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Single glass nanopore-based regenerable sensing platforms with a non-immobilized polyglutamic acid probe for selective detection of cupric ions

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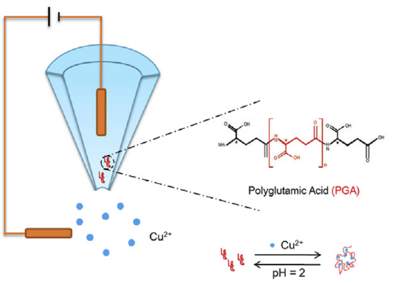
HIGHLIGHTS

- A sensing platform for cupric ions has been developed based on a single glass capillary nanopore.
- This detection is realized by the selectivity of chelation between PGA probes and cupric ions.
- Employing asymmetric salt gradients can improve the sensitivity.
- This platform shows highly sensitivity and excellent regenerability for Cu^{2+} with real samples of small volume (20 μL).

GRAPHICAL ABSTRACT



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ABSTRACT

A single glass capillary nanopore-based sensing platform for rapid and selective detection of cupric ions is demonstrated by utilizing polyglutamic acid (PGA) as a non-immobilized probe. The detection is based on the significant decrease of ionic current through nanopore and the reversal of ion current rectification responses induced by the chelated cupric ions on the probes when in the presence of cupric ions. PGA shows high selectivity for detecting cupric ions rather than other metal ions. The sensitivity of the sensing platform can be improved about 1–2 orders of magnitude by employing asymmetric salt gradients during the measurements. And the PGA-based nanopore sensing platform shows excellent regenerability for Cu^{2+} sensing applications. In addition, the method is found effective and reliable for the detection of cupric ions in real samples with small volume down to 20 μL. This nanopore-based sensing platform will find promising practical applications for the detection of cupric ions.

1. Introduction

Divalent cupric ions (Cu^{2+}), one of the most important cofactors for constituting metalloproteins, are essential ions to modulate protein functions as well as play a functional role in the forms of enzyme [1]. However, if overloaded or deficient, Cu^{2+} can give rise to a disturbance of the cellular homeostasis, which will cause serious diseases, such as Wilson disease (WD), Menkes disease (MD), and Alzheimer's disease (AD) [2–4]. In recent years, elevated levels of Cu^{2+} have also been found to cause many types of human cancers as well as infant liver damage [5,6]. Thus, it is of considerable significance to develop convenient and selective probes and methods for Cu^{2+} detection.

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So far, in addition to high-resolution atomic absorption spectrometry and inductively coupled plasma mass spectroscopy, various methods have been reported for Cu^{2+} detection by, for instance, using organic fluorophore dyes, quantum dots, nanoparticles, peptides, and so forth [7–13]. In addition, the anodic stripping voltammetry (ASV) method has also been widely used to detect heavy metal ions in electrochemical analysis with low limit of detection [14–17]. Recently, nanopore-based technology and analytics have shown their potential in ultrasensitive detections, which offer a promising way to detect target molecules in real time with sensitivity down to a single molecule level [18]. In general, to reach high sensitivity and specificity for sensing various analytes, such as metal ions, DNA, RNA, proteins as well as organic molecules, two kinds of approaches are typically used for the nanopore-based detections [19–28]. One relied on the immobilization of a suitable receptor on the nanopore inner surface. The ionic current detected through nanopore will be modulated when an analyte binds with the specific site in the nanopore interior [18,29,30]. Pourmand and coworkers had succeeded in the detection of copper ions with a prion protein modified nanopore due to the unique metal-binding properties of the prion protein [31]. A key feature of the other approach is the use of the specific mutual interaction of target-probe molecule without constructing a binding site in the nanopore interior [20,32,33]. This method opens up new avenues in developing highly-sensitive sensors for numerous targets. Wu's group had developed an α−hemolysin (αHL)-based nanopore system for the highly selective detection of Hg^{2+}, which was achieved by using thymine-containing DNA hairpins and based on the interaction between Hg^{2+} and DNA [20]. They found that the nanopore transport behaviors (event signatures), produced by the sensing probes (i.e., DNA, protein) in the absence and presence of the target metal ions, were significantly different.

Previous report confirmed the interactions of polyglutamic acid (PGA) with cupric ions by comparing the observed and simulated visible absorption spectra of Cu-PGA complex, indicating that the oxygen atom of the free carboxyl group and the nitrogen atom of the amide group of PGA can bind with the cupric ions to form Cu^{2+}-PGA chelation in aqueous solution at pH 7−8 [34]. However, attempts so far to take advantage of this property for cupric ions detection through a nanopore have not been reported. As a member of solid state artificial nanopores, glass capillary-based nanopores have their unique advantages, such as stiffness and durability, easy to be made with tunable orifice size and shape, low-costing and their facile use in biosensing [19,25], and therefore are very attractive for potential sensing applications. Herein, we presented for the first time a rapid and selective Cu^{2+} detection method that utilized polyglutamic acid (PGA) as probes based on a single conical glass nanopore sensing platform. Furthermore, we showed that the employment of asymmetric salt gradient during the nanopore experiments significantly lowered the detection limit of cupric ions. The as-prepared sensing platform was found regenerable for Cu^{2+} detections by simple immersion of the nanopore in a solution of low pH to refresh the sensing interfaces. Moreover, the method was successfully applied for the detection of copper ions in real samples with small volume down to 20 μL. The results and findings would allow us to develop a novel nanopore-based cupric ion sensor for practical applications in the analysis of real samples.

2. Materials and methods

2.1. Chemicals

Polyglutamic acid (Mw ≥ 10,000) was chemically synthesized by Beijing Hengye Zhongyuan Chemical Co. (Beijing China, Ltd.). Hydrogen peroxide (30% H_2O_2), sulfuric acid (H_2SO_4), potassium chloride (KCl), and HEPES (Free Acid) were purchased from Beijing Chemicals (Beijing China) and all of them were of analytical reagent grade. Other chemicals such as $Cu(NO_3)_2$ (99.999%), $Ni(NO_3)_2$ (99.999%), $Co(NO_3)_2$ (99.998%), $Zn(NO_3)_2$ (99.999%), $Mn(NO_3)_2$ (99.999%), $CaCl_2$ (99.999%), $FeCl_2$ (99.998%) and $Cd(NO_3)_2$ (99.999%) were bought form Alfa Aesar. The 0.1 M and 0.01 M KCl solutions buffered with 10 mM HEPES were at pH 7. All solutions were prepared by using deionized Milli-Q water (18.25 MΩ) with further purification with sterile membrane filter (0.22 μm).

2.2. Fabrication of single glass nanopores

Conical glass nanopores were fabricated by using quartz capillaries with an outer diameter of 1.0 mm and inner diameter of 0.7 mm (QF100-70-10; Sutter Instrument Co.). All quartz capillaries were cleaned directly prior to the pulling in piranha solution (3:1 98% H_2SO_4/30% H_2O_2) for about 2 h to remove organic contaminants. (Caution: piranha solution is a powerful oxidizing agent and reacts violently with organic compounds. It should be handled with extreme care.) Then the capillaries were thoroughly rinsed with deionized Milli-Q water to remove residual acid and vacuum drying at 80 °C prior to use. This process could obtain a high surface density of hydroxyl (−OH) group in the inner surface of the glass nanopores [35]. The cleaned capillaries were then pulled using a CO_2 laser-based micropipette puller (Model P−2000, Sutter Instrument Co.) preprogrammed to fabricate nanopore with an inner diameter of about 30 nm within a few seconds. Parameters used were as follows: Heat 700, Filament 4, Velocity 60, Delay 170, and Pull 180 [35].

2.3. Characterizations

The ionic currents were measured with two Ag/AgCl electrodes in a home-made electrolyte cell. One electrode setting outside in the bulk solution was used as reference/auxiliary electrode and the other connected to the head-stage of an Axopatch 200B amplifier (Molecular Devices) worked as working electrode by inserting in the quartz nanopore backfilled with solution. The ionic currents were recorded in voltage-clamp mode using a low pass Bessel filter of 5 kHz. The signals were digitized with DigiData 1440A digitizer (Molecular Devices) at 100 kHz, and viewed with Clampfit 10.2 software (Molecular Devices). The application of negative voltage (typically, −200 mV) was used to better show the ionic changes induced by the targets in current-time recordings. And the I−V curves were recorded by sweeping the voltage from −500 mV to +500 mV with 50 mV steps of voltage in 0.1 M KCl electrolyte solution, buffered with 10 mM HEPES (pH 7). The applied voltage referred to the voltage of the Ag/AgCl electrode in the nanopore interior versus another Ag/AgCl electrode in the bath solution [35,36]. Electrolyte solutions with varied concentrations were backfilled into glass capillary nanopores by using a microfill needle (World Precision Instrument, Inc.) to allow the introduction of the solution into the nanopore chamber of the tip by capillary action. All experiments were carried out at room temperature (~25 °C).

The SEM image of the glass nanopore was taken with an XL30 ESEM FEG microscope (FEI Instrument Co.) at 20 kV accelerating voltage. Glass nanopore was sputter-coated with thin gold films prior to imaging to prevent charging effect. The content of Cu^{2+} was further confirmed by Inductive coupled plasma emission spectrometer (ICP-OES, ThermoScientific iCAP6300).

3. Results and discussion

3.1. Characterization of single glass nanopores

The single conical glass capillary nanopores utilized for Cu^{2+}