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Incorporation of extra amino acids in peptide recognition probe to improve specificity and selectivity of an electrochemical peptide-based sensor

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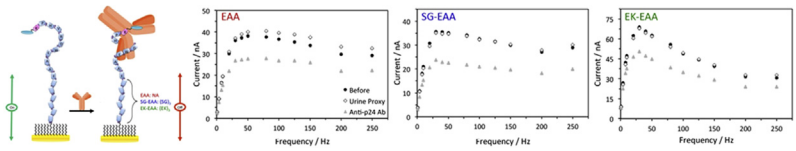
HIGHLIGHTS

- 6 additional hydrophilic amino acids were added to the peptide recognition probes.
- The resultant E-PB sensors showed improved specificity and selectivity.
- The sensors were functional in 50% synthetic saliva and 50% urine proxy.
- Sensor properties such as sensitivity and binding kinetics were minimally impacted.
- The peptide probe acts as the target recognition element and antifouling agent.

GRAPHICAL ABSTRACT



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ABSTRACT

We investigated the effect of incorporating extra amino acids (AA) at the n-terminus of the thiolated and methylene blue-modified peptide probe on both specificity and selectivity of an electrochemical peptide-based (E-PB) HIV sensor. The addition of a flexible $(SG)_3$ hexapeptide is, in particular, useful in improving sensor selectivity, whereas the addition of a highly hydrophilic $(EK)_3$ hexapeptide has shown to be effective in enhancing sensor specificity. Overall, both E-PB sensors fabricated using peptide probes with the added AA (SG-EAA and EK-EAA) showed better specificity and selectivity, especially when compared to the sensor fabricated using a peptide probe without the extra AA (EAA). For example, the selectivity factor recorded in the 50% saliva was ~2.5 for the EAA sensor, whereas the selectivity factor was 7.8 for both the SG-EAA and EK-EAA sensors. Other sensor properties such as the limit of detection and dynamic range were minimally affected by the addition of the six AA sequence. The limit of detection was 0.5 nM for the EAA sensor and 1 nM for both SG-EAA and EK-EAA sensors. The saturation target concentration was ~200 nM for all three sensors. Unlike previously reported E-PB HIV sensors, the peptide probe functions as both the recognition element and antifouling passivating agent; this modification eliminates the need to include an additional antifouling diluent, which simplifies the sensor design and fabrication protocol.

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1. Introduction

Since the 1980s, HIV (Human Immunodeficiency Virus) has been an epidemic in the United States. According to the Centers for Disease Control, approximately 48,000 people were diagnosed with HIV and 28,000 people with AIDS (Acquired Immune Deficiency Syndrome) in 2012 [1]. The most commonly used method of detecting and monitoring HIV is through the use of polymerase chain reaction (PCR). While the PCR-based viral load test is extremely sensitive and accurate, it is considered invasive since it requires the use of blood. Furthermore, this test has to be performed by trained professionals in clinics or hospitals. The availability of a user-friendly HIV self-test that could be performed in the privacy of one's home will undoubtedly lead to higher rates of testing, reducing the number of undiagnosed infection and the risk of onward transmission.

There are several home testing kits for HIV diagnosis that are based on lateral flow immunoassays, however, none of them utilize an electrochemical signal transduction mechanism. Electrochemical-based sensors have demonstrated to be well-suited for commercialization, for example, glucose biosensors accounted for approximately 85% of the world market for biosensors. Its widespread commercial success is attributed to its superb analytical performance; it is sensitive, specific and works well under a wide range of experimental conditions. More importantly, it is selective enough to be employed directly in complex matrices such as whole blood. However, for diagnosis of highly contagious diseases such as HIV, a non-invasive sensor that is functional in less transmissible and invasive matrices, such as urine and saliva, is highly desirable [2–5]. Although HIV tests that are based on the detection of anti-HIV antibodies in saliva have recently been developed [6–8], to our best knowledge, there is no equivalent electrochemical HIV sensor that is operational in saliva currently commercially available [9]. The electrochemical peptide-based (E-PB) sensor, with attributes similar to the well-characterized electrochemical DNA (E-DNA) and aptamer-based (E-AB) sensors, could potentially fill this unmet need [10–13].

While most E-DNA and E-AB sensors are functional in a wide range of realistically complex samples, this HIV E-PB sensor has only been tested in urine proxy [10]. The ability of these sensors to operate in various complex samples will likely broaden their applicability in disease diagnosis and monitoring. To date, a wide range of antifouling materials, including proteins, surfactants, and polymers, have been incorporated in the design and fabrication of amperometric and impedance sensors, as well as immunoassays [14–16]. Among all known antifouling or passivating proteins, bovine serum albumin (BSA) is the most common employed, presumably due to its effectiveness and low cost [15]. Covalently or electrostatically bound surfactants and polymers such as tween 20, polyethylene-glycol, carboxy-methyl dextran, poly-lysine, and cetyl trimethylammonium bromide, have also been shown to be capable of resisting non-specific adsorption of matrix contaminants [17–23]. Despite the fact that all these surface modification strategies have demonstrated to be effective, not all of them are compatible with E-PB sensors. Similar to the E-DNA and E-AB sensors, E-PB sensors are self-assembled monolayer (SAM)-based sensors; the sensor surface is composed of surface immobilized biorecognition probes and diluent alkanethiols [10,24–36]. Physisorption of large proteins such as BSA on top of the monolayer could affect the electron transfer between the redox-modified probe and the substrate electrode. The use of electrostatically bound antifouling molecules is one of the simplest ways to passivate a surface; however, since the molecules are not covalently attached to the sensor surface, stability of the passivating layer could be an issue, in particular, for sensors designed for use in flow-based microfluidic devices. Thus, antifouling molecules that are more permanently bound to the surface are more suitable for this class of SAM-based biosensors. To this end, we have recently succeeded in enhancing both the specificity and selectivity of a different E-PB sensor by incorporating thiolated oligothymine into the monolayer [32]. The addition of negatively-charged diluents has shown to be effective in preventing surface fouling.

In this study, we explored an equally effective strategy for surface passivation, albeit without the use of an additional thiolated antifouling diluent. The peptide recognition probes used here were modified with extra amino acids (AA) that have been shown to have antifouling properties. We compared both the specificity and selectivity of the sensors fabricated using these peptides to the one fabricated using a peptide without this addition. Our results highlight the capability of these modified peptide probes in resisting non-specific adsorption of matrix contaminants. This strategy has one major advantage over previously employed methods, in which the peptide probe functions as both the target recognition element and the antifouling coating. It is also generalizable and can potentially be used with other detection platforms that utilize surface-immobilized biorecognition probes (e.g., surface plasmon resonance (SPR) spectroscopy) [37].

2. Materials & methods

2.1. Materials and reagents

All reagents and solvents were used as purchased. Human immunoglobulin G antibodies (IgG Ab), creatinine, trizma-base, tris-hydrochloride, sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sulfuric acid, acetonitrile (ACN), and 8-mercapto-1-octanol (C8–OH) were purchased from Sigma–Aldrich (St. Louis, MO). The methylene blue (MB)-modified peptides were purchased from Xaia Peptides (Göttenborg, Sweden) (Figure S1). The three peptide sequences are shown below, the added six AA are underlined.

EAA peptide: (n) HS–$(CH_2)_{11}$-EAAEWDRVHP-K-MB (c)
SG-EAA peptide: (n) HS–$(CH_2)_{11}$-SGSGSG EAAEWDRVH P-K-MB (c)
EK-EAA peptide: (n) HS–$(CH_2)_{11}$-EKEKEK EAAEWDRVHP-K-MB (c)

The lyophilized peptides were reconstituted in 10% ACN (90% deionized (DI) water) to produce a 0.5 mM stock solution. The anti-p24 antibody (Anti-p24 Ab) was purchased from ProSpec-Tany Technogene Ltd. (East Brunswick, NJ). The sensors were interrogated in a Phys2 buffer (Phys2) containing 9.2 mM Trizma-base, 10.8 mM Tris–HCl, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ (pH 8.6). Synthetic urine solution (urine proxy) was obtained from Ricca Chemical Company (Arlington, TX); 11 mM creatinine was added to this solution. Synthetic human saliva was purchased from US Biocontract (San Diego, CA).

2.2. Instrumentation

A 1040B CH Instruments Workstation (Austin, TX) was used for all electrochemical measurements. Gold working electrodes of 2 mm diameter, Pt auxiliary electrode, and Ag/AgCl (1 M KCl) reference electrode made up the electrochemical cell. All electrodes in the electrochemical cell were also purchased from CH Instruments.

Alternating current voltammetry (ACV) was the main technique for sensor characterization. AC voltammograms were recorded from −0.15 V to −0.48 V at different frequencies and with an AC amplitude of 25 mV. Cyclic voltammetry (CV) was used to determine the probe coverage, heterogeneous electron transfer rate