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Analogous modified DNA probe and immune competition method-based electrochemical biosensor for RNA modification

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ABSTRACT

N6-methyladenosine (m6A), one of the most abundant RNA methylation which is ubiquitous in eukaryotic RNA, plays vital roles in many biological progresses. Therefore, the rapid and accurate quantitative detection of m6A is particularly important for its functional research. Herein, a label-free and highly selective electrochemical immunosensor was developed for the detection of m6A. The method is established on that the anti-m6A-Ab can recognize both m6A-RNA and m6A-DNA. An analogous modified DNA probe (L1) serves as a signal molecule, by competing with m6A-RNA for binding to Abs to broaden the linear range. The detection of m6A-RNA by this method is unaffected by the lengths and base sequences of RNA. Under optimal conditions, the proposed immunosensor presented a wide linear range from 0.05 to 200 nM with a detection limit as low as 0.016 nM (S/N = 3). The specificity and reproducibility of the method are satisfactory. Furthermore, the developed immunosensor was validated for m6A determination in human cell lines. Thus, the immunosensor provides a promising platform for m6A-RNA detection with simplicity, high specificity and sensitivity.

1. Introduction

Epigenetics is a genetics branch discipline that studies heritable changes in gene expression without gene nucleotide sequence change. In the central dogma, RNA is a bond between DNA and protein, not only for the transmission of genetic information, but also for a variety of post-transcriptional regulatory functions. Over the past decades, researchers have found more than 150 types of chemical modifications on RNA (Helm and Motorin, 2017). These modifications have greatly expanded the diversity of RNA functions and genetic information, and have a very important regulatory role in gene expression (Gilbert et al., 2016), disease occurrence (Bellodi et al., 2013), growth and development (Kan et al., 2017), immune regulation (Slotkin and Nishikura, 2013) and so on. Therefore, the detection of RNA modifications not only has very important significance to epigenetics research, but also has great potential clinical application values with respect to disease risk assessment, diagnosis, treatment and precision medical treatment (Chen et al., 2017; Nainar et al., 2016; Vu et al., 2017; Zhang et al., 2016).

N6-methyladenosine (m6A) is one of the most abundant RNA modifications, which is ubiquitous in eukaryotic RNA (Zhang et al., 2017). As is well known that DNA and proteins undergo dynamic and reversible chemical modifications that influence their functions (Meyer and Jaffrey, 2014), the modification of RNA is also dynamically regulated by many methyltransferase such as methyltransferase-like 14 (METTL14) and demethylase like fat mass and obesity-associated proteins (FTO) (Ma et al., 2017; Visvanathan et al., 2018). Accumulative evidences indicate that variant m6A levels exert diverse biological functions in mammals, such as transcription splicing (Zhao et al., 2014), nuclear RNA export (Wang et al., 2014), protein translation control (Wang et al., 2015), and cell fate determination (Geula et al., 2015). The regulatory machinery of m6A modification might be associated with human diseases or cancers (Chandola et al., 2015). In order to understand the relationship between the expression level of m6A in the gene transcripts and its basic functions, it is particularly important to rapidly and accurately detect the m6A. However, the amount of m6A in isolated RNA was estimated to be only 0.1–0.6% in Homo sapiens (Li et al., 2016b), which means only about 3–5 m6A sites in per mRNA not to mention in the total RNA (Klungland and Dahl, 2014). Low abundance of m6A in RNA (m6A-RNA) makes the precise detection difficult.

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Moreover, the methylation of adenosine does not change its ability to base pair with thymidine and uracil. Therefore, m6A cannot be detected with standard hybridization or sequencing-based methods. The m6A was first found in 1974, while the research on the biological functions of m6A is greatly lagged due to the lack of reliable and sensitive detection technique.

Some strategies for RNA m6A (m6A-RNA) detection were reported, including thin layer chromatography (TLC) (Jia et al., 2011), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Yan et al., 2013), high performance liquid chromatography (HPLC) (Narayan et al., 1994), m6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP) (Linder et al., 2015), chromatin immunoprecipitation followed by sequencing (ChIP-Seq) (Dominissini et al., 2012) and methylated RNA immunoprecipitation followed by sequencing (MeRIP-Seq) (Meyer et al., 2012). However, some of those techniques require labeling RNA with P^{32}, which is harmful to the operator's health and the environment. The other techniques need either expensive or large instruments, or complicated operation steps, or long detection time or skilled personnel, thus greatly limit their applications for rapid detection of m6A.

Electrochemical biosensors have attracted extensive attention and rapid development, due to merits of simple operation, time-saving, low-cost, miniaturized instrument, high sensitivity and selectivity. Up to now, electrochemical technique can be used in many fields, such as environmental pollution (Wang et al., 2017; Zeng et al., 2017), food safety (Lv et al., 2018; Silva et al., 2018), protein (Ji et al., 2017; Zhao et al., 2018), cell (Shen et al., 2016; Tang et al., 2018), and nucleic acid (Li et al., 2016a; Tao et al., 2017; Benvidi and Jahanbani, 2016). Especially, electrochemical technique has been applied to analyze modifications of nucleic acid, including DNA methylation (Gao et al., 2018; Jing et al., 2014). Therefore, the electrochemical method could be a potential technique for m6A-RNA detection, and several works have been done. Yin et al. has developed two electrochemical immunosensors for quantitative detection of m6A with high sensitivity (Yin et al., 2017, 2015), and Benvidi et al. has developed electrochemical impedance methods for DNA detection (Benvidi et al., 2014, 2016). It is a great encouragement for us to develop a simple and label-free electrochemical impedance method to determine m6A-RNA with high specificity.

In this work, we fabricated a simple, label-free, sensitive and selective electrochemical immunosensor for quantitative detection of m6A-RNA shown in Scheme 1. This method based on the specific interaction between antibody (Ab) and antigen (Ag), and the fact that anti-m6A-antibody (anti-m6A-Ab) can recognize both m6A-RNA and DNA m6A (m6A-DNA) (Xiang et al., 2017). First, the histidine-tagged recombinant protein G (his-PG) can firmly bind to the surface of a gold electrode through its histidine tag to form a directional layer, which promotes immobilization of Abs on the surface of the gold electrode. Abs are oriented by the specific interaction of the fragment crystallizable region (Fc) with PG that forces Ab to expose its binding sites to the environment, which can improve the recognization ability and efficiency between Ag and Ab (Casalini et al., 2015). Then m6A-DNA probes (L1), competing with the m6A-RNA in the sample for binding to the Abs, help to broaden the detection range (Nelson et al., 2003). After that, ribonuclease A (RNase A) is used to hydrolyze RNA bound to Abs. Finally the electrochemical impedance spectroscopy (EIS) signal of the electrode is detected and the intensity of the signal is inversely proportional to the amount of m6A- RNA in the sample.

2. Experimental

2.1. Reagents and materials

See Supplementary material.

2.2. Instrumentation

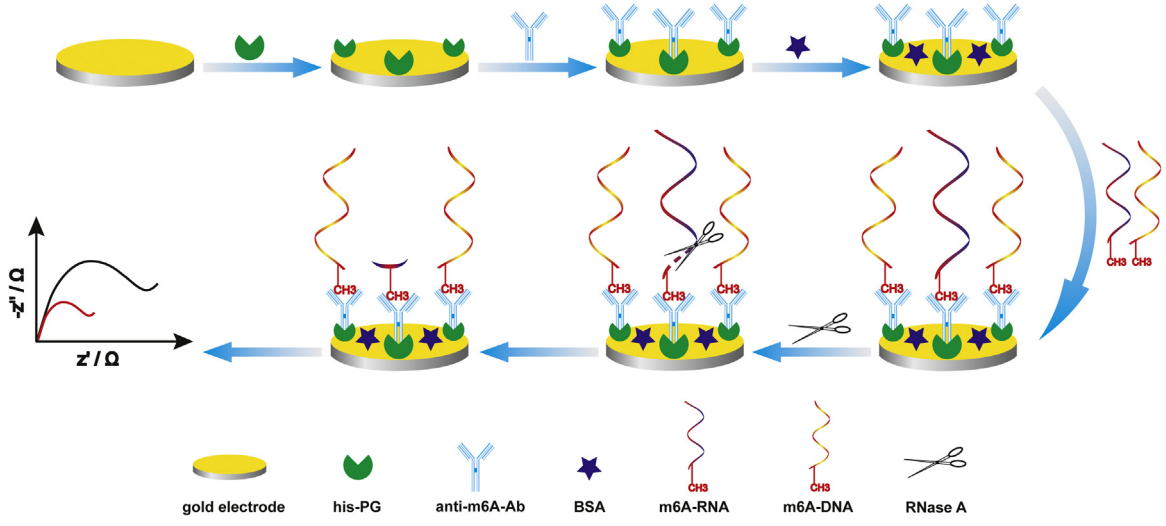
See Supplementary material.

2.3. Preparation of gold electrode

Prior to modification, the gold electrode was polished to mirror-like surface with 0.05 μm Al_2O_3 slurry. Then the gold electrode was sonicated alternatively for 5 min in ultrapure water, and anhydrous ethanol. After that, it was activated in freshly prepared piranha solution (98% H_2SO_4 / 30% H_2O_2, 7:3, v/v) for 5 min and rinsed with ultrapure water thoroughly.

2.4. Immunosensors fabrication

First, 10 μL of 20 μg/mL his-PG was added on the gold electrode and incubated overnight in a 4 °C refrigerator, then rinsed with 10 mM PBS buffer. The obtained electrode was incubated with 10 μL of 20 μg/mL anti-m6A-Ab at 4 °C for 3 h. After rinsed with 10 mM PBS buffer, the electrode was incubated with 10 μL of 0.25% Bovine serum albumin (BSA) at 4 °C for 1 h. Followed rinsing with 10 mM PBS buffer, the anti-m6A-Ab combined with m6A by drop 10 μL of mixed solution which containing different concentrations of targets and 5 nM L1 at room



Scheme 1. The procedures of fabricate immunosensor.