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Correspondence

Electrochemical Approach To Detect Apoptosis

Han Xiao,[†] Lei Liu,[†] Fanben Meng,[†] Junyi Huang,[‡] and Genxi Li*[,†]

Department of Biochemistry and National Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, P. R. China, and Laboratory of Biosensing Technology, School of Life Science, Shanghai University, Shanghai 200444, P. R. China

This paper reports an electrochemical approach for detection of apoptosis. Here we prepare a gold electrode modified with a helix peptide ferrocene (Fc)−GDGDEVDGC. Fc is used as an electroactive reporter and the peptide as a recognition and cleavage site of caspase-3, which is a special proteinase to apoptosis. Results show that this method may sensitively and specifically detect apoptotic cells with signal decline of 85%. This approach is different from the previous methods for apoptosis detection, because it does not need any fluorescent materials, expensive biological instruments, or complicated procedures.

Apoptosis, or programmed cell death, is a type of cell death that is accomplished by specialized cellular machinery, which is highly conserved to predict from observing a stereotypical morphology of cells dying either under physiological conditions or after mild injury. Programmed cell death, as important as cell division and cell migration, allows the organism to tightly control cell numbers and tissue size and to protect itself from rogue cells that threaten homeostasis.[1,2] Deregulation of the apoptotic program can lead to a variety of diseases, such as cancer, neurodegenerative diseases, autoimmune diseases, atherosclerosis, myocardial infarction, and so on.[3,4] Diagnosis of apoptosis is therefore of great importance to the early detection of therapy efficiency and the evaluation of disease progression. Traditional detection mathods of apoptosis are based on changes in the morphology and the cytoplasmic compartment of the cell,[5] which may not be very sensitive. So, development of other methods for apoptosis detection is highly necessary, and it has also received more and more attention.[6–11]

Cysteine-dependent, aspartate-specific proteinases (caspases) are a family of protease closely related to cellular apoptosis. Because they bring about most of the visible changes that characterize apoptotic cell death, caspases can be thought of as the central executioners of the apoptotic pathway. These proteinases exist in apoptotic cells and can be a key to recognize and detect apoptosis in both living cells and cell lyses. Caspase-3 is especially important in this family, because it takes part in both intrinsic and extrinsic apoptotic pathways.[12–14] Caspase-3 specifically recognizes and cleaves the N-terminus of tetra motif Asp-Glu-Val-Asp (DEVD), and this tetrapeptide motif of DEVD can be used for caspase-related detection, including apoptosis assay.[7–11] Nevertheless, these reported assay methods are based on fluorescent labeled peptide substrates, and such fluorescence-linked assays are often complicated by the requirement of an elaborate excitation, detection scheme and the broad emission bands. Based on our studies on protein electrochemistry and tumor cell detection,[15–17] we here make use of this tetrapeptide motif for the detection of apoptosis with an electrochemical method. The electrochemical method has been known to be simple, rapid, and convenient and has been used for the detection of organic species and biological molecules. We report here that apoptosis can be also detected very simply and conveniently through an electrochemical approach.

EXPERIMENTAL SECTION

Materials. The ferrocene (Fc)−peptide we designed was synthesized by Shanghai C-Strong Co., Ltd. (>95%, Shanghai, China). Ferrocenecarboxylic acid and 6-mercapto-1-hexanol were

* To whom correspondence should be addressed. E-mail: genxili@nju.edu.cn.
† Nanjing University.
‡ Shanghai University.

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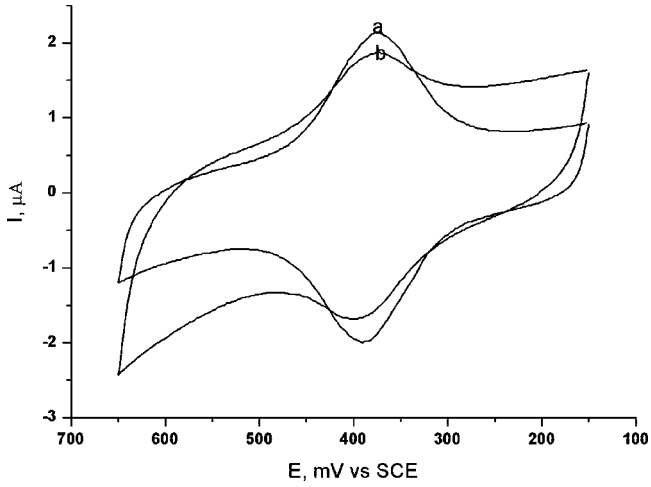

Figure 1. Cyclic voltammograms of Fc−peptide modified Au electrode in 1 M $HClO_4$ solution (a) before and (b) after the electrode was previously immersed in the apoptotic cell lysates at 37 °C for 1 h. Scan rate, 100 mV/s.

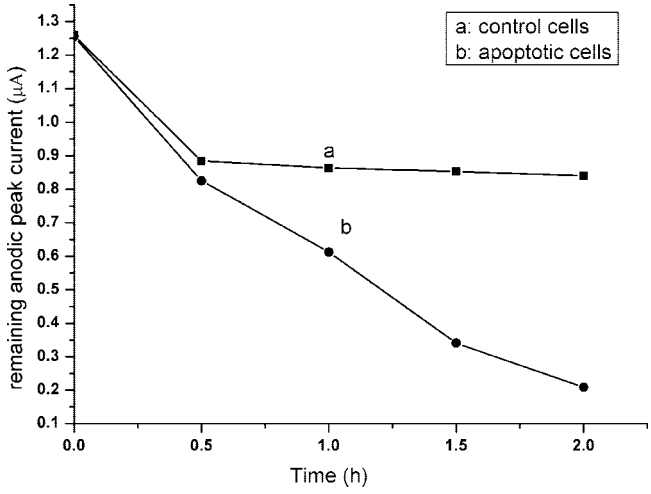

Figure 2. Relationship between the remaining anodic peak current and the time that the Fc−peptide modified Au electrode has been immersed in the cell lysates (a, control cell lysates; b, apoptotic cell lysates). Others are the same as in Figure 1.

purchased from Sigma-Aldrich Inc. Recombinant human caspase-3 was purchased from R&D systems, Inc. (>95%). HEPES free acid, CHAPS, dithiothreitol (DTT), and sucrose were obtained from Nanjing Sunshine Biotechnology Ltd. (Nanjing, China). Bovine serum albumin (BSA) was purchased from Tianxiangren Bioengineering Co., Ltd. Human lung cancer cells A549 were purchased from Institute of Biochemistry and Cell Biology in Shanghai, Chinese Academy of Sciences (Shanghai, China). Apoptosis Inducers Kit (C0005) and Tissue and Cell lysis solution (P0013) were purchased from Beyotime Institute of Biotechnology. Chemicals and solvents were analytical reagents or better and used without further purification. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead).

Peparation of Ferrocene Acetate Acid−Peptide Conjugate. The conjugate Fc−GDGDEVDGC was designed to contain a tetrapeptide sequence DEVD, and ferrocenecarboxylic acid was conjugated to an N-terminal amino group through a condensation reaction. Its C-terminal is cysteine, whose thiol group is used for covalent coupling to the Au electrode surface. The secondary structure of the Fc−peptide is a left-hand helix, confirmed by circular dichroism. The molecular weight of the purified Fc-peptide is 1077.83, which was verified by mass spectrometry analysis. The peptide was dissolved in double-distilled water (pH 5, acetic acid) without oxygen, with a concentration of 500 μM, and stored at −20 °C. The solution was diluted to 50 μM for the experiment.

Preparation of Fc−Peptide Modified Au Electrode. The substrate gold electrode (3 −m diameter) was first soaked in piranha solution (98% H_2SO_4:30% H_2O_2 = 3:1) for 5 min to eliminate the adsorbed material and then rinsed with double-distilled water. After that, the electrode was polished carefully to a mirrorlike surface with P2000 silicon carbide paper and 0.5-μm alumina slurry, respectively and sonicated for 5 min in both ethanol and water. The modification of Fc−peptide on the gold electrode was performed at 4 °C for 24 h by dipping the above pretreated gold electrode in a 50 μM Fc−peptide solution. After the modification, the Fc−peptide modified electrode was rinsed with double-distilled water and backfilled with 1 mM mercaptohexanol for 1 h. The Fc−peptide modified gold electrode was then prepared.

Cell Culture and Induction of Apoptosis. A549 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air for 2 days at a concentration of 2×10^5. Then cells were treated with Apoptosis Inducers Kit (1 μL/mL) to induce apoptosis. After 6 h, the cells were harvested, washed twice with PBS, and lysed in lysis buffer (25 mM HEPES buffer, 10% Tissue and Cell lysis solution containing 50 mM Tris-HCl (pH8.0), 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% NP40, 1 mM DTT, and 1 μL of 0.5 mM protease inhibitor) and at the same time sonicated in ice−water for 15 min. The lysates (25 mM HEPES, 10% lysis buffer) were centrifuged at 13 000 rpm for 15 min at 4 °C, and the supernatant was used immediately for apoptosis assays.

Apparatus. Electrochemical experiments were carried out on a 263A potentiostat/galvanostat (EG&G, PARC) with a three-electrode system. The Fc−peptide modified electrode was used as the working electrode. A saturated calomel electrode served as the reference electrode and a platinum wire electrode as the counter electrode. M270 electrochemical software was used for collecting and calculating data.

RESULTS AND DISCUSSION

The commonly used cyclic voltammetry can be employed to detect apoptosis through the signal change obtained at the Fc−peptide modified Au electrode. First, we have characterized the electron-transfer properties of the helix peptide.[18–21] As is shown in Figure S1 in the Supporting Information (SI), with self-assembling of the ferrocenoyl-labeled helix peptide on the gold electrode surface, the electroactive reporter Fc may display reversible redox waves, so a good electrochemical signal can be obtained due to the electron transfer through the helix peptide

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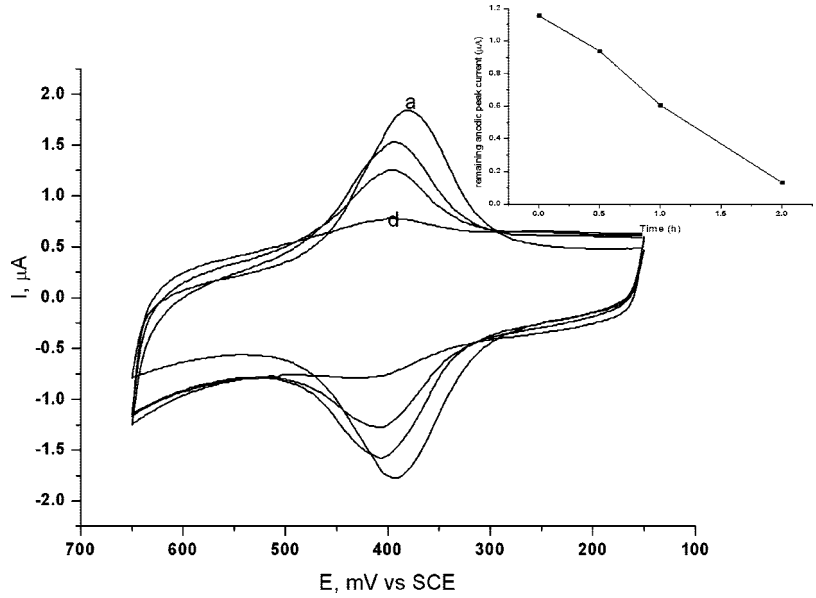
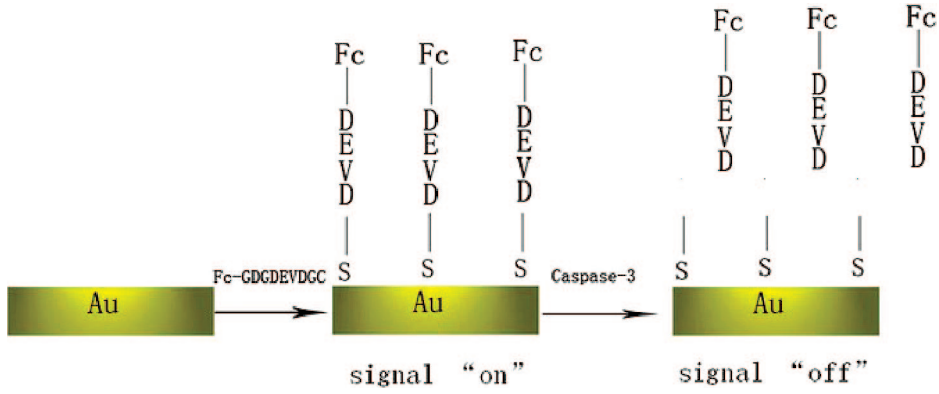


Figure 3. Cyclic voltammograms obtained at Fc−peptide modified Au electrode for 1 M $HClO_4$ solution. The electrode was immersed in recombinant human caspase-3 solution (0.15 μg/mL recombinant human caspase-3 in protease assay buffer containing 20 mM HEPES, pH 7.5, 1 mM DTT, 0.1% CHAPS) for 0, 0.5, 1, and 2 h (a−d)) at 37 °C, respectively. Scan rate, 100 mV/s. Inset shows the relationship between the remaining anodic peak current and the immersing time.

Scheme 1. Schematic Diagram for Electrochemical Detection of Apoptosis



bridge. The surface coverage (Γ) of Fc−peptide on the Au electrode is 1.64×10^{-11} mol/cm^2. And, a good linear relationship between the peaks currents and the scan rate indicates that the electrochemical response is attributed to surface-bound redox species (see SI, Figure S2).

If the Fc−peptide modified electrode has been immersed in apoptotic cell lysates for a while, the peaks currents obtained at the Fc−peptide modified electrode will obviously decrease. Figure 1 shows the cyclic voltammograms (CVs) of the Fc−peptide modified Au electrode for 5 mL of 1 M $HClO_4$ solution before and after the electrode is immersed in the apoptotic cell lysates. It can be observed that after the Fc−peptide modified electrode has been immersed in the apoptotic cell lysates for 1 h, the redox waves can be obviously observed to be decreased.

Control experiments with normal cell lysates have been performed. As we may notice from Figure 2, the redox peak currents will also decrease in the first 30 min, which is reasonable because of the unremoved physically species adsorbed on the surface of the modified electrode, which may affect the electron transfer between Fc and the electrode. However, the redox peaks will keep hardly unchanged, although the peak currents obtained at the Fc−peptide modified electrode will keep decreasing with the immersing time if the apoptotic cell lysates are employed. After 2 h treatment of the Fc−peptide modified electrode with the apoptotic cell lysates, the remaining anodic peak current will have declined to ∼15% of the initial reading. With the signal decrease (85%) for the apoptotic cells, we can conclude that the designed Fc−peptide modified Au electrode can recognize apoptotic cells and detect them very easily and simply.

In order to confirm that the decrease of the peak current is due to the recognition and cleavage of the tetrapeptide DEVD on the Fc−peptide modified electrode by caspase-3 in the apoptotic cell lysates, we further employed this modified electrode to detect recombinant human caspase-3. Figure 3 may reveal the results. Before the CVs were recorded, the modified electrode was immersed in a recombinant human caspase-3 solution (0.15 μg/mL recombinant human caspase-3 in protease assay buffer containing 25 mM HEPES, pH 7.5, 1 mM DTT, 0.1% CHAPS) for 0, 0.5, 1, and 2 h, respectively. Obviously, the peaks currents obtained at the Fc−peptide modified electrode will noticeably decrease if the electrode has been previously immersed in the

protein solution for some period, which may result from the cleavage of the peptide by caspase-3 and the consequent loss of the electrochemical reporters. The longer the electrode is immersed in the protein solution, the more obvious the decrease of the signal is. After 2-h treatment, the remaining anodic peak current of the Fc−peptide modified Au electrode is only ~10% of the initial, as shown in the inset of Figure 3.

We have also used BSA to conduct control experiments. Results show that the peaks currents obtained at the Fc−peptide modified electrode will also be somewhat decreased due to the unremoved physically species adsorbed on the electrode surface. However, even after 2-h treatment with BSA solution, the remaining anodic peak current is still ~75% of the initial. Therefore, the decrease of the peak current obtained at the Fc−peptide modified electrode can definitely denote the presence of caspase-3 or the apoptosis with the signal from "on" to "off", as illustrated in Scheme 1.

In summary, a simple, convenient, and specific approach for detection of apoptosis has been developed. In this approach, what is needed is just to immerse the Fc−peptide modified electrode in the cell lysates for ~1−2 h. If the redox peak current of the CVs is greatly decreased, it shows that these cells are apoptotic. As for the preparation of the Fc−peptide modified Au electrode, it is also very simple to an electrochemist. Therefore, the assay of apoptosis by this proposed method is very easy and simple to perform.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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