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Mini review

Research Progress in Electrochemical, Electrochemiluminescent and Photoelectrochemical Detection of Histone Acetyltransferase

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Changes in the level and activity of histone acetyltransferase (HAT) have been shown to be associated with the pathogenesis of many diseases. Electrochemical techinques have the advantages of low cost, miniaturization, real-time monitoring and good selectivity as well as sensitivity. Herein, we summarized the research progress in electrochemistry-related methods for the detection of HAT activity, including electrochemistry, electrochemiluminescence and photoelectrochemistry.

Keywords: Histone acetyltransferase; post-translational modification; electrochemistry; electrochemiluminescence; photoelectrochemistry

1. INTRODUCTION

The post-translational modifications play vital roles in eukaryotic transcription and regulating chromatin dynamics, including acetylation, methylation, sumoylation, phosphorylation, ubiquitination and ADP-ribosylation [1, 2]. Among them, the histone protein acetylation correlates largely with many basic biological processes, such as histone deposition, DNA replication and repair, and nutrient metabolism [3, 4]. In this process, histone acetyltransferase (HAT) can catalyze the transfer of an acetyl group from acetyl coenzyme A (acetyl-CoA) to the lysine residue, thus producing N-acetyl-lysine within the core histone proteins [5]. The high or low expression of histone acetylation due to the abnormal HAT activity has already been shown to be associated with the pathogenesis of many diseases, such as cancers, neurological diseases, metabolic syndromes and so on [6, 7]. Thus, HAT has been recognized as an important biomarker for the diagnosis of these diseases [8]. The activity of HAT can be determined by monitoring the formation of CoA or acetyl peptide (Fig. 1). Traditionally, the

detection of HAT activity is based on radioisotope labeling of histone substrates [9]. However, the method suffers from intrinsic hazards of radioactive materials and multistep laborious procedures [10-13]. It is highly desirable and necessary to develop sensitive and selective methods for HAT activity detection and screening of the potential inhibitors. Up to now, many radio-free biosensors have been developed for HAT activity analysis, including spectrophotometry, fluorescence and colorimetry [14-20]. However, these techniques always require expensive and large volume instrument, tedious experiment step, long detection time and/or poor sensitivity. Herein, we summarized the recent progress in electrochemistry-related methods for HAT detection, including electrochemistry (EC), electrochemiluminescence (ECL) and photoelectrochemistry (PEC).

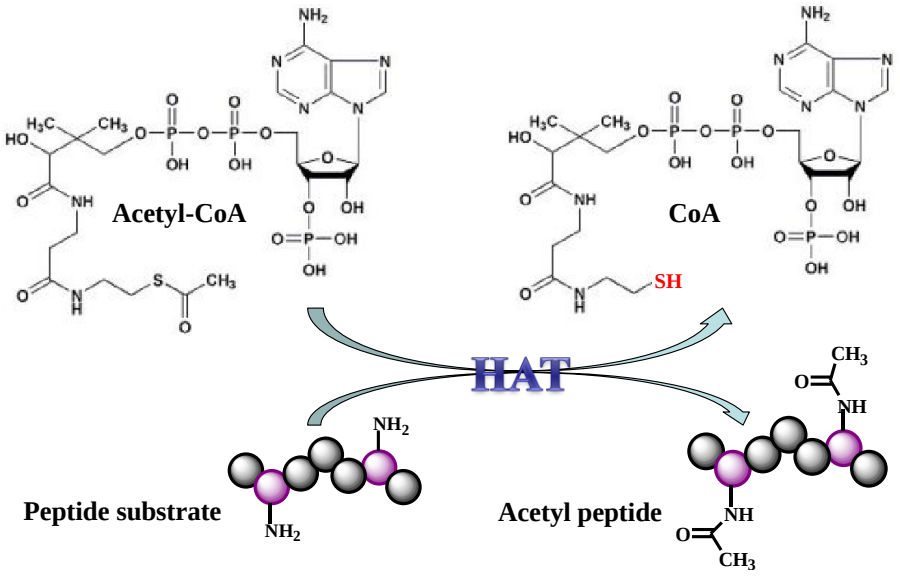

Figure 1. Chemical structures of acetyl-CoA and CoA and the catalyzed transfer of acetyl group from acetyl-CoA to the lysine residue in peptide.

2. EC METHODS

Aiming to miniaturize devices and present the potentiality of point-of-care, electrochemical techniques have attracted considerable interests in veiw of their unique apparent merits such as high anti-interference ability, low cost, and simple operation [21-24]. Peng et al. developed an ultrasensitive mushroom-like electrochemical immunosensor for probing the activity of HAT (Fig. 2A) [25]. In this work, the nanocomposite of graphene oxide (GO) and Au nanocrystals (AuNCs) were labeled with acetyl antibody (Ab_{Ac}) and methylene blue (MB). After the specific binding of acetyl and Ab_{Ac}, large amounts of MB in the nanocomposites gave rise to significant signal amplification. This immunoassay exhibited a wide linear response range (0.01 ~ 150 nM) and a low detection limit (3.6 pM) (Table 1). At the same time, Hu et al. synthesized AuPd-GO nanocomposites to carry Ab_{Ac} for the sensitive detection of HAT activity (Fig. 2B) [26]. The AuPd-GO nanocomposite with a favorable electrocatalytic property to the commercialized 3,3,5′,5′-tetramethyl benzidine (TMB) oxidation resulted in a distinct electrochemical signal.

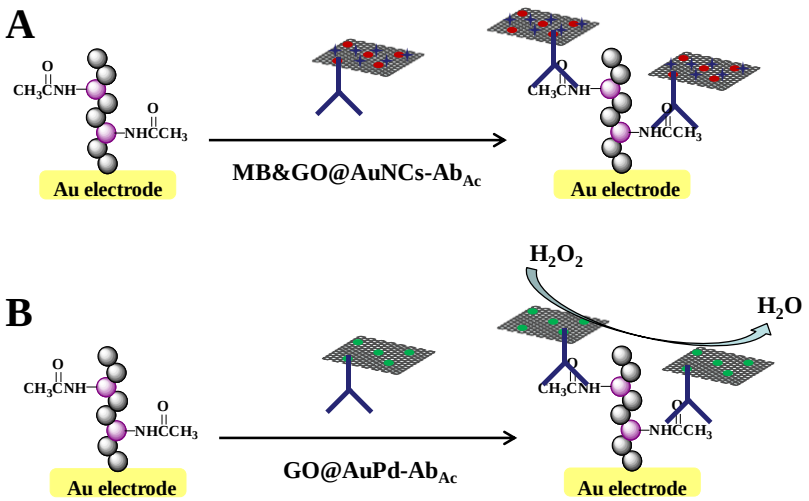

Figure 2. Schematic illustration of HAT detection by the MB&GO@AuNCs-Ab$_{Ac}$ and GO@AuPd-Ab$_{Ac}$ lables

Despite immunosensors can afford high specificity and sensitivity, the high cost and poor stability of antibodies impeded the practical applications of the immunosensors [27-30]. Therefore, other types of detection methods without the use of antibodies were developed. During the HAT-catalyzed acetylation, a large amount of CoA molecules are produced. The free thiol group in CoA can interact with some metal ions, such as Ag(I) and Cu(II). For this view, the analysis methods have been designed without the use of antibodies and tedious modifications. For example, Nie et al. reported that CoA could interact with Ag(I) to form a novel nucleic-acid mimicking CoA-Ag(I) coordination polymer (Fig. 3A), which possesses an unexpected high electrocatalytic activity towards H_2O_2 reduction [31]. Moreover, the adenine side groups on the coordination polymer facilitated the adsorption of CoA on the GO-modified electrode due to the powerful π-π interactions. This method was adapted to quantitatively detect HAT activity via electrochemical measurement of CoA generation. Recently, Cheng et al. reported an electrochemical method for HAT detection based on the semi-artificial complex of G-Quadruplex-Cu(II) (Fig. 3B) [32]. CoA, the co-product of HAT-catalyzed acetylation, competitively combined with Cu(II), and reduced the peroxidase-like activity of the metalloenzyme. This low-cost strategy exhibited a detection limit down to 0.14 nM.

Aptamer is a small oligonucleotide sequence or short polypeptide screened in vitro by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [33-35]. It can bind with the target with high affinity and strong specificity. Its emergence provides a new research platform for chemical biology and biomedicine with high efficiency and rapid recognition, and shows a good application prospect in many aspects. Wang et al. developed a label-free electrochemical stripping strategy based on the typical CoA-aptamer interaction (Fig. 3C) [36]. CoA released by HAT-based catalysis could bind with and protect its aptamer from the hydrolysis by Exo I. Then, the aptamer was extended by TdT-based catalytic reaction to generate the rich-C DNA for the formation of AgNCs, thus producing the electrochemical stripping signal. On the contrary, the absence of HAT would cause aptamer being hydrolyzed by Exo I and the amount of AgNCs was hugely reduced. The proposed strategy presented a wide concentration range from 0.01 to 100 nM with a detection limit of 2.8 pM.

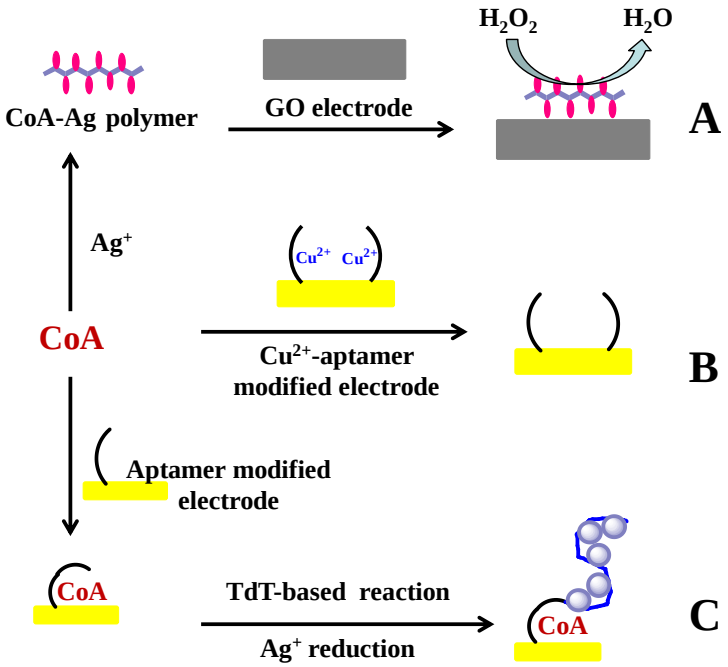

Figure 3. Schematic illustration of HAT assays by monitoring the generation of CoA by the formation of CoA-Ag polymer (A), CoA-Cu^{2+} (B) and AgCPs (C).

The HAT-catalyzed acetylation can endow the acetylated peptide with the anti-hydrolysis ability, which may be a potential way for acetylation identification. Cao et al. constructed a feasible electrochemical assay of the HAT activity [37]. Cucurbit[8]uril can interact with two Phe residues from two different peptides, thus triggering the assembly of peptide-templated silver nanoparticles (AgNPs) and substrate peptide on the electrode surface. After the HAT-catalyzed acetylation, the CPY-catalyzed digestion was blocked and large amounts of electroactive peptide-AgNPs was recruited on the electrode, thus producing a signal-enhanced electrochemical response.

3. ECL METHODS

Integrated electrochemistry with optical technique, ECL has inherit benefits of simple operation, fast response, and low background [38]. It has been shown to be a promising technique for biosensing and imaging [39]. Hu et al. established a sandwich-like immunosensor by using antibodies against HAT [40]. Two kinds of multi-functionalized GO nanomaterials, the capture antibody (Ab_1)-labeled-GO@Fe_3O_4 and the detection antibody (Ab_2)-labeled ($Ru(phen)_3^{2+}$-GO@AuNPs@tetrahedron DNA (TDN), were used. After the immune-reaction, the multi-functionalized GO nanomaterials formed a mysterious ringed Faraday-cage and extended the outer Helmholtz plane (OHP) of the modified electrode. The enhancing intercalated amounts of $Ru(phen)_3^{2+}$ and the extension of OHP drastically amplified the ECL signal. The response is linear in the concentration range of 0.005 ~ 80 nM with a detection limit of 1 pM. Moreover, a tailored DNA nano-framework-enabled ECL biosensor for quantitative detection of HAT was proposed by Zhang and co-workers [41]. In this work, DNA

nanonets were formed by Y-shape DNA as building block and Ab_{Ac}-grafted dsDNA as crosslinker following the acetylation reaction. After the capture of nanonets by the sensing electrode, terminal deoxynucleotidyl transferase (TdT) triggered renascent DNA containing abundant A and T (named as AT-dsDNA) on the DNA nanonets in the presence of NaCl. Then, a lot of $Ru(phen)_3^{2+}$ molecules were embedded inserted into the tailored DNA nano-frameworks, producing a sharply enhanced ECL signal. This method had a detection limit of 2.9 pM and a linear response in the range of 0.006-60 nM.

Li et al. developed a signal-on ECL sensor for sensitive detection of HAT based on the CoA-Ag(I) coordination complex [42]. When the formed CoA-Ag(I) coordination polymer with the RNA-like structure was adsorbed onto the GO-decorated electrode surface, it greatly improved the cathodic ECL signal of CdTe@CdS quantum dots. This label-free method showed a linear range from 0.1 to 100 nM with a LOD of 0.05 nM. In addition, Hu et al. found that the Cu(II)-quenched ECL and fluorescence signal of an electrosynthesized tris(bipyridine)ruthenium(II)-functionalized metalorganic framework (Ru-MOF) can be recorved by the produced CoA, thus realizing the analysis of HAT activity [43].

Zou et al. reported a novel ECL method for the detection of HAT by combining ECL property of silver clusters and hybridization chain reaction (HCR) signal amplification strategy [44]. In this method, the positively charged HAT substrate peptide bound with the negatively charged capture DNA (cDNA) on the electrode surface based the electrostatic interaction. With the addition of HAT, the acetylation of substrate peptide changed its charge, thus inducing the peptide to leave from the electrode surface. Then, the exposed cDNA initiated the HCR and silver ions were captured by the formed super-sandwich DNA to generate AgNCs via electrochemical reduction. The as-designed ECL method achieved a linear range of 0.5 to 100 nM and a detection limit of 0.49 nM.

RNA aptamers have also pave the way for developing various biosensors to monitor kinds of tumor markers including HAT. For example, Zhang et al. proposed an RNA aptamer-involved ECL strategy for sensitive detection of HAT [45]. During the acetylated reaction, CoA bound with the RNA aptamer and released auxiliary DNA, which was captured by three hanging arms of the constructed DNA nano-prism on the electrode surface. The auxiliary DNA can further initiate the HCR process along with continuous hybridization of two typical hairpin probes. Abundant $Ru(phen)_3^{2+}$ molecules can insert into the groove of the resulting dsDNA. This method exhibited excellent specificity and sensitivity with a detection limit of 1 pM.

Zou et al. builted a signal-off ECL biosensor for the HAT activity analysis through the resistance of acetylated peptide to trypsin hydrolysis (Fig. 4) [46]. The undamaged acetylated peptide can absorb the assembled nanoprobes consisting of gold nanoparticles and tannic acid-Fe onto the electrode by the hydrophobic and hydrogen bonding interactions. The captured nanoprobe as a mimetic superoxide dismutase consumed the reactive oxygen species and in turn the ECL signal from luminol was decreased. A good linear relationship was obtained at the concentration range of 0.1 ~ 100 nM. A detection limit of 0.074 nM was achieved.

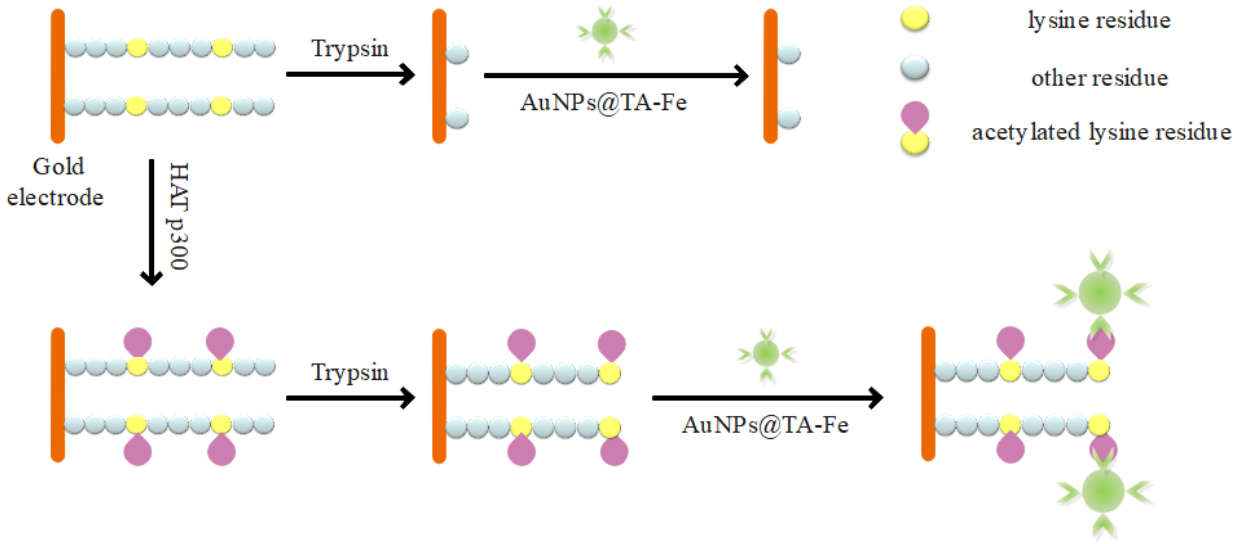

Figure 4. Schematic presentation of the ECL method for HAT determination.

4. PEC METHODS

In PEC technique, the photocurrent is generated by photoelectric conversion materials excited by light as output signal. PEC method has an ultra-low background signal and an outstanding sensitivity, resulting from the completely separated excitation light source and signal output. The technique has attracted more attentions in sensing of various targets [47, 48]. On basis of the molecule structure of CoA and the catalytic activity of *β*-galactosidase (*β*-Gal), Yin et al. proposed a PEC method for determining HAT activity (Fig. 5A) [49]. The produced CoA molecules were captured by AuNPs on the MoS_2 nanosheets and GO-modified electrode through the Au-thiol interactions. Then, the exposed phosphate group in CoA recruited phos-tag-biotin, which can be recognized by streptavidin (SA)-functionalized *β*-Gal (SA-Gal). *β*-Gal on the electrode surface promoted the hydrolysis of 4-aminophenyl *β*-D-galactopyranoside (4-APG) into 4-aminophenol (4-AP). The produced 4-AP is used as electron donor for photoelectrochemical response of MoS_2 nanosheet. The detection limit of 140 pM was obtained with a linear concentration range of 0.3 ~ 100 nM. In other work, when CoA was captured by AuNPs-WS_2 nanosheets, the phosphate groups could specifically bind with b-TiO_2, which further recruited polydopamine (PDA) based on the specific interaction between the vicinal diol of PDA and b-TiO_2 (Fig. 5B) [50]. PDA offered the electron to capture the photogenerated hole of photoactive material, thus decreasing the recombination of photogenerated electron and hole and leading to the third-order signal amplification. Similarly, succinimidyl 4-(N-maleimidomethyl) cyclohexanecarboxylate (SMCC) was used to link PDA-modified WS_2 nanosheets and CoA, in which SMCC reacted with the NH_2 group of PDA and the SH group of CoA [51]. Next, phos-tag-biotin and SA were subsequently captured by the electrode and inhibited the PEC response. This PEC strategy presented a linear relationship with a range of 0.1 ~ 200 nM. The detection limit was 0.047 nM. Besides, 3-maleimidopropionic acid was employed as a “bridge” reagent for immobilization of CoA on the amino-functionalized photoactive materials [52]. ZnO QDs as the photoelectrochemical signal inhibitors were captured by the interaction of Zn^{2+} and phosphate group.

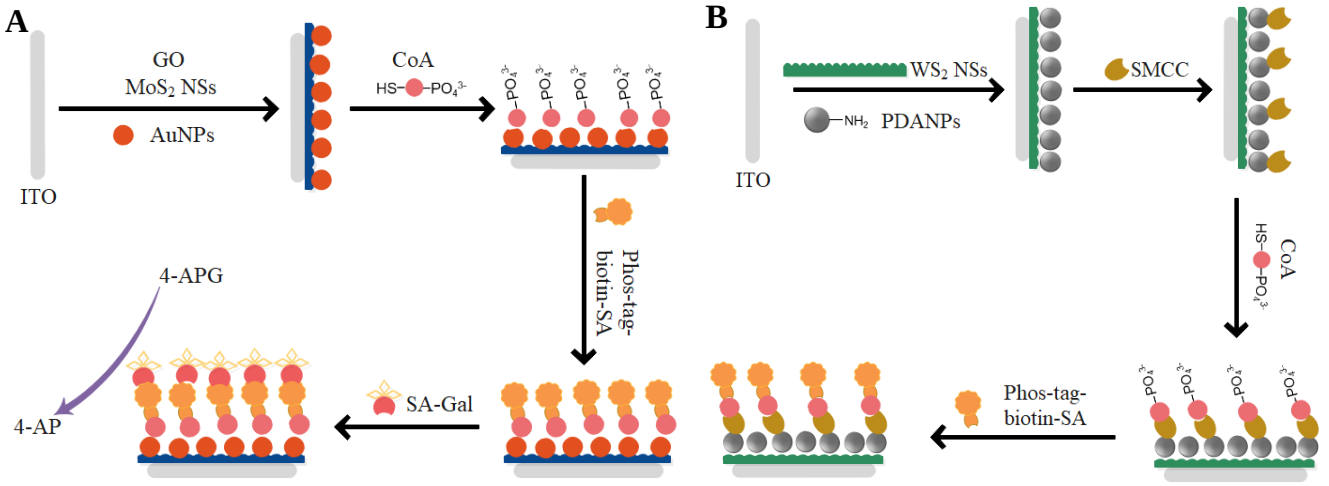

Figure 5. (A) Schematic presentation of biosensor fabrication and CoA detection. (B) Schematic illustration for HAT assay.

Table 1 Analytical performances of the EC, ECL and PEC methods for HAT detection.

Methods	Linear range	Limit of detection	Ref.
EC	0.01 ~ 150 nM	0.0036 nM	[25]
EC	1 pM ~ 1000 nM	0.5 pM	[26]
EC	0.1 ~ 100 nM	0.067 nM	[31]
EC	0.5 ~ 150 nM	0.14 nM	[32]
EC	0.01 ~ 100 nM	0.0028 nM	[36]
EC	0.1 ~ 50 nM	0.055 nM	[37]
ECL	0.005 ~ 80 nM	1 pM	[40]
ECL	0.006 ~ 60 nM	0.0029 nM	[41]
ECL	0.1 ~ 100 nM	0.05 nM	[42]
ECL	0.01 ~ 100 nM	1.5 pM	[43]
ECL	0.5 ~ 100 nM	0.49 nM	[44]
ECL	0.003 ~ 300 nM	0.001 nM	[45]
ECL	0.1 ~ 100 nM	0.074 nM	[46]
PEC	0.3 ~ 100 nM	0.14 nM	[49]
PEC	0.01 ~ 500 nM	0.0033 nM	[50]
PEC	0.1 ~ 200 nM	0.047 nM	[51]
PEC	0.01 ~ 500 nM	3 pM	[52]

5. CONCLUSION

This review summarized the recent reports on the electrochemistry-related biosensors for HAT detection. Although the methods have made exciting progress, a more sensitive electrochemical technique is still desired since the concentration of HAT in vivo is ultralow. Moreover, most of the methods require the use of antibody and enzyme, which will be prone to false positive, instability as well as batch difference. For the practical applications of HAT biosensors, it is of great importance to develop biodegradable, biocompatible and nontoxic materials.

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