analytical chemistry

Article

Cite This: Anal. Chem. 2017, 89, 11753-11757

pubs.acs.org/ac

Detection of Amphipathic Viral Peptide on Screen-Printed Electrodes by Liposome Rupture Impact Voltammetry

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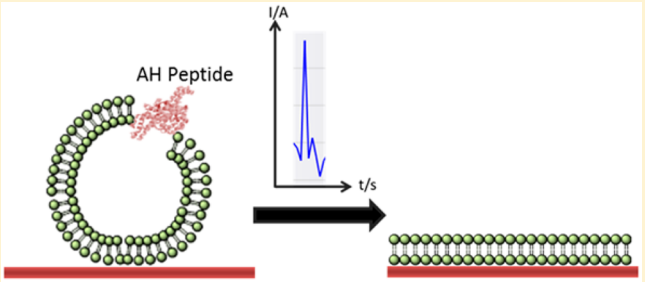
ABSTRACT: Detection of infectious viruses and disease biomarkers is of utmost importance in clinical screening for effective identification and treatment of diseases. We demonstrate here the use of liposome rupture impact voltammetry for the qualitative detection of model amphipathic viral peptide on a screen-printed electrode. This novel, proof-of-concept method was proposed for the quick and reliable detection of viruses by nonfaradaic liposome rupture impact voltammetry with the aid of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine liposomes. This provides an avenue for the development of future on-site, point-of-care detection devices for medical and biological applications.



Impact electrochemistry is a developing field which enables the study and detection of individual nanoparticles with high accuracies.[1] The bombardment of the nanoparticles onto the electrode surface at preset potentials will generate electrochemical signals which can then be processed and analyzed. Upon the striking of a particle on an electrode surface at a fixed potential, it undergoes redox reactions with the exchange of electrons which corresponds to a transfer of charge. This leads to spikes forming as the electrochemical signal. The first reported observations of impact electrochemistry were on colloidal microparticles by Micka using polarography in the 1950s[2−4] which were later explored by Heyrovsky with his studies on colloidal SnO_2 and TiO_2.[5−8] Ever since then, there has been an increasing interest and research conducted on the study of different colloidal systems and nanoparticles.[9−12] More recently, soft particles such as cells,[13] liposomes,[14−17] vesicles,[18] micelles,[19] and nanodroplets[20,21] have been studied to analyze cell exocytosis events and show potential applications in targeted cargo delivery. The signals generated from these soft particles could be from a faradaic process from the redox reactions brought about by mediators loaded into the particles or a nonfaradaic process as a result of charge transfer brought about by capacitive effect of the unloaded particles. Zutic et al. showed the interactions between phytoplankton,[22] oil droplets,[23] and phospholipid vesicles[24] at mercury electrode which generated capacitive signals due to the adhesion of the substances onto the electrode surface upon rupture or adhesion. Several similar techniques have also been developed which allow for the study of cell content[25] and exocytosis events[26] such as patch amperometry[27,28] and electrochemical cytometry.[29−32] The use of microfluidics with such techniques was successfully demonstrated with high accuracies and selectivity.[33,34]

Cho and co-workers discovered that a synthetic peptide corresponding to the amphipathic α-helix (AH) of the N-terminus of the NS5A protein of HCV[35] is able to cause lipid vesicle rupture by destabilizing the lipid bilayer of the vesicle,[36] resulting in the formation of planar bilayers onto solid surfaces such as gold and titanium dioxide, as verified by atomic force microscopy, localized surface plasmon resonance, and other surface-sensitive measurement techniques.[37−39] By making use of the rupturing capabilities of the AH peptide, we aim to establish a new methodology for the qualitative detection of the model viral peptide in a rapid and efficient approach in comparison to techniques currently available such as fluorescence spectroscopy and quartz crystal microbalance-dissipation (QCM-D).[40] This technique may be useful for future application as it is noninvasive and requires minimal sample and a simple setup. These factors are highly desirable for future on-site detection devices. Hence, we investigated the possibility of detecting AH peptide using rupture impact voltammetry on screen printed electrodes. This work can potentially be further extrapolated and applied as a sensing platform for the detection of agents which cause cell lysis or vesicle ruptures.

Received: August 16, 2017
Accepted: October 10, 2017
Published: October 23, 2017

DOI: 10.1021/acs.analchem.7b03305
Anal. Chem. 2017, 89, 11753−11757

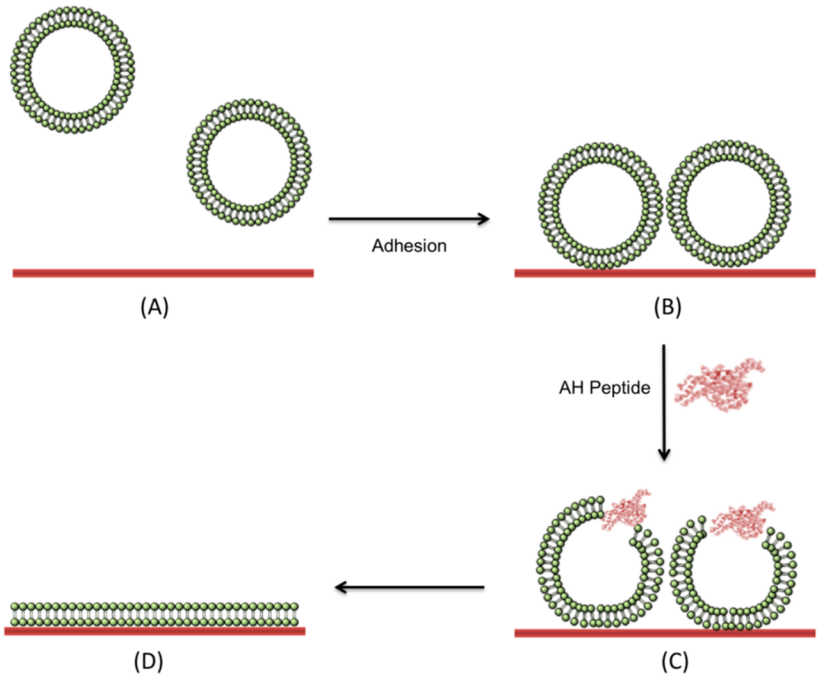


Figure 1. Illustration on the rupture and spreading of DOPC liposomes on the SPE surface: (A) migration and (B) adhesion of liposomes onto electrode surface, (C) rupturing of liposomes in the presence of AH peptide, and (D) the formation of lipid bilayer upon rupture. Figures are not drawn to scale.

EXPERIMENTAL SECTION

Materials. Potassium phosphate dibasic, sodium phosphate monobasic, sodium chloride, and potassium chloride were obtained from Sigma–Aldrich (Singapore). High quality water purified in a Milli-Q system (Millipore, MA, United States) with a resistivity of 18 MΩ cm was used.

The AH peptide (>95% purity) was produced using solid-phase peptide synthesis by Anaspec Corporation (San Jose, CA, United States); it has molecular weight of 3284.5 Da and the following amino acid sequence: H-Ser-Gly-Ser-Trp-Leu-Arg-Asp-Val-Trp-Asp-Trp-Ile-Cys-Thr-Val-Leu-Thr-Asp-Phe-Lys-Thr-Trp-Leu-Gln-Ser-Lys-Leu-Asp-Tyr-Lys-Asp-NH_2.

Screen-printed electrodes (SPEs) of 3 mm working electrode diameter were obtained from Zensor (Taiwan).

Electrochemical Measurements. Electrochemical measurements were conducted using a μAutolab Type III electrochemical analyzer (Eco Chemie, The Netherlands) controlled by NOVA 1.9 software (Eco Chemie) at room temperature (25 °C) by using a three-electrode configuration. Cyclic voltammetry (CV) experiments were performed at a scan rate of 100 mV s^{-1}. Chronoamperometry measurements were recorded at 10 ms intervals.

Liposome Preparation. DOPC liposomes were prepared at a nominal concentration of 5 mg/mL in PBS buffer by the thin-film hydration and extrusion method, and a MiniExtruder apparatus (Avanti Polar Lipids, Alabaster, AL, United States) was used to repeatedly pass the liposomes through a track-etched polycarbonate membrane containing 50 nm diameter pores. The average diameter of the extruded liposomes was around 75 nm, as determined by dynamic light scattering.

Procedure. Thirty-five microliters of 0.2 mg/mL liposome solution was dropped onto the SPE surface and allowed to set for the liposomes to adhere on the working electrode surface. Chronoamperometric scans were performed for 1000 s before AH peptide or PBS was introduced. Movement around the setup was minimized during the scans to ensure signals generated were not a result of external vibrations.

RESULTS AND DISCUSSION

From previous studies performed, Cho and co-workers discovered that the liposomes will adhere and form a single layer on the surface over a period of time.[41] Upon introduction of amphipathic α-helix (AH) peptide, it perturbs and destabilizes the lipids of the liposomes, which results in deformation and a rupture activity.[42] The lipids will form a bilayer on the electrode surface after rupturing.[43] Figure 1 shows an illustration of the rupture and spreading of liposomes upon introduction of AH peptide.

Cyclic voltammetry (CV) was first performed with SPE as a preliminary study to identify any inherent electrochemical reactions of the DOPC liposomes (Figure 2). This is to ensure

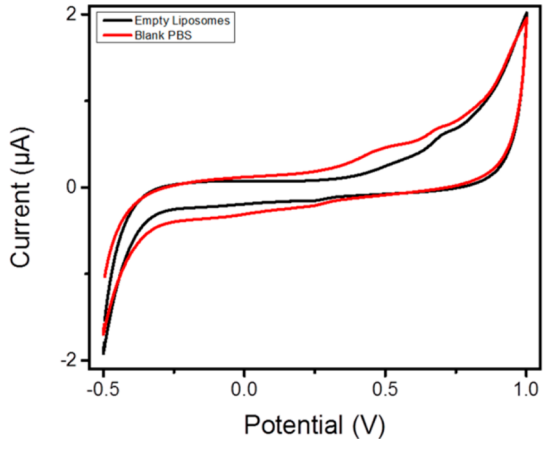


Figure 2. Cyclic voltammograms of blank PBS (red line) and DOPC liposome modified screen-printed electrode in PBS (black line).

DOI: 10.1021/acs.analchem.7b03305
Anal. Chem. 2017, 89, 11753–11757

that the signals obtained during chronoamperometry measurements will be a result of the AH peptide rupturing the DOPC liposomes and not from the inherent oxidation/reduction of the DOPC lipids themselves. The signal obtained will be a result of nonfaradaic charge transfer upon the formation of the lipid bilayer on the electrode surface upon rupture by AH peptide. In a nonfaradaic process, upon the formation of the lipid bilayer on the electrode surface, charge transfer takes place as a result of a change in capacitance. As such, a signal output will be detected. A background scan with blank phosphate buffer (PBS) was first performed (red line) in the absence of DOPC liposomes. The DOPC liposomes were then drop-coated onto the SPE surface and left to adhere before CV was performed in phosphate buffer (black line). Upon comparing the voltammograms from blank buffer and liposomes-modified SPE, the voltammograms obtained were very similar in their shapes and features. There was an absence of additional peaks in the voltammogram for the liposome-modified SPE which signified the absence of any oxidation/reduction reactions taking place. It can thus be deduced that the liposomes are not electroactive due to the absence of additional peaks in the voltammograms obtained. Any signals obtained during chronoamperometry should come from nonfaradaic capacitive charge transfer upon formation of the lipid bilayer on the electrode surface and not from the oxidation/reduction of the DOPC lipids of the liposomes (Figure 1D). As such, the spikes obtained will be from the rupturing of liposomes in the presence of AH peptide. An applied potential of +0.35 V was chosen for subsequent chronoamperometry experiments.

We next investigated the effect of AH peptide on the rupture events. The DOPC liposomes were filled with ferro/ferricyanide solution and subsequently drop-coated onto the electrode surface for adhesion to take place. AH peptide was then injected and allowed to interact with the immobilized liposomes for 20 min. The rupturing of the liposomes results in the leaking of ferro/ferricyanide solution into the buffer and will be detected by voltammetry. The response signals were subsequently analyzed with cyclic voltammetry (not shown). The signal response percentage (R%) was calculated to compare the effects before (R_b) and 20 min after (R_a) introduction of AH peptide (eq 1). A higher percentage was more favorable, which suggested better rupture activity taking place and possibly greater signal in chronoamperometry.

$$R(\%) = \frac{(R_b - R_a)}{R_b} \times 100 \quad (1)$$

Two different AH peptide concentrations were studied: 26 and 100 μM. The introduction of AH peptide at both concentrations gave positive signal response as compared to the absence of AH peptide in the system, which gave a negative R% value (Figure 3). The importance of AH peptide in the system is apparent from this observation, where it induces liposome rupture events. AH concentration of 26 μM was chosen for the preceding experiments as it gave the optimum R% and requires minimal peptide for the induced liposome rupturing to occur. This is an important parameter of consideration for effective detection through liposome rupture impact voltammetry.

The analytical performance was subsequently examined using chronoamperometry with injection of 26 μM AH peptide or PBS as control. The stability of the liposomes at the applied potential was analyzed over a period of 1000 s. The stabilization

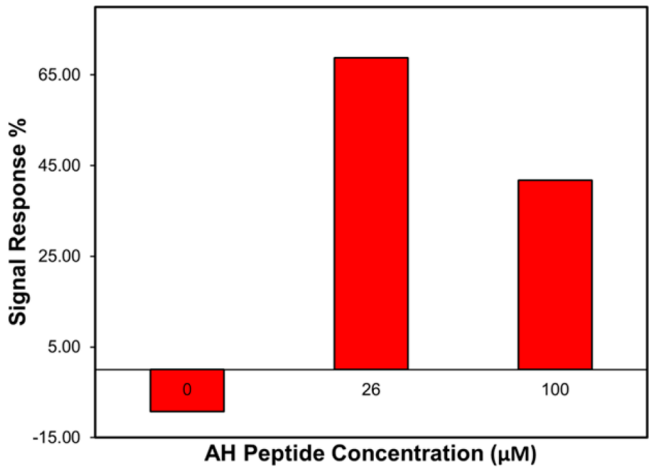


Figure 3. Effect of variable AH peptide concentration on the change in signal response.

process was performed to ascertain the stability of the liposomes at the applied potentials, and they do not rupture spontaneously. This process also allows for the background signal to be determined, which will be used for further quantification steps. From Figure 4A, minimal spikes were formed during the stabilization period apart from the signal generated by electrical background noise. The average background spike height (δ) was calculated during the 1000 s stabilization period before the introduction of AH peptide. This value serves as a reference for quantification calculations where spikes only with heights twice this value will be considered. This will also ensure the correct identification of signals in the measurements obtained. Chronoamperometry was carried out on the SPE at a standing potential of +0.35 V (vs. Ag/AgCl) to observe the rupturing of the liposomes (Figure 4A). PBS was injected as a control. From Figure 4A, a significant difference in chronoamperometric signals was observed upon injection of PBS and AH peptide. Spikes with higher frequencies and intensities were obtained in the presence of AH peptide, while no apparent spikes were formed when PBS was injected. The experiment was repeated three times, and similar observations were made (not shown). As previously mentioned, the spikes obtained were a result of nonfaradaic rupture voltammetry process. The formation of the lipid bilayer onto the electrode surface upon rupture of the DOPC liposomes results in the transfer of charge between the electrode surface and lipid bilayer. The results obtained demonstrate the ability of AH peptide to rupture the adhered liposomes, and the events can be detected by rupture impact voltammetry. The spikes obtained from the chronoamperometric measurements were then collated and analyzed accordingly. Only spikes with amplitudes twice that of the background signal (δ) were considered and were categorized according to their amplitudes (Figure 4B). From the distribution, it shows that more than half of the spikes obtained had amplitudes twice that of the background noise.

In addition to adding AH peptide to induce liposome rupture, it is also possible to trigger liposome rupture by other means such as incorporating divalent cations[44] or changing solution pH.[45] The general measurement principles of liposome rupture impact voltammetry provide a robust method to detect liposome rupture events across such possibilities, with broad potential for diagnostic and sensing applications.

DOI: 10.1021/acs.analchem.7b03305
Anal. Chem. 2017, 89, 11753−11757

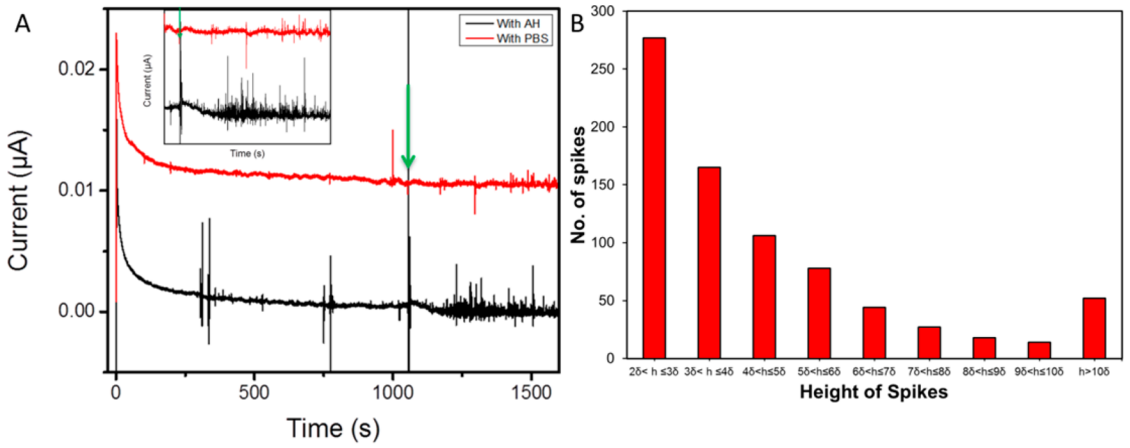


Figure 4. (A) Chronoamperometric measurements of the signals obtained. Inset shows enlarged view of spikes obtained. The scans are offset for clarity. Arrows indicate the point of injection of PBS and AH peptide accordingly. (B) Summary of spike count of liposome ruptures upon addition of AH peptide.

CONCLUSION

We demonstrated a novel proof-of-concept application of liposome rupture impact voltammetry with the aid of AH peptide. It has the potential to be an effective on-site, point-of-care detection device for preliminary detection of viral peptides in biological samples. It may prove to be more effective and requires fewer processing procedures as compared to the conventional methods presently available. It could also be extended for the detection of other biological targets such as bacteria and unicellular organisms.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

M.P. acknowledges support from Tier 1 (99/13) from Singapore Ministry of Education. We thank J.A.J and N.-J. C. for providing the DOPC liposomes and AH peptide for the project.

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