STUDY OF MOLECULAR TRANSPORT THROUGH SPECIFIC LIQUID IN BIO-MIMETIC EXTENDED NANOSPACES

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ABSTRACT

Molecular transport in 100 nm inter/intra cellular spaces such as synaptic clefts and mitochondria has important roles in biological functions. Our group has developed a powerful *in vitro* tool, i.e., bio-mimetic extended nanospace (10-1000 nm), lipid bilayer-modified extended nanochannel for mimicking the cellular spaces. In this study, we have revealed liquid viscosity and molecular diffusion in bio-mimetic extended nanospace. The diffusion of proteins and ions was much slower by increased viscosity of confined liquid, which is considered to be induced by loosely coupled water molecules within 50 nm of the surface.

KEYWORDS: Bio-mimetics, Nanochannel, Fluid, Diffusion

INTRODUCTION

State-of-the-art biology is currently focusing on behaviors of single molecules and their roles in biological systems. 100 nm inter/intra cellular spaces such as synaptic clefts and mitochondria have critical roles in signaling and energy production in biological systems. Recently, many researchers have studied molecular transport in the inter/intra cellular spaces. By *in vivo* approach, they suggested possibility of slower molecular diffusion [1]. However, further investigation has been difficult owing to complicated cell geometry and liquid components.

On the other hand, our group has established research tools for extended nanospace (10-1000 nm). Extended nanofluidic devices with size-regulated fused-silica extended nanochannel and pressure-driven flow control method have been developed. By using these tools, unique liquid properties such as higher viscosity and higher proton mobility have been revealed, and proton transfer phase with loosely coupled water molecules by hydrogen bond within 50 nm was hypothesized [2].

From these findings in extended nanospace, unique properties also can be expected in the inter/intra cellular spaces. Most recently, we have established a lipid bilayer modification method to extended nanochannel to mimic the inter/intra cellular spaces [3]. By using this *in vitro* tool, this study investigated molecular diffusion related to unique fluid property in bio-mimetic extended nanospaces.

EXPERIMENTAL

Bio-mimetic extended nanospace as illustrated in Figure 1 was constructed by lipid bilayer modification to fused-silica extended nanochannel based on vesicle fusion method [3]. For experiments, bio-mimetic- and fused-silica extended nanochannels of 200-1600 nmwere prepared. Water and phosphate buffered saline (PBS), which is a typical artificial extracellular fluid were used as liquids.

The viscosity in extended nanochannel was measured by a method using pressure-driven capillary flow, as previously developed by our group [4]. MPa order air pressure controlled by a high pressure control system was applied to extended nanochannel filled by the liquid to generate receding capillary flows (Figure 2). From a relationship between the meniscus velocity and applied pressure, the liquid viscosity was obtained.

The diffusion coefficients of protein and ion in extended nanochannel were measured by fluorescence microscopy [5]. For the protein diffusion, green fluorescent protein (GFP) 28 μM was used as a standard model. For the ion diffusion, diffusion of 1 mM calcium ion in the extended nanochannels was examined. Fluo-4 100 μM was used for calcium indicator. Diffusion from one side of the extended nanochannel to another side was observed from the fluorescence with the concentration gradients, as shown in Figure 3. From the diffusion distance, the diffusion coefficient in the extended nanochannel was estimated.

RESULTS AND DISCUSSION

Figure 4 shows the liquid viscosity as function of the channel size. The viscosity in bio-mimetic extended nanospace is significantly increased at the channel sizes smaller than 1000 nm, and becomes 4.4 times higher in 400 nm channel. The magnitude of increase in the viscosity in bio-mimetic channels is greater than that in fused-silica channels. On the other hand, the viscosity of PBS in bio-mimetic extended nanochannels is also higher than the bulk, but lower than that of water.

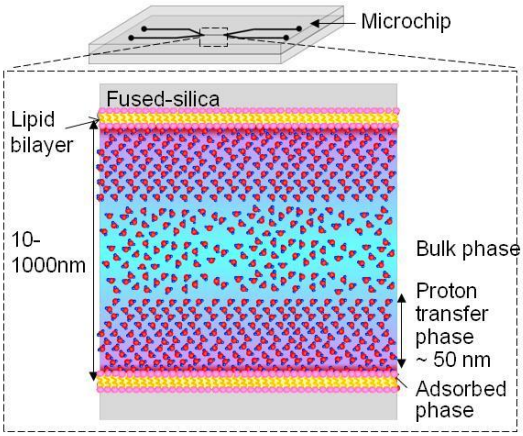

Figure 1. Schematic of bio-mimetic extended nanospace, where fused-silica extended nanochannel is modified by lipid bilayer.

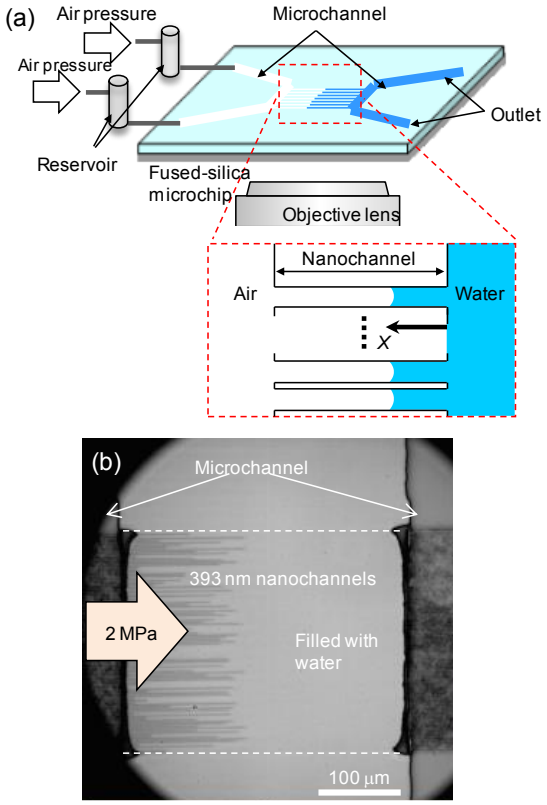

Figure 2. (a) Schematic of experimental setup for observation of capillary flow in extended nanochannels driven by high pressure control system. (b) Image of capillary flow driven by external pressure of 2 MPa.

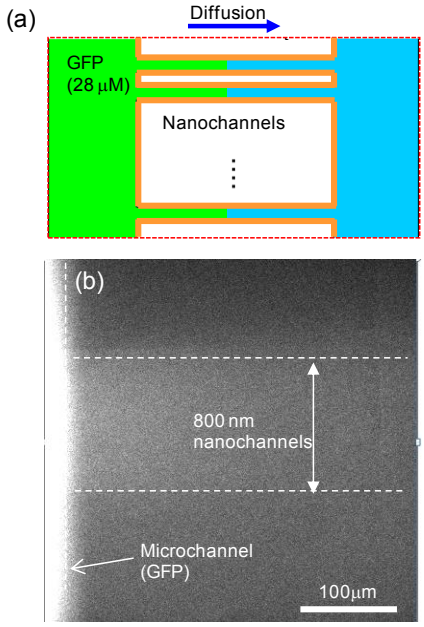

Figure 3 (a) Schematic of diffusion of GFP through extended nanochannels. (b) Image of diffusion of GFP (28 μM) in nanochannels.

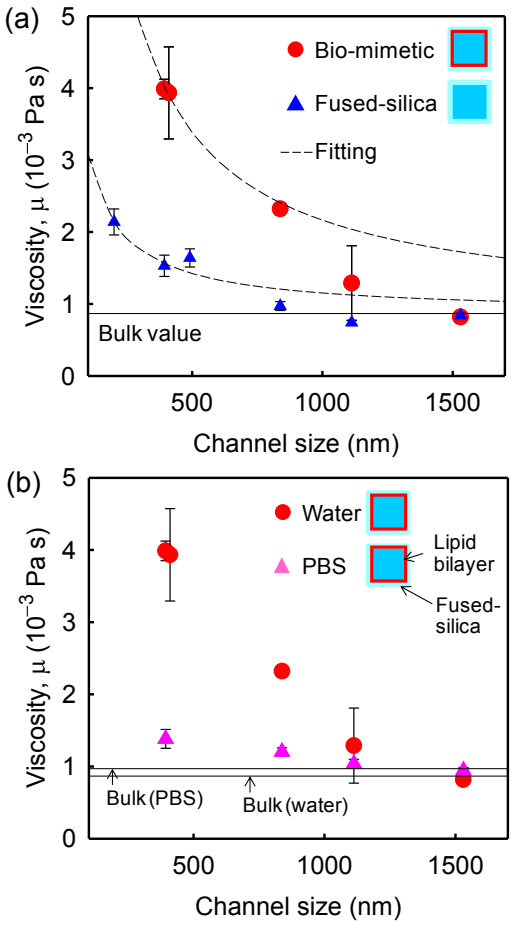

Figure 4 (a) Water viscosity as function of the size of bio-mimetic- and fused-silica extended nanochannels. (b) The viscosity of PBS and water in bio-mimetic extended nanochannels as function of the channel size.

Figure 5 shows the diffusion coefficients of GFP and calcium ion as function of the channel size. Both GFP and calcium ion show slower diffusion in the bio-mimetic channels smaller than 1000 nm compared with the bulk. In the fluid physics, the diffusion coefficients D depend on the fluid viscosity μ by the Stokes-Einstein relation ($D \propto 1/\mu$). Hence a relationship between the diffusion coefficient and the viscosity was examined, as shown in Figure 6. Since the rate of decrease of diffusion coefficients well corresponds to that of increase of viscosity, it is concluded that the decreased diffusion coefficients of protein and ion in bio-mimetic extended nanochannels are due to the increased liquid viscosity.

These results suggest that the diffusion of proteins and ions is determined by the increased viscosity of liquid in the inter/intra cellular spaces, depending on the surface properties and liquid components. Analysis by data fitting revealed the visocosity becomes higher within 50 nm of the channel wall, for both fused-silica and bio-mimetic extended nanochannels. This scale corresponds to the scale of proton transfer phase, which has been revealed in fused-silica channels. Based on the hypothesis of the proton transfer phase, for the bio-mimetic channels, phosphate groups on lipid bilayer surface can work as proton donor and induce the loosely coupled water molecules by hydrogen bonds, as silanol groups on fused-silica surface. Hence one possibility is the higher viscosity is generated by the loosely coupled water molecules within 50 nm of the wall. In this case, weaker

increase of the viscosity in PBS than that of water in extended nanochannels can be explained to be because of ion hydration effect breaking hydrogen bond network. These findings suggest that biological processes work based on

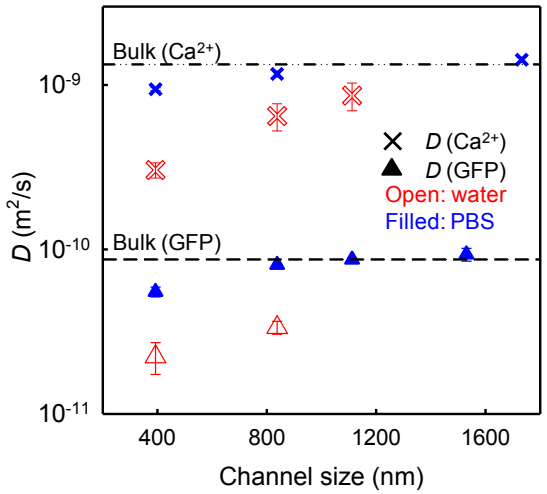

Figure 5 Diffusion coefficients as function of the size of bio-mimetic extended nanochannles.

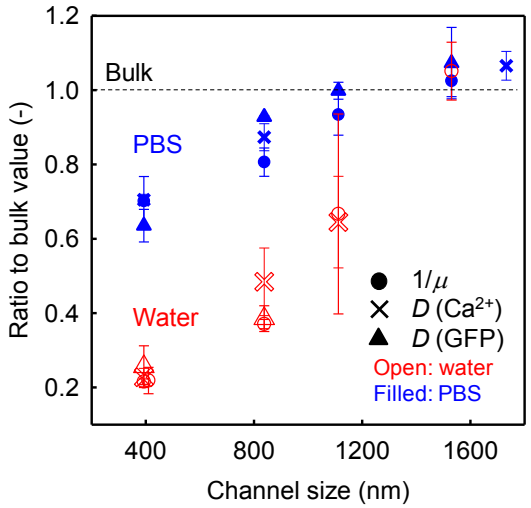

Figure 6 Diffusion coefficients D compared with inverse of the viscosities 1/μ to verify the Stokes-Einstein relation. Parameters are normalized by that in the bulk.

molecular transport by unique fluid property with microscopic structure of liquid confined in the 100 nm inter/intra cellular spaces.

CONCLUSION

Molecular transport in bio-mimetic extended nanospaces was investigated by evaluating the diffusion coefficients and the liquid viscosity. The results suggested the diffusion coefficients of GFP and calcium ion were decreased by increased viscosity of the confined liquids with microscopic liquid structure, depending on the surface properties and liquid components. This study suggests these unique transport phenomena will significantly affect the biological functions such as signaling and energy production in 100 nm inter/intra cellular space.

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