Hydrodynamic Force Depends Not Only on the Viscosity of Solution but Also on the Molecular Weights of Viscogens

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ABSTRACT: Many cellular processes, such as the diffusion of biomacromolecules, the movement of molecular motors, and the conformational dynamics of proteins, are subjected to hydrodynamic forces because of the high viscosities of cellular environments. However, it is still unknown how hydrodynamic forces are related to the physical properties of different viscogens. Here, using the atomic force microscope-based force spectroscopy technique, we directly measured the hydrodynamic forces acting on a moving cantilever in various viscogen solutions. We found that the hydrodynamic force is not only dependent on the viscosity but also related to the molecular weight of viscogens. Counterintuitively, at the same macroscopic viscosity, the hydrodynamic force rises with the increasing molecular weight of viscogens, although the local microscopic viscosity of the solution decreases. This finding provides insights into the origin of hydrodynamic forces in biomolecule solutions and could inspire many force-spectroscopy-based techniques to measure the molecular weight and conformational changes of biomacromolecules in biological settings directly.

INTRODUCTION

Hydrodynamic force refers to the drag force acting opposite to the moving direction of an object in fluid. It has been widely accepted that the hydrodynamic force is linearly proportional to the viscosity. Because of the presence of a large number of biomolecules, the cellular environment is extremely viscous.1 Many cellular processes, such as the diffusion of biomolecules in cytoplasm, the binding and interaction between different biomacromolecules, and the moving of molecular motors, are inevitably subjected to notable hydrodynamic drag forces.2,3 However, most studies on the hydrodynamic forces are focused on fluids of single components (e.g., pure water, oil, etc.). The hydrodynamic forces in solutions of biomolecules may be more complicated and remain elusive. In a solution of biomolecules, its viscosity depends not only on the concentration of solutes (called viscogens hereafter) but also on their molecular weights. Moreover, depending on the size of the viscogens, their solutions could have distinct macroscopic and microscopic viscosities.4,5 For solutions of large viscogens, the macroscopic viscosities mainly come from the friction between different viscogen molecules instead of the friction between viscogen and water. Therefore, at the same overall macroscopic viscosity, the local microscopic viscosity for a solution of small-molecule viscogens is higher than that for a solution of macromolecules.5 However, it is still unknown whether the size of the viscogens affects the hydrodynamic force. In this Letter, we used force spectroscopy by atomic force microscopy (AFM) to directly measure the hydrodynamic force on a small moving object, an AFM cantilever, in solutions containing viscogens of different molecular weights ($M_w$). We were able to quantify accurately the hydrodynamic drag forces on cantilevers at different moving speeds from force spectroscopy trajectories. We found surprisingly that the hydrodynamic forces increase at increasing molecular weight of viscogens, although the local microscopic viscosity of the solution decreases. This finding provides insights into the origin of hydrodynamic forces in biomolecule solutions and could inspire many force-spectroscopy-based techniques to measure the molecular weight and conformational changes of biomacromolecules in biological settings directly.

EXPERIMENTAL SECTION

Analytical-grade ethylene glycol (EG), glycerol (GLY), and poly(ethylene glycol) with molecular weights of 1000, 2000, 4000, and 10 000 (named PEG-1K, PEG-2k, PEG-4k, and PEG-10k), respectively, were purchased from Sigma-Aldrich. Solutions with different viscogens were prepared at least 12 h before measurements to ensure that they were in equilibrium. Each solution contained 10 mM NaCl to screen the surface charges and minimize the cantilever tip−surface interactions.

The viscosities of the solutions were measured using a HAAKE rheometer (RS6000) from Thermo Scientific at room temperature. The average viscosities from at least 10 measurements were reported.

Force spectroscopy experiments were conducted using a commercial AFM (ForceRobot3, JPK, Berlin, Germany). The experimental procedures were the same as reported previously.6 Glass coverslips were used as the substrate, and a silicon nitride cantilever (the largest triangle cantilever of MLCT, from Bruker) was used to probe the hydrodynamic forces. The spring constant of the cantilever was calibrated using the equipartition theorem. To avoid the...
calibration error of the cantilever, all data reported in this Letter were taken using the same cantilever with a spring constant of 28.6 pN nm \(^{-1}\).

## RESULTS AND DISCUSSION

Recently, the force spectroscopy mode of AFM has been widely used to study the conformational change of biomacromolecules,\(^6,8−14\) the interactions between various ligands and receptors,\(^7,15,16\) the interactions between cells and substrates,\(^17,18\) and enzymatic/chemical reactions.\(^19−22\) In force spectroscopy mode, the AFM cantilever with a biomolecule attached can move toward or against the surface to exert force on the molecule of interest (Figure 1). The force acting on the molecule can be deduced from the deflection of the cantilever, which is monitored using a laser bounced off its back and a photodiode. The distance from the cantilever tip to the substrate \((d)\) is measured using a piezoelectric positioner. The effective area of the cantilever and the effective tip height are defined as \(a_{\text{eff}}\) and \(d_{\text{eff}}\) respectively.

![Figure 1. Schematic diagram of the single-molecule force spectroscopy measurement on hydrodynamic force. A cantilever tip immersed in viscogen solution moves toward or against the substrate at a constant speed. The hydrodynamic force acting on the cantilever is measured by the deflection of the cantilever using a laser bounced off its back and a photodiode. The distance from the cantilever tip to the substrate \((d)\) is measured using a piezoelectric positioner. The effective area of the cantilever and the effective tip height are defined as \(a_{\text{eff}}\) and \(d_{\text{eff}}\) respectively.](image)

It has been well documented in the literature that the hydrodynamic forces acting on the cantilever could bias the force measurement at high loading rates.\(^23−28\) Although such an effect is unwelcome for biological applications of AFM, it provides a clean and easy-to-implement system for studying the hydrodynamic force in solution. In this experiment, we used a free cantilever moving in solutions containing various viscosogens to measure the hydrodynamic force acting on the cantilever (Figure 1). First, the cantilever was brought into contact with the substrate at a speed of \(v\) (Figure 2a). Then, it was immediately retracted at the same speed. Typical force traces for moving a free cantilever in water are shown in Figure 2a. At slow speeds (e.g., <5 \(\mu\)m s\(^{-1}\)), the approach and retraction traces are almost overlapped, indicating low hydrodynamic drag forces. However, at increasing moving speeds, the separation between approach and retraction traces becomes apparent. Because in the approach and retraction traces the cantilever moves at the same velocity but in opposite directions, the hydrodynamics forces should have the same amplitude but different signs. Therefore, the hydrodynamic force can be calculated as follows

\[
F_h(d, v) = \frac{1}{2} F_e(d, v)
\]

where \(F_e(d, v)\) is the hydrodynamic force and \(F_h(d, v)\) is the force separation between the two traces at a speed \(v\) and a given distance \(d\) away from the surface.

It is worth noting that because the cantilever has the tip on the bottom side and is asymmetric along the moving direction, the approach and retraction traces are not necessarily mirror images. To simplify our study, we focused only on the retraction traces.

As studied in detail in the literature, the hydrodynamic force on a cantilever can be described using the following equation\(^27,29\)

\[
F_h(d, v) = \frac{6\pi \eta a_{\text{eff}}^2}{d + d_{\text{eff}}} v
\]

where \(\eta\) is the viscosity of the solution; \(a_{\text{eff}}^2\) and \(d_{\text{eff}}\) are the effective surface area and the tip length of the cantilever, respectively (Figure 1). \(F_h(d, v)\), \(d\), and \(v\) are the same as defined previously.

The relationship between hydrodynamic forces and cantilever speeds at a cantilever tip-to-surface distance of 0.5 \(\mu\)m is shown in Figure 2b. Clearly, the hydrodynamic force increases linearly with respect to the cantilever speed. Moreover, the hydrodynamic force is also dependent on the separation between the cantilever and the surface. The closer the cantilever is to the surface, the larger the hydrodynamic force. The retraction traces in Figure 2a are replotted in Figure 2c and globally fitted using eq 2. Because the viscosity of water at room temperature is 1 mPa·s, \(a_{\text{eff}}\) and \(d_{\text{eff}}\) of the cantilever can be readily obtained from the fitting of the experimental data in Figure 2b,c using eq 2.

Having measured \(a_{\text{eff}}^2\) and \(d_{\text{eff}}\) of the cantilever in water, we then set out to investigate the relationship between hydrodynamic forces and viscosities of solutions containing viscosogens of various molecular weights. We added EG, GLY, PEG-1k, PEG-2k, PEG-4k, and PEG-10k to water. The viscosities of these solutions were determined using rheology and are shown in Figure S1 in the Supporting Information. The viscosities are also proportional to the amount of viscosogens added. We summarized the hydrodynamic forces at different cantilever speeds and at a tip-to-surface distance of 500 nm for various solutions in Figure 3. Clearly, adding viscosogens to water increases the hydrodynamic forces due to the increase in viscosity of the solutions. For all solutions, the hydrodynamic forces are proportional to the cantilever speeds and can be adequately regressed using a linear fit. Because \(a_{\text{eff}}\) and \(d_{\text{eff}}\) are known, in principle, the hydrodynamic forces for these solutions can be predicted using eq 2. However, we found that the experimental data significantly deviate from predictions. The ratios of experimentally obtained and theoretically predicted hydrodynamic forces at different cantilever speeds and at a given distance for different viscosogen concentrations. Such “apparent” viscosogen-dependent \(a_{\text{eff}}\) and \(d_{\text{eff}}\) are due to the limitation of eq 2. Therefore, we can add a

\[
F_h(d, v) = \frac{6\pi \eta a_{\text{eff}}^2}{d + d_{\text{eff}}} v
\]
prefactor, \( p_v \), to eq 2 to fit all experimental data, and \( p_v \) is directly related to the nature of the viscogens:

\[
F_h(d, \nu) = \frac{6 \pi \eta a_{eff}^2}{d + d_{eff}} p_v \nu
\]  

(3)

On the basis of eq 3, the relative hydrodynamic force in a solution of viscogen with respect to that in water is as follows

\[
\frac{R}{F_h} = = p_r (\text{viscosity})^R \eta
\]  

(4)

where \( \frac{R}{F_h} \) is the ratio of the hydrodynamic forces at the same pulling speed and distance to the surface for a viscogen solution and water; \( R \eta \) is the relative viscosity between the viscogen solution and water; \( p_r(\text{viscogen}) \) is the \( p_r \) for a given viscogen. If \( p_v \) is an intrinsic property of a viscogen, then the plot of \( \frac{R}{F_h} \) against \( R \eta \) should give rise to a straight line. This is indeed the case that is shown in Figure 4a. For all viscogens studied in this work, the relative hydrodynamic forces are directly proportional to the relative viscosity. \( p_v = 1 \) corresponds to the situation in which the hydrodynamic force is linearly proportional to the viscosity of the solution regardless of the size of the viscogens. This is the situation predicted by Stokes’ law. However, this condition can be realized only for objects moving through a fluid without any turbulence from viscogen molecules. With the increase in the molecular weight of viscogens, the real condition approaches this condition because the concentration of viscogens is relatively low at the same viscosity. Surprisingly, \( p_v \) is less than 1 for all viscogens, which means that the measured hydrodynamic forces are smaller than those predicted using eq 2. Therefore, without considering the viscogen-specific effect, the hydrodynamic force would be overestimated on the basis of the viscosity of the solution. Moreover, we found that \( p_v \) is directly related to the molecular weight of viscogens (Figure 4b). The higher the molecular weight, the larger the \( p_v \). Therefore, at the same viscosity, the hydrodynamic force is larger for solutions containing viscogens of higher molecular weight. However, the relationship between \( p_v \) and the molecular weight of viscogens is nonlinear. Accurate physical models that take into account the conformation of viscogens in solution are required to understand such a relationship fully. It is worth

Figure 2. Hydrodynamic force measurement using atomic force microscope-based force spectroscopy experiments in water. (a) Typical set of extended (red lines) and retracted (blue lines) trace pairs for a cantilever tip moving at different speeds. The hydrodynamic force \( (F_h) \) increases as the speed increases. (b) Summarized linear dependence of hydrodynamic force on the cantilever speed at a cantilever-tip–surface distance \( (d) \) of 0.5 \( \mu m \). The square markers are the averaged experimental data from at least 10 difference traces, and the black line is the linear fit with a slope of 6.5 ± 0.008 pN/s/\( \mu m \). (c) Retracted traces (blue) in part a can be globally fitted (red line) using eq 2 with \( a_{eff} \) and \( d_{eff} \) value of 47.37 ± 1.67 and 6.0 ± 0.3 \( \mu m \), respectively. The effective cantilever area and cantilever length that we obtained are right in the range of the size of the cantilever reported by the manufacturer.
mentioning that viscogen does not have a large effect on $\alpha_{\text{eff}}$ unless many viscogen molecules strongly adhere to the cantilever surfaces as a result of nonspecific adsorptions. This may not be the case in our measurement because the relative hydrodynamic force increases linearly with respect to the relative viscosity for the same viscogen solution (Figure 4a). Otherwise, if $\alpha_{\text{eff}}$ became larger at higher viscosities, the slope would curve upward at higher viscosities. Moreover, we performed controlled experiments by measuring the hydrodynamic force in water using a cantilever that had been used in high-concentration viscogen solutions previously. The remeasured hydrodynamic force was consistent with that measured before immersing the cantilever in high-concentration viscogen solutions. The difference was less than 2%.

Intuitively, we proposed a model to explain the molecular-weight-dependent hydrodynamic force observed in our experiments. As shown in Figure 5, viscogens of higher molecular weight would have a larger radius of gyration in solution than viscogens of lower molecular weight. Consequently, the hydrodynamic drag force could pass to longer distances via viscogen-mediated interactions. For larger viscogens, because of their larger radius of gyration, more volume in solution can be agitated through the viscogen-mediated interactions. This leads to a higher hydrodynamic drag force acting on the cantilever.
the interactions between the cantilever and larger viscogen molecules as well as the internal friction of viscogen molecules. However, for the solutions of smaller viscogens, the viscogen molecules being agitation are limited to those closed to the moving cantilever, and thus the hydrodynamic forces are weaker. Therefore, the viscogen size effect probably originates from the increase in the effective agitation area of the moving cantilever. However, further experiments are required to test this model rigorously.

**CONCLUSIONS**

In this Letter, we reported the first measurement of the relationship between the hydrodynamic force and the molecular weight of viscogens using an AFM-based force spectroscopy technique. We found that the hydrodynamic force increases for solutions containing viscogens of increasing molecular weight. This might be because the cantilever could have a larger effective agitation area in solutions of larger viscogens. However, a more quantitative physical model is yet to be provided, and the molecular origin of the different hydrodynamic forces remains to be revealed. Nonetheless, some direct applications of this finding in polymer science and biophysics could be suggested. For example, the average molecular weight of viscogens in a solution with known viscosity can be estimated by measuring the hydrodynamic forces. Moreover, the conformational transitions of biomacromolecules (e.g., protein folding and DNA condensation) can also be studied by measuring the change in the hydrodynamic forces of the solution. Exploring these applications will be our next endeavor.

**ASSOCIATED CONTENT**

* Supporting Information
Viscosity data of different solutions measured using a rheometer and the hydrodynamic force at various cantilever speeds for solutions of different viscogens. This material is available free of charge via the Internet at http://pubs.acs.org.

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**ABBREVIATIONS**

AFM, atomic force microscope  
PEG, poly(ethylene glycol)  
EG, ethylene glycol  
GLY, glycerol

**REFERENCES**


