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Sensitive cell apoptosis assay based on caspase-3 activity detection with graphene oxide-assisted electrochemical signal amplification

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ABSTRACT

This paper reports a novel approach for the simple assays of cell apoptosis using electrochemical technique. In this study, caspase-3 activity, which was detected with differential plus voltammetry (DPV) as an alternative to conventional spectrometry approach, was employed as an indicator of cell apoptosis and, while an acetylated peptide Ac-GGHDEVDHGGGC was used as the blocked substrate. In the presence of casepase-3, the hydrolysis of blocked peptide might release active amine groups, which could covalently conjugate with graphene oxide. Therefore, electroactive methylene blue molecules could be further attached to the electrode surface through π–π stacking and electrostatic interactions. Using this proposed new method, a very sensitive detection of caspase-3 could be achieved with a low detection limit of 0.06 pg/mL, and a new method for sensitive detection of cell apoptosis was developed. Moreover, we have successfully used this new method to detect cell apoptosis with human pulmonary carcinoma A549 cell after apoptosis inducing.

1. Introduction

Apoptosis, the so-called programmed cell death, is a highly regulated biological process that is associated with the cell fate. It conducts to eliminate the unwanted cells from tissues during normal physiological metabolism, help develop and function the immune system and remodel tissues. Abnormality of apoptosis regulation process is involved with pathogeny and chemotherapy response for a variety of diseases such as cancer, neurological disorders, atherosclerosis, myocardial infarction, and autoimmune diseases (Favaloro et al., 2012; Haunstetter and Izumo, 1998). Therefore, detection of apoptosis is critically essential not only for biological science but also for the evaluation of disease progression, medical treatment, pharmaceutical development, and food safety management (Brunelle and Zhang, 2010; Carson and Ribeiro, 1993; Pan et al., 2008), etc. So, many of the proteins that are critical to apoptosis have been identified. Among them, the caspase family (cysteine-dependent, aspartate-specific proteinases) is revealed to play an important role in cellular apoptosis. They arise in apoptotic cells and can be the critical key to identify and determinate apoptosis in both viable cells and cell lysate (Boatright and Salvesen, 2003; Li and Yuan, 2008; Shi, 2002). Caspase-3 is especially important because it takes part in both intrinsic and extrinsic apoptosis pathways. Therefore, caspase-3 is the most used target to detect apoptosis (Du et al., 2004; Porter and Janicke, 1999). Based on the fact that the tetra-peptide substrate DEVD (Asp-Glu-Val-Asp) can be specifically recognized and cleaved at the N-terminal of the motif by caspase-3, several fluorometric and colorimetric assaying methods have been established (Boeneman et al., 2009; Jun et al., 2009; Pan et al., 2012; H. Wang et al., 2011; Zhou et al., 2014), and commercially kits are now available for caspase-3 based apoptosis assays. Nevertheless, applications of these photometric approaches may be hindered because they require complicated laborious extraction process to maintain the expected specificity and sensitivity (Takano et al., 2014). Additionally, these photometric approaches are confronted with insufficient sensitivity due to the technical limitation of themselves. Our group have reported a novel caspase-3 sensor with excellent sensitivity by using surface plasmon resonance (SPR) technique (Chen et al., 2013). However, it still has problems in terms of the cost of equipment and specially trained operator.

As an alternative technique to photometry, electrochemical approach potentially provides a more attractive method for apoptosis assessment. Electrochemical detection of noble metal ions (Bojdi et al., 2014a, 2014b), small bioactive molecules

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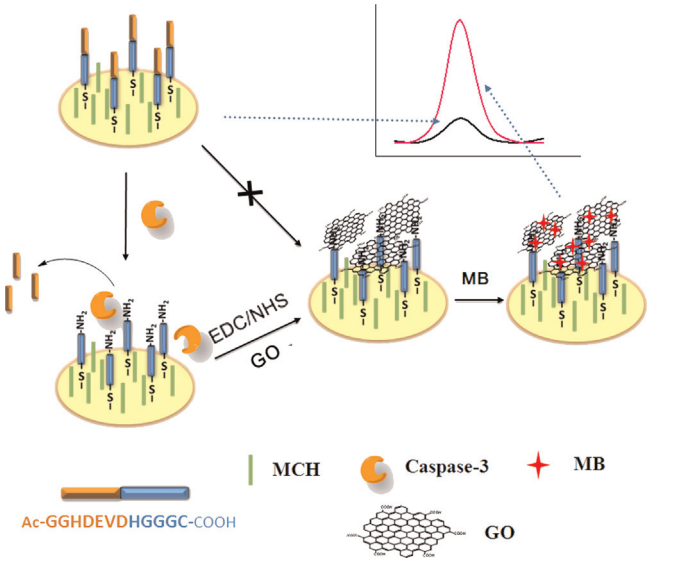

Scheme 1. Schematic illustration of the modified electrode surface and the electrochemical strategy for sensing caspase 3 activity.

(Hosseini et al., 2014; Zhao et al., 2013), DNA (Cao et al., 2012; Li et al., 2011), enzymes (J. Wang et al., 2011; Yang et al., 2012) and cancer marker proteins (Elshafey et al., 2013; Li et al., 2014; Song et al., 2014; Zhao et al., 2012) have been reported with ultrahigh specificity and sensitivity. Electrochemical method is also cost efficient and easier to be miniaturized in a compact device, so it has received great interests. However, there are only a limited number of reports that have been published on electrochemical approach for apoptosis assays (Takano et al., 2014; Zhang et al., 2011). In this work, we have proposed a simple and efficient, label-free and highly sensitive electrochemical method for caspase-3 detection. Based on the detection of caspase-3, an electrochemical method for the apoptosis assay is developed. As presented in Scheme 1, an N-terminal blocked (acetylated) peptide Ac-Gly-Gly-His-Asp-Glu-Val-Asp-His-Gly-Gly-Gly-Cys is employed in this work as the substrate. After being covalently modified onto the gold electrode surface, the blocked peptide cannot be attached with graphene oxide (GO) through the formation of amide bonds due to the unavailable amine groups. However, in the presence of caspase-3, the cleavage of peptide results in the exposure of a new free N-terminal amine group after washing off the acetylated pieces. Following the activation by the EDC/NHS chemistry, GO can be covalently attached to the electrode. As the caspase-3 concentration rising, more amine groups are available for the EDC/NHS chemistry, thus more GO can bind to the electrode. Consequently, more electroactive molecule methylene blue (MB) can be assembled on GO through π–π stacking and electrostatic interactions. Therefore, electrochemical signal can be obtained due to the redox reaction of MB. In the meantime, signal amplification is achieved due to the large surface to volume ratio of GO and the absorption of numerous MB. Our designed system can give a very sensitive detection of caspase-3 with a low detection limit of 0.06 pg/mL.

2. Material and methods

2.1. Materials

Recombinant human caspase-3 (*E. coli*-derived) was purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Bovine serum albumin (BSA), thrombin, trypsin, carboxypeptidase, 6-hydroxy-1-hexanethiol (MCH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), DL-dithiothreitol (DTT), GO dispersion (2 mg/mL) and methylene blue were purchased from Sigma-Aldrich (St. Louis, MO, USA). The designed peptide (Ac-Gly-Gly-His-Asp-Glu-Val-Asp-His-Gly-Gly-Gly-Cys, > 95%) was synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). An Apoptosis Inducers Kit and cell lysis solution were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Other chemical reagents were all of analytical-grade. 25 mM HEPES (pH 7.5) solution was used to prepare caspase-3 stock solution. The peptide was dissolved in 20 mM HEPES (pH 6.0) with 10 mM TCEP with a stock concentration of 20 μM. The washing buffer was 20 mM HEPES containing 0.05% Tween-20, pH 6.0. All solutions were prepared with deionized water purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of 18 MΩ cm.

2.2. Electrode preparation and modification

The gold electrode (3.0 mm diameter) was firstly cleaned with piranha solution (concentrated H_2SO_4: 30% H_2O_2=3: 1) for 5 min to eliminate the adsorbed material and then rinsed with deionized water. After that, the electrode was polished carefully with 1, 0.3 and 0.05 μm alumina slurry, respectively. The residual alumina powder was removed by sonicating the electrode sequentially in both ethanol and deionized water sequentially. Afterward, the electrode was cleaned electrochemically to remove any remaining impurities with 0.5 M H_2SO_4. Finally, the electrode was dried by purging with nitrogen.

To prepare SAM with substrate peptide, the gold electrode was firstly incubated with 100 μL of peptide solution (5 μM, in 20 mM HEPES, containing 10 mM TCEP, pH 6.0) for 16 h at 4 °C. TCEP was used here to prevent terminal cysteine of the substrate peptides from forming disulfide bonds. The remaining peptide was removed by rinsing thoroughly with the washing buffer for 3 times. The peptide-modified gold electrode was then immersed in 100 μL of MCH solution (1 mM) for 30 min to obtain the well-aligned peptide monolayers.

2.3. Cell culture and inducing apoptosis

The human pulmonary carcinoma A549 cell was maintained in DMEM (penicillin G 80 U/mL, streptomycin 80 μg/mL) supplemented with 10% fetal bovine serum at 37 °C in a humid atmosphere of 5% CO_2 for 2 days, then treated with Trypsin–EDTA digestion solutions and suspended with different concentrations of 2×10^5 and 1×10^6 cells per mL. Afterward, A549 cell with different concentrations were respectively treated with the Apoptosis Inducers Kit (1 μL/mL) to induce apoptosis for 6 h.

2.4. Caspase-3 digestion and cell lysate treatment

Caspase-3 catalyzed cleavage was performed by adapting the manufacturer's protocol. Briefly, the caspase-3 stock solution was diluted with desired concentrations in the reaction buffer solution (25 mM HEPES containing 10 mM DTT, 0.1% (w/v) CHAPS, pH 7.5). The substrate peptide-modified electrode was then immersed in 100 μL of caspase-3 solution for 30 min at 37 °C in a humidified chamber. After that, the reaction was terminated by rinsing the electrode thoroughly with the washing buffer. And for the control groups BSA, thrombin, trypsin and carboxypeptidase were employed instead of caspase-3 following the same procedures.

As for the cell lysate treatment, after inducing apoptosis for 6 h the cells were harvested, washed twice with phosphate buffer solution (PBS), and lysed in the lysis buffer (25 mM HEPES buffer,

pH 7.5, 10% cell lysis solution containing 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% NP40 and 1 mL of a 0.5 mM protease inhibitor) accompanied sonication in ice water for 15 min. The lysates were centrifuged at 13,000 rpm for 15 min at 4 °C, and the supernatant was collected and used immediately for apoptosis assays.

2.5. GO conjugation and MB absorption

After rinsing with washing buffer, the caspase-3 treated electrode was immediately immersed into 100 μL GO solutions (0.4 mg/mL) for 30 min. The graphene oxide was pre-activated by EDC/NHS chemistry (40 mM/10 mM) for 20 min. The unconjugated GO was removed by rinsing thoroughly with the washing buffer for 5 min. Then 50 μL 100 μM MB solutions (20 mM HEPES, 0.1% Tween-20, pH 6.0) was drop-casted on the electrode for 30 min at room temperature.

2.6. Electrochemical measurements

The electrochemical measurements by using cyclic voltammetry, differential pulse voltammetry and electrochemical impedance spectroscopy (EIS) were performed on a model 660a Electrochemical Analyzer (CH Instruments) with a conventional three-electrode cell at room temperature in the stirred condition. A saturated calomel electrode (SCE) was used as the reference electrode, and a platinum wire electrode as the counter electrode. Cyclic voltammograms (CVs) were performed over a scan range from 0.1 to −0.3 V at the scan rate of 100 mV/s. Differential pulse voltammograms (DPVs) were obtained by scanning from 0.1 to −0.3 V with a step potential of 4 mV, pulse amplitude of 50 mV, and a pulse period of 0.2 s at a scan rate of 10 mV/s. EIS data was obtained within the frequency range from 0.1 Hz to 100 kHz, the amplitude was 5 mV, and the direct current potential was limited at the formal potential of the redox pair $[Fe(CN)6]^{3-/4-}$ (0.224 V versus SCE). The electrolyte solution for EIS measurements was 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ solution containing 0.1 M KCl. All electrolyte solutions were thoroughly purged with high purity nitrogen over 15 min before the measurements, to avoid the interference from the dissolved oxygen.

2.7. Atomic force microscopy (AFM) characterization

The AFM images were obtained using an Agilent 5500 instrument (Santa Clara, CA). The tapping mode was conducted with a scan range of $10 \times 10\ \mu m^2$. A microscope cover glass (Matsunami, Japan) was used for the substrate chip. Au film (about 50 nm thickness) was deposited on the cover glass by the sputter coating system (E5000, Polaron Co., U.K.) under conditions of 2.0×10^{-2} mbar and 20 mA for 18 s.

3. Results and discussions

3.1. Characterization of peptide modification and enzyme reaction on the gold electrode

Scheme 1 illustrates the principle of this approach for electrochemical detection caspase-3 activity. The substrate peptide (Ac-GGHDEVDHGGGC) contains the cleavage sequence of DEVD. A C-terminal cysteine was employed to immobilize substrate peptide on the gold surface self-assembly *via* the covalent bond between Au and thiol group of cysteine as described everywhere followed backfill with MCH. The N-terminal of the substrate peptide was particularly blocked with acetyl. In the absence of caspase-3, the peptide remains to be blocked. Therefore, there are no free-amino groups used to covalently conjugate with GO through the EDC/NHS chemistry. However, if caspase-3 is added to the system, the blocked substrate peptide is hydrolyzed into a shortened peptide with a free N-terminal amino group. In this case, GO is covalently immobilized onto the surface of electrode through the amidation which is activated by the EDC/NHS. Sequentially, the added electrochemical active molecule MB is attached to electrode through the π–π stacking and electrostatic interactions between GO and MB (D. Wang et al., 2011; Yang et al., 2011). Then, electrochemical measurements are conducted for the qualitative and quantitative assay of caspase-3 activities. A "signal-on" approach is achieved for detection of apoptosis.

To prove the principle of the detection system, we have firstly checked the interface properties of the substrate peptide the gold modified electrode. Fig. 1A shows the Nyquist plots of EIS for the electrode at different modified stages. The impedance spectrum contains a semicircular portion at higher frequencies and a linear part at lower frequencies. The increase of the semicircle diameter reflects the increase in the interfacial charge transfer resistance. As shown in Fig. 1A, the impedance spectrum of the bare gold electrode includes almost no semicircle domain. After the immobilization of substrate peptide (pI ≈ 4.36), a semicircle can be observed due to the formation of a negatively charged interface at the measurement condition of pH 7.4, which may electrostatically repel the negatively charged redox species $[Fe(CN)_6]^{3-/4-}$. When the electrode is further treated with MCH, the diameter of the semicircle continues to be increased, indicating the further formation of significant steric hindrance between $[Fe(CN)_6]^{3-/4-}$ and electrode. This may result from the formation SAM of peptide after backfilled with MCH. Besides, Fig. S1 depicts the ATR-IR spectra

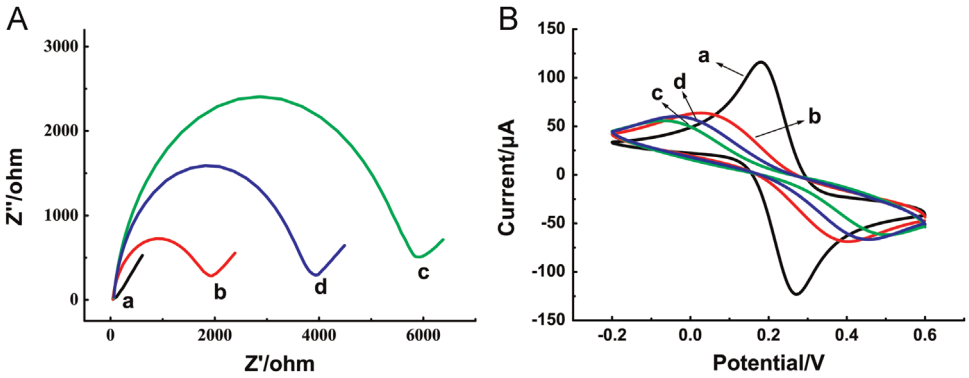

Fig. 1. (A) Nyquist plots and (B) CVs corresponding to ((a) black line) bare gold electrode, ((b) red line) peptide modified electrode, ((c) green line) peptide modified electrode after blocking with MCH, ((d) blue line) the electrode treated with caspase-3 (30 pg/mL). Electrochemical species: 5 mM $[Fe(CN)6]^{3-/4-}$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

before and after the substrate peptide immobilized onto the electrode. The characteristic absorption peaks of amide I band, attributed to the C=O stretching vibration of the peptide backbone, and amide II band, assigned to the coupling of the N–H in-plane bending and C–N stretching vibration of the peptide backbone, was evidenced by the peaks at 1647.1 and 1575.8 cm^{-1}, respectively (Yang et al., 2014).

Meanwhile, it should be mentioned that MCH was used to occupy the spare space on the electrode surface and induce peptide chain to orientate orderly, also avoid the nonspecific adsorption of caspase-3 onto the gold electrode. After treated with caspase-3, the diameter of the semicircle has a decrease which indicates the cleavage of substrate peptide. The decrease may due to the remaining peptide (HGGGC) with a higher pI ≈ 7.36 which is nearly neural at the measurement condition, and also the weakened steric hindrance after washing off the hydrolyzed fragment (Ac-GGHDEVD). Both allow more $[Fe(CN)_6]^{3-/4-}$ molecules to access the electrode surface. Additionally, CVs are also conducted. As shown in Fig. 1B, prior to the immobilization of substrate peptide the bare gold electrode presented a well-defined redox peak of $[Fe(CN)_6]^{3-/4-}$ species. Followed by modified with peptide and further backfilled with MCH, a continuous shift of the redox peak and decrease in peak value are observed. These can be also caused by the electron transfer hindrance between $[Fe(CN)_6]^{3-/4-}$ and electrode which accounts for the formation of a negatively charged interface and the significant steric hindrance. To the contrary, a slight recovery of the redox peak is observed when treated with caspase-3. The recovery also reveals the cleavage of substrate peptide. All these impedance spectra and CVs are coherent as predicted.

After treated with caspase-3 and washing off the blocked cleavage fragment, the electrode is functionalized with amino groups. And then the electrode is immersed into GO suspensions which contain EDC and NHS with a molar ratio of 4:1. Therefore, GO is covalently immobilized onto the electrode through the EDC/NHS chemistry. The AFM pictures (Fig. S2) show the surface morphology of the homemade chip at different stages. As shown in Fig. S2A, the bare gold film has a relatively smooth morphology. Meanwhile, the peptide modified chip (Fig. S2B) shows lots of highlight spots distributed in the full scale. The height profiles reveal an increased height from 1.0 nm to 4.0 nm after the immobilization of substrate peptide, indicating the covalent immobilized peptide. In the case of caspase-3 treated chip, after GO conjugation some highlight stripes can be observed in Fig. S2C, which height rise to 15 nm and may be caused by the folded GO after dried on the surface. This visually reveals that GO is successfully conjugated onto the surface of gold film.

3.2. Graphene oxide-assisted electrochemical signal amplification

Fig. 2 shows the CVs for the electrode before and after MB absorption. For the treatment of caspase-3 (Fig. 2Aa) and GO conjugation (Fig. 2Ab), there are no redox peak detected in 20 mM Tris buffer (pH 7.4). However, a characteristic redox peak is observed after MB absorption and washed off three times with the washing buffer (Fig. 2Ac). To enhance the effectiveness of our design, a more sensitive electrochemical technique, differential pulse voltammetry, has been utilized to obtain the direct electrochemical signal for the detection of caspase-3. Fig. 2Ba depicts the differential pulse voltammogram (DPV) obtained upon analyzing 30 pg/mL caspase-3. Compared to the control group with no caspase-3 treatment (Fig. 2Bb), an apparently increased signal can be observed due to the cleavage of blocked peptide; thus, GO is conjugated to the electrode through the new exposed amino groups. Consequently, abundant MB molecules are allowed to access the surface of electrode and electrochemical measurement is available. In contrast, in the presence of caspase-3 but without conjugation with GO (Fig. 2Bc), the DPV signal shows no change to the control group, indicating that the conjugation of GO to electrode is essential not only for the sensitive detection but also for the caspase-3 triggered "signal-on" strategy of the detection.

3.3. Optimization of sensing conditions

Then several important aspects involved in the detection were examined. Firstly, considering the caspase-3 triggered "signal-on" strategy is based on cleavage of the blocked substrate peptide, we have checked the required minimum caspase-3 hydrolyzation time by measuring DPVs in the presence of 30 pg/mL caspase-3. Experimental data (Fig. S3) reveals that at least a 30-min incubation time is required to get equilibrium. So, 30 min is chosen for the caspase-3 hydrolyzation time in this work. Secondly, as the sensitive detection would be relied on the conjugation of GO to electrode, we have explored the effects of different concentrations of GO. As shown in Fig. S4A, a higher concentration of GO in the range from 0.05 to 0.3 mg/mL would be better for the sensitivity. Nevertheless, considering that a mild excess amount of GO is highly desired and 0.4 mg/mL is enough for the sensitive detection, we have chosen a concentration of 0.4 mg/mL for the following detection. Thirdly, because the electrochemical signal is directly related to the capacity of MB molecules attached to the electrode, the desired concentration of MB was examined as well. Fig. S4B shows the calibration curve of DPVs at different concentrations of MB. A saturated platform is observed at the concentration of 50 μM. There is no further increase even at a doubled

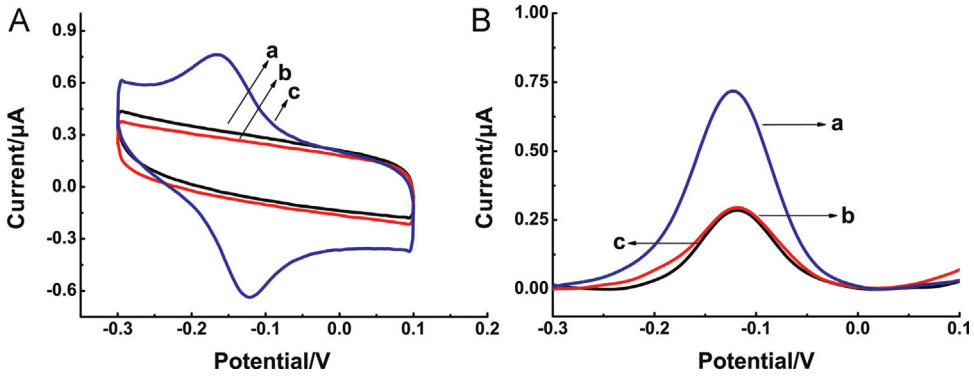

Fig. 2. (A) CVs corresponding to (a) the peptide modified electrode after treated with caspase-3 (30 pg/mL), (b) caspase-3 treated electrode after GO (0.4 mg/mL) conjugation and (c) GO conjugated electrode after MB (50 μM) absorption. (B) DPVs corresponding to (a) caspase-3 (30 pg/mL) treated and GO (0.4 mg/mL) conjugated electrode, (b) caspase-3 (30 pg/mL) treated and without GO conjugated electrode, (c) the control electrode without caspase-3 treatment. Electrolyte: 20 mM HEPES (pH 7.0). Scan rate: 0.1 V/s.

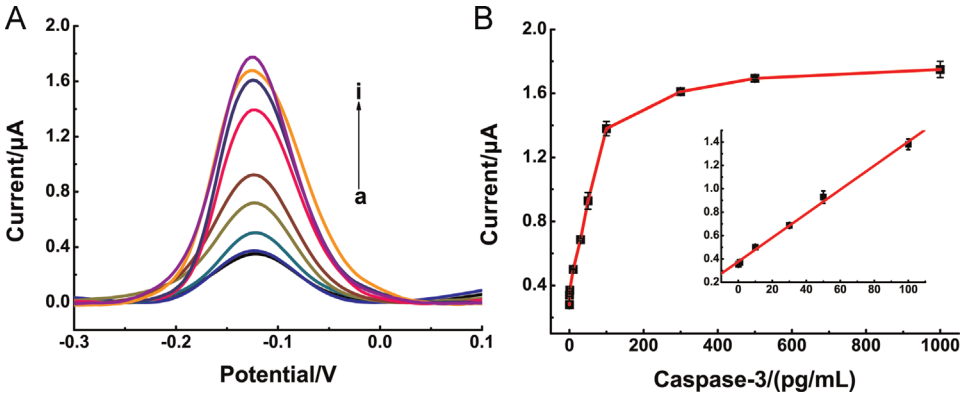

Fig. 3. (A) DPVs for the measurements of caspase-3 at different concentrations (a) 0.1, (b) 1.0, (c) 10, (d) 30, (e) 50, (f) 100, (g) 300, (h) 500, and (i) 1000 pg/mL. (B) The corresponding calibrated curve of different concentrations of caspase-3 from 0.1–1000 pg/mL. The intert shows the linear relationship between the DPV peak current and the caspase-3 concentration. Error bars represent standard deviations of measurements ($n=3$).

concentration of MB. So, 50 μM is employed as a constant concentration of MB and applied in the experiments. Besides, the calculated RSD of the background signal obtained from control groups (0 pg/mL caspase-3) is 1.24% ($n=5$), indicating that the signal is highly reproducible and thus can hardly affect the detection system.

3.4. Sensor's sensitivity and selectivity

Fig. 3A displays the DPVs recorded upon analyzing caspase-3 at different concentrations. The DPV peak increases in response to caspase-3 with a mounting concentration within the range from 0.1 to 1000 pg/mL. And a well linear response can be observed in the range of 0.1 ~ 100 pg/mL (Fig. 3B, insert). For each concentration, the measurement has been repeated at least for three times independently. An average coefficient of variation of 1.36% is obtained, indicating that the precision and reproducibility of this approach are highly acceptable. A low detection limit is found with 0.06 pg/mL (3 S/N), which is 10^3–10^5 times more sensitive compared to other reports (Table 1). Therefore, our designed approach shows excellent superiority due to its simplicity and sensitivity to some extent.

To evaluate the specificity of the detection method, several proteins such as thrombin, trypsin, carboxypeptidase and BSA have been explored. The DPV results depict that the responses of non-target proteins can only get the same level as which of the blank control (Fig. 4A), implying the high selectivity of our approach.

Table 1
Comparison of different caspase-3 sensing system.

Method	System	Detection limit (pg/mL)
Fluorescence	GO–peptide conjugates (H. Wang et al., 2011)	7272.7(0.4 nM)
	QDs-fluorescent protein (Boeneman et al., 2009)	363.6 (0.02 nM)
Absorption	DNAzyme–peptide conjugates (Zhou et al., 2014)	16181.8 (0.89 nM)
	Gold nanoparticles and peptide (Pan et al., 2012)	10000
Electrochemistry	DEVD-pNA (Takano et al., 2014)	1090.9 (0.01 U/mL)
	This study	0.06

3.5. Detection of caspase-3 in Human pulmonary carcinoma A549 cell

We have conducted real cell apoptosis assays employing our detection approach. For the measurement, human pulmonary carcinoma A549 cell at different concentrations were employed and treated with the Apoptosis Inducers Kit. The control group was examined as the same cell concentration expect for the treatment with Apoptosis Inducers Kit. As shown in Fig. 4B, the DPV peak of the experiment group is obviously higher than the control group, indicating that the system has a well response to the real cell apoptosis. With the cell number increased from 2×10^5 to 1×10^6 mL, the accompanying elevated signal of experiment group further verifies the practicability and effectiveness of the system. Consequentially, the expected cell apoptosis assays were realized with above approach.

4. Conclusions

In summary, we have proposed an electrochemical approach in this work for the detection of cell apoptosis based the caspase-3 triggered signal-on strategy with GO-assisted amplification. As the target protein, caspase-3 can be detected in a range of 0.1–100 pg/mL. A low detection limit of 0.06 pg/mL was obtained with our approach. It is 10^3–10^5 times more sensitive compared to other reports. Besides, our system also shows well response to the real samples, indicating its potential application. Furthermore, this approach has also demonstrated some other advantages. On one hand, the approach makes use of the specific cleavage triggered signal-on strategy and is highly resistant to non-target proteins, thus resulting in excellent specificity. On the other hand, because any target protein of peptidase with no free amino group in the binding sequence can be translated in principle to the study of the very commonly employed peptides, this approach may be extended to the assays of numerous targets. Certainly, there still are some issues that may limit the extended application of the proposed approach. For instance, this system may displays a false positive signal and broad noise background if the substrate peptide is highly charged positively or negatively, which will take the edge off the sensitivity and specificity of the detection. Nevertheless, with elaborate and optimized substrate and condition, this approach will be extended to the assays of more species of proteins in the future.

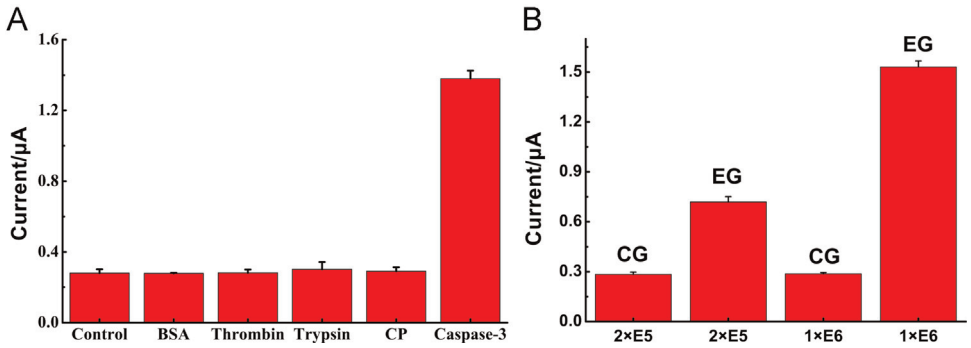

Fig. 4. (A) DPV peak currents responses of the assay for different proteins: (a) blank control without caspase-3, (b) 1 ng/mL BSA, (c) 1 ng/mL thrombin, (d) 1 ng/mL Trypsin, (e) 1 ng/mL carboxypeptidase and (F) 100 pg/mL caspase-3. Comparing to caspase-3 the other proteins with a 10-fold concentration get the same response as the blank control. (B) DPV peak currents response to the cell lysate which as real sample. The A549 cell concentration of 2×10^5 and 1×10^6 mL^{-1} were employed. Comparing to the control group (CG) the experiment group (EG) was treated with Apoptosis Inducers Kit. Error bars represent standard deviations of measurements ($n=3$).

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.02.007.

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