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Potentiometric Biosensing of Proteins with Ultrasensitive Ion-Selective Microelectrodes and Nanoparticle Labels

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We report here for the first time on the use of potentiometry for ultrasensitive nanoparticle-based detection of protein interactions. In particular, a silver ion-selective electrode (ISE) is used as an effective transducer for sandwich immunoassays in connection to the capture and silver enlargement of gold nanoparticle tracers. It is anticipated that this approach may form the basis for highly sensitive bioaffinity assays.

Nanoparticle-based electrochemical detection of proteins has recently received considerable attention. These include the use of gold[1] and semiconductor[2] nanocrystal tracers, as well as carbon-nanotube-derived amplification of the recognition and transduction events[3] and colloidal gold labels with carbon paste electrode substrates.[4] These nanoparticle-based electrochemical immunoassays commonly rely on anodic stripping voltammetry (ASV) due to its intrinsic preconcentration step that allows one to achieve ultratrace level detection limits.[5]

Recent improvements in the detection limits of ion-selective electrodes based on polymeric membranes containing selective receptors (ionophores) yielded sensors for the direct measurement in the subnanomolar concentration range.[6] This was made possible by minimizing passive zero current ion fluxes from the membrane into the sample solution.[7,8] Recent approaches include the development of trace level potentiometric sensing membranes backside contacted with conducting polymers as ion-to-electron transducers,[9] monolithic columns as membrane supports,[10] and polymeric membranes with optimized aqueous inner solutions.[6] It is now possible to use miniaturized ISEs for detecting femtomole amounts of ions in microvolume samples[11,12] since there is no expected deterioration of the signal or detection limit as the sample volume is reduced. We show here that such microsensors may be very attractive systems for the ultrasensitive detection of bioassays when coupled to nanoparticle amplification labels.

Scheme 1 outlines the new particle-based biosensing protocol. It is based on a sandwich immunoassay where the target mouse IgG antigen is captured by the primary anti-mouse IgG antibody modified gold substrate, followed by adding a secondary anti-mouse IgG antibody conjugated to gold nanoparticle tags and by catalytic silver enlargement onto the gold labels. The precipitated silver is oxidatively dissolved with hydrogen peroxide to yield dilute

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Supporting Information Available: Experimental details, instrumentation, reagents, and construction of the silver ion-selective microelectrode. This material is available free of charge via the Internet at http://pubs.acs.org.

electrolyte backgrounds for the potentiometric detection of the released silver ions with a polymer membrane silver ion-selective microelectrode. The detection here is made without a preconcentration step typically used in other electrochemical techniques.[13] As was illustrated recently,[11] further reduction of the sample volume for the final measurement may yield proportionally lower ISE detection limits in terms of total assessable concentration, and hence the concept presented here should give even lower protein detection limits upon further miniaturization.

To amplify the mouse IgG signal, silver enhancement was used after the immunoassay. Such enhancement aims at catalytic deposition of silver on the gold tags, while avoiding spontaneous deposition onto other components of the assay. Because analogous optical methods rely on a threshold amount of silver for visualization, excess silver ions are not of major concern. In contrast, such adsorption of silver ions on the captured probe can lead here to undesired contributions to the background. These blank signals can be reduced with a sodium thiosulfate fixer solution that transforms the silver cation into a $Ag(S_2O_3)_35^-$ anion[14] and by controlling the silver precipitation time and the concentration of silver enhancer solution. Precipitation periods shorter than 10 min assured that only the gold tags are coated with silver;[15] a 5 min period offered the best tradeoff between high sensitivity and selectivity. The concentration of silver enhancer solution was optimized by studying different dilution ratios.[4,16] A 20-fold dilution was found to best reduce the undesired background while improving the target-to-control signal ratio. The targets were additionally rinsed with nanopure water to remove all unbound salt to avoid the autonucleation of silver.

A silver ion-selective microelectrode (Ag-ISμE) is used to quantify the silver ions released after the catalytic deposition of silver onto 10 nm diameter gold tags. This silver is here oxidatively dissolved for detection purposes with hydrogen peroxide because reagents, such as nitric acid, previously used in conjunction with ASV[15] compromise the detection limit in potentiometry. The effective dissolution of silver ions by H_2O_2 was confirmed by transmission electron microscopy (TEM; see Supporting Information) and demonstrated that catalytic deposited silver can be efficiently oxidized when the concentration of hydrogen peroxide and reaction time are adequately optimized.

The potentiometric measurements were performed in a 1 mL cell with a sodium-selective electrode as pseudo reference since the background sodium concentration was known (10^{-5} M $NaNO_3$) and kept constant. The Ag-ISμE used as indicator was characterized before measurements and confirmed to give a detection limit of 1 nM Ag^+. The concentration of silver ions in the range of 1–10 nM was subsequently used for indirect quantification of the IgG target.

Figure 1A shows the corresponding potentiometric response of the silver ion-selective microelectrode for the control solution (a), the response for two different nontarget proteins, myoglobin (b) and lysozyme (c), and for 2.5 and 5 μg/mL mouse IgG (as complementary target) after dissolution of the silver precipitate. The well-defined silver signal of the 5 μg/mL target (~60 mV) is substantially greater than that of the control (~20 mV; a vs e). A 4-fold excess (20 μg/mL) of two noncomplementary proteins yielded signals similar to those of the control (b,c vs a). Such behavior indicates that the ISE transducer offers high sensitivity and selectivity. The response of Figure 1B also reflects the good discrimination against an excess of unwanted constituents, with similar signals observed for 2.5 μg/mL mouse IgG and for 2.5 μg/mL mouse IgG mixed with 20 μg/mL lysozyme (b vs c). The minimization of nonspecific binding (i.e., the low “control” signal) reflects the efficient washing steps and dense surface coverage with a co-assembled monolayer.

Figure 2A displays the corresponding potentiometric response of the silver ion-selective microelectrode to increasing concentrations of mouse IgG (0.5–7.5 μg/mL; a–f). Well-defined signals are observed for each target concentration. The resulting plot of the response versus log[IgG target] (shown as inset, B in Figure 2) is linear and offers a well-defined concentration dependence suitable for quantitative protein analysis (with a slope of 26.0 mV mL/μg). Also shown in Figure 2C is the response for a 0.25 μg/mL IgG target solution, which indicates a detection limit of around 12.5 pmol of IgG in the 50 μL sample. This approaches those observed for analogous amperometric detection with enzyme labels and for adsorptive stripping voltammetry with metal nanoparticles.[1] The protocol described here may therefore be most attractive with even smaller volumes of detection. A series of five repetitive measurements of 2.5 μg/mL mouse IgG target (data not shown) was used for estimating the precision. This series yielded reproducible signals with a relative standard deviation of 4%.

We demonstrated here for the first time a polymer-membrane-based potentiometric method for nanoparticle-based protein detection. The optimization of each parameter made possible a successful methodology that will be added to the existing ones. Silver ions were detected after multiple washing steps that remove the potentially interfering halide and sulfide ions. The detection was achieved by direct potentiometry, without any ion accumulation used in analogous ASV measurements of nanoparticle tags.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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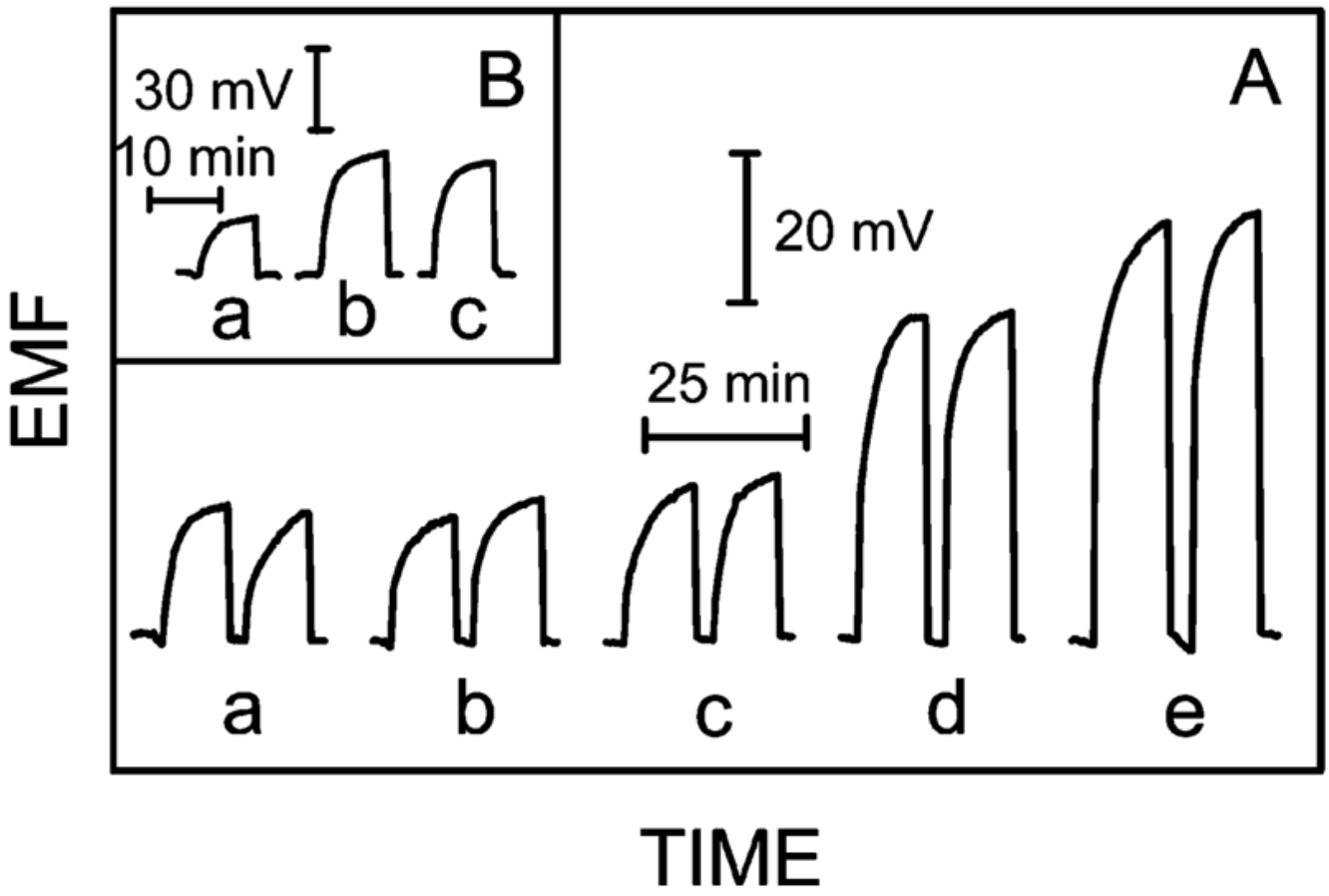


Figure 1.
Potentiometric responses of the silver ion-selective electrode for the (a) control (zero target), (b) 20 μg/mL myoglobin, (c) 20 μg/mL lysozyme (as a noncomplementary targets), (d) 2.5 μg/mL mouse IgG, and (e) 5 μg/mL mouse IgG (as complementary targets) after sandwich immunoassay with 5 min silver enhancement (A). Inset B: potentiometric responses of silver ion-selective electrode to (a) 20 μg/mL lysozyme, (b) 2.5 μg/mL IgG solution containing 20 μg/mL lysozyme, and (c) 2.5 μg/mL mouse IgG.

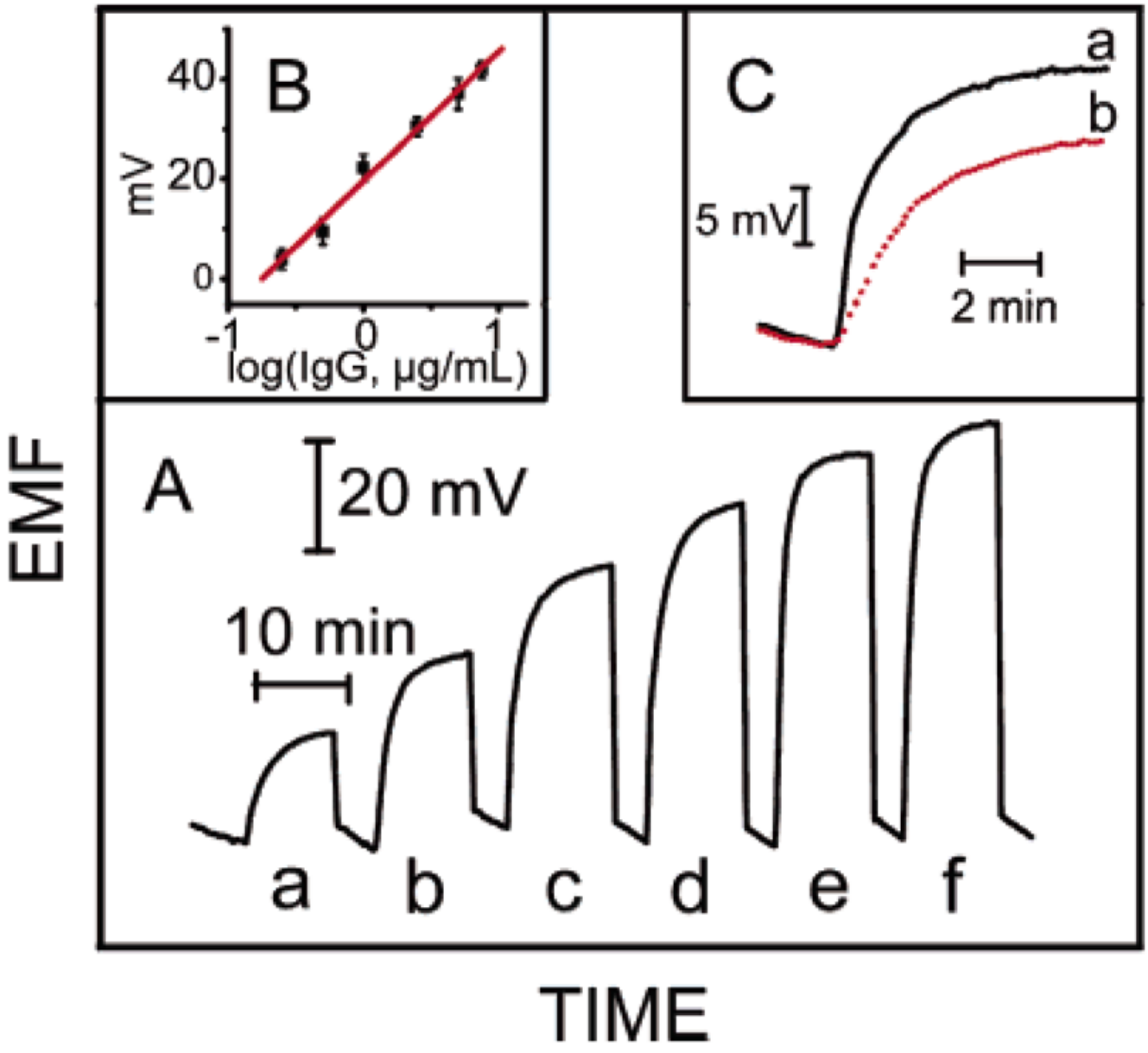
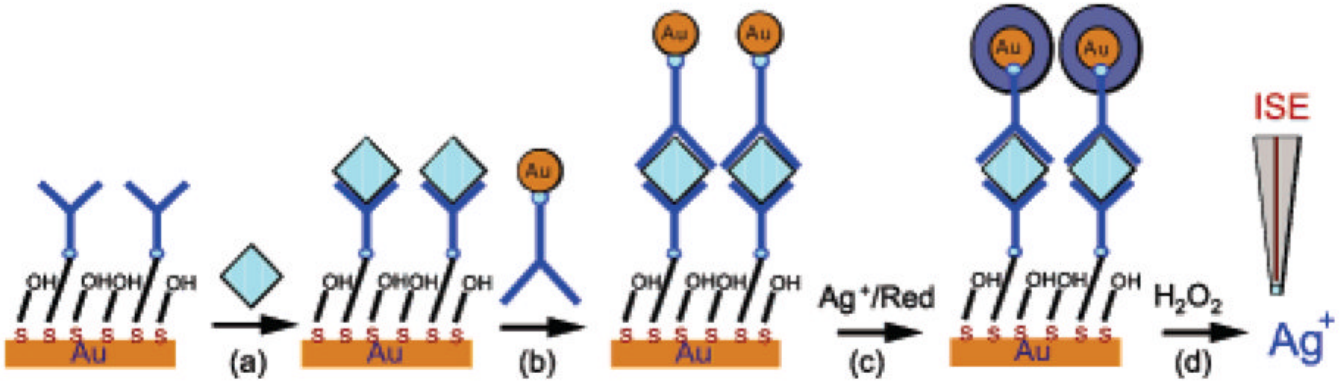


Figure 2.

Potentiometric responses of silver ion-selective electrode for increasing levels of the mouse IgG target: 0 (a), 0.5 (b), 1 (c), 2.5 (d), 5 (e), and 7.5 (f) μg/mL mouse IgG (A). Inset: calibration curve of the silver ISE responses versus log[IgG] (B), and the potentiometric responses of silver ion-selective electrode for (a) 0.25 μg/mL mouse IgG and (b) control (C).



Scheme 1.

Potentiometric Detection of Sandwich Immunoassay[a]

[a] Conditions: (a) antigen addition, (b) capture of the gold nanoparticles labeled anti-mouse IgG antibody, (c) catalytic deposition of silver ions on the conjugated Au nanoparticles, (d) silver dissolution and potentiometric detection using a Ag^+-selective electrode (ISE).