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Peptide-Mediated Electrochemical Steric Hindrance Assay for One-Step Detection of HIV Antibodies

Sahar Sadat Mahshid,[†,⊥] Sara Mahshid,[∥] Alexis Vallée-Bélisle,[*,‡] and Shana O. Kelley[*,†]

[†]Department of Pharmaceutical Science, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario M5S 3M2, Canada

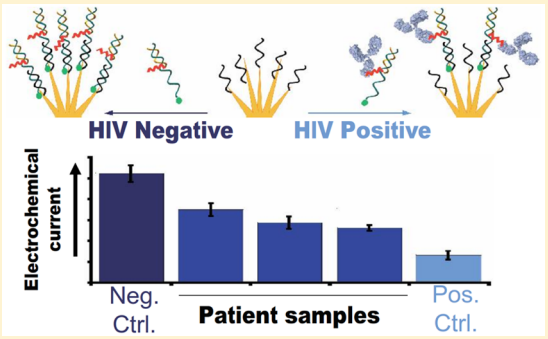
[⊥]Biological Sciences Department, Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, Toronto, Ontario M4N 3M5, Canada

[∥]Department of Bioengineering, McGill University, Montréal, Québec H3A 0C3, Canada

[‡]Département de Chimie, Université de Montréal, Montréal, Québec H3T 1J4, Canada

Supporting Information

ABSTRACT: Diagnosis of infectious disease in patients, including human immunodeficiency virus (HIV) infection, can be achieved through the detection of specific antibodies produced by the immune system. We have previously shown that macromolecules such as antibodies can be efficiently detected in complex biological samples by sterically inhibiting the hybridization of conjugated complementary DNA strands to electrode-bound DNA strands. Here, we report a peptide-mediated electrochemical steric hindrance hybridization assay, PeSHHA, specially for the detection of antibodies against the gp41 protein of HIV-1. We show that the sensitivity of this PeSHHA can be significantly enhanced using nanostructured electrodes and demonstrate the rapid, one-step detection of HIV-1 antibodies directly in clinical samples.



Rapid diagnosis of infectious diseases caused by viruses or bacteria remains key to reduce disease transmission and improve survival rates.[1−3] Early diagnosis of patients infected with the human immunodeficiency virus (HIV), for example, improves patient outcomes and limits transmission of the infection.[4] HIV diagnosis is typically performed either through nucleic acids analysis (viral load measurement) or through the detection of HIV antibodies.[5,6] The main obstacle to achieving rapid diagnosis, particularly in developing countries, relates to the fact that current methods for the quantitative detection of viral loads and antibodies are not accessible enough since they typically rely on specialized technicians in sophisticated clinical laboratories.

Several sensing technologies are available for the detection of antibodies that are typically based on the readout of antibody−antigen interactions in sandwich assays such as the enzyme-linked immunosorbent assay (ELISA).[7,8] The multiple steps required for ELISA, however, makes this approach time and reagent intensive. Combining ELISA with new advancements in nanotechnology and microfluidic systems as well as the development of new homogeneous detection assays holds great promise for the development of practical diagnostic systems.[9−14] Of note, electrochemical detection platforms combined with immobilized biorecognition agents offer many potential alternatives for rapid and sensitive detection of biological molecules including antibodies.[15−18] As well, the introduction of nanostructured electrodes has improved the sensitivity and speed of electrochemical biomolecular detection.[19,20]

Electrochemical steric hindrance hybridization assays (eSHHAs) represent an important advance for the one-step detection of proteins and small molecules directly in complex biological samples such as whole blood.[21,22] In these assays, the steric effects of a large target macromolecule inhibit the hybridization of redox-active DNA strands to their complementary strands attached on the surface of an electrode. We recently combined the simplicity of eSHHA with the high sensitivity of nanostructured electrodes to improve the performance of the steric hindrance mechanism.[23,24] This combined approach not only improved the level of signal gain for eSHHA[25] but also decreased the limit of detection of this DNA-based sensing assay down to the picomolar concentration regime.[26]

Here, we adapt eSHHA for the detection of HIV antibodies in clinical samples. To do so, we developed a peptide-mediated steric hindrance hybridization assay, PeSHHA, that employs peptides as an important class of broadly applicable biorecognition elements.[27−29] Using a peptide−peptide nucleic acid (PNA) conjugate, we developed a signaling strand that

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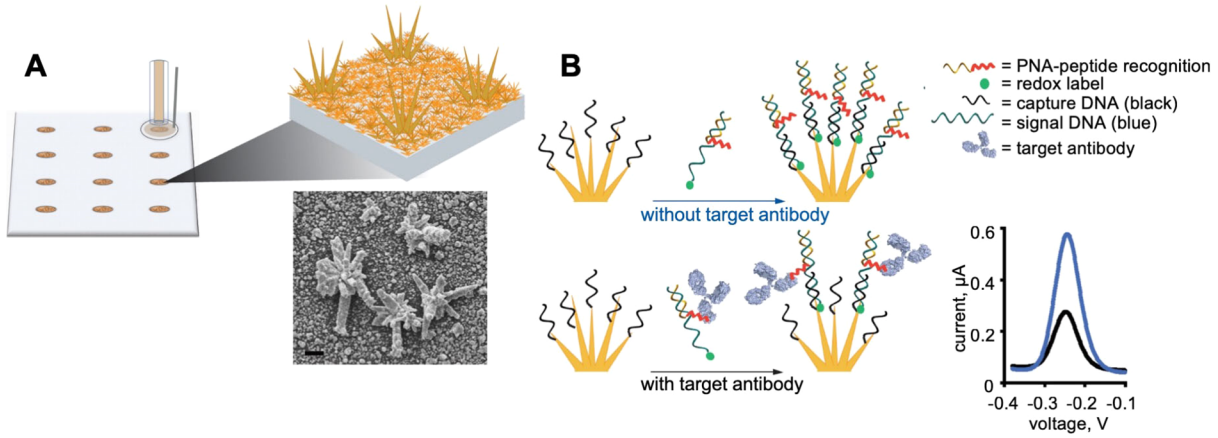

Figure 1. PeSHHA carried out on nanostructured electrodes. (A) Schematic illustration of ITO substrates with 12 addressable wells each containing ITO functionalized with gold nanostructured electrodes (top) and the SEM image of the electrode surfaces (bottom). (B) Schematic illustration of the PeSHHA: the PNA–peptide construct binds to the redox-labeled signal DNA, and its hybridization to the electrode-bound capture DNA reflects if a target antibody is bound. The magnitude of the electrochemical signal then reports on the presence (black) or absence (blue) of the antibody. SEM scale bar is 1 μm.

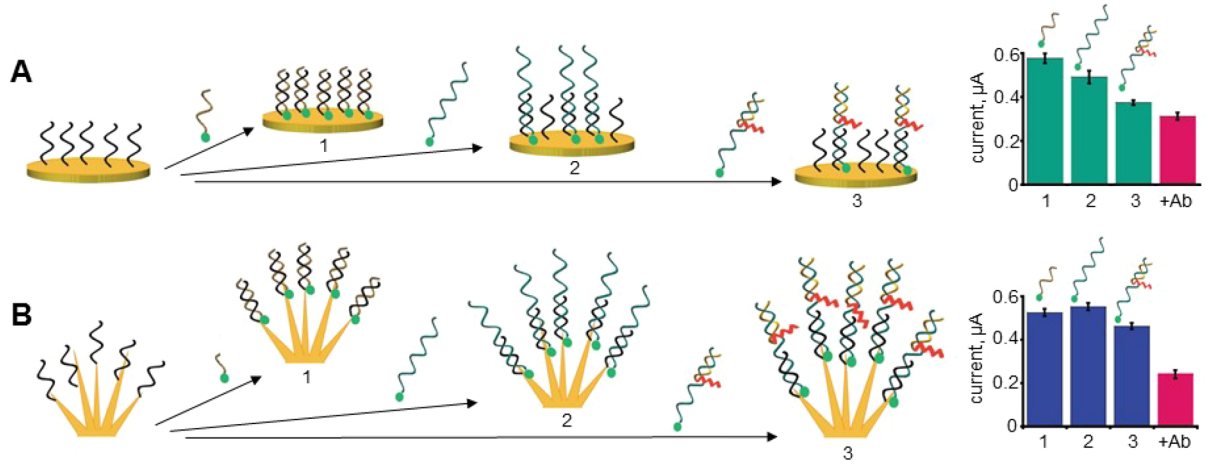

Figure 2. Modifications introduced to the steric hindrance platform to reduce the background and improve the signal readout. Investigation of the responses of PeSHHA platform on (A) a flat electrode compared to (B) a nanostructured electrode for (1) the original design where the signaling and capturing strands are 16-base constructs, (2) a modified design where the signaling strand is a 32-base construct, and (3) another modified design where the 32-based signaling strand is hybridized to the peptide–PNA complex construct. Current signals generated for 1, 2, and 3 are compared to the response of the platform in the presence of target antibody on both electrodes (A and B), demonstrating the importance of nanostructured electrodes for the reduction of background currents in PeSHHA (see also Figure S5).

can specifically capture human antibodies and reduce hybridization between this strand and a complementary surface-bound capture strand (Figure S1). When using nanostructured gold deposited on indium tim oxide (ITO) as a sensing electrode (Figure 1), we show that HIV antibodies can be sensitively detected in model samples as well as a panel of clinical specimens.

RESULTS AND DISCUSSION

Development of PeSHHA. We developed a PeSHHA that enables the detection of the 4E10 antibody that is a recombinant version of a human monoclonal antibody produced by humans in response to the presence of the protein gp41 of HIV-1. This antibody recognizes the specific NWFDIT epitope of gp41.[30,31] We attached this peptide to a peptide nucleic acid (PNA) sequence that hybridizes to a 32-nucleotide DNA molecule (Figures 1b and S1).[18,32] This complex is responsible for signal transduction by responding to the absence or presence of the target antibody via a steric hindrance effect that alters the efficiency of hybridization with an electrode-bound capture probe.

As shown in Figure 1B, the 32-base signaling strand carries at one end the redox label, methylene blue (MB), which is proximal to the surface upon hybridization to the surface-attached capture strand. A maximal steric hindrance effect is achieved when employing a high density of capture DNA strands attached to the electrode surface and the inclusion of enough signaling DNA to saturate all capture sites on the electrode surface (Figure S2).

We first determined how the gain of the assay is affected when the 16-nucleotide duplex is attached to the peptide via the PNA linkage. The efficiency of the steric hindrance signaling mechanism relies on a large size differential between the signaling strand with and without an attached target antibody.[21,22] The larger the overall size of the signaling strand, the smaller the antibody gain should be. We found that the

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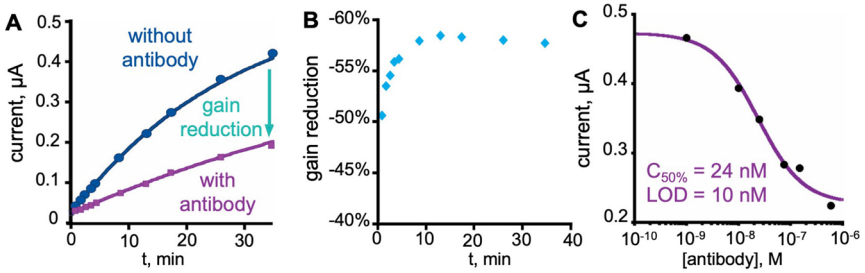

Figure 3. Detection of a monoclonal antibody using PeSHHA. (A) Kinetic response curves in the absence (blue line, 0.035 min^{-1}) and the presence (purple line, 0.014 min^{-1}) of target antibodies (4E10 antibody) show a decrease in hybridization efficiency for the antibody-bound signaling strand, (B) resulting in an average gain reduction of 55% in the first minutes. (C) A dose−response curve confirms that this assay is quantitative over a two-order of magnitude linear dynamic range (1−100 nM). Experiments were performed with the 4E10 antibody spiked into PBS.

electrochemical current (hybridization efficiency) is reduced by 16% when increasing the size of the signaling strand from 16 to 32 nucleotides and by 35% when this 32-nucleotide DNA is attached to the PNA (peptide located in the middle of the signaling strand) (Figure 2A). Upon binding to the antibody, the hybridization efficiency of this peptide-labeled signaling strand is only reduced by 17%, which represents a relatively weak gain for an assay. We also tested the steric hindrance mechanism when attaching the peptide at the extremity of the signaling strand and found that this specific conformation reduces the overall steric hindrance effect produced in the presence of antibody (Figure S3).

PeSHHA on Nanostructured Electrodes. Motivated by our recent finding that nanostructured electrodes improve the performance of the steric hindrance mechanism,[23,24] we also tested PeSHHA using nanostructured electrodes. Here, we explored the use of ITO as a substrate that could support the display of gold nanostructures over larger surface areas. The gold nanostructures were generated on the ITO substrate via electrodeposition (see Material and Methods in the Supporting Information). We applied linear voltammetry in the potential range of 0−0.6 V to create nanostructures within addressable wells.[33] SEM imaging of the substrates (Figure 1A) confirmed the generation of nanostructured surfaces inside the wells and uniformity of electrodeposition. When performed on these nanostructured electrodes, PeSHHA produced a strong 55% gain reduction in the presence of target antibody (Figure 2B), much larger than the 17% reduction observed on 2D electrodes (Figure 2A).

We tested the time and concentration dependence of PeSHHA with a monoclonal antibody. When nanostructured electrodes were used to perform PeSHHA, we observed a gain reduction of >55% within the first 5 min of the hybridization experiment (Figure 3A,B). This is attributed to the low hybridization kinetics for the antibody-attached signaling strand on the nanostructured electrode (2.5-fold reduction).[23] This gain reduction is also quantitative, achieving a typical 100-fold linear range with a $C_{50\%}$ of 24 nM (Figure 3C) (see also Supporting Information).

Detection of Antibodies in HIV Patient Samples. We then demonstrated the performance of PeSHHA for the detection of HIV-1 antibodies in patient specimens. Eleven banked patient blood plasma samples were tested using Western blot (Figure 4A; see Table S2) and the PeSHHA platform (Figure 4B). Each patient sample was tested three times. Positive and negative control samples consisted of a negative human plasma sample with and without spiked 4E10-antibody (300 nM), respectively. The five patient samples that tested positive for HIV-1 on the Western blot (1, 3, 4, 5, 7) displayed low electrochemical current levels (<0.3 μA) comparable to the positive control. Six other patient samples (8, 9, 11, 12, 14, 15) displayed high electrochemical current levels (>0.5 μA) comparable to the negative control. These samples were determined to be either negative or indeterminate for HIV-1 using the Western blot but positive for HIV-2 for five of the patients (8, 9, 11, 12, 14). It is worth noting that, while the Western blot requires multiple preparation steps, expensive reagents (antibodies), and several hours of analysis, PeSHHA successfully detected a specific human antibody directly in human plasma in less than 10 min.

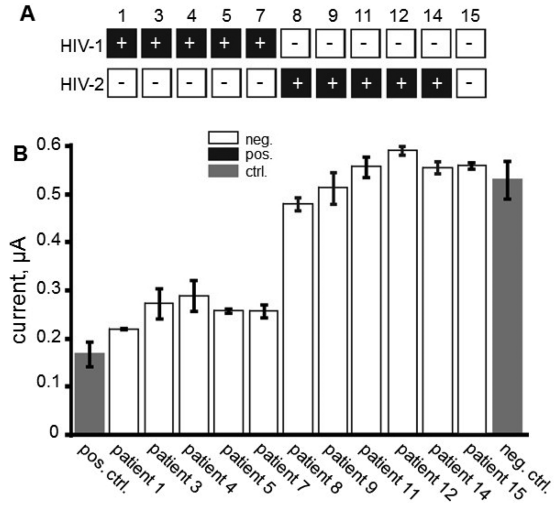

Figure 4. PeSHHA platform accurately identifies patient samples containing HIV-1 antibodies within 10 min directly in unprocessed blood plasma. (A) Summary of Western blot results and (B) PeSHHA results. Error bars represent the standard error deviation of three measurements. Positive and negative control samples consist of a negative human plasma sample with and without spiked 4E10-antibody (300 nM), respectively.

CONCLUSIONS

In this study, we developed an electrochemical steric hindrance hybridization assay (eSHHA) for the rapid detection of specific HIV-1 antibodies directly in blood plasma samples. This peptide-mediated assay, called PeSHHA, combines a robust DNA-based signaling mechanism with the high selectivity/specificity of peptide recognition elements to capture HIV-1 antibodies. Given the versatility and modularity of PNA as a

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linker joining peptide antigens with DNA strands, we believe that PeSHHA may be readily adaptable for the detection of any specific antibody for which we possess a known peptide epitope.[27−29] We also showed that PeSHHA conducted on nanostructured electrodes is quantitative within a dynamic range (1−100 nM) that enables the detection of clinically relevant antibodies.

PeSHHA represents a significant improvement when compared to other similar technologies for the detection of human antibodies such as ELISA and Western blot. It is a rapid one-step technique that does not require specialized technicians and sophisticated laboratory facilities. The ability to generate readout signal 10 times larger than other electrochemical biosensors for the detection of antibodies[18,34,35] and execute a one-step reagent-less process in undiluted blood plasma without signal drift or decay[36,37] makes it an ideal candidate for adaptation into a portable diagnostic device.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b00648.

Materials, electrode fabrication, estimation of the optimal concentrations of signaling, capturing and recognition DNA/PNA constructs, and PeSHHA response with shorter signaling DNA strands and on the surface of flat electrodes (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: shana.kelley@utoronto.ca.

*E-mail: a.vallee-belisle@umontreal.ca.

ORCID

Sahar Sadat Mahshid: 0000-0002-0834-8787

Sara Mahshid: 0000-0003-4203-819X

Alexis Vallée-Bélisle: 0000-0002-5009-7715

Shana O. Kelley: 0000-0003-3360-5359

Notes

The authors declare no competing financial interest.

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