Published in final edited form as:

Analyst. 2009 August ; 134(8): 1614–1617. doi:10.1039/b902171j.

Nanoparticle-induced potentiometric biosensing of NADH at copper ion-selective electrodes

Karin Y. Chumbimuni-Torres and Joseph Wang^{*}
Dept Nanoengineering, UCSD, La Jolla, CA, 92093, USA.

Abstract

We demonstrate the first example of using potentiometry at ion-selective electrodes (ISEs) for probing in real-time monitoring of biometallization processes. A copper ISE is used for real-time monitoring of the NADH-mediated reduction of copper in the presence of gold nanoparticle seeds. Such potentiometric detection of NADH is not susceptible to surface fouling common with analogous amperometric measurements of this co-factor. Biosensing of ethanol is illustrated in the presence of alcohol dehydrogenase and NAD⁺, along with potentiometric detection of the NADH product at the copper ISE. The concept can be readily expanded to the monitoring of various biometallization processes in connection to different enzymatic transformations and ISE, and used for ultrasensitive detection of bioaffinity interactions in connection to common enzyme tags.

Introduction

Nanoparticle-induced biometallization processes have been exploited recently for optical monitoring of enzymatic reactions.¹ Such biometallization sensing protocols commonly rely on the intrinsic property of metal nanoparticle seeds to catalyze the reduction of metal ions by an enzymatically liberated reducing agent. For example, the hydrogen-peroxide stimulated growth of gold nanoparticles with gold was employed for optical measurements of glucose in the presence of glucose oxidase.² Similarly, the L-Dopa induced enlargement of gold nanoparticles was used for probing optically the activity of the enzyme tyrosinase in the presence of the tyrosine substrate.³ While most of these biometallization efforts have focused on colorimetric bioassays, little attention has been given to analogous electrochemical measurements.^{4,5} The latter has involved stripping-voltammetric⁴ or chronocoulometric⁵ measurements of the enzymatically-deposited metal.

We report here on the first example of using direct potentiometry for monitoring nanoparticle-induced biometallization processes. Potentiometry at ion-selective electrodes (ISEs) has been widely used for several decades for monitoring the activity of metal ions.⁶ Recent efforts in the field of ISEs have led to dramatic improvements in the detection limits of direct potentiometric measurements.^{7,8} Such high sensitivity and selectivity of ISEs are coupled with their wide dynamic range and a real-time monitoring capability with no sample perturbation. These properties make ISE extremely attractive for direct probing of biometallization processes by monitoring directly and rapidly the depletion of enzymatically-precipitated metal ion from the bulk solution. The new potentiometric probing strategy is illustrated here with the use of a copper ISE for monitoring the NADH-mediated reduction of copper ions in the presence of gold nanoparticles (Fig. 1). NADH is involved as a cofactor in hundreds of enzymatic reactions of NAD⁺/NADH-dependent dehydrogenases and hence the new protocol could be applied for

the potentiometric biosensing of numerous important substrates. Shlyahovsky *et al.*⁵ illustrated the use of NADH as reducing agent for the catalytic deposition of copper on the gold nanoparticles, with the amount of copper deposited being proportional to the NADH concentration. As will be illustrated in the following sections, recently developed solid-contact ISEs⁹ are extremely useful for probing such catalytic reduction of copper in the presence of gold nanoparticle seeds (Fig. 1) and to offer convenient potentiometric monitoring of related NAD⁺-dependent dehydrogenase reactions. While the concept is illustrated here in connection to the NADH-stimulated catalytic reduction of copper it could be readily expanded towards the potentiometric monitoring of different enzymatic transformations using various ISE, and to the transduction of different biomolecular interactions in connection to different enzyme labels.

Experimental

Reagents

The ionophore *N,N,N',N'*-tetracyclohexyl-3-thiaglutaric diamide (Copper (II) ionophore (IV)), lipophilic cation exchanger sodium tetrakis[3,5-bis(trifluoromethyl) phenyl]-borate (Na-TPPB) and the lipophilic salt tetradodecyl ammonium tetrakis(4-chlorophenyl)borate (ETH 500) were purchased in Selectophore[®] or Puriss grades from Fluka (Milwaukee, WI, USA). Methylene chloride was obtained from Fisher (Pittsburgh, PA, USA). Poly(3-octylthiophene) (POT) was synthesized following the procedure of Jarvinen *et al.*¹⁰ and was purified according to the patent application.¹¹ The synthesis of methyl methacrylate–decyl methacrylate (MMA–DMA) copolymer matrix was based on Qin *et al.*¹² 1,4-Dihydro- β -nicotinamide adenine dinucleotide (NADH), gold nanoparticles (10 nm diameter, 0.01% HAuCl₄, G1527), alcohol dehydrogenase (EC 1.1.1.1.; 180 U mL⁻¹) and the copper ion standard solution (1000 ppm; AA Standards) were purchased from Sigma–Aldrich. The phosphate saline buffer was obtained from Invitrogen (New York, USA). All stock solutions were prepared using double deionized water (18.2 M Ω cm).

Apparatus

Potentiometric measurements were performed with a multifunctional data acquisition board (779026-01 USB-6009 14 Bit, National Instruments, Austin, TX, USA) connected to a six-channel high Z interface (WPI, Sarasota, FL, USA). Amperometric measurements employed an electrochemical Analyzer (CH 1232A Instruments, Austin, TX, USA) connected to a personal computer.

Potentiometric and amperometric measurements

Potentiometric measurements were carried out in a 2 mL sample using the solid-contact Cu ISE and a commercial Ag/AgCl double junction electrode as reference (Type 6.0729.100, Metrohm AG, 9101 Herisau, Switzerland). All potentiometric measurements were performed at room temperature. Activity coefficients of copper were calculated according to the Debye–Hückel approximation and the potential values were corrected for liquid-junction potentials with the Henderson equation. Amperometric measurements were carried out in a 5 mL cell containing a glassy carbon (GC) working electrode, an Ag/AgCl reference and a platinum wire counter electrode.

Preparation of the copper ion-selective electrode

The Cu²⁺-ISE membrane contains the copper (II) ionophore (IV) (0.52 wt%, 12 mmol kg⁻¹), NaTPPB (0.45 wt%, 5 mmol kg⁻¹), ETH 500 (1.13 wt%, 10 mmol kg⁻¹), and the copolymer MMA–DMA (97.85 wt%). These membrane components (60 mg total) were dissolved in CH₂Cl₂ (0.8 mL). The resulting mixture was degassed by purging it with N₂ before casting it

onto the POT-coated gold wire microelectrode (200 μm diameter). The solid-contact Cu^{2+} micro ISE was prepared according to a previously described procedure.¹³ The resulting ISE was conditioned first in 10^{-3} M $\text{Cu}(\text{NO}_3)_2$ (1 day) and subsequently in a 10^{-7} M $\text{Cu}(\text{NO}_3)_2$ and 10^{-3} M $\text{Ca}(\text{NO}_3)_2$ mixture (overnight).

Results and discussion

We demonstrate here that ISEs are extremely attractive for monitoring directly the nanoparticle-induced enzyme-mediated reduction of metal ions. The new potentiometric biosensing concept is illustrated below using a solid-contact copper ISE for monitoring the NADH-mediated reduction of copper ions in the presence of gold nanoparticles. Such seed-mediated growth of nanoparticles involves the formation of a Cu^0 shell on the gold nanoparticles.⁵ This ISE is based on the copper ionophore *N,N,N',N'*-tetracyclohexyl-3-thiaglutaric diamide along with the POT conducting polymer and the MMA-DMA matrix support. The solid-contact Cu^{2+} ISE was tested first in a copper calibration experiment (using a 10^{-3} M $\text{Ca}(\text{NO}_3)_2$ background), where it yielded good linearity (E vs. $\log [\text{Cu}^{2+}]$) over a wide concentration range of 10^{-8} to 10^{-3} M copper with a slope of 29.8 mV per decade, along with a low detection limit of 8×10^{-9} M (not shown).

The ability of the new electrode to monitor NADH-stimulated copper reduction in the presence of gold nanoparticles is illustrated in Fig. 1. This figure displays a potential-time potentiometric tracing obtained at the Cu ISE (in the presence of gold nanoparticles and 10^{-5} M Cu^{2+}) for successive 10^{-5} (a), 10^{-4} (b), 10^{-3} (c) and 10^{-2} (d) M additions of NADH. Such changes in the level of NADH result in well-defined potentiometric signals, reflecting the decreasing concentration of the copper ion. The ISE allows real-time monitoring of such biometallization process, as it responds continuously and instantaneously to the changes in the level of the copper ions. The potential decreases rapidly at first within the first min, and then more slowly, reaching its steady-state value within *ca.* two min. Common optical or voltammetric probing of biometallization processes^{2–5} lack such real-time monitoring capability and cannot be used for probing the kinetics of the nanoparticle-induced biometallization processes. The well-defined response to 10^{-5} M NADH (a) indicates convenient measurements of micromolar concentrations of the cofactor and reflects the coupling of the amplification feature of the biometallization process with the high sensitivity of the Cu^{2+} ISE. While the nanoparticle-catalyzed reduction of copper ions into copper metal by NADH follows the general mechanism shown in Fig. 1, the observed change in the copper concentration (*ca.* 1.5 decade, based on the ~ 45 mV potential change, a–d) indicates that the reaction mechanism is more complex. This is reflected also by the relative concentrations of NADH and copper ions. The complex growth mechanism associated with the biocatalytic enlargement of gold nanoparticles was discussed by Willner's team.²

Factors affecting the new potentiometric biosensing process were evaluated and optimized. For example, we examined different copper concentrations over the 10^{-6} to 10^{-3} M range and found an optimal NADH detection in the presence of 10^{-5} M copper (not shown). The importance of the gold nanoparticles for the new potentiometric probing is illustrated in Fig. 2 that compares the NADH/copper response in the absence (A) and presence (B) of gold nanoparticles. A substantially higher (>5 -fold) sensitivity is observed in the presence of the gold nanoparticle seeds, reflecting their catalytic role. As indicated from the inset of Fig. 2, the enhanced sensitivity associated with such catalytic precipitation is coupled to a linear dependence between the potential signal and the $\log[\text{NADH}]$ over a wide concentration range. These data support the role of the gold nanoparticles in the enzyme-mediated reduction of copper ions.

The copper-based potentiometric response to NADH is also highly stable. This represents a significant advantage over common amperometric measurements of NADH which are prone to surface fouling effects associated with the accumulation of reaction products.¹⁴ Fig. 3 compares the amperometric (A) and potentiometric (B) response to 1×10^{-5} M NADH, as recorded over a continuous 50 min period. As expected, the glassy-carbon amperometric electrode displays a rapid decay of the signal, with up to 24, 55 and 75% current depressions after 10, 30 and 50 min, respectively, indicating a nearly complete inhibition of the oxidation process. In contrast, the NADH response of the Cu ISE remains highly stable throughout the entire experiment, with no apparent potential diminutions.

The NADH-mediated deposition of copper enabled the development of a potentiometric bioassay for ethanol in the presence of the enzyme alcohol dehydrogenase (ADH), its NAD⁺ cofactor, and copper ions. Fig. 4 displays the potentiometric response of the copper ISE to increasing ethanol concentrations over the 1 to 50 mM range (b–e). The ADH biocatalytic reaction of ethanol leads to the formation of NADH that acts as reducing agent for the catalytic reduction of copper. Since the amount of the enzymatically-generated NADH (and hence the concentration of the copper ion) is controlled by the concentration of ethanol, the potential signal of the Cu ISE is proportional to the substrate concentration. The response thus decreases rapidly upon adding the ethanol substrate, reaching steady-state values within 1.0–1.5 min. The resulting linear calibration plot (E vs. $\log[\text{Ethanol}]$) is also shown in the inset of Fig. 4. The concept could be readily expanded to the monitoring of other dehydrogenase transformations.

Conclusions

In conclusion, we demonstrated for the first time the use of ion-selective electrodes (ISEs) for probing nanoparticle-induced biometallization processes. The new potentiometric route allows direct real-time probing of the biometallization process through monitoring of the depletion of the deposited metal ion from the bulk solution. The concept can be readily expanded to the monitoring of different biometallization processes in connection to various ISEs and enzymatic transformations. The new approach would thus enable potentiometric measurements of a wide range of substrates or inhibitors of different enzymatic reactions. It should also enable ultrasensitive potentiometric detection of bioaffinity interactions in connection to common enzyme tags (*e.g.*, alkaline phosphatase) generating a reducing agent. We are also exploring the ability of the real-time potentiometric monitoring for probing the kinetics of the nanoparticle-induced biometallization processes.

Acknowledgments

Financial support from the National Institutes of Health (RO1 EB002189) and the National Science Foundation (CHE 0506529) is gratefully acknowledged.

References

1. Willner I, Baron R, Willner B. *Adv. Mater* 2006;18:1109.
2. Zayats M, Baron R, Popov I, Willner I. *Nano Lett* 2005;5:21. [PubMed: 15792406]
3. Baron R, Zayats M, Willner I. *Anal. Chem* 2005;77:1566. [PubMed: 15762558]
4. Hwang S, Kim E, Kwak J. *Anal. Chem* 2005;77:579. [PubMed: 15649056]
5. Shlyahovsky B, Katz E, Xiao Y, Pavlov V, Willner I. *Small* 2005;1:213. [PubMed: 17193433]
6. Wang, J. *Analytical Electrochemistry*. 3rd edn. New York: Wiley; 2006.
7. Bakker E, Pretsch E. *Anal. Chem* 2002;74:420A. [PubMed: 11811417]
8. Bakker E, Buhlmann P, Pretsch E. *Electroanalysis* 1999;11:915.

9. Chumbimuni-Torres KY, Rubinova N, Radu A, Kubota L, Bakker E. *Anal. Chem* 2006;78:1318. [PubMed: 16478128]
10. Jarvinen H, Lahtinen L, Nasman J, Hormi O, Tammi A-L. *Synth. Methods* 1995;69:299.
11. Xiao S, Qui C, Qui CX. U.S. Pat. Application. 2004 20040254336.
12. Qin Y, Peper S, Bakker E. *Electroanalysis* 2002;14:1375.
13. Chumbimuni-Torres K, Calvo-Marzal P, Wang J, Bakker E. *Anal. Chem* 2008;80:6114. [PubMed: 18570385]
14. Wang J, Angnes L, Martinez T. *Bioelectrochem. Bioenerg* 1992;29:215.

Fig. 1. Potentiometric response of the Cu ISE to different NADH concentrations: 1×10^{-5} (a), 1×10^{-4} (b), 1×10^{-3} (c) and 1×10^{-2} (d) M. Conditions: 1×10^{-5} M copper solution (2 mL) containing 50 μL of 10 nm Au-nanoparticles (0.01% HAuCl_4).

Fig. 2. Influence of the Au-nanoparticle seeds upon the response of the Cu-ISE to increasing concentrations of NADH in steps of: 1×10^{-5} (a), 1×10^{-4} (b), 1×10^{-3} (c) and 1×10^{-2} (d) M. Potentiometric response in the absence (A) and presence (B) of Au-nanoparticles. Inset: the corresponding calibration curve for B. Other conditions, as in Fig. 1.

Fig. 3.

Amperometric (A) and potentiometric (B) response to 1×10^{-5} M NADH using: (A) 1×10^{-3} M phosphate saline solution (5 mL, pH 7.4) containing 1×10^{-5} M copper ion, and a glassy carbon electrode held at +0.75 V; (B) 2 mL of a 1×10^{-3} M phosphate saline solution containing 1×10^{-5} M copper ion and Au-nanoparticles 50 μL , using a copper ISE.

Fig. 4. Potentiometric response of copper ISE for different ethanol concentrations: 0 (a), 1×10^{-3} (b), 5×10^{-3} (c), 1×10^{-2} (d), and 5×10^{-2} M (e), in the presence of NAD^+ (10 mM) ADH (180 units mL^{-1}), 1×10^{-5} M copper ion and 50 μL of gold-nanoparticles, in PBS (1 mM, pH 7.4). Inset, the corresponding calibration curve for ethanol.