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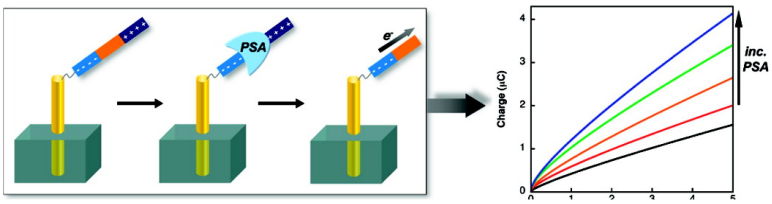

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Ultrasensitive Detection of Enzymatic Activity with Nanowire Electrodes

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The identification of specific proteins correlated with the presence and progression of cancer now provides the opportunity to use these biomarkers for accurate and early diagnosis.[1,2] Realizing the full utility of cancer biomarkers will require the development of methods that are suitable for implementation into clinical diagnostic devices. Many protein detection methods rely on affinity-based methods that report on the presence of an analyte rather than activity,[3,4] an approach that can limit the specificity and predictive value of a diagnostic strategy.

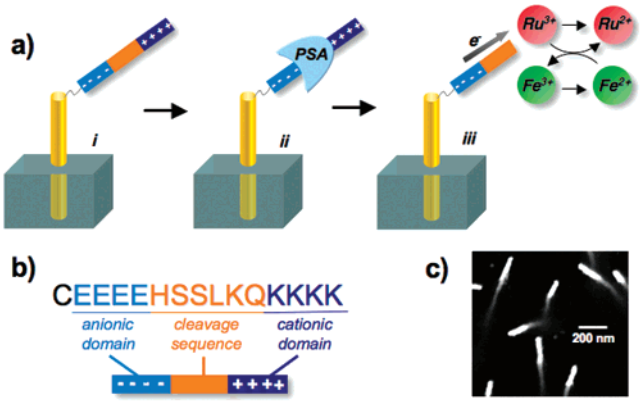
We have developed an electrochemical strategy that relies on nanostructured electrodes to detect protein activity. Electrochemical readout for protein detection, while shown to have great promise in early studies,[5] has been limited primarily to examples involving redox-active proteins[6] or the development of binding-based assays.[7−9] Here, using a nanoscale electrode platform and electrocatalytic readout, we demonstrate an activity-based approach for the ultrasensitive detection of enzymes. This approach represents a significant advance because it allows for the quantitation of a specific enzymatic activity, rather than a binding event.

A validated cancer biomarker, the prostate-specific antigen (PSA),[10,11] was used as a target for the novel electrochemical protein detection system. The presence of PSA at elevated levels in serum has been correlated with prostate cancer, and quantitation of this protein by immunoassay is used for routine prostate cancer screening.[10,11] The effectiveness of the screening test has been called into question, however, as the test appears to lack sufficient specificity to eliminate high-false positive rates.[12] The biological function of PSA involves the proteolytic cleavage of specific protein sequences in seminal fluid;[13] we therefore targeted this activity (Scheme 1a) to obtain an assay for PSA that would be more specific than that currently used in the clinic, which relies on antibody/PSA complexation.

The nanoscale platform for the PSA sensor consisted of an electrode composed of an ensemble of nanowires made by templated electroless deposition within a polycarbonate membrane.[14] About 100−200 nm of wire (d = 10 nm) is exposed from the membrane surface using oxygen plasma etching, providing three-dimensional structures as sensing elements (Scheme 1b). This electrode platform was previously used for electrochemical nucleic acids hybridization,[15,16] and has been shown to provide excellent sensitivity.

To use nanowire electrodes for PSA detection, a peptide sequence was designed that would transduce the cleavage chemistry catalyzed by PSA into an electrical signal (Scheme 1b). A thiol-containing cysteine residue was incorporated at the end of the peptide to facilitate adsorption on gold (see Supporting Information for all experimental details concerning peptide immobilization). For electrical signal transduction, a cleavage site (HSSLKQ) known to be recognized and cleaved by PSA in vivo[17] was flanked by an anionic (EEEE) and cationic (KKKK) sequence that would serve to modulate the efficiency of an electrocatalytic reaction[15,16] controlled by electrostatics (Scheme 1a). The electrocatalytic reporter system uses $Ru(NH_3)_6^{3+}$ as a primary electron acceptor; this redox-active compound is electrostatically attracted to negatively charged molecules and will preferentially bind to the immobilized peptide when the cationic portion has been cleaved by PSA. An excess of a secondary electron acceptor, $Fe(CN)_6^{3-}$, is used to amplify the current flowing to $Ru(NH_3)_6^{3+}$ by chemically oxidizing the electrochemically reduced Ru(II). Because of its negative charge, $Fe(CN)_6^{3-}$ does not access the surface of the electrode masked by the partially anionic peptide and therefore does not react directly. This design allows for readout of the presence of the protease through an increase in electrocatalytic current; this "signal-on" approach should minimize false positive results that could be caused by electrode stripping or fouling.

Scheme 1 [a]



[a] Electrocatalytic readout of enzyme activity. (a) Schematic illustrating electrocatalytic detection of PSA activity on a peptide-derivitized gold nanowire. A cysteine-containing sequence is immobilized on a gold nanowire electrode (i), and then incubated with PSA (ii), which recognizes and cleaves the sequence shown in orange (iii). The cleavage of the peptide is detected by using an electrocatalytic reporter reaction that yields an increased electrochemical current in the presence of the negatively charged monolayer generated by the cleavage event. Please note that the illustration is not drawn to scale and that many peptide molecules would be immobilized on each gold nanowire. (b) Sequence of peptide used to transduce the activity of PSA into an electrical signal. (c) Scanning electron micrograph image of an electrode consisting gold nanowires (d = 10 nm). The nanowires shown are prepared by electroless gold deposition within a polycarbonate membrane, followed by oxygen plasma etching to expose 100−200 nm of nanowire.

As shown in Figure 1, time-dependent chronocoulometric measurements of electrocatalytic charge at the peptide-derivatized nanoscale sensor are responsive to the presence of PSA (see Supporting Information for all experimental details). While signals collected at nanowire electrodes derivatized with an inert monolayer of cystamine do not exhibit any change in the presence of PSA (see inset), the designer peptide promotes electrocatalysis once cleaved by the protease. Concentrations as low as 1 pM can be

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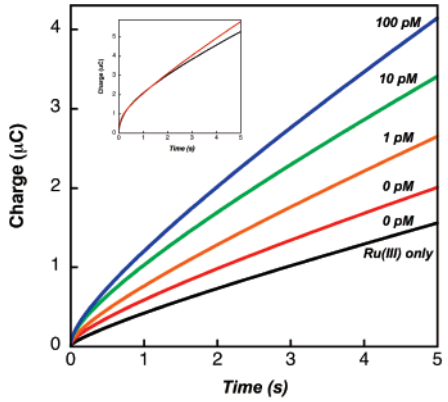

Figure 1. Electrocatalytic detection of PSA in buffer using nanowire electrodes. Samples of PSA were incubated with peptide-modified electrodes for 15 min and then electrocatalytic currents were measured by applying a −450 mV potential step for 5 s in a solution containing 100 μM $Ru(NH_3)_6^{3+}$, 1 mM $Fe(CN)_6^{3-}$, and 25 mM sodium phosphate/25 mM NaCl (pH = 7). The noncatalytic signal (black line) in the presence of Ru(III) only is shown for reference. The catalytic signal (red line) is obtained once $Fe(CN)_6^{3-}$ is added. Upon the addition of PSA, this catalytic signal increases in intensity. The inset shows control experiments performed with cystamine-derivatized electrodes. Identical experiments to those described were performed with cystamine-derivatized electrodes. A typical response observed in the absence of PSA (black) and the presence of 1 μM PSA (red) is shown. The signal changes recorded in these controls were within the error margins of the experiment.

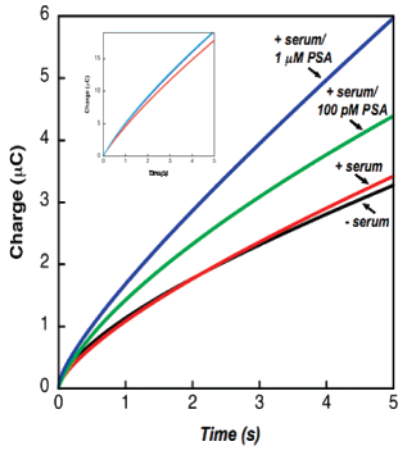

Figure 2. Detection of PSA in serum samples. Electrocatalytic charge was measured in buffer (black) and compared to human serum (red) after 15 min incubations to assess levels of nonspecific peptide cleavage. None was observed. The serum used in these experiments contained negligible levels of PSA as determined using a conventional immunoassay. In the presence of 100 pM PSA (green) and 1 μM PSA (blue), significant increases in electrocatalytic signal were observed. The inset shows control experiments: cystamine-derivatized electrodes were exposed to buffer (red), serum (light blue), and serum + 1 μM PSA (dark blue). See Supporting Information for quantitation of data and standard deviations.

visualized using this method, a detection limit well below physiologically relevant PSA levels.[7]

The performance of the nanowire-based PSA detection assay was also tested in serum (Figure 2). The introduction of serum lacking PSA does not produce a significant change in the electrocatalytic signal generated, which confirms the specificity of the assay and that serum proteases (e.g., other kallikreins, plasminogen, thrombin, or carboxypeptidase N)[18] other than PSA do not react with the electrode. Upon addition of serum containing 100 pM PSA, the amount of this antigen present in healthy individuals, a significant signal increase is detected, indicating that the activity of PSA can be detected specifically in a complex sample. With 100 pM PSA, a change in the electrocatalytic charge of 36 ± 3% was observed (versus a change of −4.5 ± 11% at a control cystamine monolayer) and with 1 μM PSA, the electrocatalytic charge increased by 90 ± 11% (versus a change of 3.3 ± 7% at a control cystamine monolayer). These signal changes, possessing low error levels and excellent reproducibility in multiple trials, indicate that this method is robust enough for use with biological samples. It is noteworthy that experiments using conventional macroscale electrodes for the detection of PSA did not produce the same robust and reproducible signal changes (data not shown) corresponding to the presence of the enzyme. Thus, as noted in studies of nucleic acids detection performed with gold nanowire electrodes,[15] this platform exhibits a significant advantage for electrochemical protein biosensing.

The measurement of protein activity using a simple electrochemical approach is enabled through the use of a novel electrode composed of nanostructured elements. This straightforward approach provides the basis for a new family of assays for enzymatic activity. A high level of sensitivity is attained with this method in a concentration range that would enable protein activity to be detected in clinical samples. This system therefore represents a promising platform for the development of a molecular diagnostic system, and indeed, may represent a general approach to the detection of enzymes in biological media.

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Supporting Information Available: Electrode fabrication and modification protocols, electrochemical analysis methods, and information on materials used in experiments described. This material is available free of charge via the Internet at http://pubs.acs.org.

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