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Short communication

Electrochemical impedance spectroscopy study of proteolysis using unmodified gold nanoparticles

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ABSTRACT

Proteases are involved in numerous cell functions and abnormal proteolysis may lead to a diversity of serious diseases. Herein, a simple electrochemical method is developed to study proteolysis by employing unmodified gold nanoparticles (AuNPs). Substrate of a protease is modified on a gold disk electrode, forming a barrier for electrochemical species and reflecting a significant charge transfer resistance (R_{ct}). After the proteolysis process, the substrate can be cleaved coupled with the decline of R_{ct}. The electrical properties of the substrate residues on the electrode may also change, leading to the subsequent adsorption of AuNPs. Due to the excellent electrical conductivity of AuNPs, R_{ct} can be further decreased, which can be used to reveal the proteolysis process. The proposed method allows the determination of the model protease, trypsin, with desirable sensitivity and specificity. It may also hold great potential use in the study of other proteolysis processes and some biomedical applications in the future.

1. Introduction

Proteases, also termed as proteolytic enzymes, catalyze the hydrolytic degradation of other peptides, proteins or themselves [1,2]. They constitute an estimated 2% of human proteome, and have attracted considerable attention [3]. Proteases can be classified into six groups including aspartate, cysteine, glutamate, metallo, serine, and threonine proteases [4], which play critical roles in different cell functions such as nutrient digestion and immune system cascade amplification [5]. Abnormal proteolysis may lead to a diversity of serious diseases, such as acquired immunodeficiency syndrome (AIDS) [6], pancreatitis [7] and rheumatoid arthritis [8]. Recent studies also show that proteases are involved in tumor evolution and suppression [9]. Moreover, many commercially used proteases have been developed for food industry and medical therapeutics [10,11]. Therefore, there is an urgent need to develop simple and sensitive protocols for the detection of proteolysis in molecular mechanism investigation, medical diagnosis and manufacturing process control.

Currently, a number of methods have been developed for the study of proteolysis including colorimetric [12], fluorimetric [13], chromatographic [14], mass spectrometry assays [15], as well as electrochemical methods [16]. However, some of these methods may use sophisticated instrumentations and complicated experiment processes, some may cost expensive reagents, and some show good utility for quantitative analysis but are not suitable for enzyme activity determination.

To overcome these problems, we herein present a simple electrochemical method to study proteolysis using unmodified gold nanoparticles. Trypsin, a kind of serine protease, is used as the model protease and a peptide is designed as the substrate of proteolysis. The negatively charged peptide is firstly modified on a gold electrode surface, blocking the penetration of electrochemical species. The proteolysis process then cleaves the peptide and decreases the charge transfer resistance (R_{ct}). Unmodified gold nanoparticles (AuNPs) with excellent electrical conductivity [17] can be further adsorbed on the electrode surface due to the change of the electrical properties of the remaining substrate, which contribute to a further decline of R_{ct}. Electrochemical impedance spectroscopy (EIS), a powerful tool to investigate the interfacial properties at solid/liquid interfaces is employed to monitor the proteolysis caused R_{ct} fall in this work [18]. The proposed electrochemical method shows fairly good utility for the study of trypsin catalyzed proteolysis and can be used as a general assay to detect other protease activity by simply changing the substrate on the electrode surface.

2. Experimental section

2.1. Materials and chemicals

Substrate peptide (Cys-Ala-Gly-Arg-Ala-Asp-Ala-Asp-Ala-Asp-Ala-Asp, CAGRADADADAD) was synthesized by China peptides Co., Ltd.

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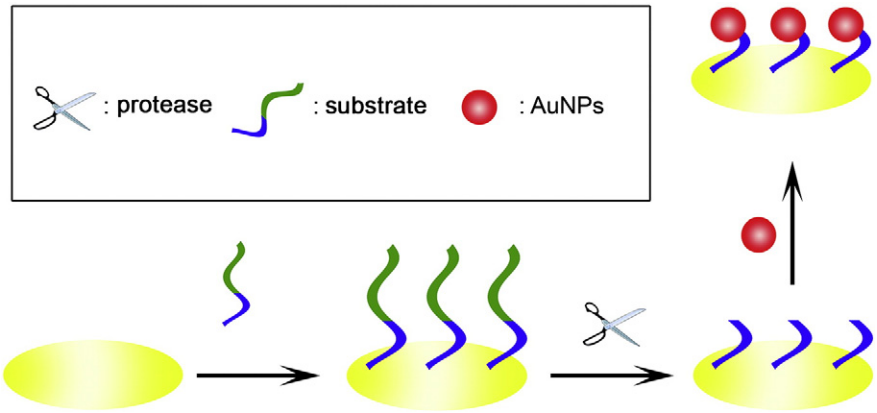

Fig. 1. Schematic representation of the electrochemical method for the study of proteolysis.

(Shanghai, China). Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) was purchased from Shanghai Jiushan Chemicals Co., Ltd. (Shanghai, China). Mercaptohexanol (MCH), trypsin (EC 3.4.21.4), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), Tris-HCl, and Bowman–Birk inhibitor (BBI) were ordered from Sigma (USA). All the other chemicals were of analytical grade and used as received. Water used was previously purified with a Milli-Q purification system (18.2 MΩ cm).

2.2. Preparation of peptide modified electrode

A gold electrode (2.0 mm diameter) was soaked in piranha solution (98% H_2SO_4 / 30% H_2O_2 = 3:1) for 5 min, polished with P3000 silicon carbide paper and 1, 0.3, 0.05 μm alumina slurry, subsequently. After further sonication in ethanol and water for 5 min each, the electrode was treated with nitric acid (50%) for 30 min and then electrochemically cleaned with 0.5 M H_2SO_4. The electrode was dried and immersed in peptide solution (1 mM, 20 mM HEPES, 10 mM TCEP, pH 7.0) at 4 °C for 16 h, followed by a 0.5 h incubation with 1 mM MCH [19].

2.3. Preparation of AuNPs

AuNPs (13 nm) were prepared by citrate reduction of $HAuCl_4$ according to the previous work [20]. Briefly, $HAuCl_4$ solution (100 mL, 0.01% (w/v)) was mixed with trisodium citrate (3.5 mL, 1% (w/v)) under stirring and boiling for 15 min. Afterwards, the heating was removed while stirring was continued for 30 min. Then, the solution was cooled down. Excess reagents were then removed by centrifuging at 10,000 rpm for 20 min.

2.4. Proteolysis and AuNPs adsorption on the electrode surface

The peptide electrode was digested by trypsin solution (50 mM Tris-HCl, 20 mM $CaCl_2$, pH 8.5) at 37 °C for 1 h. The concentrations of trypsin was from 0 to 2 unit mL^{-1}. After that, the electrode was washed by the buffer solution (20 mM Tris-HCl, 5 mM $MgCl_2$, 0.1 M NaCl, 1.0% Tween-20, pH 7.0) to terminate the reaction and block nonspecific binding [21]. The digested electrode was further incubated with 10 nM AuNPs (pH 4.2) at room temperature for 0.5 h. Finally, it was washed with water and EIS was applied to obtain the signal responses.

2.5. EIS measurement

EIS was recorded by an electrochemical analyzer (CHI660D, CH Instruments, China) at room temperature. The employed three electrode system consisted of a saturated calomel reference electrode (SCE), a platinum auxiliary electrode and the peptide modified electrode as the working electrode. 5 mM $Fe(CN)_6^{3-/4-}$ with 1 M KNO_3 was used to probe the electrochemical behavior since the electrolyte was sensitive to surface chemistry [22]. Experimental parameters

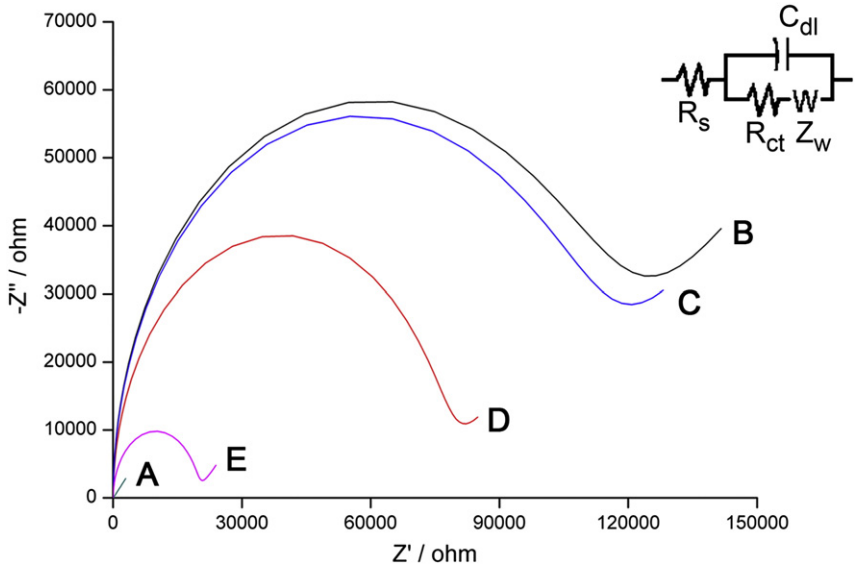

Fig. 2. Nyquist diagrams of impedance spectra for (A) bare gold electrode, (B) peptide modified electrode, (C) peptide modified electrode after AuNPs incubation, (D) peptide modified electrode after trypsin digestion, (E) peptide modified electrode after trypsin digestion and then AuNPs incubation. Inset is the electrical equivalent circuits.