Reversing current rectification to improve DNA-sensing sensitivity in conical nanopores

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Abbreviations: APTES, 3-aminopropyl-triethoxylsilane; **Glu**, glutaraldehyde; **PAH**, poly(allylamine hydrochloride); **RNase H**, ribonuclease H

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Abstract

Herein, we report the ultrasensitive DNA detection through designing an elegant nanopore biosensor as the first case to realize the reversal of current rectification direction for sensing. Attributed to the unique asymmetric structure, the glass conical nanopore exhibits the sensitive response to the surface charge, which can be facilely monitored by ion current rectification curves. In our design, an enzymatic cleavage reaction was employed to alter the surface charge of the nanopore for DNA sensing. The measured ion current rectification was strongly responsive to DNA concentrations, even reaching to the reversed status from the negative ratio (-6.5) to the positive ratio (+16.1). The detectable concentration for DNA was as low as 0.1 fM. This is an ultrasensitive and label-free DNA sensing approach, based on the rectification direction-reversed amplification in a single glass conical nanopore.

1 Introduction

The concept of nanopore stems from biological ion channels, which are formed by functional protein complexes in cells. By regulating the transmembrane transport of molecules and ions, these biotic nanopores play a pivotal role in life and cell activities [1-3].

Contrasting with biological nanopores, solid-state nanopores, could not only simulate the transport property as their biological nanopores counterparts, but also exhibit better stability and mechanical properties [4]. Consequently, a wide range of applications have been developed using solid-state nanopores such as biomimetic ion channels, biosensors, and nanofluidic devices [5-7].

During the past two decades, nanopore based sensing techniques as well as relevant applications have sparked great interest among the researchers. Several signal detection methods have been developed including ionic-current blockade [6,8-12], ion current rectification [13-16] and tunneling current [8]. Among these, ion current rectification is a highly sensitive approach for monitoring the change of surface charge in asymmetric nanopores. This method, first discovered in quartz nanopipettes and later in conical nanopore, is generally stirred by uneven distribution of surface charge in the nanopore [17]. As the tip size is equivalent to the thickness of electric double layer on the inner surface of the nanopore, conical nanopore exhibits special ionic transport property, i.e., ion current rectification, being manifested by a nonlinear *I-V* curve [14,15,18,19]. The generation of ion rectification phenomena is mainly affected by internal factors (i.e., nanopore shape, surface charge, material and wettability) and external factors (i.e., pH, electrolyte concentration, pressure gradient). [20,21] Among these, nanopore size and surface chemistry are two key factors affecting the ion rectification characteristics, and have great significance for the

sensitive sensing ability of nanopores [22,23]. Once a nanopore is fabricated, its size is difficult to change. Therefore, the ionic rectification characteristics are mainly affected by the surface chemistry of the nanopore.

A series of sensing platforms based on ion current rectification of conical nanopore channels have been introduced [24-27]. For instance, Baker et al. designed a sensing device that could detect DNA, using a dendrimer-modified nanopore [28]. In 2010, Ali et al. reported a single conical nanopore modified with uncharged peptide nucleic acid for sequences specific detection of DNA [29]. Jiang et al. constructed a nanofluidic sensor, based on DNA supersandwich structures [30] and obtained the detection limit of 10 fM for DNA and 1 nM for ATP. Recently, our group designed a single glass conical nanopore, that was conditioned by conformational and surface charge synergy for ATP detection with a detection limit as low as sub-pM in 2016 [7]. Zhang et al. developed a method for detecting DNA by monitoring the ion current rectification upon the hybridization of target DNA with probe DNA by the nanopore [31]. They achieved a detection limit of 10 fM for the target DNA. Likewise, A simple sensing strategy based on phosphodiamide morpholino oligonucleotide (PMO) modified nanopore was also proposed for ultrasensitive detection of miRNAs [32]. The limit of miRNAs detection attained by the researchers was 1fM in PBS and 10 fM in serum samples. The basic mechanism of these strategies of nanopore DNA biosensing was attributed to the immobilization of complementary DNA or PNA on the inner surface of the

nanopore as a probe. In such cases, the sensing was achieved by monitoring the current-voltage curves when target DNA was hybridized to probe DNA, which was ultimately bound to the inner wall of the nanopore. Once biological recognization happened, the density of surface charge of the inner surface of the nanopore as well as the effective diameter of the nanopore was changed, causing the change in the observed ion current rectification.

In those previous work, when target DNA hybridizes the probe DNA, it stimulates an increment in the negative charge of the interface. However, since the probe DNA is inherently negatively charged, the amount of change in the interface charge with the same negative polarity is usually limited, which hinders the increase of sensitivity of the sensor. If the surface charge can be efficiently changed from the negative status to the positive status, further improved sensitivity can be reasonably expected. In this work, we combined the properties of ion current rectification of a nanopore with the enzymatic cleavage reaction to construct an ultrasensitive DNA sensor, based on poly(allylamine hydrochloride (PAH) modified glass conical nanopore. In our design, an enzymatic cleavage reaction [33] involving the ribonuclease H (RNase H) was utilized to specifically cleave RNA strands that are complementary bound to DNA in a cyclic manner, which leads to a change of the charge polarity of nanopore, and thus presents a reverse ion rectification phenomenon. Importantly,

this reverses of the ion rectification direction can amplify the change in ion rectification ratio and significantly improve the sensitivity of DNA detection.

2 Materials and methods

2.1 Fabrication of conical nanopore

A single glass conical nanopore was prepared by reference method (see ESI for details) [34]. The size of the nanopore was estimated by measuring the steady-state limiting diffusion current of the platinum disk electrode, before removing the platinum wire for the estimation of the tip radius of the nanopore. The tip radius of the nanopore used herein was about 9 nm (Fig. S1, ESI).

2.2 Poly (allylamine hydrochloride) (PAH) immobilization

A series of functional modifications on a single glass conical nanopore were performed. The specific modification process used, is as follows:

Firstly, the nanopore was cleaned with a piranha solution (H_2SO_4 / H_2O_2, 3:1 (v / v), 80℃), and dried at 110℃ for 30 min. Secondly, the nanopore in (1) was sequentially immersed in APTES ethanol solution (5%, 30 min), the aqueous solution of glutaraldehyde (2.5%, 12h) and PAH aqueous solution (1mg/ml, 1 h). After each soaking, it should be cleaned and dried at 110℃ for 30 min. With this step, the chemical functional modification process of the inner surface of the nanopore was completed.

2.3 DNA and RNA hybridization

RNA (1 μM), RNase H (1 U/μL) and the corresponding concentration of DNA were mixed well and incubated at 37℃ for 1 h. All the nucleic acid solutions were prepared under aseptic conditions. The DNA and RNA reaction solution was injected into the PAH-modified nanopore. After soaking for 30 min, the current-voltage curve was measured.

2.4 Ion current measurement

The ion rectification ratio [I ("on" state) / I ("off" state)] used in this work is defined as the ratio of the larger absolute value of the current as compared to the smaller one recorded in the two voltage polarities (± 1V) [35] . Notably, when the absolute value of the current under the negative voltage was higher than the current under the positive voltage, the ion rectification ratio was marked as (-). Likewise, when the absolute value of the current under the positive voltage was greater than the current under the negative voltage, the rectification ratio was marked as (+). The linear sweep volt-ampere characteristic curve (scan voltage: from -1 V to 1 V; scan rate: 100 mV/s) was measured using a CHI 660E electrochemical workstation in KCl solution (formulated in PBS buffer, 1 mM, pH=7.4).

3 Results and Discussion

3.1 Surface modification of nanopore

The modification of PAH on the inner wall of the nanopore was materialized by covalent chemical method (Fig. 1a). Siwy has emphatically demonstrated the fact that the ion current rectification is closely related to the polarity as well as density of the charge on the inner surface of single conical nanopore [14]. Therefore, the modification process of each step could well be monitored by measuring the *I-V* curve of the nanopore. Herein, the *I-V* curve measurement of the nanopore was performed in KCl buffer solution (1 mM, pH=7.4), and a linear sweep voltammetry curve was recorded.

When the pH was maintained as 7.4, the *I-V* curve measured by the bare nanopore turned out to be nonlinear, indicating a negative ion rectification phenomenon and the negative charge polarity of the inner surface of the nanopore. The aforementioned phenomenon is due to the deprotonation of the silicon hydroxyl groups on the inner surface of the nanopore. Having undergone silane chemical reaction, the amino protonation of the inner surface of the nanopore became positively charged, which led to the reverse of the ion rectification direction from negative to positive. Subsequently, the amino group was converted into an aldehyde group in a cross-linking reaction with glutaraldehyde. Since the aldehyde group offered no charge under neutral condition, the measured *I-V* curve appeared to be almost a straight line, with no signs of ion rectification. Finally, the ion current rectification was restored with PAH modification of the inner surface of the nanopore. A positive ion rectification phenomenon was observed, demonstrating the positively charged

inner surface of the nanopore. This positive charge was transmitted by a large amount of amino protonation of PAH successfully immobilized on the inner surface of the nanopore (Fig.1b).

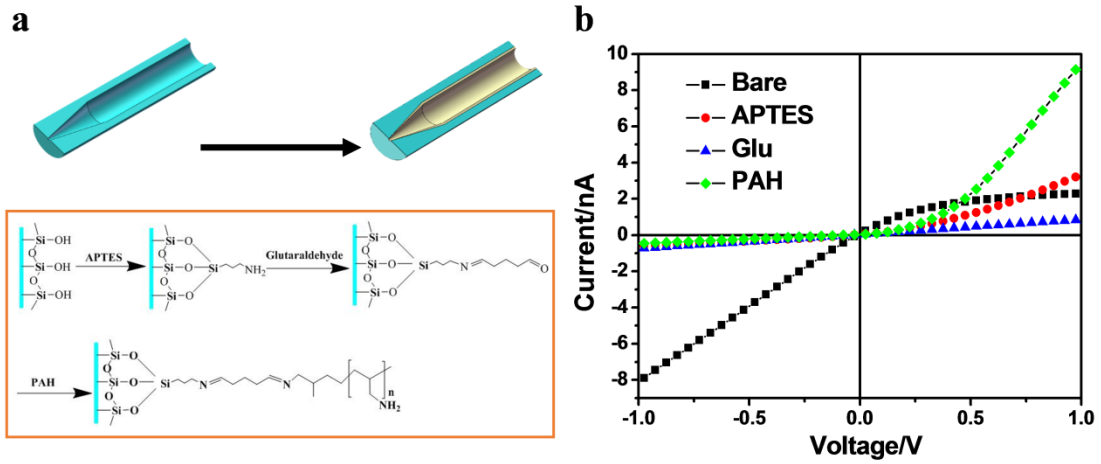

Fig.1 (a) Schematic description of the functionalization of the PAH on the surface of glass conical nanopore. (b) Measuring the *I-V* curve in KCl solution (1 mM, pH=7.4) to monitor the process of chemical modification on the inner surface of the glass conical nanopore.

3.2 DNA detection

Sensing is carried out by combining the characteristic of ion current rectification and the reaction of ribonuclease cleavage (Fig.1). Attributed to the unique structural properties, a single glass conical nanopore exhibits high sensitivity towards inner surface charge, which can be facilely characterized by ion current rectification. In the design of this biosensor, enzymatic cleavage can capacitate the ribonuclease (RNase H) to specifically cleave RNA

strands which are complementary to DNA. For the DNA, RNA and RNase H system being tested, in circumstances when the DNA under investigation can not hybridize with RNA, the PAH-modified nanopore electrostatically adsorbs the RNA strand. This adsorption paves for the increase of the negative charge on the inner surface, resulting in a negative ion rectification phenomenon. On the contrary, when the DNA to be tested specifically hybridizes with RNA, the RNA in the DNA-RNA hybrid strand is cleaved specifically upon treatment with RNase H. At this point, the negative charge on the inner surface of the nanopore undergoes decline, as the released DNA continues to hybridize with the RNA strand in the solution, triggering a new round of degradation. As the RNA is cleaved cyclically, the polarity of the charge on the inner surface of the nanopore becomes positive, representing a positive ion rectification phenomenon. This sensing strategy actually reverses the direction of ion rectification from negative to positive, which amplifies the change in ion rectification ratio and significantly improves the sensitivity of DNA detection.

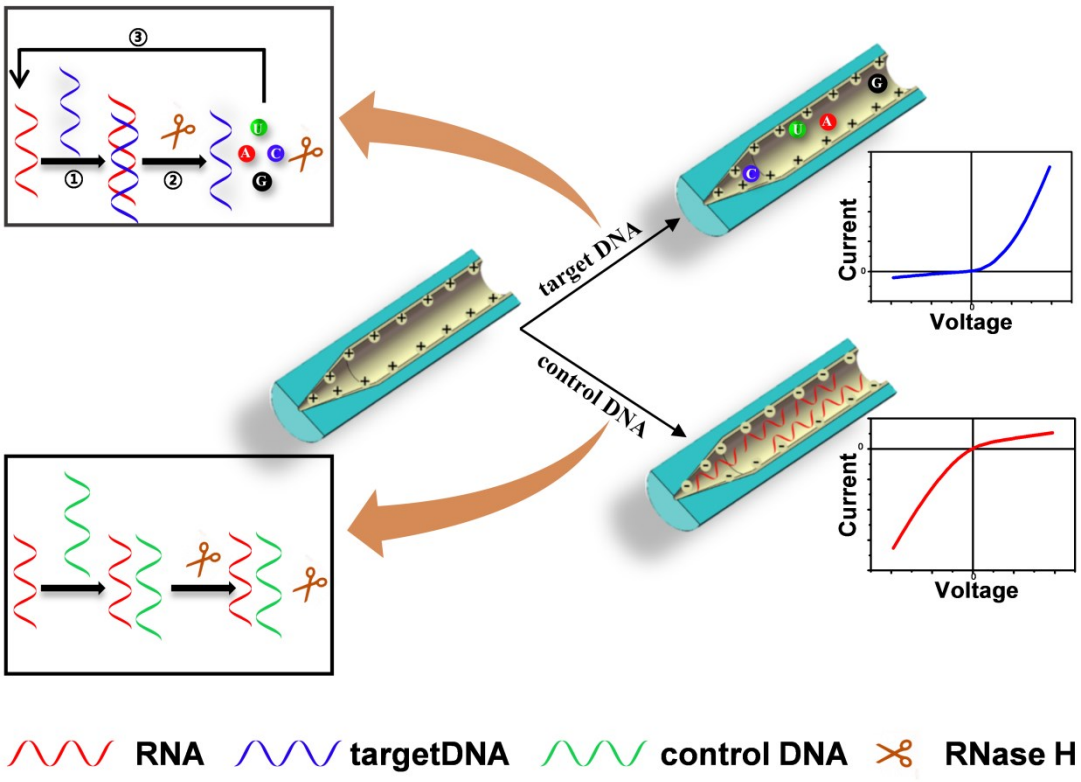

Fig 2. Schematic description of the DNA detection based on PAH-modified single glass conical nanopore.

In order to obtain the maximum amount of interfacial charge change on the inner surface of the nanopore, the ion rectification ratio and the difference of each set of experiment were calculated (Fig. S2, ESI). The obtained results are shown in Table S1. From the data, it could be inferred that when the concentration of RNA was at 1 μM, the difference in the rectification ratio was the largest.

The PAH-modified nanopore was then used for DNA detection. For this purpose, a verifying experiment using different DNA solutions was conducted. Target DNA (100 pM) and control DNA (100 pM) were added to the RNA solution (1 □M), respectively, followed by adding RNase H (1 U/□L). The reaction solution was then injected into the PAH-modified nanopore and the current-voltage curve was measured under KCl solution (1 mM, pH=7.4) (Fig. 3a). The ion current rectification ratio obtained, was 16.1 for the detection in target, and -6.5 for control detection, respectively (Fig. 3b). And the value of -6.5 was close to the one (-6.2) solely tested by RNA (Fig. S2, ESI). The given data further clarifies the fact that the RNA in the DNA-RNA hybrid strand was specifically cleaved by the enzyme. That is why the polarity of the charge on the inner surface of the nanopore was reversed.

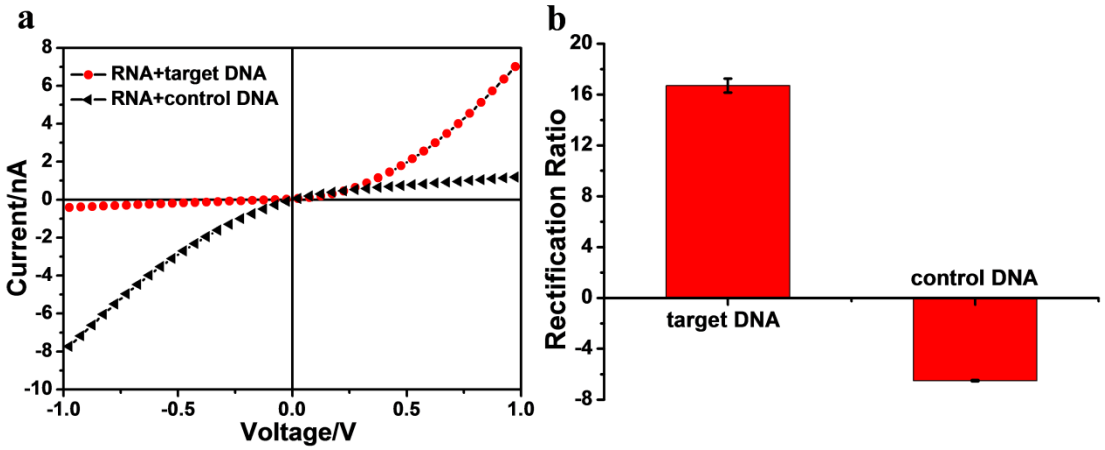

Fig.3 *I-V* curves (a) and corresponding rectification ratios (b) of the PAH-modified nanopore responding to target (100 pM) and control DNA (100 pM), respectively. The measurement was in KCl (1mM, pH=7.4) solution.

3.3 Response to DNA concentrations

Fig.4a displays the *I-V* curves of the PAH-modified nanopore, measured by spiking various concentrations of target DNA (from 0.1fM to 100 pM). The ion rectification ratios resulting from the added concentrations were -5.8, -3.7, -1.1, 3.2, 5.6, 10.9, and 16.1 respectively. The aforementioned results further revealed the fact that the ion rectification ratio increased significantly with increase in the concentration of DNA，exhibiting a linear range from 0.1 fM to 100 pM (y=0.686lg(x)+7.23) on a logarithmic scale (Fig. 4b). The ion rectification ratio calculated for blank sample was -6.2, while upon the addition of target DNA of 0.1 fM, the rectification ratio turned out to be -5.8. Based on the above experiment, 0.1 fM was regarded as the lowest detectable DNA concentration by the sensor. This concentration is much lower than those reported by previous ion-rectifying nanopore sensors (10 fM) [30,31].

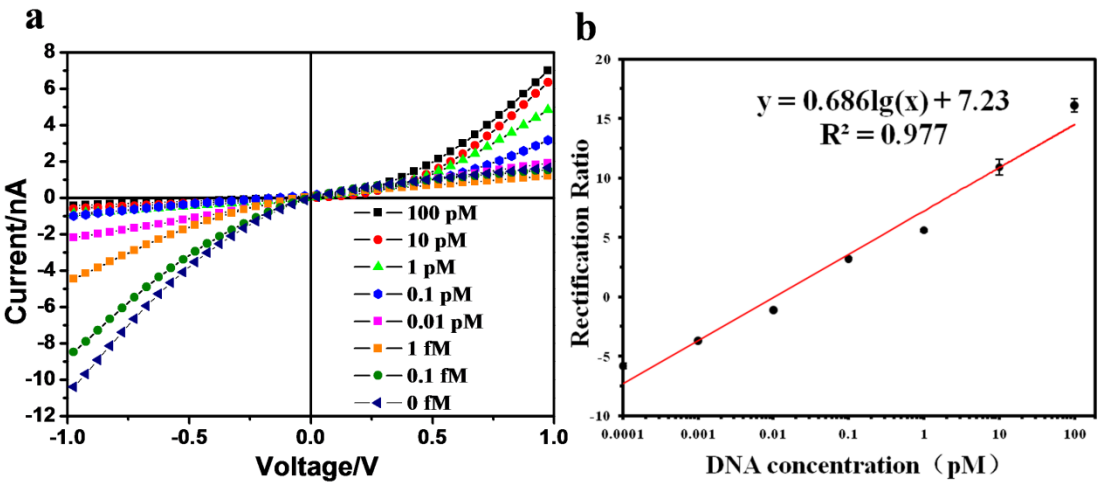

Fig. 4 (a) *I-V* curves of the PAH-modified single glass conical nanopore in KCl (1 mM, pH=7.4) aqueous solution with spiked target DNA concentrations. (b) Relationship between the rectification ratio of nanopore and the target DNA concentrations.

3.4 Reusability of the nanopore sensor

The reusability of the proposed sensor was investigated by injecting the NaOH (0.5 M) and HCl (0.4 M) solution in sequence into the nanopore to remove the RNA adsorbed on the inner surface of the nanopore. As shown in Fig. 5, the current at a voltage of -1 V could be repeated for at least 4 cycles of the addition of 1 μM of RNA and the cleaning of the NaOH solution. Moreover, no significant change in the response current was observed after RNA adsorption. This indicated the fact that the nanopore sensor had excellent reusability.

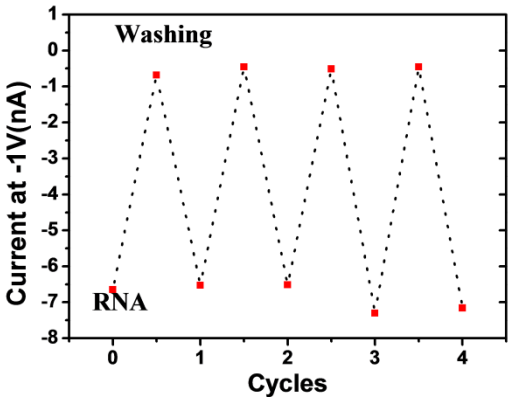

Fig. 5 The reusability of the nanopore-based DNA sensor. The binding RNA were removed by using NaOH (0.5 M) and HCl (0.4 M) solution in sequence for 10 min.

4 Concluding remarks

In summary, we have designed a novel biosensor for label-free and ultrasensitive detection of DNA. The proposed biosensor involves PAH-modification of nanopore and is based on the principles of ion current rectification and enzymatic cleavage reaction. For the biosensor development, firstly, PAH along with a host of amino groups were modified onto the inner surface of the nanopore by virtue of a series of covalent modifications. Later, the process of enzymatic cleavage reaction was employed, in which ribonuclease H specifically cleaves RNA strands that are complementary to DNA. Ultimately, RNA cleavage paved the way towards the reversal of the ion rectification direction. Thus, the changes of ion rectification ratio was amplified, significantly improving the sensitivity of the sensor. The detectable concentration achieved by the developed nanopore-based biosensor is as low as

0.1 fM. It is also worth mentioning that the robust sensor exhibits excellent reusability. We believe that the presented work would provide opportunities to inspire the new biosensing platform based on nanopore.

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The authors have declared no conflict of interest.

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