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Sensitive and selective determination of caspase-3 based on calixarene functionalized reduction of graphene oxide assisted signal amplification

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ABSTRACT

Apoptosis is a crucial event for the efficacy of anticancer drug. Caspase-3 plays a critical role in cell signaling pathways of apoptosis. The calixarene derivatives display high affinity for various kinds of biomolecules by host-guest recognition own to their unique cavity structure. The calixarene functionalized reduction of graphene oxide exhibits stronger molecular recognition ability and a higher electrochemical response to biomolecules than unmodified graphene oxide. In this work, caspase-3 recognizes and cleaves N-terminal blocked peptide containing tetra-peptide substrate Asp-Glu-Val-Asp. p-sulfonatocalix[6]arenes sodium modified graphene oxide (pSC_6-rGO) recognizes exposed N-terminal amine group assembled on the electrode. Due to the large surface area to volume ratio of rGO, numerous electrochemical active methylene blues (MB) can be absorbed through host-guest recognition. As a result, sensitive caspase-3 detection was achieved with a low detection limit of 0.0167 pg/mL by this electrochemical signal amplification strategy. This approach was also applied to measure apoptosis in the practical cell samples and shows good performance.

1. Introduction

Apoptosis, also known as cell program necrosis, is a kind of orderly death in order to maintain the stability of the inner environment. Apoptosis has important implications in exploring many biological processes, including tumor formation, embryonic development, and neurodegenerative diseases. It can eliminate defective cells from the tissue in the normal metabolic process. Abnormality occurs during apoptosis regulation, which is associated with chemotherapy and pathogenic reaction of some diseases such as atherosclerosis, myocardial infarction, autoimmune diseases, neurodegenerative diseases, and cancer [1–4]. Recently, some key proteins have been found to be closely related to apoptosis. Among them, the caspase family, like cysteine-dependent aspartate-specific protease, shows an important role in apoptosis. They are produced in apoptotic cells with the ability to identify and determine apoptosis in cell lysates and living cells [5–7]. Especially, caspase-3 is particularly crucial since it is involved in intrinsic and extrinsic apoptotic pathway. Thus, caspase-3 is the most commonly used to detect apoptosis target.

In recent years, commercial kits have been produced for apoptosis assay of caspase-3 by means of fluorescence and colorimetric detection. According to the literature, the detection line of the fluorescence method is 363.65 pg/mL (20 pM), and the detection line of the colorimetric method is 0.89 nM (16.18 ng/mL) [8–11]. The photometric methods are difficult to use in practical applications for the reason that they need a laborious extraction process. In our previous study, a novel caspase-3 sensor has been reported for excellent sensitivity through surface plasmon resonance (SPR) [12]. Certainly, there are several problems with the cost of the laboratory equipment and the experimenter training. As an emerging

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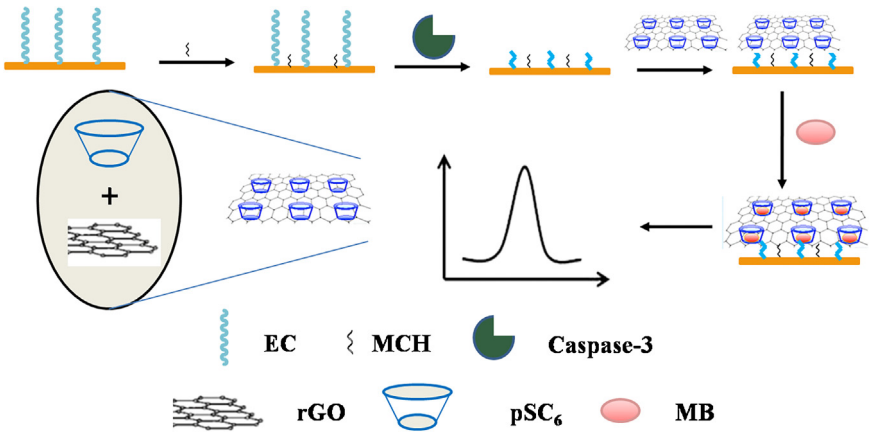

Scheme 1. Schematic illustration of the modified electrode surface and the electrochemical strategy for sensing caspase-3 activity. (EC is Ac-Gly-Gly-His-Asp-Glu-Val-Asp-His-Gly-Gly-Gly-Cys, MCH is 6-hydroxy-1-hexanethiol, pSC_6 is p-Sulfonatocalix (6) arenes sodium, rGO is reduction of graphene oxide, MB is methylene blue.).

and outstanding biosensor technology, electrochemistry obtains ultra-high specificity and sensitivity, which can be used to detect noble metal ions [13], small bioactive molecules [14], DNA [15], cell [16] and cancer marker proteins [17]. Accordingly, much attention has been paid to electrochemical technology for its relatively low experimental cost and easy operation. Some electrochemical caspase-3 sensors have been fabricated [18–21]. The sensitivity and selectivity were improved by specific digestion identifies the polypeptide sequence and signal amplification of host-guest recognition.

Reduced graphene oxide (rGO) is a honeycomb planar film formed by carbon atom in sp2 hybridization, and it is a new type of nanomaterial discovered in recent years. Owing to its excellent strength, conductivity, thermal conductivity, optical properties, and high specific surface area [22], rGO has been developed by leaps and bounds in physics [23], materials science [24], electronic information [25], computer, biosensing [26] and other fields.

As the third-generation host compound after the crown ether and cyclodextrin, calixarene can form host-guest complexes with a variety of inorganic, organic and biological guest molecules through host-guest recognition [27–29], showing very high molecular selectivity and binding ability. It is noteworthy that the selective combination of calixarenes and target molecules is available for developing a variety of sensors based on multifunction of calixarenes and the strong recognition [30,31]. Diao et al. reported p-sulfonatocalix[4,6,8]arenes sodium and graphene oxide modified glassy carbon electrode with enrichment capabilities and high supramolecular recognition, and displayed enhanced electrochemical responses to dye molecules and biomolecules [32].

In our work, we present an efficient, simple, highly sensitive, and label-free electrochemical method for caspase-3 detection. As presented in Scheme 1, caspase-3 recognizes tetra-peptide substrate DEVD (Asp-Glu-Val-Asp) and cleaves at the N-terminal motif. As a substrate of caspase-3, the N-terminal blocked peptide Ac-Gly-Gly-His-Asp-Glu-Val-Asp-His-Gly-Gly-Gly-Cys is used. After covalent modification to the surface of the gold electrode by Cys, the blocked peptide cannot react with pSC_6-rGO due to the formation of host-guest recognition. Whereas, the cleavage of peptide leads to the exposure of a new free N-terminal amine group after elution of the acetylated tablet in the presence of caspase-3. With the caspase-3 concentration rising, more pSC_6-rGO can bind to the surface of gold electrode. Therefore, through π-π stacking, electrostatic interaction, and host-guest recognition, a large number of active molecules of MB can be assembled on rGO, and the electrochemical signal can be obtained by redox of MB. At the same time, signal is amplified by a large surface area to volume ratio of rGO contributing to absorbing numerous MB via the host-guest recognition. This system is very sensitive to caspase-3 with a low detection limit.

2. Experimental

2.1. Materials and apparatus

Recombinant human caspase-3 derived from Escherichia coli was provided from R & D Systems, Inc. (Minneapolis, MN, USA). Sodium citrate tribasic dehydrate, graphene oxide (GO), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 6-mercapto-1-hexanol (MCH), methylene blue (MB), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), albumin from bovine serum BSA, thrombin, trypsin, DL-dithiothreitol (DTT), 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), and Dulbecco's modified eagle medium (DMEM) were purchased from Sigma–Aldrich, Inc. (St. Louis, USA). The substrate peptide was synthesized by Sangon Biotech Co. Ltd. (Shanghai, China): An N-terminal blocked (acetylated) peptide Gly-Gly-His-Asp-Glu-Val-Asp-His-Gly-Gly-Gly-Cys, (Ac-GGHDEVDHGGGC EC, >96.4%). p-Sulfonatocalix[6]arenes sodium was also provided from Sangon Biotech Co. Ltd. (Shanghai, China). The UV–vis spectra were recorded on Shimadzu UV-2450 PC UV–vis spectrophotometer using quartz cuvettes with a volume of 50 μL and a path length of 10 nm. Electrochemical detections were conducted on a Metrohm electrochemical analyzer (Metrohm Autolab B.V., the Netherlands). FI-TR spectra were performed by a Bertex 70 (Bruker, Germany). Scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS) investigations were carried out with a FEI Nova NanoSEM 450 instrument respectively. The Milli-Q purification system (Branstead, USA) was employed to prepare deionized water for all solutions used in this work at a resistivity of 18 MΩ cm. All of the above chemical reagents are of analytical grade.

2.2. Synthesis of pSC_6-rGO

The pSC_6-rGO was synthesized according to the literature [32]. GO (10 mg) and pSC_6 (20 mg) were dispersed in deionized water (20 mL) by ultrasonic bath, and the mixture was stirred at room temperature for 12 h. The mixture was continued to vigorously stir at 75 °C for 14 h after adding hydrazine hydrate (100 μL) and ammonia solution (200 μL). Finally, the mixture became a black dispersion by using centrifugation and sonication with 30 min. After washing with deionized water for three times, pSC_6-rGO (1.0 mg/mL), easily dispersed in water by re-ultrasonic treatment,