Reagentless electrochemical immunoassay using electrocatalytic nanoparticle-modified antibodies†

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We describe a new approach for reagentless electrochemical immunoassay sensing in which Au/Pd NPs can be "loaded" onto antibodies to create an electrocatalytic antibody that is sensitive to the oxygen reduction reaction.

Metal nanoparticles (NPs) have been the subject of much interest as labels for biorecognition events.[1] Different properties associated with NPs such as optical,[2] electrochemical,[3] and their use as mass labels[4] have been exploited for sensing applications. Recently, the Willner group introduced the use of nucleic acid-functionalized Pt-NPs as catalytic labels to detect biomolecules using the electrocatalyzed reduction of H_2O_2,[5] and the Pt-NP generated chemiluminescent signal in the presence of luminol/H_2O_2.[6] In these works NPs effectively serve as inorganic enzymes generating a signal analogous to that obtained from enzymatic turnover with significant advantages over enzymes including increased thermal and environmental stability. In this study we further the utility of catalytic NPs for bioanalysis by introducing an electrocatalytic nanoparticle-modified antibody that is sensitive to the oxygen reduction reaction (ORR).

The nanoparticle catalyzed ORR has been extensively studied for electrochemical energy conversion/storage, corrosion, and fuel cell applications,[7] but not for sensing applications. This process can occur as either a one step four electron reduction of oxygen to H_2O, a two step process consisting of the two electron reduction of oxygen to H_2O_2 followed by the two electron reduction of H_2O_2 to H_2O, or a combination of the two processes. While the ideal size range of the NP for the NP catalyzed ORR is 2–20 nm, the material composition of the NPs on a given electrode is also of the utmost importance. Yang *et al.* reported that upon studying the catalytic activity of four different metal NPs on screen printed carbon electrodes the lowest catalytic peak potentials observed went in the order of Pd < Pt < Au < Ag.[8]

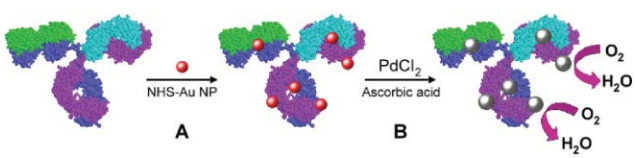

Fig. 1 Scheme depicting the preparation of Au/Pd modified antibodies. (A) Mono-NHS 1.4 nm Au-NPs are covalently attached to anti-mouse TNF-α antibody. (B) Pd is deposited onto the Au-NP seeds.

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The preparation of the NP-modified antibody is presented in Fig. 1 (see ESI†). Mono-Sulfo-NHS 1.4 nm Au particles were reacted with anti tumor necrosis factor (TNF-α) antibody to create an Au-NP-modified antibody (Fig. 1A). The single functional unit on the NP is necessary to prevent cross-linking and aggregation of antibodies. The Au-NP was then used as a seed in the presence of $PdCl_2$ and ascorbic acid to create Au/Pd core-shell particles on the antibody surface (Fig. 1B).

Growth of the Pd shell on Au-NPs can be monitored with transmission electron microscopy (TEM), as presented in Fig. 2. Fig. 2A shows a 1.4 nm Au-modified antibody with approximately 25 NPs. This was typical for antibodies functionalized under these experimental conditions. After 15 min of Pd deposition (Fig. 2B) 3–5 particles have grown to a diameter of 2–2.5 nm. It is believed that these particles are the most accessible to the reaction solution as folds of the antibody structure might limit solution accessibility to some NPs. The presence of Pd was confirmed by X-ray microanalysis (EDAX) measurements. After 30 min of deposition the size distribution of particles grew to 2–8 nm (Fig. 2C).

The catalytic activity of the NPs was studied using linear sweep voltammetry on a glassy carbon electrode (GCE) surface treated with NP-modified antibody in 0.1 M phosphate buffer saline (PBS), pH 7.4, (Fig. 3). As expected, the 1.4 nm Au-modified antibody showed no catalytic activity with little current response from 100 to −500 mV and a small oxygen reduction wave at −650 mV (brown). A GCE coated with Au/Pd-NP antibody (30 min Pd deposition) showed a decrease in the overpotential of oxygen reduction with a very broad cathodic wave at −400 mV (green) due to the presence of Pd. An electrode prepared from a 1 h deposition did not produce a significant change in the shape and peak potential of the voltammogram (orange) leading to the conclusion that the catalytic activity is not due to the NP structures, but instead resembles a response more characteristic of bulk Pd. This could be due to the asymmetry and proximity of the produced NPs as well as possible non-spontaneous deposition onto the antibody itself at longer deposition times. The 2–2.5 nm Au/Pd NPs from a 15 min deposition time showed excellent

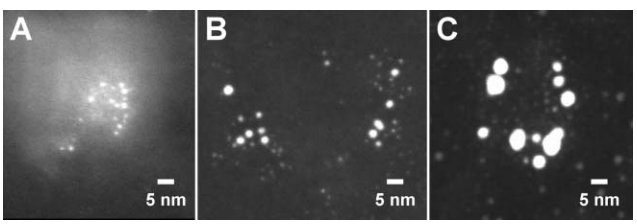

Fig. 2 TEM pictures showing 1.4 nm Au-NP-modified antibody (A) after 15 and 30 min of Pd deposition (B and C respectively).

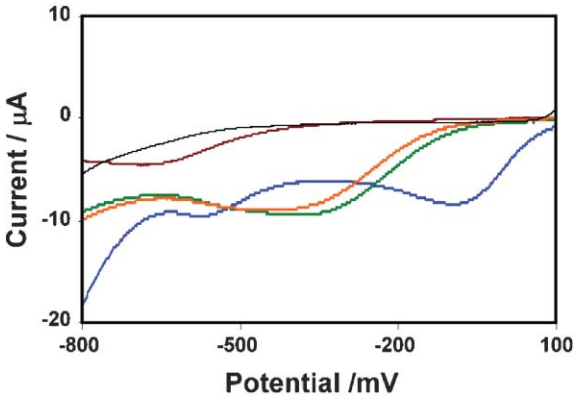

Fig. 3 Linear sweep voltammograms of GCE modified with NP-antibody at 0, 15, 30, and 60 min Pd deposition (brown, blue, green, and orange lines respectively) and 15 min deposition after degassing (black line). Scan rate: 100 mV s^{-1}. Ag/AgCl reference and Pt counter electrodes respectively.

catalytic activity with a low potential peak at −160 mV and a slightly smaller peak at −570 mV (blue line) which are assigned the one step two electron reduction of oxygen to H_2O_2 and the one step two electron reduction of H_2O_2 to H_2O respectively. No peaks were observed upon degassing with Ar (black), and a reduction wave appeared at ~−550 mV upon subsequent addition of 1 mM H_2O_2 (data not shown).

The low potential of the oxygen reduction peak when compared to the non-catalytic antibody (Fig. 3 brown) makes the prospect of an electrocatalytic antibody extremely attractive for sensing purposes. The "sandwich" immunoassay using an antibody-tagged label is one of the most common detection schemes used today due to the exceptional specificity of the antibody/antigen complex and the ability to make antibodies complementary to a wide range of possible analytes. Although other biological recognition ligands exist with inherent advantages over antibodies for detection (*e.g.* peptides, aptamers), they have yet to be developed against many biologically relevant molecules, such as the cytokine TNF-α. As some biomolecules lack electroactive groups for electrochemical detection, electrochemical tags for antibodies are typically used. The two most common electrochemical tags are enzymes that produce redox-active products, and electrochemiluminescent labels. Both require additional reactants to produce signals such as substrate, and electron donating molecules respectively. The ubiquitous nature of oxygen makes its role as a reactant for the electrocatalytic antibodies a possible advantage in sample preparation and device fabrication.

Catalytic nanoparticles are very sensitive to interfacial electron transfer and maximum catalysis is achieved when the nanoparticle is close to the electrode.[5] The probe immobilization method therefore has a profound impact on the electron transfer resistance between the nanoparticle and the electrode. Our initial immunoassay experiments with NP-modified detection antibodies employed an aminobenzoic acid layer that was electrpolymerized onto a GCE followed by EDC/NHS crosslinking of the antibody probes (see ESI†). This surface significantly impaired electron transfer providing only a small ORR wave upon saturation of the electrode with target protein (500 ppb TNF-α). This led us to pursue an alternative protein immobilization method with improved electron transfer properties. The modification of proteins with aryl diazonium salts and subsequent immobilization by electrodeposition has recently been shown to be a very convenient method for the construction of protein surfaces.[9] Our work in this area has shown that the antibody surface, prepared from the electrodeposition of the diazonium-modified protein, acts as a porous film with individual pinhole sites and is highly suitable for immunosensing platforms.[10] A complete sandwich immunoassay consisting of diazonium-antibody probe immobilization, exposure to target, and capture of nanoparticle-antibody label (see ESI†) is presented in Fig. 4. Upon increasing concentration of TNF-α a cathodic current appears with a broad wave centered at ~−400 mV due to the reduction of oxygen. The two step ORR is suppressed, due to additional blocking caused by the antibody probe and captured target protein layers. However a strong signal dependence upon concentration is observed from 1 ppt to 100 ppb TNF-α. The corresponding analytical signals taken from the current at −400 mV (inset) show that the technique is highly suitable for quantitative work with a detection limit of 1 ppt. While the basis of a logarithmic dependence of the signal-*versus*-concentration is not understood, it has been observed in other solid-state DNA sensors.[11]

In conclusion we describe a new approach for electrochemical immunoassay sensing in which Au/Pd NPs can be deposited onto an antibody to create an electrocatalytic antibody. The use of catalytic NPs provides a promising alternative to conventional enzymatic antibody tags. Electrocatalyzed oxygen reduction can lead to sensing devices that do not require excess reagents simplifying device fabrication, while antibody specificity can provide recognition to a wide variety of possible analytes.

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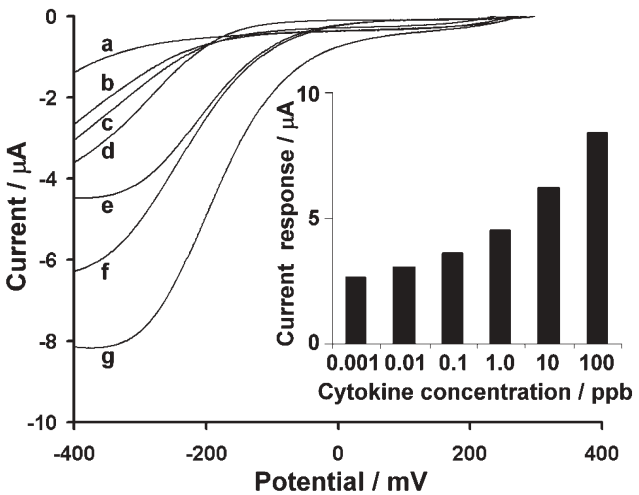

Fig. 4 Linear sweep voltammetric response upon increasing concentrations of TNF-α: 0, 0.001, 0.01, 0.1, 1, 10 and 100 ppb; a–g respectively. 0.1 M phosphate buffer, pH 7.4. Scan rate: 100 mV s^{-1}. Ag/AgCl reference and Pt counter electrodes respectively. Inset: Current response taken at −400 mV for increasing concentrations of TNF-α.

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