Ultrasensitive Determination of Cysteine Based on the Photocurrent of Nafion-Functionalized CdS–MV Quantum Dots on an ITO Electrode

Yi-Tao Long,* Cong Kong, Da-Wei Li, Yang Li, Somenath Chowdhury, and He Tian

As a fundamental building block for functional and structural components of many proteins and enzymes, cysteine, a sulfur-containing nonessential amino acid, plays a critical role in many biological processes. This critical residue helps to fold and maintain a stable structure of protein, contributes towards enzymatic reactions and detoxification processes, and participates in numerous posttranslational modifications. Diseases like Alzheimer’s disease and acquired immune deficiency syndrome (AIDS) were observed to be accompanied by a deficiency of cysteine. Thus, a rapid, selective, and sensitive detection of cysteine is of great interest. Various techniques for detecting this critical amino acid have been developed using spectroscopic, electrochemical, chromatographic, and electroluminescence methods. Recently, nanomaterials were readily explored for sensitive detection of cysteine utilizing the specific interaction between the surface of the nanomaterials and the cysteine that leads to absorption or fluorescence change. Most of the methods involve the use of disposable sensing reagent or nanoparticles. Demand for an alternative method to develop an economical, environmentally friendly sensor led us to the investigation of a reusable sensor for this critical amino acid.

The photoelectrochemical detection method is a promising analytical tool of high sensitivity and low cost. The different forms of excitation (light) and detection (current) could reduce the undesired background signals, to allow a highly sensitive detection performance. Despite this fact, scarce work has been done to apply photoelectrochemical properties in analytical fields. Quantum dots (QDs) have shown excellent photoelectrochemical properties including size-controlled photoluminescence, high fluorescence quantum yields, and impressive stabilities against degradation and photobleaching. They can interact with many dyes to change their photoelectrochemical properties, thereby resulting in photoinduced charge or energy transfer, which implies the feasibility of these signals for use in the analysis of chemical agents. Thus, nanostructured devices based on QDs, such as CdS QDs and CdSe/ZnS QDs, could be typically used in the photoelectrochemical detection method.

Up to now, no method for the detection of cysteine based on this highly sensitive photoelectrochemical method has been developed. Herein, we demonstrate the ultrasensitive detection of cysteine by exploiting a photoelectrochemical method based on efficient charge transport sequentially from cysteine to methyl viologen (MV)-coated CdS QDs, and then to an electrode, which is triggered under light excitation of the electrons in the valence band of CdS QDs. In the present study, Nafion film was used as the reactive matrix to confine a stable spatial distribution of MV-coated CdS QDs through electrostatic interaction on an indium tin oxide (ITO) electrode.

To investigate the photoinduced electron transfer on the modified ITO electrode, the photoresponse of Nafion film incorporated with CdS, MV, or CdS–MV complex was studied by using a xenon light source equipped with a monochromatic filter at a given potential. The Nafion/MV-coated ITO electrode showed a slightly higher photoresponse than the bare electrode (Figure 1, curves a, b) upon irradiation by light. A marginally higher photocurrent was obtained on the Nafion/CdS QDs-coated ITO electrode (Figure 1, curve c), probably due to the strong light-harvesting ability of the CdS QDs. However, a strongly enhanced photocurrent response (Figure 1, curve d) was observed on the Nafion/CdS–MV-coated ITO electrode. The enhancement on the electrode is attributed to the efficient electron transfer through proper arrangement of photosensitizers (CdS QDs) and electron acceptors (MV), where photocurrent is generated with a fast charge separation and a slow charge recombination upon irradiation. Briefly, electrons are excited from the valence band (VB) of CdS QDs to the conduction band (CB) under irradiation, and then transferred onto MV to form methyl viologen radicals (MV•+) which provide an electron relay, while cysteine gives electrons to CdS QDs to refill the valence band, thereby resulting in efficient charge transport into the electrode at a given potential. The photoinduced electron-transfer process that has occurred on the functionalized ITO electrode is depicted in Figure 1. The photoresponses on the electrode and different batches of...
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**Figure 1.** Schematic representation of the photoinduced electron-transfer process on a Nafion/CdS–MV-coated ITO electrode. Bottom right: the photocurrent response of a) bare ITO, and further coated with b) Nafion/MV, c) Nafion/CdS, and d) Nafion/CdS–MV in the presence of cysteine (CysH, 0.6 μM), at an applied potential of 100 mV versus Ag/AgCl, under the irradiation of a xenon light source.

modifications were almost identical upon repeated cycles of light switching on-off at the same intensity, thus indicating the stable photoelectrochemical feature.

Based on this electron-transfer process, the CdS QDs in the Nafion matrix serve as the electron source, where electron excitation from the valence band of the nanoparticles should depend on the energy of exciting light. The consistent behavior of the photocurrent with the absorption of CdS QDs (Figure S4; see the Supporting Information) proves that the photocurrent originates from electron–hole generation on the CdS QDs under suitable light excitation, which further confirms the electron-transfer process. It is noted that the intensity of the photocurrent is consistently reinforced by increasing concentrations of cysteine, thus promising the quantitative determination of cysteine.

The anodic or cathodic photocurrents were observed at a potential higher than ~100 mV or lower than ~200 mV versus Ag/AgCl on the functionalized electrode (Figure S5; see the Supporting Information). When the photoexcited electrons were transferred onto the MV acceptors from CdS QDs, the positive electrode would oxidize the MV⁺⁺⁺⁺ rather than refill holes of the CdS QDs, which was achieved by a sacrificial electron donor (cysteine), thus leading to a photoinduced anodic current.[26] Vice versa, a negative electrode would not allow electron transfer through but out, giving a cathodic current, and cysteine does not participate in the process.

To obtain a high photocurrent response from cysteine, we chose an appropriate applied potential of 100 mV, so that the more positive potential did not lead to a higher response and also to avoid large background current in the dark and minimize the electrochemical stress of the CdS QDs on the electrode under a given light intensity.[27]

To verify the performance of the photoelectrochemical sensor in the determination of cysteine, its photocurrent response with increasing concentration of cysteine (from 0 to 3 μM) was measured (Figure 2). The relationship between the cysteine concentration and photocurrent response in the range from 0.2 to 2.8 μM was fitted linearly with the equation (R² = 0.9940). A lower detection limit of 0.1 μM has been defined. As the concentration continues to increase, the photocurrent response shows saturation behavior, which implies a highly efficient surface electron-transfer process depending on the quantitative electrochemical relationship between CdS QDs and cysteine. Five electrodes made by the same procedure independently showed an acceptable reproducibility with a relative standard deviation of 5% for the photocurrent response to the same concentration of cysteine (0.6 μM). These results promise an excellent performance of the functionalized ITO electrode in the quantitative analysis of cysteine.

The selectivity of the photoelectrochemical sensor was confirmed by measuring its photoresponse toward cysteine and other protein-forming amino acids as well as another common thiol compound, glutathione (GSH; Figure 3). The cysteine gave the highest response on the electrode, while other amino acids in the experiment gave little response compared with the response of the blank solution. GSH also responded positively in the experiments, but to a quite lower extent than that toward cysteine. The difference in response between these two thiol compounds may be reasonably attributed to the different electron-donating ability. Moreover, the Nafion as an anionic perfluorinated polymer with sulfonate groups is chemically and photochemically inert, and has been extensively explored to assemble organic molecules and nanomaterials on surfaces and to give a permselective membrane through its unique ion-exchange, discriminative, and highly biocompatible properties.[33,36] In this study, the film would be less permeable to the molecule of high
molecular weight (GSH) than to the one of low molecular weight (cysteine), because spatial occupation results in a more difficult approach of GSH than cysteine. The above two factors, reduction ability and film permeability, result in the different responses between cysteine and GSH. Nonetheless, at pH 7.4, when it is negatively charged, the Nafion film may close out the anion molecules through electrostatic interaction. Despite that, the Nafion film is permeable to the negatively charged GSH and cysteine. One explanation is that the thickness of the film may influence its permeability to both molecules. The present result shows that it could be permeable to molecules of small molecular weight regardless of their charge properties, thus implying that the coulombic interaction on the film is not responsible for the permselectivity. Potential-reducing compounds (glucose, nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), ascorbic acid) have also been tested for their photocurrent response under the same conditions with cysteine. Response to glucose rather than other compounds was observed.

To verify the electron-transfer process, a spectroscopic investigation was carried out in a solution containing the CdS QDs, MV, and the above-mentioned reducing compounds. The time evolution of the UV/Vis absorption spectrum of the solution was recorded in real time and in situ (Figure S6; see the Supporting Information). After continuous irradiation for 2 min, two strong absorption peaks were observed at 396 and 606 nm in the solution containing cysteine, GSH, or glucose, which indicated the formation of MV+. These phenomena confirm the involvement of an effective electron-transfer process with the cysteine, glucose, and GSH rather than other compounds in the testing experiment.

An about 20% higher response signal was observed after the addition of 5 equiv of both glucose and GSH, with their interferences in the response to cysteine (Figure S7; see the Supporting Information). These results show the slight disturbance at the presence of glucose and GSH in the solution in the determination of cysteine. The reproducibility of the functionalized electrode was examined; three batches of electrodes were made under the same procedure independently, with each batch including three electrodes. Results showed an acceptable reproducibility with a relative standard deviation of less than 5% for the photocurrent response to the same concentration of cysteine (0.6 μM). The photocurrent responses were quite stable within ten cycles of continuous measuring in the same solution, and the response in the blank solution went back nearly to the background level after 2 min of careful rinsing with pure water. Within detection ten times alternately between the cysteine solution and the blank solution, the reversibility was acceptable and further measurement using the same functionalized electrode showed a decreased photocurrent response (Figure S8, Table S1; see the Supporting Information).

In summary, a novel photoelectrochemical electrode for the detection of cysteine has been prepared. Nafion was used for the easy and simple assembly of the MV-coated CdS QDs complex for the readout of the photoelectrochemical signal on the functionalized surface. Photoelectrochemical analysis of cysteine shows a linear response of 0.2–2.8 μM and a lower detection limit of 0.1 μM. The surface assembly of the Nafion/CdS-coated ITO electrode proves to be effective in the detection of cysteine with high sensitivity, good selectivity, and fast response. Furthermore, this simple and effective QD assembly method on a sensing surface may be applied as a good design principle in the preparation of other molecular sensors.

**Experimental Section**

Preparation of CdS QDs: Ultrapure water (30 mL) and CdCl₂ (14 mg) were added to a 125-mL flask with stirring, and the pH of the solution was adjusted to approximately 2.8 with dropwise addition of thioglycolic acid (TGA). Then, the reaction solution was titrated with concentrated NaOH (1 M) until the pH increased to ≈8.5. Aqueous Na₂S·9H₂O solution (8.25 mL, 10 M) was added to the reaction flask. Subsequently, the reaction solution was stirred for 4 h at room temperature. Finally, TGA-capped CdS QDs were precipitated by addition of acetone and then the mixture was centrifuged to collect the precipitate. The TGA-capped CdS QD powder was obtained after drying in a N₂ atmosphere. Then the TGA-capped CdS QDs were characterized by UV–visible and fluorescence spectroscopy.

Electrode Preparation: Prior to the use of ITO electrodes, they were cleaned by swabbing and sonification with detergent/deionized (DI) water, rinsing in DI water, and finally rinsing in acetone and methanol. For the preparation of Nafion/CdS–MV-coated ITO electrodes, TGA-capped CdS QDs (200 μL, 0.25 μM) and MV chloride (20 μL, 50 mM) were mixed together, the Nafion perfluorinated solution (200 μL, diluted to 0.5% with water) was added to the CdS–MV complex solution, and the final well-distributed solution (20 μL) was dropped onto the ITO electrode. The modified ITO electrode was dried at room temperature, with N₂ protection, and then a compact transparent film formed on the electrode. For the preparation of Nafion/CdS QDs-coated ITO electrodes, TGA-capped CdS QDs (200 μL, 0.25 μM) and Nafion perfluorinated solution (200 μL, diluted to 0.5% with water) were mixed together, and the mixed
solution (20 μL) was dropped onto the ITO electrode. The modified ITO electrode was dried at room temperature, with N₂ protection, and then a compact transparent film formed on the electrode. For the preparation of NaFon/MV ITO electrodes, MV chloride (20 μL, 50 μm) and NaFon perfluorinated solution (200 μL, diluted to 0.5% with water) were mixed together, and the mixed solution (20 μL) was dropped onto the ITO electrode. The modified ITO electrode was dried at room temperature, with N₂ protection, and then a compact transparent film formed on the electrode.

**Experimental Setup for Photocurrent Measurement:** All electrochemical experiments were performed at room temperature with a CHI 1232a electrochemical workstation (Shanghai Chengu Co. Ltd., China). A xenon lamp light source (LE-SP-LS-XE 500, Shenzhen Leo-photoelectric Co. Ltd., China) was used. The surface of the functionalized ITO electrode in the cell was irradiated with a xenon lamp operated at 500 W, through a homemade light chopper with the shutter chopping frequency controlled manually. The functionalized electrode surface was oriented facing the incident light (front irradiation). The photoresponse of the functionalized ITO electrode as a working electrode was measured using the amperometric i-t curve technique with an Ag/AgCl reference electrode and a Pt wire counter electrode, in sodium phosphate buffer (0.2 M, pH 7.4) was continuously illuminated by a xenon lamp light source (LE-SP-LS-XE 500, Shenzhen Leo-photoelectric Co. Ltd., China) equipped with a monochromatic filter (365 ± 6 nm, Shenyang HB Optical Technology Co. Ltd., China), in a horizontally perpendicular direction to the UV/Vis light path. The photocurrent action spectrum was obtained in the UV/Vis spectrum of the solution could be recorded in situ and in real time.

**Characterization of Electrodes:** Fluorescence spectra were obtained on a Shimadzu Cary Eclipse (Varian) fluorometer. The NaFon membrane incorporated with CdS–MV complex was imaged by a Veeco DI 3100 AFM instrument with a Nanoscope V controller.

**X-ray Photoelectron Spectroscopy:** An Axis-165 (Kratos Analytical) photoelectron spectrometer equipped with a monochromatic Al Kα X-ray source (1486 eV) with an operating power of 210 W was used to collect photoemission spectra. The base pressure during measurements was maintained at 5 × 10⁻¹⁰ Torr. The high-resolution spectra were measured at a pass energy of 40 eV and a step increment of 0.1 eV. The peaks were fitted using the publicly available XSPEAK v. 4.1, Shirley and linear functions were used as a background and Gaussian–Lorentzian cross-products were used to fit the individual peaks.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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