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pH-modulated ion-current rectification in a cysteine-functionalized glass nanopipette

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ABSTRACT

The unique properties of nanopores depend largely on the functionalization of the pore surface, which has always been a great challenge. In this study, a facile method for functionalizing the surface of a glass nanopipette to establish thiol-based linkage without involving Au film coating was successfully developed by utilizing the thiol–maleimide click reaction which works rapidly and with high specificity. Cysteine was employed as a model molecule to functionalize the nanopipette, resulting in pH-modulated ion-current rectification behavior which showed a good reproducible response to pH. Due to the widespread existence of thiol groups in nature, this nanopipette functionalization method has the potential and versatility to be used in a wide range of applications, including mimicking biological ion channels and fabricating sensing devices.

1. Introduction

Synthetic nanopores have attracted much attention due to their potential use as nanostructures emulating biological ionic channels in fundamental research and various applications. The unique properties of these nanopores in controlling mass and charge transport allow them to be used for ion perm-selectivity, ion concentration polarization, and ion current rectification (ICR) [1–4]. Synthetic nanopores have thus been successfully utilized in analyte preconcentration, ionic-conductance-based single molecule determination and ICR-based sensing [5–12]. In the ICR phenomenon, an asymmetric diode-like current–voltage behavior can be observed in which the ionic current can be manipulated by the polarity of the applied voltage [13]. ICR has been achieved using nanopores fabricated from various materials including silica [14,15], poly(ethylene terephthalate) (PET) foil [16–19], polyimide film [20] and glass membranes [21]. Among these, glass- and quartz-based nanopores are most frequently used due to their ease of fabrication, low dependence on expensive equipment such as photolithography, ion-beam and e-beam, and suitability for use in single cell studies [22]. Modification of the nanopore surface is of great importance since the performance of nanopores is mainly dependent on their surface functionalization. For instance, the specificity of ICR-based nanopore sensors for molecule detection is enabled by a wide range of surface modifications [8,9,23–28]. Moreover, ICR-based nanopores can also respond to single/multiple external stimuli such as pH, light, temperature, etc. through functionalization with stimulus-responsive molecules or groups [29,30].

For silica-based materials, two general functionalization strategies are commonly used: physical adsorption and covalent attachment. Though physical adsorption is the easiest way to functionalize a surface, it is likely to suffer from leakage, lack of immobilization specificity and susceptibility to ambient conditions such as pH, temperature and ionic strength [31,32]. In comparison, covalent attachment produces a much more stable functionalized surface which is more suitable for nanostructured devices [33]. To initiate covalent attachment, it is necessary to activate the surface of silica-based materials in order to introduce reactive groups, due to the presence of inert silanol groups (Si-OH) on the surface. Various organosilanes including 3-aminopropyltrimethoxysilane (APTES), 3-mercaptopropyltrimethoxysilane (MPTS), 3-glycidoxypropyltrimethoxysilane (GOPS) and 3-(triethoxysilyl)propylsuccinic anhydride (TESP-SA) have been employed to create a surface layer with reactive amino, mercapto, epoxy and carboxyl functionalities, respectively [34,35]. However, when functionalizing a glass nanopore the covalent coupling is mostly based on the amide condensation reaction [9,24–26,36–40]. Meanwhile, thiol groups exist in a vast range of chemical compounds and biomolecules, and have

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been used as anchor groups for molecule immobilization due to their ability to bind with a Au surface [41,42]. To functionalize a glass nanopipette using thiol bearing molecules, it has been necessary to first generate an Au film on the inner surface of the nanopipette [43,44]. However, it is challenging to grow an ultrathin Au film with homogeneous coverage since Au is apt to form non-consecutive nanoparticles on the surface [45]. On the other hand, thiol-based linkages can be established using the Michael addition reaction, which is a modular click reaction with the ability to produce highly stereospecific and regiospecific products under facile conditions [46]. A series of thiol–Michael addition reactions with rapid kinetics and high specificity have been developed, including thiol–vinyl sulfone, thiol–acrylate, thiol–maleimide, and thiol–yne systems. Among these systems, the thiol–maleimide Michael addition reaction has been widely used in biological systems due to its selectivity in aqueous conditions, rapid reaction kinetics and product stability.

In a previous study, we have produced an ion-current rectification-based customizable pH response in glass nanopipettes via silanization [47]. In this case the highest pH response in ICR was achieved by modification with equal concentrations of amino and carboxylic groups. Therefore, in this study, we added the same quantity of amino and carboxylic groups by immobilizing cysteine on the inner surface of the nanopipette to produce pH-modulated ICR behavior. The thiol group in cysteine can react with the maleimide group in N-(2-aminoethyl)maleimide trifluoroacetic acid via the thiol–Michael addition reaction — see scheme in Fig. 1(a). The pH-modulated ICR behavior of the cysteine-modified nanopipette is then successfully demonstrated.

2. Experimental

2.1. Materials and apparatus

L-Cysteine, 3-(triethoxysilyl)propylsuccinic anhydride (TESP-SA), N-(2-aminoethyl)maleimide trifluoroacetic acid (AEMTFA) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from J&K Scientific Ltd. Sodium chloride, sodium phosphate dibasic and citric acid were obtained from Sinopharm. Phosphate/citrate buffers with pH values of 2.24, 3.00, 4.00, 5.05, 6.02, 7.02 and 8.00 were prepared by varying the ratio of sodium phosphate dibasic to citrate. All the solutions used for the electrochemical characterizations contained 20 mM phosphate/citrate and 0.1 M sodium chloride and were prepared from deionized water (18 MΩ cm, Millipore). All chemicals were used as received without further purification. Quartz capillaries with 1.0 mm outside diameter and 0.7 mm inside diameter were purchased from Sutter Instrument Co. The SEM images of the nanopipettes were taken using a Helios G3CX FESEM (FEI) with an accelerating voltage of 5 kV without Au coating. The pH of the solution was measured by a FE20/EL20 laboratory pH meter (METTLER TOLEDO). Linear sweep voltammetry was performed on a CHI 830D electrochemical workstation (CH

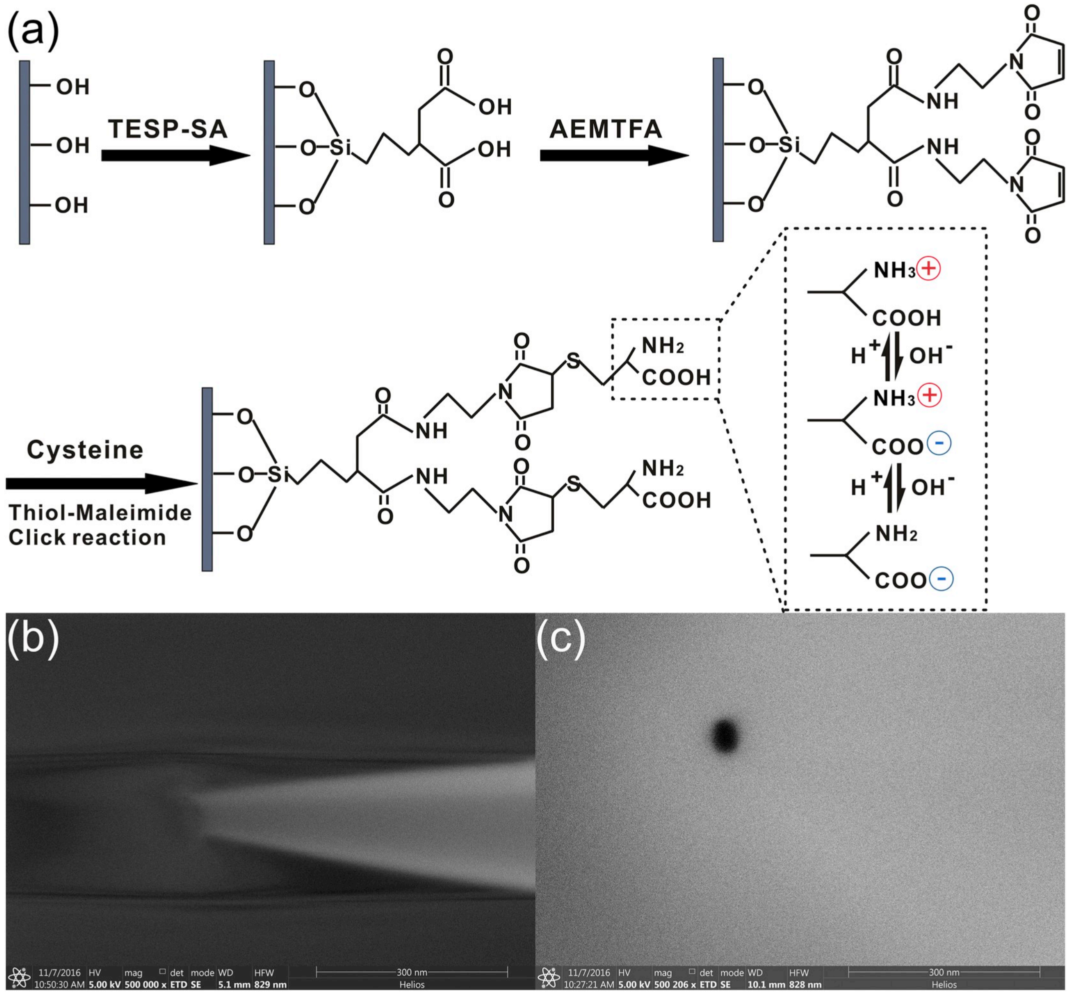

Fig. 1. (a) Schematic diagram of cysteine functionalization of the glass nanopipette via the thiol–maleimide click reaction for pH-modulated ICR behavior. (b), (c) SEM images of the nanopipettes: (b) side view and (c) end-on view.