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Surface charge modulated aptasensor in a single glass conical nanopore

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ABSTRACT

In this work, we have proposed a label-free nanopore-based biosensing strategy for protein detection by performing the DNA–protein interaction inside a single glass conical nanopore. A lysozyme binding aptamer (LBA) was used to functionalize the walls of glass nanopore via siloxane chemistry and negatively charged recognition sites were thus generated. The covalent modification procedures and their recognition towards lysozyme of the single conical nanopore were characterized via ionic current passing through the nanopore membrane, which was measured by recording the current–voltage (*I*–*V*) curves in 1 mM KCl electrolyte at pH=7.4. With the occurring of recognition event, the negatively charged wall was partially neutralized by the positively charged lysozyme molecules, leading to a sensitive change of the surface charge-dependent current–voltage (*I*–*V*) characteristics. Our results not only demonstrate excellent selectivity and sensitivity towards the target protein, but also suggest a route to extend this nanopore-based sensing strategy to the biosensing platform designs of a wide range of proteins based on a charge modulation.

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1. Introduction

The transport of ions and molecules regulated by biological ion channels are of great importance in various cellular and biological processes (Hille 1978; Hucho and Schiebler, 1977; Perozo et al., 2002). Biological nanopores such as α-hemolysin have been widely researched in the applications for analysis of nucleic acids (Clarke et al., 2009; Hurt et al., 2009; Wang et al., 2011b), proteins (Madampage et al., 2010; Wang et al., 2011a; Ying et al., 2012; Zhao et al., 2009), and small molecules (Howorka and Siwy, 2009; Wu and Bayley, 2008). However, such protein based nanopores together with their embedding lipid bilayers are unstable, fragile and impressionable to the external environments (Ali et al., 2010; Tahir et al., 2013; Tian et al., 2013). These drawbacks make them unsuitable for practical applications. Recently, solid-state nanopores have been rapidly developed with the advantages over their biological counterparts in terms of stability, robustness, and control over pore shape, diameter and the pore surface properties (Ali et al., 2011; Gyurcsanyi, 2008; Hou et al., 2011). And the broad applications such as biosensing (Choi et al., 2006; Gyurcsanyi, 2008; Tian et al., 2012; Wei et al., 2012; Schibel and Ervin, 2014), DNA sequencing (Fologea et al., 2005; Lagerqvist et al., 2006; Yan and Xu, 2006), molecular separation (Martin et al., 2001; Savariar et al., 2008), and mimicry of biological channels (Hou et al., 2011; Hou and Jiang, 2009; Zhang et al., 2011, 2010), have been explored.

Until now, two basic methods including resistive-pulse sensing (Luan and Zhou, 2012; Niedzwiecki et al., 2010; Sexton et al., 2007, 2010) and ion-current rectification (Ali et al., 2011, 2012, 2010, 2008; Tian et al., 2013; Wang and Martin, 2008; Yusko et al., 2010; Zhao et al., 2013) have been proposed for target analysis via synthetic nanopores. For the resistive-pulse sensing technique: when a molecule or particle was driven through a nanopore of comparable size, an electrical signal can be measured under the influenced of an applied voltage (Dekker, 2007; Li et al., 2012). However, as described by Ali et al. (Ali et al., 2010), this technique faces the limitation when considering the fast molecule translocation and concomitant electronic noise in more practical applications.

On the other hand, due to their comparable pore diameter with the electric double layers and excess surface charge on the pore walls, synthetic conically-shape nanopores can behave the special ion transport property called ionic current rectification, showing nonlinear current voltage curves (Zhang et al., 2011; Zhao et al., 2013). Once a nanopore channel was fabricated, its shape was hard to change, so that the ionic rectified characteristics will be mainly determined by the surface chemical properties of the nanopore. The rectified properties of synthetic nanopores can be utilized for the label-free detection of various analytes based on the change of

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charge polarity or density on the pore surface, induced by the recognition of target molecules. Generally, to construct a nanopore-based biosensing platform, it is essential to introduce a suitable functional group acting as the recognition site to the sensible tip side of the conical nanopores.

In recent years, nucleic acids that act as molecules for self-assembly of molecular nanostructure and also as a material for building machinelike nanodevices have become important building blocks for bottom-up nanotechnology (Krishnan and Simmel, 2011). Motivated largely by the rapid development of the DNA nanotechnology and nanopore technology, researchers have paid great efforts in integrating DNA molecules into the synthetic nanopore systems and achieved a number of nucleic-acid-based nanopore sensing elements (Actis et al., 2011; Fu et al., 2009; Liu et al., 2013; Tian et al., 2013). Martin and co-workers have reported a DNA-functionalized nanotube membrane which showed an ability to selectively recognize the single-base mismatch DNA strands (Harrell et al., 2004; Kohli et al., 2004). Ali et al. demonstrated the design and construction of peptide nucleic acid (PNA)-modified synthetic ion channels for the sequence specific detection of single-stranded DNA oligonucleotides (Ali et al., 2010). Recently, Jiang and co-workers have developed a series of DNA conformational transformation-based biomimetic nanochannels and paved the way for constructing nanopore gating of pH (Xia et al., 2008), potassium (Hou et al., 2009) and mercury(II) ions (Tian et al., 2013). However, the design and construction of robust and inexpensive nucleic acid-based nanofluidic devices for highly sensitive and selective detection of various target analytes still remains great challenges in life science and materials science.

Among the enormous nucleic acids libraries, aptamers are extremely promising components that can act as biospecific recognition site for a wide range of target molecules with advantages over traditional antibodies, such as stability, small size and chemical simplicity, ease of synthesis and the general availability for almost any given protein (Krishnan and Simmel, 2011; Xiao et al., 2013). The previous research concerning the design of nanopore-based aptasensing paradigms (Abelow et al., 2010; Actis et al., 2011; Ding et al., 2009; Rotem et al., 2012; Zhang et al., 2015) has mostly focused on the conformational change induced by binding events, causing a pore blockage for signal detection. However, the important property that conical nanopores are highly susceptible towards the surface charge received little attention in nanopore-based aptasensing design. It is worth noting that some aptamers such as lysozyme binding aptamer (LBA) could undergo recognition-induced reversal of the charge with a proper pH control. This property has been utilized for constructing electrochemical sensors using both electrochemical impedance spectroscopy (EIS) (Rodriguez et al., 2005) and cyclic voltammetric (CV) (Cheng et al., 2007) methods.

Herein, we demonstrated a proof-of-concept that aptamer–protein interaction induced neutralization of the surface charge in a single glass conical nanopore, accompanied by a decrease in the rectified currents, can be used to develop a nanopore-based biosensing platform for the detection of target proteins with high selectivity and sensitivity. The LBA was first introduced to the nanopore channels via a covalent modification process. At proper pH value, the aptamer strand was negatively charged while the lysozyme (pI=11) molecules were positively charged. The biospecific interaction between proteins and aptamers induced the partial neutralization of negative surface charge, which led to a sensitive change in the rectified ionic current of the single conical nanopores. The monitoring of covalent modification of LBA and the recognition events were characterized by recording the change of ionic current of the single conical nanopore.

2. Materials and methods

2.1. Chemicals and Materials

Prism glass capillary (outer diameter ~1.35 mm, inner diameter ~0.95 mm, Hirschmann, Germany), platinum wire (diameter 25 μm, from Alfa Aesar), Tungsten wire (0.25 mm, 99.95%, Alfa Aesar); Ferrocene (Fc, 99%, Alfa Aesar); Tetra-n-butylammonium hexafluorophosphate ($TBAPF_6$, 98%, Alfa Aesar), 3-aminopropyl-triethoxylsilane (APTES, 99%, Sigma-Aldrich), Glutaraldehyde (25% solution in water, Acros); Lysozyme (Boyun); BSA (97%, Boyun); Pepsin (Boyun); Cytochrome C (98.89%, Calbiochem); The lysozyme binding aptamer (LBA) was purchased from Shanghai Sangon Co., Ltd (Shanghai, China). The 42-mer aptamer used for functionalization was amino-terminated at its 5′ end, that is 5′-$(CH_2)_6$-ACT TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′. The counterpart Control DNA strand was also a 42 base numbers with amino-terminated (Control DNA), 5′-$(CH_2)_6$-ACT ATA CGT GCA TAT ACA GCT AGA GAT GCT AGG AGT ACT ATG-3′.

2.2. Preparation of single glass conical nanopore channels

We prepared single glass conical nanopore channels from glass capillaries, according to the method reported by White and coworkers with slight modifications (Zhang et al., 2006). Firstly, a platinum wire was electrochemically etched in 15% $CaCl_2$ to obtain a sharpened tip (see SEM images of the Pt tips in Fig. S1a and b). Then, the sharpened tip was sealed into a glass capillary. Finally, the Pt wire sealed in glass was pulled out and etched in the boiled aqua regia solution for 4 h to obtain the single conical glass nanopore channels. The geometry of the nanopore channel was observed from the fluorescence image by injecting 1 μM Rhodamine B solution into the pore (Fig. S1c). The pore radius was determined by measuring the steady-state diffusion-limited current of the Pt disk electrode (Fig. S1d) prior to etching according to the Eq. (1) (Zhang et al., 2004, 2006). It has been demonstrated that the relative uncertainty in r is within 20% compared to the results obtained from SEM (Zhang et al., 2004).

$$i_d = 4nFDC_b r \quad (1)$$

where i_d is the steady-state limiting current of the nanodisk electrode measured in 5.0 mM Ferrocene and 0.1 M Tetra-n-butylammonium hexafluorophosphate acetonitrile solution, n is the number of electrons transferred per molecule, F is the Faraday constant, D is the diffusion coefficient (2.4×10^{-5} cm^2/s), C_b is bulk concentration of the redox molecule, and r is the radius of Pt nanodisk, respectively.

2.3. Immobilization of LBA onto the single glass conical nanopore surface

Fig. 1 shows the schematic diagram of the modification process of LBA to the glass nanopore through siloxane chemistry. Firstly, the nanopore channel was treated with piranha acid (concentrated H_2SO_4/ 30% H_2O_2, V:V=3:1, 80 °C, 30 min), followed by washing with ultrapure water and absolute ethanol to obtain clean silica hydroxyl group on the interior surface. Then 5% APTES in absolute ethanol was used to react with the interior pore surface for 30 min, followed by rinsing with absolute ethanol and baking at 120 °C for 30 min. Afterwards, the resulting nanopore channel was treated with 2.5% glutaraldehyde aqueous solution overnight, followed by rinsing with ultrapure water thoroughly. Finally, the tris (hydroxymethyl)aminomethane hydrochloride solution (Tris–HCl, 20 mM, pH=7.4, containing 100 mM NaCl, 5 mM $MgCl_2$) with 5′-

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