Electrochemical sensing for caspase 3 activity and inhibition using quantum dot functionalized carbon nanotube labels†

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Received 26th August 2010, Accepted 11th October 2010
DOI: 10.1039/c0cc03494k

A novel electrochemical sensing platform for sensitive determination of caspase 3 activity and inhibition was developed by combining the site-specific recognition and cleavage of the DEVD-peptide with quantum dots as signal amplification.

Apoptosis is an important biological process that is associated with pathogeny and chemotherapy response for a variety of diseases.1 This intracellular process is mediated, at least partially, by a series of cysteine-dependent, aspartate-specific proteases.2 Caspase 3 is the most frequently activated cysteine protease during the apoptosis process, and has been identified as a key mediator and a well-established cellular marker of apoptosis.3 Therefore, sensitive detection of caspase 3 activity and its inhibition by potential inhibitors has become an important subject in apoptosis diagnosis to guide therapeutic choices and monitor treatment.4

The analytical technique for caspase 3 activity screening primarily includes western blotting,5 flow cytometric-based analysis,6 and colorimetric/fluorometric assay.3a,7 Although promising, most of them, unfortunately, either are time-consuming and labor-intensive, or require highly technical expertise and sophisticated instrumentation. Recently, fluorescence resonance energy transfer (FRET) technology has been introduced into sensing caspase 3 activity; it mainly relies on the specific recognition and cleavage of the fluorophore-tagged Asp-Glu-Val-Asp (DEVD) peptides by active caspase 3.4a,8 However, the performance of these FRET-based sensors was mainly dependent on the efficiency of energy transfer between the fluorescence donor and acceptor, which may limit their sensitivity and applicability. Therefore, it is beneficial to develop a highly sensitive and convenient detection approach for caspase 3 activity.

The electrochemical technique offers attractive advantages in high sensitivity, inherent simplicity, and low cost, and has been widely used in bioassay.9–12 Wang et al.9c developed a carbon nanotube-based electrochemical sensor for assay of salivary cholinesterase enzyme activity. Xiao et al.12 reported an electrochemical approach for detection of apoptosis using a ferrocene-labeled DEVD peptide. On the other hand, with the advance of nanobiotechnology, quantum dots (QDs), which exhibit sharp and well-resolved stripping voltammetry signals as the metal components, are well recognized as electroactive species for signal amplification.10 Herein we developed a novel electrochemical sensing platform for profiling caspase 3 activity and inhibition by combining the advantages of QDs-based signal amplification with the specific recognition and cleavage of DEVD-peptide by active caspase 3. To the best of our knowledge, this is the first work on the use of quantum dots for electrochemical detection of caspase 3 activity and inhibition.

Fig. 1 outlines the design of the caspase 3 activity assay. First, a smart, caspase-3-responsive, and biotinylated DEVD-peptide (biotin-Gly-Asp-Gly-Asp-Glu-Val-Asp-Gly-Cys) was designed and immobilized on the gold electrode surface through the chemical adsorption of the thiol group of the terminal Cys residue. This peptide-modified electrode (biotin-DEVD/Au) was then backfilled with 6-mercapto-1-hexanol (MCH) to prevent the nonspecific adsorption of the peptide chain. Such a nonspecific adsorption resulted in a flat-lying peptide chain that became inaccessible to the enzymes.11 Moreover, considering the fact that caspase 3 is mainly assayed in cell lysates, the presence of MCH monolayer could also efficiently prevent the nonspecific adsorption of abundant non-target proteins in cell lysates, which may passivate the sensing layer by blocking the access to the DEVD-peptide substrate. Subsequently, the MCH/biotin-DEVD/Au was immersed into the apoptotic cell lysates containing active caspase 3, which could specifically recognize and cleave the N-terminus of DEVD. This cleavage event led to the loss of the biotin label, which diffused away from the electrode surface. The ratio of the uncleaved biotin-DEVD on the electrode surface was related to the activity of caspase 3 in cell lysates. Prior to evaluating the ratio, a signaling probe

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† Electronic supplementary information (ESI) available: Experimental details and Fig. S1–S5. See DOI: 10.1039/c0cc03494k

Fig. 1 (A) Preparation process of CNTs-QDs-SA bioconjugates via LbL assembly. (B) Schematic illustration of the electrochemical strategy for sensing caspase 3 activity.
composed of CdTe QDs, carbon nanotubes, and streptavidin was prepared via the layer-by-layer (LbL) assembly approach (Fig. 1A). The synthetic procedure is detailed in the ESI.† Of note, carbon nanotubes were employed as the carrier to load a large amount of electroactive QDs with a diameter of 3.77 nm (Fig. S1†) for signal amplification, which could be confirmed by the TEM image of CNTs-QDs (Fig. S2†). After being coupled with streptavidin through Schiff-bonds, the obtained CNTs-QDs-SA bioconjugates could effectively recognize the uncleaved biotin-DEVD through the streptavidin–biotin interaction. Finally, the captured CNTs-QDs-SA was dissolved in HNO3, and the cadmic component acting as the detector target was quantified by anodic stripping voltammetry (ASV). The produced electrochemical signal was related to the amount of uncleaved biotin-DEVD on the electrode surface, which depended on the activity of caspase 3 in cell lysates. Consequently, the activity of caspase 3 could be evaluated by monitoring the QDs amplified electrochemical signal.

The fabrication process of the electrochemical sensor was first characterized by AFM as shown in Fig. 2. As compared with the bare Au electrode (A), light dots with some loosely-packed agglomeration were observed on the electrode surface (B), which was attributed to the biotin-DEVD. The backfill of MCH resulted in a more dense film (C), while the caspase 3 cleavage led to a uniform film with obviously decreased height. After reacting with CNTs-QDs-SA, the surface was covered with loosely tube-like particles, indicating effective recognition of CNTs-QDs-SA to biotin-DEVD. The success of the assembly process was also confirmed by EIS (Fig. 2F). The bare Au electrode revealed a very small semicircular domain (a), implying a very low electron transfer resistance. With the immobilization of the biotin-DEVD (b) and MCH backfilled to the electrode (c), the electron transfer resistance gradually increased because of the poor conductivity of biotin-DEVD and MCH. However, after the cleavage of biotin-DEVD by active caspase 3, the EIS showed a lower resistance (d), which was due to the partial loss of biotin labels. Finally, the capture of the CNTs-QDs-SA further decreased the resistance (e), owing to the good electronic conductivity of CNTs-QDs. These results were consistent with the fact that the electrode was fabricated as expected.

For validation of the methodology, human leukemic HL-60 cells were chosen as a model and exposed to apoptosis inducer for the activation of caspase 3 (see ESI†).12 Time-dependent cleavage of biotin-DEVD by active caspase 3 in the lysates from cells treated with apoptosis inducer for 12 h was investigated by ASV (Fig. S3†). A well-defined peak for the oxidation of Cd was observed at around −0.7 V, and the peak current decreased gradually with the increase of immersion time from 0 to 60 min, and then leveled off after 60 min. Thus, 60 min of immersion could lead to an efficient cleavage. Meanwhile, the time course of caspase 3 activation by apoptosis inducer is also presented in Fig. 3A. In the absence of apoptosis inducer, caspase 3 was not activated, leading to a maximum current response (a). After incubation with apoptosis inducer, the current response decreased gradually (b–f). The dependence of peak currents on the incubation time of apoptosis inducer is shown in Fig. 3B. As expected, with the increasing incubation time, the current decreased gradually and reached a minimum value at 12 h. The results were consistent with the observation from a colorimetric method using a caspase-3 cellular activity assay kit (Fig. S4†). Thus, the proposed strategy can be used for monitoring the caspase 3 activity during cell apoptosis.

To further assess the specificity of the sensor, a common caspase 3 inhibitor Ac-DEVD-CHO was employed. As expected, pretreatment with the inhibitor could notably inhibit the activation of caspase 3 (Fig. S5†).13 Correspondingly, the current response with regard to the caspase 3 activity in the

![Fig. 2 AFM images of the bare Au electrode (A), biotin-DEVD/Au (B), MCH/biotin-DEVD/Au before (C) and after (D) the caspase 3 cleavage, and followed by the capture of CNTs-QDs-SA bioconjugates (E). (F) Nyquist plots corresponding to the different Au electrodes from (A) to (E).](#)

![Fig. 3 Time course of caspase 3 activation by apoptosis inducer and concentration-dependent caspase 3 inhibition by Ac-DEVD-CHO. (A) Anodic stripping voltammetry measurements for caspase 3 activity after the HL-60 cells were incubated with apoptosis inducer for (a) 0, (b) 2 h, (c) 4 h, (d) 6 h, (e) 12 h, and (f) 24 h. (B) Plot for the dependence of peak currents on the incubation time of apoptosis inducer. (C) Anodic stripping voltammetry measurements for caspase 3 inhibition. HL-60 cells were pre-incubated with (a) 0, (b) 5 µM, (c) 17 µM and (d) 21 µM Ac-DEVD-CHO for 30 min, before addition of the apoptosis inducer for 12 h; (e) HL-60 cells without treatment were used as control. (D) Relationship between the remaining percentage of peak currents (A) and the concentration of caspase 3 inhibitor Ac-DEVD-CHO. Error bars represent one standard deviation for three independent measurements.](#)
respectively. The value of inhibitor and apoptosis inducer, and cells without treatment, for the current response measured for cells treated with both concentrations of etoposide (b–d). The standard calibration of etoposide (a), and decreased gradually with increasing activation by etoposide. The current response in the presence of inhibitor was much higher than that in the absence of inhibitor (Fig. 3C). Moreover, the response showed an activation by etoposide. The current change was indeed due to the catalytic presence of inhibitor (Fig. 3C).

Fig. 4 displays the concentration-dependence of caspase 3 activation by etoposide. The current response in the presence of etoposide (b) was much lower than that in the absence of etoposide (a), and decreased gradually with increasing concentrations of etoposide (b–d). The standard calibration curve for etoposide detection is shown in Fig. 4B. The normalized current increased gradually with the etoposide concentrations between 7.14 μM and 50.0 μM, and the detection limit was 7.14 μM. Altogether, these results suggest that the developed QDs-based electrochemical assay can lead to a more efficient manner to effectively screen caspase 3 inhibitors and anticancer drugs in vitro.

In summary, we developed a novel electrochemical sensing platform that combines the action of enzyme cleavage with quantum dots-based signal amplification to enable sensitive determination of caspase 3 activity and inhibition during cell apoptosis. This electrochemical strategy exhibited attractive advantages of ease of performance, high sensitivity and specificity. An important feature of this method is that it allows the assessment of drug action in activating caspase 3 within a short time at a low concentration. Sequentially, it presents a significant tool for efficient screening of potential caspase 3 inhibitors and anticancer drugs, suggesting promising applications in cancer research.

This research was financially supported by the National Natural Science Foundation of China for the Key Program (20635020, 20821063) and National Basic Research Program of China (2006CB933201, 2011CB933502).

Notes and references