Force field measurements within the exclusion zone of water

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Abstract Water molecules play critical roles in many biological functions, such as protein dynamics, enzymatic activities, and cellular responses. Previous nuclear magnetic resonance and neutron scattering studies have shown that water molecules bind to specific sites on surfaces and form localized clusters. However, most current experimental techniques cannot measure dynamic behaviors of ordered water molecules on cell-size (10 μm) scale. Recently, the long-distance effect of structured water has been demonstrated by Pollack and his colleagues. Namely, there is a structured water layer near the hydrophilic surface that can exclude solutes (Zheng et al, *Adv Colloid Interface Sci* 127:19–27, 2006; Pollack 2006, *Adv Colloid Interface Sci* 103:173–196, 2003). The repelling forces of water clusters inside this exclusion region are investigated in this study. With a laser tweezers system, we found the existence of an unexpected force fields inside the solute-free exclusion zone near a Nafion surface. Our results suggest that the water clusters could transduce mechanical signals on the micrometer range within the exclusion zone. This unexpected inhomogeneous force field near the hydrophilic surface would provide a new insight into cellular activities, leading to a potential new physical chemistry mechanism for cell biology.

Keywords Water · Exclusion zone · Nafion · Laser tweezers

1 Introduction

Water molecules play critical roles in many aspects of life, such as protein dynamics and cellular activities [4]. Nuclear magnetic resonance and neutron scattering measurements

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have shown that water molecules can form localized molecular clusters on protein surfaces [5]. Recent studies also showed that solutes are excluded from the structured water layers immediately adjacent to various gel and biological surfaces. The solute-exclusion (EZ) zone is considerably larger than anticipated by conventional theories: nominally on the order of 100 μm or more [1]. The identification of the EZ zone provides unique opportunities to understand the basic mechanism of biomolecular interactions within water structures and to explore the importance of water organization in life [6].

The concept of layered or “structured” water has a long history, which began with a hypothesis put forth a century ago by Fischer and Moore [7]. The idea was strongly advocated by the Hungarian biophysics school [8]. Gilbert Ling suggested that structuring originated at charged protein surfaces, to which water molecules would associate because of their polar nature [9]. Once a single layer adhered, additional layers would adhere one upon another to form a layered network [10, 11]. Structured water was also the centerpiece of Albert Szent-Gyorgyi’s classic book (1972), in which interactions between macromolecules and surrounding water were argued to be the living state’s dominant feature [12].

Modern biophysical approaches have begun addressing water layers in a more quantitative way, particularly using the surface-force apparatus and atomic force microscopy [13]. Classical (such as DLVO) theory predicts that as surface-to-surface separation diminishes, the force will increase monotonically (ultimately diminishing sharply as the surfaces get extremely close together). But experimental results indicate that the force is oscillatory. Different solvents have been used and in each instance the spatial period of the oscillation was equal to the diameter of the sandwiched (solvent) molecules [13, 14]. These experiments confirm that layering of solvent molecules between closely juxtaposed, charged surfaces is possible, although the exact mechanism of layering is still unsettled and may not involve dipolar structuring [15].

Since Ashkin established the laser-based optical trapping in early 1970s, the applications of laser tweezers have been demonstrated in many biophysical studies [16–23]. In the single beam laser tweezers systems, most platforms are based on modified microscopes. Briefly, with different optical designs and settings [20–23], the parallel laser beam passed through a high numerical aperture microscopy objective is highly focused on the sample plane. The targeted samples are trapped within the high gradient energy field generated by the focused laser beam. Two different theoretical models (Mie scattering and Rayleigh scattering) have been developed to interpret the trapping mechanism, and mainly depends on the diameters of trapped samples [21, 22]. Near the central equilibrium region of the laser beam, the force applied on the trapped sample is a function of the displacement of the sample from the gradient centers, refractive index of the sample and surrounding solution, laser power, and the gradient of the focused laser beam [18, 21]. By measuring the sample displacement, the displacement can be converted into the force applied on the targeted samples. Because of the high spatial resolution ($\sim\text{nm}$) and precise force measurement (pN), this non-destructive tool has been intensively applied in various biophysical studies, such as kinesin stepping, DNA structure, enzyme affinity, etc [19, 21, 23–25].

Although the EZ zone has been demonstrated as a general phenomenon in the vicinity of various hydrophilic surfaces [1, 26], the properties of force-caused exclusion is not yet understood. Moreover, the establishment of a feasible platform to explore the molecular interactions in structured water networks (EZ zone) is critical and necessary. Hence, we investigate the potential of an optical tweezers system to measure the force field in the vicinity of hydrophilic surfaces. The force vectors within the EZ zone were analyzed with

different surface-modified probes. Due to the importance of force translation in cell biology, these measurements within EZ zone provide a new insight for the role of water dynamics in cellular functions.

2 Materials and methods

2.1 Laser tweezers system

To measure structured water's mechanical properties with minimum disturbance, we used a dielectric microsphere (2 μm diameter) as the force probe. The infrared laser tweezers system can be operated as a two-dimensional force measuring apparatus, applying a specific magnitude of force load to a trapped microsphere moving in designed directions [20, 27]. The applied force on the microsphere can be calculated by the microsphere's relative movement and the laser power. An inverted microscope (TE-2000E, Nikon) was modified to integrate with other optical components (Fig. 1). To achieve more precise spatial movement measurements two different lasers were set separately for trapping (1,064 nm, Nd:YVO₄, BL-106C, Spectra-Physics, Newport, Irvine, CA, USA) and position detection (828 nm, LDS-series, Point Source, Optodyne, Compton, CA). The quadrant photodiode (QPD, SPOT9-DM1, UDT, San Diego, CA) was adapted to collect the movement of the probe with 20 k bandwidth. Data analysis and force calibration were performed using custom software developed using LabVIEW[®] (National Instruments, Austin, TX, USA) and MATLAB[®] (MathWorks, Natick, MA, USA) [27].

Fig. 1 Layout of IR laser tweezers system. A trap laser (1,064 nm) and a separate detection laser (828 nm) were used in our system. An acousto-optical modulator was used to steer the trap laser beam. Laser output beams were expanded to overfill the back aperture of the objective. A telescopic lens was used for manually steering the position of the optical trap in the specimen plane. The laser beam was highly focused by an oil immersion objective lens (100 $\times$ /N.A 1.40) to form the optical trap in the specimen plane. A computer controlled three-axis piezo stage was incorporated to provide precise specimen positioning. Trapped samples were imaged with a camera, and the forward scattered light signals were coupled onto the position detector on the back focal plane of the condenser. Scattering signals were translated into sample displacements and converted into force units with post signal processing

2.2 Exclusion zone

In previous studies, the long-impact exclusion zone was observed on the Nafion surface in aquatic environment. The width of this solute-free zone is usually several hundred micrometers from the various hydrophilic surfaces [1]. Various ions, such as imidazole or TES (2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid), and different ion concentrations are applied to influence the formation of the EZ zone [28]. Similar EZ zones have been reported in polar solvents as well [26]. Here, we placed a Nafion film into the glass slide chamber with 10 mM imidazole solution as described previously [1, 26].

2.3 Experimental methods

To measure the force field within the exclusion zone, we trapped single microsphere in the laser beam center and dragged the microsphere toward the hydrophilic surface. Force calibration can be achieved with determined stiffness of the optical trap and measured displacement from the equilibrium trap position [21]; through Hooke's law $F = -\alpha\chi$, where F is the applied force, α is the stiffness, and χ is the displacement. The stiffness of the optical trap mainly depends on the laser power, light wavelength, and trapped bead diameters. In each set of experiments, laser power and other parameters were kept constant, and 2 μm polystyrene microspheres with differ surface modifications were used as our scanning probes [21]. Once microspheres of known radius are trapped, the physics of Brownian motion in a harmonic potential can be used to obtain the stiffness. The thermal fluctuations of the trapped bead can be described as a Lorentzian spectrum [21]. The power spectrum can be fit with a rolloff frequency, from which the stiffness can be calculated [21]. The applied force can be determined by measured displacements from the equilibrium trap position multiplied by the known stiffness.

3 Results

A distinct EZ zone formed next to the surface 5 minutes after Nafion surface was placed in 10 mM imidazole solution (Fig. 2). To measure the mechanical force in the EZ zone, 2 μm non-functionalized polystyrene microspheres were used as the force sensing probe. To calibrate the force probe, we set a lower optical trap stiffness ($\sim 8.2 \times 10^{-6}$ N/m), which is more sensitive to the force changes induced by the environment. Then, the laser beam was moved 1 mm away from the Nafion surface, and the microsphere was trapped at the equilibrium position within the laser beam. The piezo stage was adapted to preciously control the distance between the force probe and the Nafion surface. Under different distance settings, the displacements of the microsphere from the equilibrium center were recorded and converted into the force applied by the EZ zone. The results showed that the repelling force decreases from 3 to 0 pN as the distance increases from the surface to 60 μm . The exclusion range was consistent with previous studies [1, 26]. However, the data demonstrated the force field is not homogeneous throughout the exclusion zone, but is a rather a gradient field across the region (Fig. 3). Once the microsphere was dragged closer to the surface (~ 10 μm), the force applied by the optical trap was not sufficient to overcome the repelling force from the EZ zone. The trapped microsphere was pushed out from the equilibrium position and escaped from the laser beam. An impenetrable barrier exists close to the hydrophilic surface (~ 10 μm).

Fig. 2 The exclusion zone under fluorescence microscopy. Nafion film is placed in the glass slide chamber with 10 mM imidazole solution: the microsphere ($2\ \mu\text{m}$) exclusion formed in the vicinity of the Nafion surface and lasted for more than 1 h. Force field measurements were performed under the same condition

To further study the impenetrable barrier, under the same experimental settings, we increased the stiffness of the optical trap ($1.78 \times 10^{-5}\ \text{N/m}$) with a higher laser intensity (100 mW). The results showed the repelling force increases from 0 to 0.5 pN at $60\ \mu\text{m}$ away from the surface. The observed EZ expansion phenomenon here was consistent with a previous observation [29]. With stronger applied force ($\sim 3.5\ \text{pN}$), the trapped microsphere can be pushed much closer to the surface ($\sim 5\ \mu\text{m}$).

In our study, various microspheres, $2\ \mu\text{m}$ polystyrene microspheres with non-functionalized, amine-functionalized and carboxyl-functionalized surfaces, were used as force probes. We measured the magnitude of the repelling (+)/attracting (−) force applied on the trapped microsphere within the exclusion zone with micrometer spatial resolution. The results showed the different force response with three different surface modifications of the microspheres (Fig. 4). The repelling force, ranging from 1 to 5 pN, applied on non-functionalized/carboxyl-functionalized microspheres. In contrast, there was an attracting force (approximately -1 to $-3\ \text{pN}$) pulling positive-charged microspheres toward the surface. Though the force response curves are variable, similar impermeable barriers ($\sim 10\ \mu\text{m}$ from the surface) were observed under all experimental conditions with the same probe stiffness.

Fig. 3 The force response curves of hydrophobic microspheres as a function of the distance from the Nafion surface. The distance shown on the x-axis indicates the microsphere's relative position to the film surface, and the exclusion force extended to tens of micrometers under both laser tweezers stiffness settings

Fig. 4 The different force response curves of microspheres with three different surface modifications. On the y-axis, we defined the force direction as positive while a microsphere experienced the repelling force opposite to the Nafion surface

4 Discussion

The EZ zone has been identified near many types of surfaces, including various biological or synthetic gel surfaces [1]. Our results clearly demonstrate the existence of forces as a function of distance from the surface within the EZ zone. We also confirmed that the range of this force extends more than 50 μm from the surface (Fig. 3), and that the gradient profiles are relatively consistent with the stable charge potential profile demonstrated in a previous study [1]. The negative potential gradients sustained within the EZ zone were proposed to result from the formation of organized water networks and induced dipoles on water molecules (Figs. 4 and 5).

Fig. 5 Water molecules in the exclusion zone “imprint” the charge distribution by dipole arrangement. Charged microspheres are excluded to show the charge-distribution information preserved in the exclusion zone by water molecules

Through the interaction of dipole–dipole interactions, surface charges and hydrogen bonds, various simulation models have been developed to investigate the orientation and dynamics of water networks in the vicinity of various surfaces [30–33]. Though the mechanism of the long-distance force gradient is elusive, we hypothesize that the force transformation within the EZ zone is relative to the water structure and induced dipoles (Fig. 5). Our results show that different charged microspheres experienced different force fields near a Nafion surface (negative surface). For neutral and negative microspheres, the gradient repulsive force field was recorded up to 50 μm away from the Nafion surface. The repelling force may from the exclusive effect for water network formation. The larger force field applied on the carboxyl-functionalized microsphere may come from a synergistic effect of the negative induced dipoles and water structure. In the region far from the surface, the negative charge gradient may potentially dominate the attractive forces found for positive microspheres within the EZ zone; however, due to the compact formation of structured water networks, positive microspheres are excluded from this region closer to the Nafion surface ($\sim 10\ \mu\text{m}$) (Fig. 5).

Water structures play critical roles on different biological scales. On a biomolecular level, water molecules attached to specific sites of biomolecules and influence their activities [34, 35]; the water molecules form specific organization that are proposed to influence the cytoskeletons [15, 34]; a long distance of repulsion was also observed in the vicinity of rabbit psoas muscle samples. However, the lack of adequate tools limits the investigation of biomolecular interactions in water structures (networks). In our study, we demonstrated the specific directional pico-Newton scale force field recorded by laser tweezers systems that also have been successfully applied to various biomolecular studies, such as enzyme kinetics and DNA structure folding [23, 24]. In addition to the demonstration of the feasibility of optical tweezers systems in structured water studies, our results also provide much-needed experimental evidence for the exclusive forces within the EZ zone.

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