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Short communication

A Sensitive Electrochemical Method for Detection of Histone Deacetylase with the Signal Amplification of Ferrocene-Capped Gold Nanoparticle/Streptavidin Conjugates

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This work reported the amplified voltammetric detection of histone deacetylase (HDAC) using ferrocene (Fc)-capped gold nanoparticle/streptavidin (SA-AuNPs) conjugates. The acetylated peptide substrates immobilized on the gold electrodes were deacetylated by HDAC, resulting in the appearance of free amine groups on electrode surface. The exposed primary amines were then derivatized with biotin for the attachment of Fc-capped SA-AuNPs conjugates. The voltammetric responses were found to be proportional to the HDAC concentrations ranging from 0.1 to 30 nM with a detection limit of 20 pM.

Keywords: Histone deacetylases; Electrochemical biosensors; Gold nanoparticles; Signal amplification; Ferrocene

1. INTRODUCTION

Post-translational modification (PTM) of proteins plays a key role in the regulation of various cellular processes by regulating the activity, localization and interaction with other cellular molecules such as proteins, nucleic acids, lipids, and cofactors [1]. Systematic studies of PTM (e.g. glycosylation, proteolysis, phosphorylation, acylation, adenylation, farnesylation, ubiquitination, and sulfation) by enzymes in disease have been summarized in databases containing disease associated mutations and human polymorphisms [2,3]. PTM enzymes have thus attracted great interest from researchers in multidisciplinary fields because of their central role in major cell-signaling pathways and their aberrant activity serving as the bio-markers and the most important class of drug target in a variety of human

diseases [4]. Furthermore, sensitive, selective, and high-throughput assays of PTM enzymes are important for both academic research and pharmaceutical discovery.

The acetylation of histones is reversibly controlled by histone acetyltransferase (HAT) and histone deacetylase (HDAC) [5]. Among them, HDAC is an enzyme that hydrolyzes the neutral ε-acetamido groups of acetylated lysines to produce positively charged lysines and has been implicated to function in the downregulation of gene expression [6]. Traditional method for assessing the activity of HDAC is radiometric assays, relying on transfer of radioactive element to the specific substrate peptides and quantification by scintillation counting. The radiometric method is general and versatile but hampered by the complicated multi-step procedure and unhealthy radioactive waste [7,8]. Therefore, it is crucial to develop novel sensitive and selective method for assessing the activity of HDAC. Recently, electrochemical biosensors have gained considerable interests because of their high sensitivity, simple instrument, low endogenous background, small analyte volumes, and excellent compatibility with array format and miniaturization. At present, the increasing demand for detection of ultralow amount of analytes is pushing the enhancement of sensitivity by selecting different signal amplification strategy [9]. Because of the unique combination of chemical and physical properties, gold nanoparticles (AuNPs) as labels have been widely used in diagnostics and detection. Normally, AuNPs can be coated with biological recognition elements (e.g. antibody, natural or synthetic receptors and aptamer) and redox tags (e.g. ferrocene (Fc) and thionine (Th)) for the molecular recognition and signal amplification [10-12]. In this work, we reported the amplified voltammetric detection of HDAC using ferrocene (Fc)-capped gold nanoparticle/streptavidin (SA-AuNPs) conjugates. The analytical merits of this method (e.g. reproducibility, selectivity, sensitivity and detection limit) were demonstrated.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Tris(carboxyethyl)phosphine (TCEP), 6-mercapto-1-hexanol (MCH), biotin N-hydroxysuccinimideester, 6-ferrocenyl-1-hexanethiol ($Fc(CH_2)_6SH$), gold nanoparticle/streptavidin (SA-AuNPs) conjugates, alkaline phosphatase (ALP), bovine serum albumin (BSA), KH_2PO_4, K_2HPO_4 and tris-(hydroxymethyl)aminomethanehydrochloride (Tris–HCl) were obtained from Sigma-Aldrich (Changhai, China). The acetylated peptide with a sequence of Ac-CPPPPEEGIHK(Ac) was synthesized and purified by China Peptides Co., Ltd. (Shanghai, China). Histone deacetylase was obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). Prostate specific antigen (PSA) was obtained from Linc-Bio Science Co. Ltd. (Shanghai, China). The preparation of the Fc-capped SA-AuNP conjugates followed the method described in the previous report [13]. All aqueous solutions were prepared freshly with a Millipore system (Simplicity Plus, Millipore Corp.).

2.2 Procedure

Gold disk electrode with a diameter of 2 mm was polished with diamond pastes down to 3 μm and alumina pastes down to 0.3 μm, and then sonicated in ethanol and water. The peptide-covered

electrode was prepared by immersing the cleaned gold electrode in a phosphate-buffered saline solution (PBS buffer, 10 mM, pH 7.4) containing 0.1 mM peptide and 0.5 mM TCEP in the darkness for 12 hours. This step was followed by washing the electrode thoroughly with water and soaking the electrode in 1 mM MCH solution for 30 min. After the peptide/MCH film had been formed, 30 μL of Tris−HCl buffer (20 mM, pH = 8.0) containing 0.1 M NaCl, 2 mM KCl, 1 mM $MgCl_2$ and a given concentration of HDAC was cast onto the electrode surface. Again, the electrode was rinsed with water to rid any non-specifically adsorbed substance. To attach Fc-capped SA-AuNPs conjugates, the electrode was first allowed to react with 20 μL of 0.5 mM biotin N-hydroxysuccinimide ester for 15 min and then rinsed with a copious amount of 50% ethanol/water. The electrode was then exposed to 5 μL of Fc-capped SA-AuNPs conjugates for 10 min. After the electrode had been rinsed with water, voltammetric determination was performed on a CHI 660E electrochemical workstation (CH Instrument, Shanghai, China) using a homemade plastic electrochemical cell. A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively.

3. RESULTS AND DISCUSSION

3.1 Detection principle

A schematic representation of the HDAC detection via the oxidation of Fc tags on the AuNPs is illustrated in Fig. 1. The self-assembled monolayers (SAMs) of acetylated peptide/MCH were first formed on the gold surface. Insertion of the PPPPEE segment between the substrate and the cysteine residue could enhance the hydrophilicity of peptide and help position the peptide away from the electrode surface so that a higher deacetylation efficiency can be attained [4,14]. In the presence of HDAC, the acetyl group in the peptide would be removed, resulting in the appearance of free amine groups on electrode surface. The exposed primary amines were then derivatized with biotin for the attachment of Fc-capped SA-AuNPs conjugates. Consequently, a facile electron-transfer reaction between the Fc tags and the electrode surface takes place. As described previously, each gold nanoparticle is capped with a large number of Fc molecules [13]. Thus, the number of redox tags attached to the electrode will be greater than the number of peptide substrate immobilized on the electrode and the signal intensity is enhanced. The peak currents would be dependent upon the amounts of HDAC in the solution.

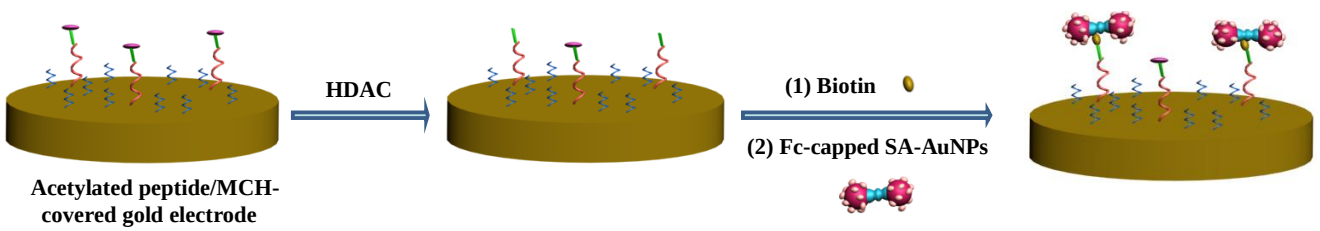

Figure 1. Schematic representation of the HDAC detection on the acetylated peptide/MCH-covered gold electrode by the following amplified voltammetric detection via the oxidation of the Fc tags on the SA-AuNPs conjugates. For clarity, the molecules and AuNPs are not drawn to scale.

3.2 Feasibility for HDAC detection

Curve a in Fig. 2A is a representative cyclic voltammogram (CV) collected at the acetylated peptide/MCH covered electrode after exposed subsequently to HDAC, biotin N-hydroxysuccinimide ester and Fc-capped SA-AuNPs conjugates. The couple of well-defined redox waves with Epa = 0.359 V and Epc = 0.342 V were attributed to the oxidation/reduction of the Fc moieties. The voltammogram exhibits a characteristic diffusional "tailing" of the peaks, due to the repulsive interaction between the ferrocenium moieties confined at the electrode surface [13]. For comparison, the same procedure was implemented with the electrode without having exposed to HDAC (curve b). The current dropped to the background level, indicating that the attachment of Fc-capped SA-AuNPs conjugates is dependent on HDAC. Furthermore, we also investigated the dependence of i_{pa} on the incubation time of HDAC. The oxidation current of Fc tags increased sharply within the modification time before 45 min but began to level off beyond 45 min (Fig. 2B), indicating that the HDAC-catalyzed deacetylation reaction completed within 45 min.

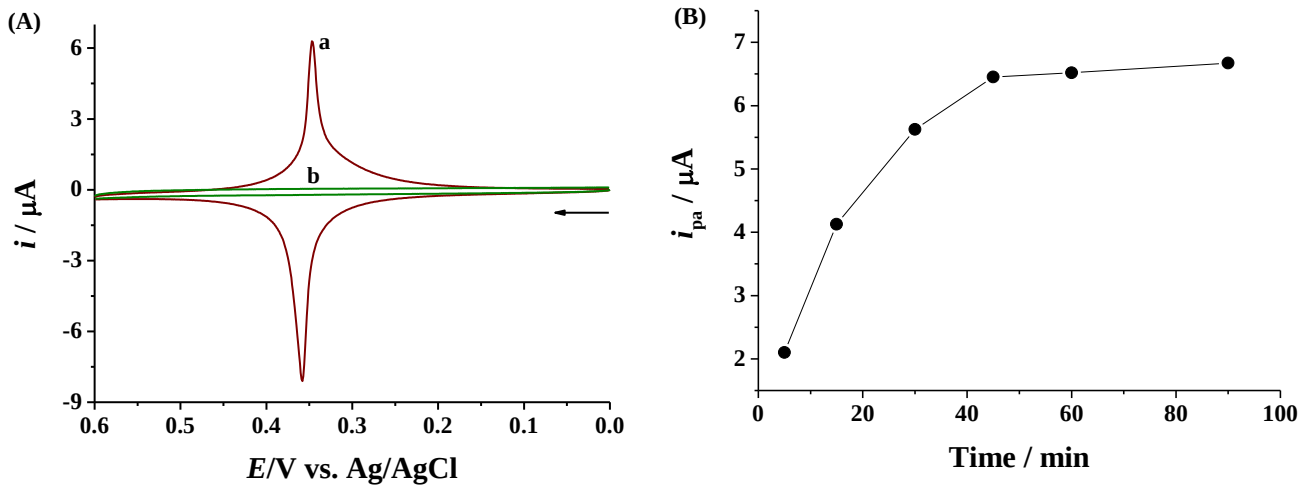

Figure 2. (A) Cyclic voltammograms (CVs) collected at the acetylated peptide/MCH covered electrodes with (curve a) and without (curve b) exposed to 30 nM HDAC, followed by incubation with biotin N-hydroxysuccinimide ester and Fc-capped SA-AuNPs conjugates. (B) Plots of the oxidation peak current (i_{pa}) against the incubation time of HDAC.

3.3 Sensitivity and selectivity to HDAC

We assessed the analytical merits of this method, such as reproducibility, selectivity, sensitivity and detection limit. The dependence of the oxidation current on the HDAC concentration is presented in Fig. 3A. The relative standard deviations (RSDs) shown as the error bars in Fig. 3 are all less than 10%, indicating the good reproducibility of the method. The oxidation current of Fc tags increased sharply within the concentration range of 0.1 – 30 nM but began to level off beyond 30 nM. The plateau exhibited by the curve is indicative of the deacetylation of all the peptide substrates by HDAC. The current increased linearly with the concentration of HDAC between 0.1 and 30 nM, which can be

expressed by i_{pa} (μA) = 0.22[HDAC] (nM) + 0.32 (R^2 = 0.984). The detection limit (3σ) of the method was estimated to be 20 pM (n = 11). The lower detection limit is a result of the combined effect of high redox activity of Fc and signal amplification of AuNPs.

The specificity of this method was studied by using serum protein (BSA), protease (PSA) and dephosphorylase (ALP) at the concentration of 100 times higher than that of HDAC. As shown in Fig. 3B, three of the tested interferences induced negligible changes in the current, indicating that they can not promote the deacetylation of substrate peptide. Moreover, to demonstrate the viability of the sensor in biomedical samples, its performance was investigated in the presence of blood serum. The change of the peak current induced by HDAC in 10% serum was the same as in the buffer, demonstrating that serum did not interfere with the assay. Because the serum sample may show strong light scattering effect, we believe that the electrochemical method presented here will be more suitable for the determination of HDAC in a biological matrix.

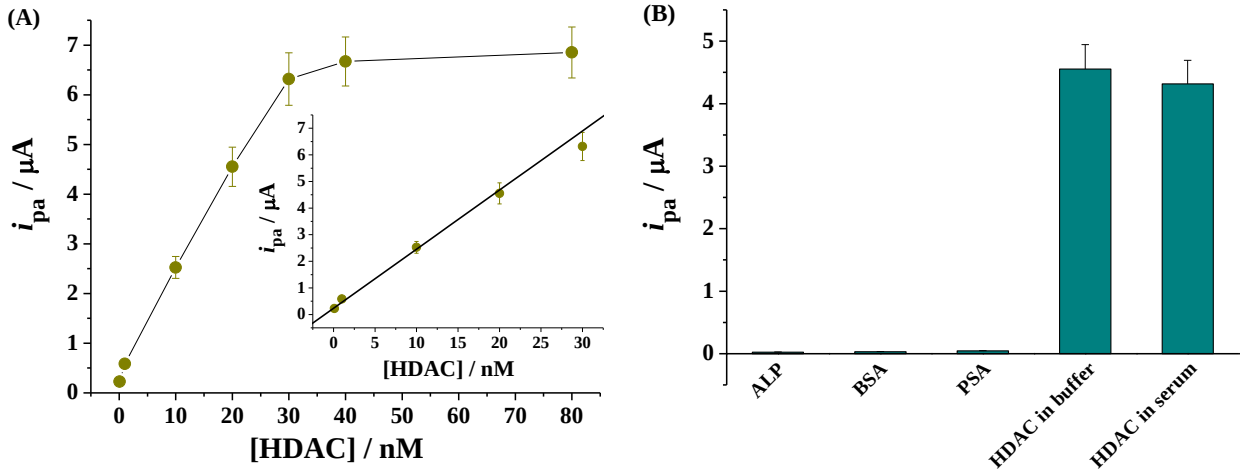

Figure 3. (A) Plots of the i_{pa} against the HDAC concentration (0.1 – 80 nM). The inset shows the linear plots at concentrations of 0.1, 1, 10, 20, and 30 nM. (B) Selectivity of the sensing protocol to 20 nM HDAC.

4. CONCLUSION

In conclusion, this work reported a sensitive electrochemical strategy for the detection of HDAC with the signal amplification of Fc-capped SA-AuNPs conjugates. The deacetylation of substrate peptides by HDAC led to the expose of free amine groups and allowed for the follow-up biotinylation, which facilitated the capture of Fc-capped SA-AuNPs conjugates through the biotin-SA interaction. The concentration of HDAC was determined by measuring the oxidation current of Fc tags. As a result, a low detection limit (20 pM) was achieved. We believe that this work could be valuable for the design of electrochemical biosensors for detection of various PTM enzymes and likely lead to many detection applications in a biological matrix.

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