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Voltage-controlled metal binding on polyelectrolyte functionalized nanopores

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Abstract

Most of the research in the field of nanopore-based platforms is focused on monitoring ion currents and forces as individual molecules translocate through the nanopore. Molecular gating, however, can occur when target analytes interact with receptors appended to the nanopore surface. Here we show that a solid state nanopore functionalized with polyelectrolytes can reversibly bind metal ions, resulting in a reversible, real-time signal that is concentration dependent. Functionalization of the sensor is based on electrostatic interactions, requires no covalent bond formation, and can be monitored in real time. Furthermore, we demonstrate how the applied voltage can be employed to tune the binding properties of the sensor. The sensor has wide-ranging applications and, its simplest incarnation can be used to study binding thermodynamics using purely electrical measurements with no need for labeling.

The stability and ability to mimic biological channels make solid state nanopores ideal for studying (bio)molecular interactions. Compared to biological nanopores, solid state nanopores are very stable, their diameter can be controlled through the fabrication process and they can be integrated into devices and arrays¹. Furthermore, their surface properties can be tuned by chemical functionalization, thus allowing the development of chemical and biochemical responsive nanopores². Martin's group pioneered the field of resistive-pulse sensing with conically shaped nanopores³. More recently, his group exploited signal changes induced by selective binding of drugs on the surface of a track etched nanopore to develop sensors for hydrophobic analytes⁴. We recently developed a sensing technology using a functionalized quartz nanopore at the tip of a nanopipette. One of the key features of this technology is that nanopipettes can be easily, inexpensively and reproducibly tailored at the bench by laser-pulling a quartz capillary. This technology relies on a simple electrochemical readout that transduces, in a label-free manner, binding events at the nanopore⁵.

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Supporting Information

I–V curves of chitosan/PAA functionalized nanopipettes, influence of amplitude and frequency of an applied AC voltage on signal generated upon binding of Cu²⁺, and response of a bare nanopipette to DC voltage. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

Antibodies⁶⁻⁷, DNA⁸ and aptamers⁹ have been used as recognition elements demonstrating the versatility of this platform for biorecognition. The high impedance of the nanopipette tip confines the sensitivity of the device, making the dimension and geometry of the tip crucial for the sensor performance⁵. Nanopipette technology can be easily integrated with piezoactuators to generate a sensor with high spatial resolution. As a nanopipette approaches a surface, the ionic current will decrease due to “current squeezing”, a well known effect, exploited to great benefit in Scanning Ion Conductance Microscopy (SICM)¹⁰. Besides sensing, nanopipette based platforms have been used to investigate single-molecule biophysics¹¹, for the controlled delivery of molecules inside a single cell¹², and to image cells at the nanoscale¹³.

Recently, Baker and collaborators¹⁴ showed for the first time that metal ion sensing can be performed with a functionalized nanopipette. Having functionalized the sensor with an imidazole-terminated silane, they showed a response to Co²⁺ ions. To go from this proof-of-concept to a usable analytical technique, development of such sensors must address selectivity, reproducibility, and sensitivity. Herein we show that a nanopipette functionalized with chelating biopolymers can be used to measure binding affinity with Cu²⁺ ions. The all-electrical and label-free detection of Cu²⁺ ions is rapid and reversible, and occurs at neutral pH.

As a model system, we demonstrate the reversible binding of cupric ions on quartz nanopipettes electrostatically modified with chitosan and polyacrylic acid (PAA) multilayers. Physisorption of polyelectrolytes on a quartz nanopipette causes the modification of its permselectivity and introduces metal-binding properties. The chelating properties of chitosan¹⁵ and PAA¹⁶ are well known and chitosan in particular, is widely employed for the removal of metals from ground and waste water^{15, 17}.

The conical geometry of a solid state nanopore as well as the nanometer size pore generates an interesting electrochemical behavior. For example, charged nanopores respond to a symmetric input voltage with an asymmetric current output, an effect referred to as current rectification. The origin of this effect in nanopipettes has been extensively described in a recent review published by our group⁵. Briefly, when the diffuse electrical double layer thickness is comparable with the pore size, the electrostatic interactions between fixed charged species on the nanopore surface and ionic species in solution alters ion transport properties¹⁸. In order to quantify the extent of the rectification, the rectification coefficient (r) has been introduced as a useful parameter, sometimes referred to as the degree of rectification, which is defined as the logarithm of the ratio between the current measured at particular positive voltage and the current measured at the same voltage but with the opposed polarity.

$$r = \log_{10} \frac{I_+}{I_-}$$

Quartz nanopores, being negatively charged, show a negative current rectification ($r < 0$). The rectification can be inverted ($r > 0$) by modifying the nanopore surface with cationic functional layers such as poly-L-lysine¹⁹, dendrimers⁸, aminosilane²⁰, and carboxymethyl-chitosan²¹.

We monitored in real time the electrostatic physisorption of chitosan and polyacrylic acid (PAA) on a quartz nanopipette by simple electrochemical measurements (Fig. 2). At acidic pH, the positively charged amino groups from the chitosan backbone allow the physisorption of the polyelectrolyte on the negatively charged nanopipette surface and invert

the permselectivity of the nanopipette. Similarly, carboxylic groups of polyacrylic acid (PAA) confer to the polymer a negative charge at neutral pH allowing the physisorption on the positively charged chitosan nanopipette.

We monitored the deposition of every polyelectrolyte layer on the nanopipette by electrochemical measurement (Fig. 3). Here we employed the rectification coefficient at ± 500 mV, as an indication of the nanopipette surface charge, using it to quantify the effect of the layer-by-layer assembly on the nanopore.

Interestingly, the multilayer assembly increased the rectification properties of the nanopipette: the rectification coefficient at pH=7 increased from -0.1 , for a bare nanopipette to -0.8 after the physisorption of 5 layers of chitosan/PAA, and plateaued afterwards. Similarly, at pH=3 the rectification coefficient increased from 0 to 0.65 after 5 layers. In addition to plateau on the rectification coefficient at five layers, we also observed that there was no change in the overall current (Fig. S1, supporting information) upon attempted addition of further layer of PAA or chitosan, indicating that no further polyelectrolyte was deposited on the sensor surface that was already fully covered with a chitosan/PAA mixed layer.

These results contrast with those described by Ali et coworkers²² who showed that the surface charge of a single asymmetric nanochannel in a PET membrane decreases dramatically with the number of layers assembled into it. We hypothesized that this behavior could be explained by the imperfect multilayer formation that led to a mixed layer rather than a layer-by-layer assembly. Our studies of the pH response of the chitosan/PAA modified nanopipette corroborate this assumption (Fig. 4).

Chitosan has an intrinsic pK value of ~ 6.5 ²³, while PAA has a pK of 4.3²⁴. Assuming a perfect layer-by-layer assembly, if PAA is the outermost layer, the nanopipette should be neutral at pH<4.3 and negatively charged at pH>4.3. Likewise, if chitosan will be the outermost layer, the nanopipette should be positively charged at pH<6.5, and neutral at higher pH. The rectification coefficient of the chitosan/PAA nanopipette, however, is positive at pH<5, and negative and pH>5. This indicates that at pH<5, the nanopipette permselectivity is governed by the protonated amino groups of chitosan while, at pH>5, by the negatively charged carboxylic groups of the PAA.

Furthermore, according to Rusu-Balaita et al.²⁵, at pH=5 between 50 and 75 % of the chitosan amino group should be protonated, while for PAA at least 50% of the carboxylic groups should be negatively charged. This is consistent with the experimental data that show a slight negative rectification at pH 5 and demonstrates the mixed layer formation.

The physisorption of chitosan and PAA layers on a quartz nanopipette gives it reversible metal binding properties that are not observed with the bare sensor. Chitosan binds several metal ions, however, it shows a stronger affinity for cupric ions at pH>5²⁶. Thus we chose to study, as a model system, the complexation of Cu^{2+} to chitosan/PAA functionalized nanopipettes. The addition of Cu^{2+} in the reservoir immediately affects the permselectivity of the sensor causing a decrease in the ionic current (Fig. 2). The binding is completely reversible and nanopipettes are regenerated up to 5 times without any loss of performance (Fig. 5). Regeneration is performed by immersion of the sensor into a pH3 buffer for 60 seconds. Acidic pH protonates the chitosan amino groups, thus causing the release of the cupric ions in solution. Alternative methods of regeneration such as immersion into citrate buffer at neutral pH or 0.1% EDTA, showed equal success.

The layer-by-layer assembly enhances the metal binding properties and the stability of the nanopipette sensor with respect to a monolayer deposition. Notably, the observed effect on

Cu^{2+} binding is due to the presence of combined polyelectrolytes. When the nanopipette was functionalized with chitosan or PAA only, we observed little variation in the output current upon addition of cupric ions in the bulk solutions. Furthermore, the interface between the polyelectrolytes and the quartz nanopipette was not stable as the sensors were regenerated only once before a complete loss of the Cu^{2+} binding property. But, when mixed layers of chitosan and PAA were constructed on a nanopipette, the interface showed extremely reproducible pH response, high signal variation upon binding of Cu^{2+} , and stability over numerous regeneration cycles. Similarly, Vasiliu et al.²⁷ demonstrated that multilayer adsorbed onto chitosan beads increase dramatically their stability in acidic pH. Through FTIR measurements, Wang and coworkers²⁸ demonstrated that $-\text{NH}_2$, $-\text{OH}$ and $-\text{COOH}$ groups were all involved in the Cu^{2+} adsorption by chitosan/PAA attapulgitite composites. We speculate that a similar chelation mechanism takes place in the chitosan/PAA functionalized nanopipette enhancing the metal binding ability.

We then investigated the response of the sensor to different concentrations of cupric ions (Fig. 6) The nanopipette responds linearly to increasing Cu^{2+} concentrations (Fig. 6, inset). The variation of the normalized current vs. Cu^{2+} plot is analogous to a Langmuir adsorption isotherm.

The current was normalized according to:

$$I_n = 1 - \frac{I_s}{I_b}$$

Where I_s is the signal after addition of cupric ions in solution, and I_b is the baseline signal measured in pure buffer.

Assuming that the binding of Cu^{2+} is an equilibrium process with independent binding sites and the variation of the normalized current is proportional to the number of cupric ions bound to the nanopipette sensor, one can estimate the thermodynamic affinity constant K for Cu^{2+} binding to the sensor using the following equation:

$$\frac{1}{I_n} = \frac{1}{I_{\max}} + \frac{1}{I_{\max} K c_{\text{Cu}^{2+}}}$$

Where $I_{\max}$ is the I_n value at maximal surface coverage, and c is the concentration of cupric ions in solution. From the linear fit of figure 6, one can extrapolate a K value of $4 \times 10^4 \text{ M}^{-1}$. This value is in good agreement with those calculated with different platforms for cations adsorption to chitosan²⁹. The sensitive range of the chitosan/PAA functionalized nanopipette (4×10^{-6} to $1 \times 10^{-4} \text{ M}$) falls in the range of drinking water standards³⁰. More sensitive techniques have been developed using different signal transduction mechanisms^{31–32}, but a rapid, reversible, and label-free nanopipette sensor may find applications in the field of continuous ion sensing.

The applied voltage across the nanopore plays a crucial role on the detection mechanism. The effect of voltage and waveform frequency on ion permselectivity in conical nanopores has been investigated^{33–34}, and it is known that ion current rectification decreases at high frequency waveforms. A higher voltage applied gives a larger change in the output current upon chelation of cupric ions by the chitosan/PAA functionalized nanopipette (Fig. 2, supporting information). For an equal Cu^{2+} concentration (20 μM), I_n is .95 at 1V, while only .52 at 50mV. Interestingly, the higher the frequency of the applied sinusoidal voltage the smaller was the change measured upon binding of Cu^{2+} by the sensor. For a 20 μM Cu^{2+}

concentration in the bulk solution, I_n is .32 of its initial value at 1 KHz frequency while to .42 at 0.5 Hz. This indicates that the signal is relatively weakly dependent on the frequency of the applied signal. This allows higher frequencies to be used for measurements, potentially negating confounding effects which may be associated with DC voltages, such as electrode consumption, and nanopipette clogging.

Having characterized the response of the sensor to an AC voltage, we investigated the effect of a DC voltage. The binding of Cu^{2+} on the sensor can be controlled by the applied voltage (Fig. 7). When a positive voltage is applied, cations are depleted from the nanopipette tip due to the electrophoretic flow. To leverage this effect, the binding of Cu^{2+} can be triggered by controlling the applied voltage. Upon applying a positive voltage, no binding occurs as cupric ions are depleted from the nanopipette tip; as soon as the voltage is switched to negative, binding occurs, causing a decrease in the ion flow, a change that is reflected on the next positive step. The current at negative potential has a poorer S/N ratio compared to the same positive potential before the addition of Cu^{2+} ions. Similar effect was observed on DNA functionalized nanotubes³⁵ and we speculate that the increase in the noise level is due to physical movement of polyelectrolyte chains in response to a negative bias, as this effect is not observed with bare nanopipettes (Fig. 3, supporting information).

Conclusions

In conclusion, we have demonstrated the reversible Cu^{2+} binding to nanopore sensors modified with chitosan/polyacrylic acid multilayer. We showed the analytical application of chemically functionalized solid-state nanopores for ion sensing. We optimized the frequency and the waveform of the applied voltage to maximize the signal-to-noise ratio, showing that the applied voltage can trigger the Cu^{2+} binding on the sensor at neutral pH. Such reversible nanopore-based sensors can be calibrated and used for continuous monitoring. Furthermore, the ability to temporally and spatially direct the binding of molecules can allow the development of precise biosensing devices capable of studying thermodynamic and kinetic properties of the analyte-receptor interaction. Nanopipette sensors can be integrated with piezoactuators and positioned over any surface with nanometer precision. We can speculate that the high spatial resolution of the reversible nanopipette sensor will allow the functional mapping of biological surfaces to investigate local variation of pH and ion concentration.

Materials and Methods

Reagents

Chitosan is the only pseudo natural polysaccharide based on $\beta(1-4)$ D-glucosamine partly N-acetylated on the C-2 position. The sample used is a chitosan Kitomer provided by Marinard (Canada). It is characterized by an average degree of acetylation DA~0.2 and a weight-average molecular weight $M_w=500,000$ at CERMAV. Polyacrylic acid (35wt. %, 5523925, Aldrich, MO). Copper Sulfate 99.99+, Tris HCl and Sodium Citrate were purchased from Sigma Aldrich (Saint Louis, MO). PBS solutions at pH 7.4 were prepared using standard method. Aqueous reagents were prepared using ultrapure water with $>18\text{M}\Omega\text{ cm}^{-1}$ resistance.

Nanopipette fabrication

Nanopipettes were fabricated from quartz capillaries with filaments, with an outer diameter of 1.0 mm and an inner diameter of 0.70mm (QF100-70-5; Sutter Instrument Co.).

The capillary was then pulled using a P-2000 laser puller (Sutter Instrument Co.) preprogrammed to fabricate nanopipettes with an inner diameter of 50 nm. Parameters used

were: Heat 625, Fil 4, Vel 60, Del 150, and Pul 192. The resulting nanopipette tips had inner diameters ranging from 37 to 82 nm, with the mean diameter of 56 nm³⁶.

Measurement Setup

All measurements were performed in a two electrode setup since the current flowing through the nanopipette is too small to polarize a reference electrode¹⁸. The nanopipette, acting as the working electrode, was backfilled with 0.1M KCl, 10 mM TrisHCl (pH 7), and a Ag/AgCl electrode was inserted. The filling buffer of the nanopipette was kept constant throughout all the measurements as its pH did only marginally affect the overall permselectivity of the nanopipette. Another Ag/AgCl ground electrode was placed in bulk solution acting as auxiliary/reference electrode. Both electrodes were connected to a MultiClamp 700B (Molecular Devices) amplifier with a DigiData 1322A digitizer (Molecular Devices), and a PC equipped with pClamp 10 software (Molecular Devices). The system remained unstirred for the duration of the measurement, which was conducted at room temperature.

Polyelectrolyte physisorption

Aqueous solutions of chitosan and polyacrylic acid (PAA) were each prepared with a concentration of 5 mg/mL. Chitosan, being insoluble at neutral pH, was dissolved into a pH 3 HCl solution. Nanopipettes were filled with a pH 7 solution (0.1M KCl, 10mM Tris HCl) and immersed into a pH 3 solution. We applied a sinusoidal voltage (500mV, 5Hz) to the nanopipette while adding 10 μ L of the chitosan stock solution in the reservoir. Chitosan was allowed to physisorb on the negatively charged quartz nanopipette for 1 minute. With the same applied voltage, the nanopipette was then immersed into a pH 7 bath where 10 μ L the PAA stock solution were added. After 1 minute the nanopipette was moved to a pH 7 solution to measure the rectification coefficient. The procedure was repeated until the desired numbers of layers on the sensor was reached.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic representation of the electrochemical configuration and the reversible binding of cupric ions on the chitosan/PAA nanopipette.

Figure 2.

Monitoring of the functionalization of a nanopipette with chitosan/PAA. Solutions: pH 7 (0.1M KCl, 10mM Tris HCl), pH 3 (0.1M KCl, 10mM phosphate/citrate). Nanopipette filled with 0.1M KCl, 10mM Tris HCl (pH 7).

Figure 3.

Variation of the rectification coefficient vs. numbers of chitosan/PAA layers deposited at pH 3 and 7. Solution: 0.1M KCl, 10mM phosphate/citrate buffered to the desired pH. Nanopipette filled with 0.1M KCl, 10mM Tris HCl (pH 7).

Figure 4. pH response of a bare nanopipette (black squares) and chitosan/PAA nanopipette (red triangles). Measurements were carried out in a 0.1M KCl solution, buffered with 10 mM phosphate/citrate to the desired pH. Error bars were calculated from at least four different pH measurements with the same nanopipette. Nanopipette filled with 0.1M KCl, 10mM Tris HCl (pH 7).

Figure 5. Variation of the rectification coefficient after recycling of the nanopipette. Cu^{2+} concentration: 100 μM (pH =7). The sensor was regenerated by immersing the sensor into a pH=3 solution for 1 minute. Nanopipette filled with 0.1M KCl, 10mM Tris HCl (pH 7).

Figure 6. Response of the chitosan/PAA nanopipette to various concentration of Cu^{2+} in 0.1 M KCl, 10mM Tris HCl, pH=7. The inset shows the linear fit (R : 0.997). The ratio I_s/I_b was calculated from the negative peaks of a sinusoidal waveform of 500 mV amplitude and 5 Hz frequency.

Figure 7.

Role of the waveform on the Cu^{2+} detection by a chitosan/PAA functionalized nanopipette.

a) Cartoon depicting the role of electrophoresis on the interaction of cupric ions with a nanopipette b) Output current, the arrow indicates the addition of Cu^{2+} ions (final concentration in solution 150 μM). No change is detected while applying a positive voltage, while an immediate response occurred upon switching to a negative potential which caused a variation on the following positive step.