, for this particular pore, the average force changes on the order of ten percent per μm of distance to the pore membrane. To provide a complete picture including all our measurements on dsDNA and dsRNA in different nanopores, we took the following approach: for each molecule for which force curves (Figure 2b) could be recorded at minimally 3 different z positions, we calculated the slope $\partial f(z)/\partial z$, normalized it to the average and expressed it into percent change per micrometer and binned it in a histogram (Figure 3c). A maximum likelihood Gaussian fit to the values gives a mean value of $2 \pm 17\%$ (the error is the standard deviation). We therefore conclude that within experimental error there is no dependence of the force on the distance z to the membrane, even at distances as close as 250 nm from the surface. This result ensures that it is possible to measure on even shorter trapped molecules, such as very short duplexes or even single-stranded RNA, without having to correct the values of the measured forces.

Above, we have discussed the force measured on dsRNA in a 22 nm nanopore and found this to be roughly equal to the force on dsDNA in the same pore. However, for a thorough comparison, the forces must be probed in nanopores of different transverse sizes. This is because the nanopore size influences the net electro-osmotic force on the captured molecule^{29,31} and hence the net force measured in our experiments. In a recent publication by Van Dorp et al.,¹¹ this effect was demonstrated at low ionic strength (<50 mM KCl), where the total force on dsDNA was found to decrease as the size of the nanopore was increased. This was attributed to a hydrodynamic coupling of the dsDNA counterions to the nanopore surface; close to this surface, the nonslip condition on the water molecules causes a transfer of part of the counterions' momentum to the molecule. Therefore, the nanopore geometry influences the magnitude of the drag force exerted by these counterions on the nucleic acid molecule inside the pore. Specifically, for larger pores this coupling is less pronounced, and the drag force of the counterions is mostly directed against the nucleic acid molecule, leading to a lower net force measured in the optical tweezers with increased pore size.

To get a complete picture of the forces on dsRNA molecules, we have therefore investigated the dependence of this force on the size of the nanopore, additionally probing dsDNA as a control. Measurements of the average force per voltage $\langle f \rangle$ on both dsRNA (blue) and dsDNA (black) over a 10-fold variation in nanopore sizes from 3.5 to 35 nm in diameter are shown in Figure 4. Each point in the graph is

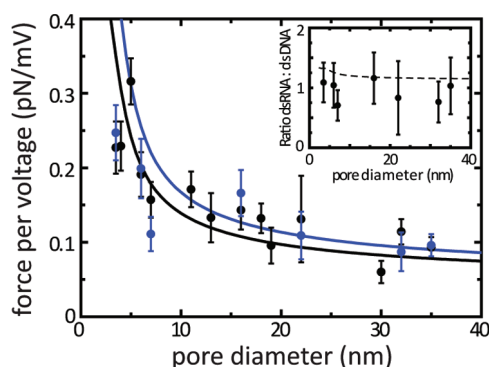


FIGURE 4. Average force per voltage $\langle f \rangle$ on dsRNA (blue) and dsDNA (black) versus nanopore diameter. Points indicate the experimentally measured data, while the solid lines are numerical calculations.¹¹ The inset shows the ratio $\langle f \rangle_{\text{dsRNA}} / \langle f \rangle_{\text{dsDNA}}$, where the black dotted line is the ratio expected from the numerical calculations. In all cases the error bars derive from the uncertainty in the determination of the trap strength (typically around 15–20%, see Supporting Information, Methods).

the mean force per voltage $\langle f \rangle$, averaged over molecules on different beads probed at different distances z from the nanopore membrane (the latter is justified by the independence of f on z , see Figure 3). We observe that as the nanopore size increases, the average force decreases monotonically for dsRNA, as it does for dsDNA. The solid lines in Figure 4 correspond to numerically calculated values using the model from van Dorp and co-workers; these can be seen to capture the trends in the experimental data well. The fact that the dsRNA follows the same trend as the DNA also serves as an additional validation of the theoretical assumptions underlying these calculations. However, exact quantitative agreement between model and data is not expected, given that the mean-field theory underlying this model becomes less reliable at high ionic strength. Particularly for experiments conducted in very small nanopores, we start to see clear deviations of the model from our data, where the measured forces lie below the predicted values. This is not surprising, as for such small pores finite ion sizes will start to become important, which is not accounted for in the model.

To directly compare the forces on dsRNA and dsDNA we compute the ratio $f_{\text{dsRNA}}/f_{\text{dsDNA}}$ for all measurements in which both dsRNA and dsDNA were probed in the same nanopore (Figure 4, inset). For this ratio, numerical simulations (indicated by the dashed line) predict a value just above unity over the entire range of pore sizes. On average, we indeed find the force on dsRNA molecules to be very similar to that on dsDNA with the ratio between the two values falling slightly below unity (data points in Figure 4, inset; $f_{\text{dsRNA}}/f_{\text{dsDNA}} = 0.95 \pm 0.18$, where the error indicates the standard deviation). This experimentally established similarity between the forces on the two molecules could be convenient for future experiments involving RNA. For instance, this would allow the use of hybrid DNA-RNA constructs in which the DNA could serve as a stiff double-stranded handle for a short RNA.

The slight discrepancy between the experimental data and the numerical model may reflect an inherent oversimplification of assumptions in the model compared to the experimental situation. For instance, the model approximates the molecules as cylinders with a uniformly surface charge. The highly localized nature of the charge groups on both dsRNA and dsDNA, however, may lead to the localization of at least a fraction of the counterions. As the dsRNA helix is significantly different in structure from that of dsDNA (the RNA bases being strongly tilted in the helix), the contribution of this effect to the net force may be quite different for the two molecules. For the same reason, the electro-osmotic drag force from the counterions may well be more significant for the dsRNA helix than for dsDNA, leading to a larger reduction of the purely electrical force acting on the phosphodiester backbone. As such structural differences are not captured in the model, it would tend to overestimate the force on dsRNA. The lower values for the forces on dsRNA measured in the experiments can be viewed in this light.

CONCLUSIONS

With the measurements presented here we have expanded the force measurements in solid-state nanopores to include individual dsRNA molecules. The dsRNA molecules are quite long given their required synthesis via in vitro transcription, but are 4-fold shorter than dsDNA molecules previously studied. We demonstrate that our versatile instrument integrating optical tweezers and nanopores can reliably measure forces for these shorter molecules, even in very small nanopores down to 3.5 nm in diameter. For dsRNA we find the force per applied voltage to be on the order of, but slightly lower than, the force per applied voltage on dsDNA, convenient for experiments involving possible hybrid dsDNA-RNA constructs. Furthermore, by demonstrating that force measurements are still reliable at very close distances to the nanopore membrane, these experiments open up the way for the use of even shorter molecular constructs, the biochemical synthesis of which is typically much easier. As the interest in RNA continues to grow this technique could provide valuable information on the structure of RNA molecules and their interactions with proteins.

Acknowledgment. We thank S. W. Kowalczyk, A. R. Hall, M. Y. Wu, and V. Svehnikov for assistance in nanopore fabrication, A. J. te Velthuis and S. Donkers for assistance with RNA preparation, and U. F. Keyser, J. van der Does, and J. Beekman for advice on instrument construction. We also wish to thank S. van Dorp and S. Lemay for valuable discussions. N.H.D. acknowledges financial support from the TU Delft, The Netherlands Organisation for Scientific Research (NWO), Nanoned, and from the European Science Foundation via an EURYI award.

Supporting Information Available. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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