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Copper Sensing with a Prion Protein Modified Nanopipette

Paolo Actis^{*,a}, Alex McDonald^{*,b}, David Beeler^b, Boaz Vilozny^a, Glenn Millhauser^b, and Nader Pourmand^a

^aDepartment of Biomolecular Engineering, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064

^bDepartment of Chemistry and Biochemistry, University of California, Santa Cruz, 1156 High Street, Santa Cruz, CA 95064

Abstract

Protein-metal interactions determine and regulate many biological functions. Nanopipettes functionalized with peptide moieties can be used as sensors for metal ions in solution.

Solid-state nanopores are a class of nanomaterials that have been successfully applied to the study and detection of numerous of biomolecular interactions^{1–2}. A nanopore can be simply described as a nanometer-sized opening separating two chambers filled with electrolyte solutions. When a voltage is applied across a nanopore, an ionic current is established with a magnitude that depends on size, geometry, and charge of nanopore opening. Biomolecular analysis can be performed when analytes translocate through the nanopore, giving rise to temporary blockades of the ionic current characteristic of the size and charge of the analyte being studied. This configuration, called resistive pulse sensing, enables the analysis of single molecules, but lacks specificity, as biomolecules of similar size and charge cannot be discriminated. An alternative experimental configuration relies on the immobilization of a receptor on the nanopore surface. The interaction of the receptor with its target analyte (if present in the solution) causes permanent current blockade. Our group pioneered the applications of a particular class of nanopores, called nanopipettes for the study of proteins^{3–4}, toxins⁵ and metal ions^{6–7}. In a nanopipette, the nanopore is situated at the tip of a very sharp quartz needle, which is easily fabricated at the bench using a laser puller.

In this paper we describe the development of functionalized nanopipettes to study peptide-metal interactions. As a model system, we take advantage of the unique metal-binding properties of the prion protein (PrP). This protein is responsible for a class of infectious neurodegenerative diseases termed transmissible spongiform encephalopathies (TSEs)^{8–9}. TSEs are spread via the misfolding of cellular PrP (PrP^C) into the infectious scrapie form, PrP^{Sc}, in the process forming PrP^{Sc} amyloid fibrils. It is now well established that PrP is a copper binding protein with an ability to take up several Cu²⁺ equivalents in its flexible N-terminal segment. Uptake of copper in this domain drives several physiological processes, including PrP^C trafficking and endocytosis¹⁰.

The PrP copper binding domain is composed of four tandem repeats of the highly-conserved eight amino-acid sequence PHGGGWGQ, termed the octarepeats¹¹. The four octarepeats coordinate a single Cu²⁺ ion at low occupancy with a K_d of 0.12 nM as determined by

electron paramagnetic resonance (EPR). The histidine from each octarepeat forms a square-planar coordination environment around the Cu^{2+} ion. At higher Cu^{2+} concentration, however, each octarepeat coordinates its own Cu^{2+} ion with a K_d of 7–12 μM^{12-13} . In addition, the octarepeats can weakly coordinate a single Zn^{2+} ion in a tetrahedral geometry with a K_d of $\sim 200 \mu\text{M}^{14}$. Herein, we describe the immobilization of the prion peptide on quartz conical nanopipettes to probe its interaction with metal ions in solution, in order to develop an all-electrical Cu^{2+} sensor amenable to *in vivo* studies.

The prion peptide was immobilized by physisorption onto the negatively charged nanopipette walls and the functionalization was followed in real time by chronoamperometry, as described in the supporting information. During immobilization, addition of the prion peptide to the bath solution causes the ionic current to instantaneously drop by $\sim 35\%$ of its initial value due to the interaction of the positively charged lysine residues at the n terminus of the peptide with negatively charged carboxylic group of the nanopipette walls (Fig 2A). The electrostatic immobilization appeared to be stable and the peptide was removed only by treatment with diluted hydrochloric acid (data not shown). The 4-octarepeat is known to bind Cu^{2+} with micromolar affinity, as demonstrated with mass spectrometry¹⁵ and electron paramagnetic resonance¹⁶. We then added 6 μM Cu^{2+} to the bath and measured in real-time the evolution of the ionic current (Fig 2B). The current dropped by 15% in less than a minute, as Cu^{2+} ions were chelated by histidine moieties of the peptides. A bare nanopipette showed no response to Cu^{2+} up to mM concentrations. As expected, the binding is reversible by addition of EDTA to the bath solution (Fig. S1). Nanopipette sensors were regenerated up to 6 times with no loss in sensor performance (Fig. 3).

The current response is concentration dependent (Fig. 4A). We used the functionalized nanopipette to study response of the prion peptide to metal ions. The nanopipette responds linearly to increasing Cu^{2+} concentrations (Fig. 4A, inset). The modulation of normalized current vs. Cu^{2+} concentration is analogous to a Langmuir adsorption isotherm. Assuming that copper binding is an equilibrium process with independent binding sites and the variation of the normalized current is proportional to the number of Cu^{2+} bound to the nanopipette, the thermodynamic affinity constant K for Cu^{2+} binding can be estimated using the following equation:

$$\frac{1}{I_n} = \frac{1}{I_{max}} + \frac{1}{I_{max}Kc} \quad (1)$$

Where I_n is the signal normalized to the one measured in pure buffer, I_{max} is the signal when all the binding sites are occupied, and c is the concentration of Cu^{2+} . The plot of $1/I_n$ vs $1/c$ is linear (Fig.4 a inset) and the affinity constant K for Cu^{2+} binding can be calculated from the slope. We obtained a value of 1 μM^{-1} which is in excellent agreement with values reported in the literature¹⁷. This result demonstrates that the immobilization of the prion peptide on the nanopipette walls doesn't affect its binding properties. We then evaluated the specificity of the binding compared to other divalent metals. As expected, neither Ca^{2+} nor Mg^{2+} showed any interaction with the prion peptide (data not shown). By contrast, Zn^{2+} and Mn^{2+} did interact. We found an affinity constant K of 500 μM^{-1} and 600 μM^{-1} for zinc and manganese, respectively (Fig. S2). These values are consistent with values reported in the literature^{14, 18-19} confirming Zn^{2+} and Mn^{2+} bind to the prion peptide but with a much lower affinity than Cu^{2+} .

Jeremy Lee's group pioneered the applications of nanopores to study the interaction of the prion peptide with metals²⁰, and antibodies²¹. Lee's group employed a biological nanopore, α -haemolysin, to study metal-induced conformational changes in the prion peptide. They

employed resistive-pulse sensing to monitor the change of the translocation rate of prion peptides upon interaction with metal ions. Their approach has the advantage of qualitatively studying peptide-metal interactions at the single-molecule level, but it cannot be used for analytical screening. Our approach, which relies on the immobilization of the prion peptide on nanopipette walls, allows the quantitative monitoring of peptide-metal interactions and the extrapolation of thermodynamic affinity constants. Furthermore, nanopipettes can be easily integrated with nanomanipulators to comprise a scanning ion conductance microscope. This allows the positioning of the nanopore sensor over living cells with nanometer resolution^{22–24}. We propose that with further improvements, these nanopipette sensors could be used to monitor in real time the extra- and intra-cellular concentrations of metal ions in and around living cells.

In conclusion, the electrostatic immobilization of the prion peptide on a conical quartz nanopipette allows the monitoring of peptide-metals interactions. The sensor takes advantage of the unique properties of the PrP octarepeat domain and can be regenerated several times by EDTA treatment without any significant loss of performance, demonstrating the stability of the peptide bond with nanopipette walls. Our findings pave the way for the development of peptide-encoded nanopipette sensors. As shown by Scott Banta's group, peptides selected from phage display can be used as recognition elements in biosensing experiments²⁵, which expands the applications of nanopipette sensors in biomolecular analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig.1. SEM micrographs of the conical nanopipettes used in this study. A) top view showing a 35 nm nanopipette pore and B) side view showing the conical shape. C) Three-dimensional rendering of the prion protein with copper ions included (cyan spheres).

Fig. 2.

a) Interaction of the prion peptide with the nanopipette surface causes a decrease in the normalized ionic current. 20 μg of the prion peptide was added to the bath. b) Addition of 2 μM Cu^{2+} causes a further 15% drop in the ionic current, indicative of the interaction of copper with the prion peptide. No change in current is seen when copper is added to an unmodified nanopipette.

Fig.3. Reversibility of the Cu^{2+} interaction with the peptide-modified nanopipette. Copper binding was reversed by addition of 1 mM EDTA, and solution was exchanged with fresh buffer. Cu^{2+} concentration 5 μM .

Fig.4. Dose-response and metal screening a) Dose-response of the peptide-modified nanopipette to increasing concentrations of Cu^{2+} . Inset shows linear fit to the Langmuir isotherm ($R^2=0.99$). b) Dose-response to other divalent metals (Mn^{2+} , Zn^{2+}) compared to Cu^{2+}