An electrochemical peptide-based biosensing platform for HIV detection†

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Received (in Austin, TX, USA) 14th September 2009, Accepted 5th November 2009
First published as an Advance Article on the web 25th November 2009
DOI: 10.1039/b919070h

We have fabricated a potentially generalizable electrochemical peptide-based (E-PB) sensor for the detection of HIV anti-p24 antibodies. The E-PB sensor is sensitive, specific and fares well even when challenged in a realistically complex medium such as human urine proxy.

There are growing demands for point-of-care medical diagnostics and rapid methods for the specific detection of biological analytes in a variety of complex samples. As a result, a number of peptide and protein-based sensing methods have been described in the literature, many of which feature impressive sensitivity and generalizability.[1–9] However, the vast majority of these approaches require the addition of exogenous reagents or utilize changes in relatively non-specific properties, such as mass, charge, or electron density, to generate an observable signal. This, unfortunately, makes many of these approaches time-consuming, reagent-intensive and thus not ideal for field applications.[3] Electrochemical detection methods, known for speed, sensitivity, and low cost/mass/power requirements, have attracted increasing attention in recent years, leading to the development of various electrochemical protein sensing approaches.[10,11] Specifically, the Plaxco group and others have developed a label-free, reusable, near real-time electrochemical protein sensing platform.[12–14] The electrochemical aptamer-based (E-AB) sensor is label-free, specific, operationally convenient and selective enough to be employed directly in realistically complex samples.[12–15] However, while all E-AB sensors have demonstrated sensitivity and selectivity that favourably rival their optical counterparts,[15] the choice of target analytes remains limited to targets for which aptamers have been selected against. The development of a peptide-based sensor that is analogous to the E-AB sensors could significantly extend the range of potential targets to include diagnostically-relevant antibodies.

Here we report the first electrochemical peptide-based (E-PB) sensor for the detection of HIV anti-p24 antibodies. Our approach to the design of this sensor is to combine binding-induced changes in the dynamics of the polypeptides with the well-established electrochemical biosensing platform. The rationale behind this biosensing approach is that binding of the target induces a significant change in the dynamics of the sensing probe (*i.e.*, the rate at which the redox label collides with the electrode surface), which slows the electron transfer rate to the methylene blue (MB) reporter of reacted probes so significantly that it is effectively prevented from carrying out efficient electron transfer (Fig. 1).[16–18] An alternative explanation of the sensing mechanism is that the bulky antibody engulfs the MB upon binding, thereby fully obstructing electron transfer to the methylene blue label. Of note, the E-PB sensing platform is potentially generalizable and can be adapted to the detection of a wide variety of disease-relevant antibodies, which undoubtedly will expand the real world applicability of this class of sensors.

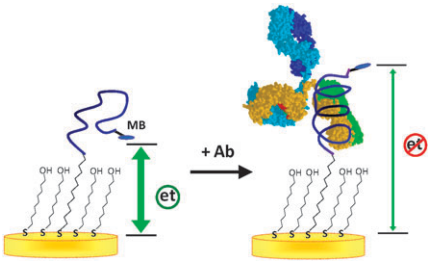

Fig. 1 E-PB sensor construct and signaling mechanism. The signaling event of the E-PB sensor is analogous to the linear probe E-DNA sensor. In the absence of target, the peptide probe is unstructured and highly flexible, facilitating efficient electron transfer to and from the redox label *via* collisional electron transfer. Binding of the target induces a significant change in the dynamics of the peptide probe (*i.e.*, the rate at which the redox label collides with the electrode surface), therefore producing a readily detectable reduction in the current.

The recognition peptide we employed is a higly antigenic epitope from the HIV-1 capsid protein, p24, with the sequence EAAEWDRVHP,[19] which has been well-characterized and is therefore ideal for our prototype peptide-based sensor.[20,21] This epitope is confined to a predominantly α-helical structure in the native state; the short peptide used in this study, however, lacks defined secondary structure.[20] We presume binding of the target antibody induces the formation of a more rigid complex. The N-terminus of the probe is modified with an 11-carbon thiol for direct attachment to the gold electrode while the C-terminus is modified with a redox label, MB, *via* the side chain of the added lysine residue (Fig. S1, ESI†). Fabrication of this sensor involved direct adsorption of the thiolated peptide probe, followed by passivation of the remaining area of the electrode with diluent molecules, 9-mercapto-1-nonanol (C9-OH) or 6-mercapto-1-hexanol (C6-OH). Independent of the choice of diluent molecules, sensor preparation time for both sensors is ~24 h. A sensor with C9-OH passivation displays an average surface probe density of 1.7×10^{10} molecules per cm^2 (RSD < 7%, $n = 3$), whereas a sensor with C6-OH passivation shows an average density of 1.22×10^{12} molecules per cm^2 (RSD < 10%, $n = 3$).

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† Electronic supplementary information (ESI) available: Experimental procedures. See DOI: 10.1039/b919070h

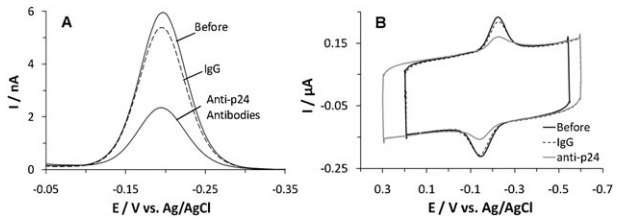

Fig. 2 Sensor interrogation in (A) AC voltammetry and (B) CV with 43 nM IgG, followed by an interrogation with 43 nM anti-p24 in a Phys2 buffer. Each interrogation was allowed to proceed for 1 hour. The AC voltammograms shown are averages of three different sensors with similar probe density.

Sensor performance was first assessed using alternating current (AC) voltammetry by scanning from 0 to −0.5 V (*vs.* Ag/AgCl/3 M KCl) in a physiological buffer (Phys2, pH 7.4). In the absence of the target, the E-PB sensor fabricated with C9-OH shows a sharp, well-defined AC voltammetric peak at ∼−0.2 V, a potential consistent with the reduction potential of the methylene blue (MB) redox moiety in this medium (Fig. 2A). The sensor responds specifically to its antibody target (anti-p24 antibodies). In the presence of 43 nM of anti-p24 antibodies, we observe a 57% decrease in the MB reduction current. An 11% signal decrease is evident with the same concentration of random IgG antibodies, suggesting the sensor's specificity (Fig. 2A). The majority of the response is achieved within 60 min although complete signal saturation time is ∼100 min, which is reasonable owing to the size of the antibody (∼150 kD) (Fig. S2, ESI†). Attempts to regenerate the sensor with reagents that have been previously used in electrochemical and surface-plasmon resonance-based sensors have only been marginally successful, presumably due to the strength and complexity of antigen–antibody interactions.

To understand how diluents affect sensor performance, C6-OH passivated E-PB sensors were also fabricated. The shorter diluent could, presumably, improve binding efficiency and kinetics by improving the flexibility of the probe. However, while no significant improvement in the binding kinetics is evident, less signal suppression is observed under the same experimental condition (Fig. S3, ESI†). The reduction of sensor sensitivity is probably due to the enhanced flexibility of the probe and thus the feasibility of collisional electron transfer even in the "bound" state. Another adverse effect of utilizing shorter diluents in sensor fabrication is the stability, in which the C6-OH passivated sensor is relatively unstable (*i.e.* ∼20% loss of MB current in 1 h) and is therefore not used in the rest of this study.

While AC voltammetry has been the interrogation method of choice for most folding-based electrochemical biosensors,[12,15] cyclic voltammetry (CV) has also been demonstrated to be an effective sensor interrogation method.[22] As shown in Fig. 2B, CV scans obtained at 1 V s^{-1} display a characteristic MB redox peak pair at −0.21 V and −0.15 V ($E_{1/2}$ = −0.18 V). This value falls within the typical redox potential range of methylene blue. Modulating the cyclic voltammetric scan rate provides further evidence that MB-modified peptide probes are confined at the electrode surface. Peak currents of the MB redox reaction (I_p) are directly proportional to scan rates (Fig. S4, ESI†), consistent with a surface-confined electrochemical reaction.[23,24]

The cathodic segments of the voltammograms are used to allow direct comparison between CV and AC voltammetry. In the presence of 43 nM of IgG, an 8.3% signal reduction in the peak height is evident, whereas a significant peak current suppression (55%) is observed when challenged with equal concentration of the anti-p24 antibodies. The change in the peak height is also reflected in the change in the integrated peak area (*i.e.* charges). In this case, a loss of 5.9% and 57% of the peak area is evident in the presence of equimolar quantities of IgG and anti-p24 antibodies, respectively (Fig. 2B).

The C9-OH passivated E-PB sensor responds to its target antibody in a concentration-dependent manner, with a linear response in the semi-log domain (Fig. 3). The calibration curve displays the data averaged from three independent experiments, in which three electrodes were interrogated with successively higher concentrations of anti-p24 antibodies, suggesting overall sensor performance reproducibility despite the minor differences in the surface probe density. To date, the E-PB sensor has shown a detection limit (10 nM or 1.5 μg mL^{-1}) lower than the reported detection limit (33 nM or 5 μg mL^{-1}) obtained *via* surface-plasmon resonance spectroscopy, a relatively complex and expensive analytical technique.[25] The concentration of anti-p24 antibodies in blood serum and urine, depending on the patient's immune system and the time elapsed post infection, generally ranges from tens to hundreds of nanomoles.[25,26] Even without optimization, this sensor has a dynamic range that includes the typical concentration range commonly observed with HIV infected patients.

While serum-based detection methods are more commonly implemented for HIV diagnosis, urine-based analysis is non-invasive, safe, and cost-effective. Thus motivated, we challenged the E-PB sensor in a realistically complex medium, human urine proxy (Fig. 4).[27,28] We observe a ∼2% initial signal reduction when the sensor is introduced to the 50% urine proxy (when compared to the peak current observed in a Phys2 buffer) (Fig. 4A). A very small peak potential shift in the MB redox potential (∼7 mV) is observed, owing to the minor differences in the pH (pH 7.4 *vs.* 7.6). More importantly,

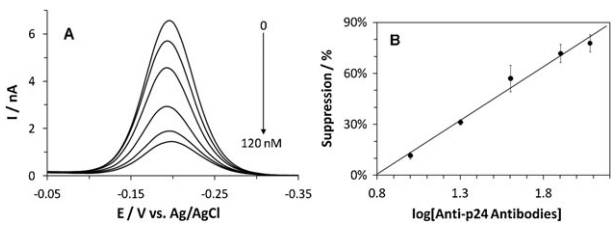

Fig. 3 (A) AC voltammograms for the E-PB sensor in the presence of anti-p24 antibodies at 0 nM, 10 nM, 20 nM, 40 nM, 80 nM, and 120 nM (from top to bottom). The interrogation time was fixed at 60 min. (B) A calibration curve demonstrating peak height *versus* target concentration; E-PB sensor response increases linearly with the log of the target concentration. The error bars represent one standard deviation from the mean, as calculated from three sensors with similar probe density.

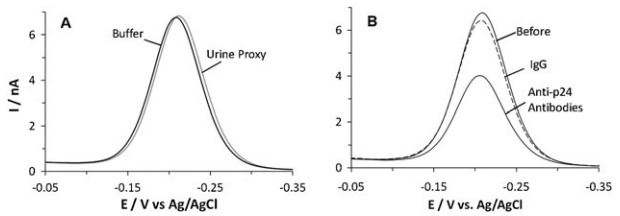

Fig. 4 The E-PB sensor can detect anti-p24 antibodies even in a realistically complex sample. (A) Sensor signal in target-free Phy2 buffer and 50% urine proxy. (B) Sensor interrogation in AC voltammetry with 43 nM IgG, followed by interrogation with 43 nM anti-p24 in 50% urine proxy. Each interrogation was allowed to proceed for 1 hour. The voltammograms shown are averages of three different sensors with similar probe density.

in the presence of 43 nM of random IgG, we observe a mere 6% decrease in the MB reduction current, whereas a 43% signal suppression is evident upon addition of 43 nM of anti-p24 antibodies.

Although the observed signal suppression in the presence of the anti-p24 antibodies in urine proxy is lower than that obtained in the buffer (57% *vs.* 43%), the specificity of the sensor is improved. As observed, a 6% signal suppression is evident in the presence of 43 nM of IgG in the urine proxy, whereas an 11% signal reduction is observed when interrogated in the Phys2 buffer (Fig. 2A and 4B). We presume that this signal suppression originated from non-specific binding of the IgG to the peptide probe and the presence of urea, a chaotropic agent, increases the stringency of binding and thus minimizes non-specific interactions.[29]

In conclusion, we have fabricated the first E-PB sensor that is analogous to the well-characterized E-AB sensors. The E-PB sensor is sensitive, specific and selective enough to be employed directly in a realistically complex medium. Further improvement in this sensor's sensitivity, specificity and selectivity will be achieved by utilization of longer peptide epitopes[20] and diluents that have known resistance to biofouling and these prospects are currently under investigation in our laboratory.[30,31] This sensor, in conjunction with the recently developed gold-plating technology,[32] can potentially be implemented as a non-invasive and cost-effective HIV diagnostic test.[33]

The authors acknowledge the University of Nebraska-Lincoln and Nebraska ESPCoR for financial support. The authors also acknowledge Weiwei Yang and Socrates J. P. Caňete for insightful discussions.

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