DEVELOPMENT OF VISUALIZATION TECHNIQUE FOR HYDRODYNAMIC STRESS FIELD UTILIZING FLUORESCENT FORCE PROBE

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ABSTRACT

This study investigates the fluorescence response of a molecular force probe called FLAP (flexible and aromatic photofunctional systems) by measuring spatially averaged fluorescence spectra and two-dimensional fluorescence images of FLAP solution in microchannel flow. We aim at developing a technique for visualizing the hydrodynamic stress field through fluorescence imaging of FLAP. FLAP is composed of two rigid fluorescent aromatic wings fused with a flexible eight-membered ring. This molecule undergoes stress-induced conformational change between V-shape and planar shape and emits blue or green fluorescence depending on its conformation. We used three types of FLAP whose molecular ends were chemically functionalized with PEG (polyethylene glycol) chains of different molecular weights (5000, 10000, and 20000). Each FLAP-PEG was dissolved in a dimethyl sulfoxide (DMSO) /glycerol mixture, and the solution was supplied to a sudden contraction microchannel at constant flow rates in the range of 2.2–446 μL/min. These flow rates correspond to the elongational stress of 0.1–21 kPa at 10 μm upstream of the entrance of the contraction part. The spectral measurements showed that the intensity ratio of green (529 nm) to blue (494 nm) fluorescence increased monotonically with the flow rate, which indicates the response of FLAP to hydrodynamic elongational stress. In addition, FLAP bearing the longest PEG chains showed the highest increasing rate of the intensity ratio, which suggests the possibility of adjusting the stress sensitivity of FLAP-PEG by changing the chain length. In fluorescence imaging, two-dimensional distributions of the intensity ratio were visualized around the entrance of the contractions part. The intensity ratio distributions showed a characteristic pattern in the region where the velocity gradient was relatively large in the streamwise direction.

INTRODUCTION

From scientific and engineering perspectives, hydrodynamic stress is an essential quantity to analyze the dynamic characteristics of flow fields. For example, wall shear stress is important in designing and developing various fluidic systems since it is directly related to the flow resistance and required power for fluid transport. Wall shear stress is also one of the key parameters in biomedical field which inhibits or aggravates the pathogenesis of cardiovascular disorders(1). It is also important to know hydrodynamic stress in fluid as well as at channel wall, for understanding the unique behavior of non-Newtonian fluids and complex fluids. Therefore, various measurement techniques have been proposed to determine the hydrodynamic stress(2,3).

For the measurement of wall shear stress, a flush-mounted wall-element called floating-element is often used. This element is moveable in the plane of the wall-shear stress and its movement is detected by resistive or optical methods. Although a MEMS-fabricated floating-element has been developed recently, the spatial resolution is still restricted to its characteristic size (~0.1 mm) and it is difficult to obtain two-

dimensional distribution. In addition, installing the element might disturb the flow field. Other methods, such as hot-wire anemometer, hot-film method, Preston and Stanton tubes, for instance, determine the wall shear stress based on another measured quantity, i.e., velocity, heat transfer rate, or pressure. These methods rely on the certain assumption concerning the flow field (such as law of the wall) and/or require extensive calibration for accurate measurement. On the other hand, no well-established technique can be found for the measurement of hydrodynamic stress in fluid. However, optical elasticity imaging utilizing birefringence phenomenon in polymer solution has recently been studied as a promising approach[4].

For developing a non-invasive visualization technique for hydrodynamic stress field, the present study proposes an optical-based method utilizing a unique molecular force probe called "FLAP (flexible and aromatic photofunctional systems)[5]". In order to examine the feasibility of FLAP as a stress probe, we investigate the fluorescence response of FLAP to hydrodynamic elongational stress by measuring spatially averaged fluorescence spectra and two-dimensional fluorescence images of FLAP solution in microchannel flow.

PRINCIPLE

The molecular structure and the fluorescence characteristics of FLAP are depicted in Fig. 1. FLAP is a fluorescent molecule which is composed of two rigid fluorescent aromatic wings fused with an eight-membered ring[5,6]. FLAP changes its conformation flexibly between V-shape and planar shape and emits blue or green fluorescence depending on its conformation. FLAP dissolved in solution takes the V-shape conformation in the ground state and emits blue fluorescence after excited by ultraviolet (UV) light. If FLAP is subjected to an external tensile force in the ground state, its conformation is altered from V-shape to planar shape. FLAP in the planar shape is excited by the same UV light and emits green fluorescence. Therefore, the intensity ratio of green to blue fluorescence can be used to quantify the force applied to the molecule. Since the force and the time required for the conformation change are on the order of 100 pN and a few nanoseconds, respectively, FLAP can be potentially applied to real-time visualization of small stress distributions in various fields. Based on this principle, FLAP has been used to visualize the tensile force distribution during polymer deformation[7].

In the present study, we examine the fluorescence response of FLAP to hydrodynamic elongational stress. We used three types of FLAP whose molecular ends were chemically functionalized with PEG (polyethylene glycol) chains with different molecular weights (5000, 10000, and 20000 for each end). The total molecular weights (MW) of these molecules are 12000, 22000 and 42000. Hydrodynamic force is expected to be exerted on the PEG chains, which are in coil state in stationary fluid, and stretch the FLAP under flow condition. Since the critical elongational rate at which the polymer chain shows the initiation of coil-stretch transition generally decreases as increasing the molecular weight of polymer[8], the difference in the molecular weights of PEG can change the stress sensitivity of FLAP functionalized with PEG chains (hereafter referred to as FLAP-PEG).

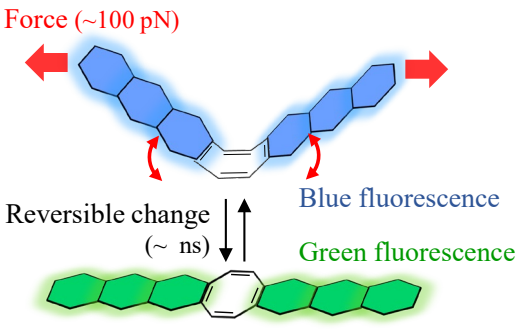

Fig. 1. Reversible change in molecular conformation and fluorescence color of FLAP in response to external force.

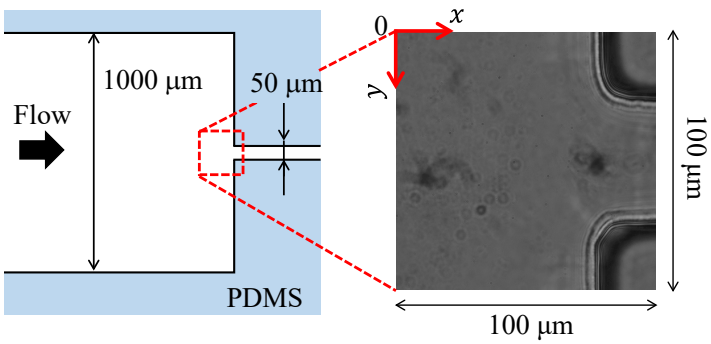

Fig. 2. Schematic of a sudden contraction microchannel and a transmission image of the measurement area of fluorescence imaging.

EXPERIMENTAL SECTION

Apparatus

The measurement system was mainly comprised of a microchannel, fluid supplying system, inverted fluorescence microscope (Olympus, IX-73), and detector. Ultraviolet LED (Thorlabs, M365LP1) was used as excitation light source. The fluorescence from the FLAP-PEG solution flowing in the microchannel was collected by 100x objective lens (Olympus, UPlanSApo). Fluorescence mirror unit (Olympus, U-FUW) and multichannel spectrometer (Ocean Optics, Frame-S) were used for spectral measurement. On the other hand, fluorescence filters (Semrock, FF01-458/64 for blue and FF02-534/30 for green fluorescence) and CMOS camera (Andor, Zyla 4.2 plus) were used for fluorescence imaging.

A sudden contraction microchannel was used to evaluate the fluorescence characteristics of FLAP probe in flow with elongational stress. Figure 2 shows the schematic of the microchannel composed of a polydimethylsiloxane (PDMS) chip and a cover glass. The width of the main channel was 1000 μm and that of the contraction part was 50 μm. The channel height was 74.3 μm. A syringe pump (Nihon Koden, CFV-3200) was used for supplying FLAP-PEG solution to the microchannel at constant flow rate.

Experimental condition

FLAP-PEG was dissolved into dimethyl sulfoxide (DMSO)/glycerol mixture (40:60 in volume) at the concentration of 5 or 10 μM. The viscosity of the solution was 85.4 mPa·s at 296 K. The solution was supplied to the microchannel at constant flow rates in the range of 2.2–446 μL/min. These flow rates correspond to the cross-sectional average velocity of 10–2000 mm/s at the contraction part. The focal plane of the objective lens was positioned at the middle height of the microchannel and the measurement area was set at the entrance of the contraction part (Fig. 2). The exposure time of the spectrometer or the camera was 1s and 30 spectra/images were collected for each condition.

Prior to the measurement, we conducted a numerical simulation of flow field using a computation software (COMSOL Multiphysics 4.3a) and estimated the elongational stress along the center line of the microchannel based on the velocity gradient in the flow direction. The elongational stress was estimated to be 0.1–21 kPa at 10 μm upstream of the entrance of the contraction part for the above flow rate.

RESULTS AND DISCUSSION

Spectral measurement

Figure 3 shows the fluorescence spectra of FLAP-PEG (MW=22000) solution at each flow rate, which is normalized by the peak intensity at 494 nm. The legend shows the cross-sectional average velocity at the contraction channel. The green fluorescence band (490–600 nm) increased with flow rate, whereas that of blue fluorescence band (420–490 nm) decreased. This trend was also observed in the spectra of FLAP-PEG with molecular weights of 12000 and 42000.

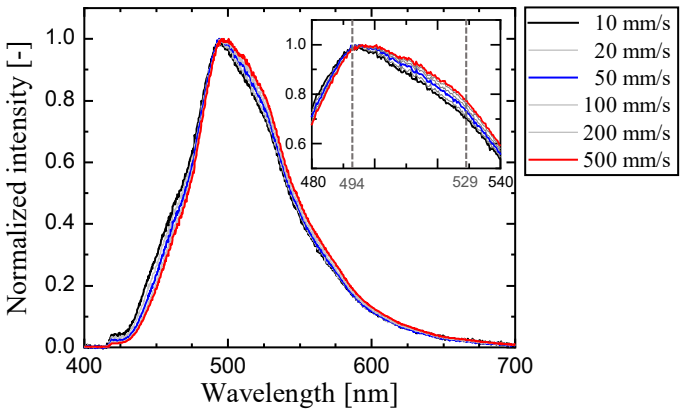

Fig. 3. Normalized fluorescence spectra of FLAP-PEG (MW=22000) in DMSO/glycerol mixture at each flow rate.

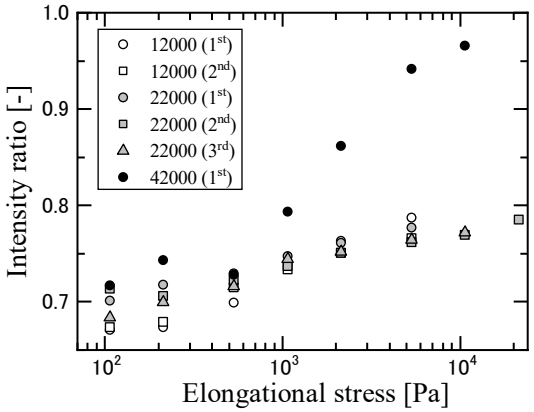

Fig. 4. Relationship between the measured fluorescence intensity ratio and the elongational stress estimated by simulation.

The intensity ratio of green (529 nm) to blue (494 nm) fluorescence of the measured spectra was plotted against the elongational stress estimated by numerical simulation in Fig. 4. The legend represents the molecular weight of FLAP-PEG and the measurement order in brackets. The intensity ratio increased monotonically with the elongational stress for all types of FLAP-PEG. This indicates that FLAP was planarized by the flow stress and the number of FLAP in planar shape emitting green fluorescence increased with increasing the stress. This result suggests the potential application of FLAP as a hydrodynamic stress probe. In addition, FLAP bearing the longest PEG chains (MW=42000) showed the highest increasing rate of the intensity ratio, which agrees with our expectations. However, the increasing rate of molecular weight 12000 was slightly larger than that of 22000. In addition, the number of measurements is still limited since the measurements were sometimes disturbed by channel clogging and leakage. Further measurement should be made to ensure the measurement reproducibility and verify the range of the stress sensitivity of FLAP-PEG by changing the PEG chain length.

Two-dimensional fluorescence imaging

To examine the possibility of flow stress visualization using FLAP-PEG, two-dimensional fluorescence imaging was performed at the measurement area shown in Fig. 2. FLAP-PEG with molecular weight of 22000 was used for the experiment. The flow rate was adjusted in the range 2.2–111 μL/min so that the cross-sectional average velocity was 10–500 mm/s in the contraction channel. Fluorescence images of blue and green bands were measured by switching the fluorescence filters corresponding to these spectral bands after the flow reached the steady state at each condition.

Figures 5(a)–(c) show the distributions of the green to blue fluorescence intensity ratio calculated from the measured images at the condition of 10, 50 and 100 mm/s, respectively. The white area in the figure represents the channel wall. The intensity ratio increased in all area with increasing the flow rate. The intensity ratios averaged over the area approximately 10 μm upstream of the entrance (indicated by red square in Fig. 5(a)) are 0.64, 0.71, 0.76 and 0.82 at the condition of 10, 50 100, and 500 mm/s, which corresponds to the elongational stress of 0.1, 0.5, 1.0, and 5.2 kPa, respectively. The intensity ratio increases almost linearly with the elongational stress in logarithmic scale. This is consistent with the spectral measurement result in Fig. 4. Focusing on the distribution, all figures exhibit a similar fan-like pattern, in which the area of high intensity ratio is expanding from the entrance of the contraction to the upstream area. Since large velocity gradient is generated in the streamwise direction upstream of the entrance, it is reasonable that the intensity ratio is high in the region. However, area of heigh intensity ratio can be observed also downstream of the entrance (i.e., in the contraction part). The velocity gradient in the flow direction decreases rapidly in the region and the fluorescence intensity ratio should decrease

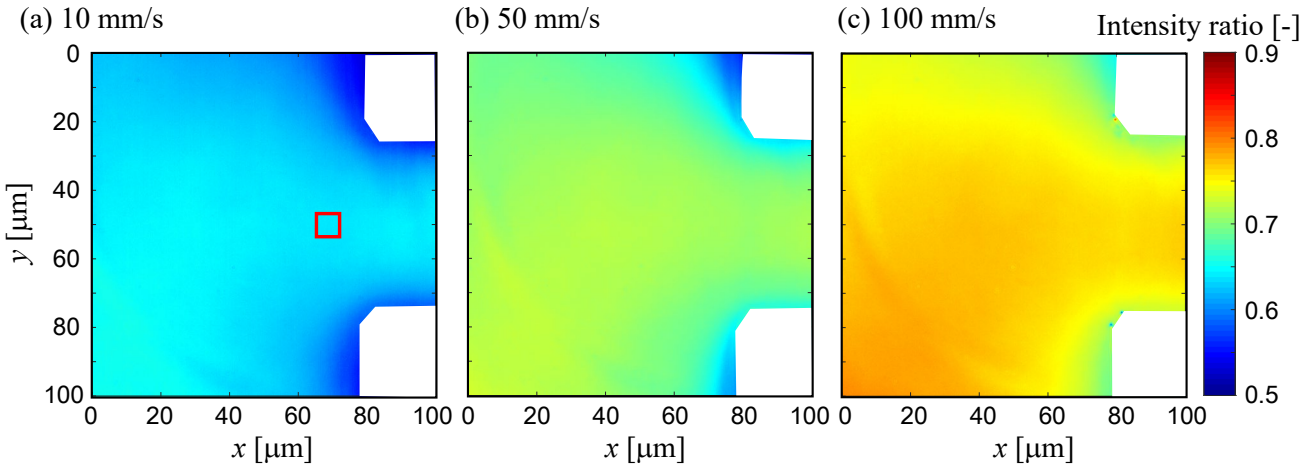

Fig. 5. Distributions of the intensity ratio of green (529 nm) to blue (494 nm) fluorescence measured at different flow rates. The cross-sectional average velocity in the contraction channel was (a) 10 mm/s, (b) 50 mm/s, and (c) 100 mm/s.

as well. In addition, some influence of the intensity profile of the excitation light is observed in the results as can be seen in the lower left region of the figures. We will further analyze the intensity ratio distributions in flow fields with various channel geometries and compare with numerical simulation result to examine if the intensity ratio distribution has some correlation with stress distribution.

CONCLUSIONS

The present study investigated the change of fluorescence wavelength of FLAP-PEG in response to hydrodynamic elongational stress in order to develop a non-invasive visualization technique for stress distribution in channel flow. The spectral measurement result in the sudden contraction microchannel showed that the intensity ratio of green to blue fluorescence monotonically increased with the elongational stress and the highest increasing rate was observed for FLAP-PEG with the highest molecular weight. The two-dimensional distributions of the intensity ratio exhibited a characteristic pattern around the entrance of the contraction part where the relatively large velocity gradient was generated in the flow direction. In addition, the intensity ratio in the whole measurement area increased with the elongational stress. These results indicate the response of FLAP-PEG to hydrodynamic stress and support the potential use of the molecule for stress visualization in flow field.

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