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Scrutiny on Significant Factors of Hemin Catalyzed Ag Nanoparticle as Biosensor for RNA

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Abstract: A responsive electrical detection technique of nucleic acids has been demonstrated on submicrogapped biosensor. This method contain immobilizing of Peptide Nucleic Acid (PNA) probes in the gap areas of a pair of interdigited microelectrodes and subsequently hybridizing with their complementary target RNA. After hybridization, hemin molecules were introduced into the RNA strand via zirconium-phosphate and zirconium-carbonate chemistries. The newly attached hemin molecules act as a catalyst to accelerate reducing ammoniacal Ag ion to form Ag nanoparticles, which span the gap of the interdigitated microelectrode. The conductance of the Ag nanoparticles directly correlated with the number of the hybridized RNA molecules. Nearly 1 fM sensitivity was achieved under optimal conditions.

Key words: Sensitive detection, nanotechnology, RNA, biochip, sub-microgap

INTRODUCTION

There is a universal attempt towards the expansion of bioanalytical strategy that can be used for determination, of definite substance species counting nucleic acid molecules (Boztok and Cokuysal, 2006; Cokuysal *et al*., 2006). For example, it is a significant issue in analytic of genetic, bacterial and ecological observing of microorganisms. The majority regularly used manner for determining the existence and concentration of nucleic acids include fluorescence, chemiluminescence as well as electrical procedures (Odentahl and Gooding, 2007). Distinctive nucleic acid detection methods rely on marking of the nucleic acid to be inspected. Following hybridization by complementary RNA detains molecules in a uniform phase; the markers are perceived at the bound nucleic acids positions by one of the aforementioned manner. These procedures are extremely insightful and well reputable but furthermore demonstrate many disadvantages, such as photo blanch of fluorophore labels, high costs, the prerequisite of large device and extended diagnostic processing time. For this cause, there is motionless a require for a straightforward, dependable and gainful detection method for fast nucleic acid analysis. Here have been several modus operandi obtainable for label-free detection, these method canister be electrochemistry, such as label-free electro chemiluminescence (Wei *et al*., 2007) and optical methods, such as silicon-on-insulator microring resonator means (Vos *et al*., 2007) and surface colloid communication detection (Sun *et al*., 2007) and nanogapped sensors. Numerous years ago, a study by Lee *et al*. (2002) was published relating to the RNA detection by measuring its capacitance modify earlier than and following the RNA hybridization. The gap aloofness is 50 nm. It is a label-free method, no required to use fluorescent label, nevertheless, it is very hard to manufacture a nanogap sensor with the gap aloofness only 50 nm wide and for the meantime the signal dissimilarity earlier than and after hybridization is not very high. In 2004, there is a report (Moreno-Hagelsieb *et al*., 2004) to use interdigited electrodes for RNA sensing, Its detection limit can get to 0.2 nM and this technique can be well-matched with micro array knowledge. This collection in Belgium supplementary urbanized their way and the detection limit can reach to 50 pM. A comparatively speedy and immediate analysis can be achieved by means of electrical and electrochemical biosensors. There are previously some rumor recounting the use of electrical technique detection, such as conductivity measurements (Park *et al*., 2002). Recently, the probable of metal nanoparticles as an substitute label for ultra sensitive detection of nucleic acids has been shown (Haick, 2007). Velev and Kaler (1999) established the formation of arrays of biosensors by in site assemblage of colloidal particles onto micro patterned electrodes. Their investigative consequences demonstrate that a way of interfacing colloidal get-together with electronic path could be used to generate useful plans with sensitivities analogous to those of scientific examine. It can be assume that their technique detained huge assure for generate throwaway on-chip arrangement of greatly perceptive minuscule sensors for specific proteins, RNA wreckage, or other bimolecular.

Hemin (hydroxyferriprotoporphyrin) is the firm, oxidized form of the free heme core of the enzyme, HRP. In

contrast to HRP, hemin is an economical iron-porphyrin molecule that does not hold any amino acid remains and for this reason has considerably senior firmness in a wider range of pH circumstances. Consequently, by launch a compound hemin to the analytical system to postpone HRP, a regular bustle can be continued for Ag statement. In this study, a tale, fast electrical detection way for nucleic acids on sub-microgapped biosensor is urbanized.

MATERIALS AND METHODS

Manufacture of sub-micro gapped biochip: Sub-micro gapped chips used in this experiment were made-up by an ignition method. A solitary chip contained 5×5 interdigited microelectrodes every shaped of 120 pair of 140 μm long and 600 nm broad fingers; the gap distance between each pair of finger-like electrodes was 500 nm.

Surface was treated according to a method described elsewhere (Liu and Bazan, 2005) with a few amendment. Temporarily, the chips were methodically cleaned with chloroform and acetone to remove any possible organic contaminants, then were dried with nitrogen and rendering to 8 min oxygen plasma, previous to being washed in isobutanol and absolute ethanol and dried. then they were washed amid absolute ethanol and permitted to dried out under gentle nitrogen flow. Unreacted PNA detain probes were detached in the subsequently stride by a methodical washing by water and methanol. finally, the chips were treated with a dimethylformamide solution of ethanolamine and diisopropylethylamine to passivity their surface.

Hybridization and electrical detection: Hybridization was carry out in a 10 mM HCl, 1.0 mM EDTA and 0.1 M NaCl buffer for 50 min at room hotness. After that, the hybridized RNA strand was stimulated via zirconium-phosphate chemistry. By doing so, hemin molecule was attached to the RNA strand, to proceed as catalysts in the resulting step of Ag ion reduction producing Ag nanoparticles. A newly prepared solution of mixed Ag acetate and hydroquinone in the ratio of 2:1 (both dissolved in sodium nitrate buffer, pH 4) was added to the chip surface and nurture for 6 min, to make a permanent Ag nanowire, which would electrically petite the electrodes. The descriptive diagram for the quantity mechanism is shown as Fig. 1. Conductance measurements were achieved under ambient circumstances by using Alessi REL-6100 probe station (Cascade Microtech.) prepared with an Advantest R8340A ultrahigh resistance meter (Advantest Corp., Tokyo, Japan). Data were reported as a regular of the comeback from 10 measurements.

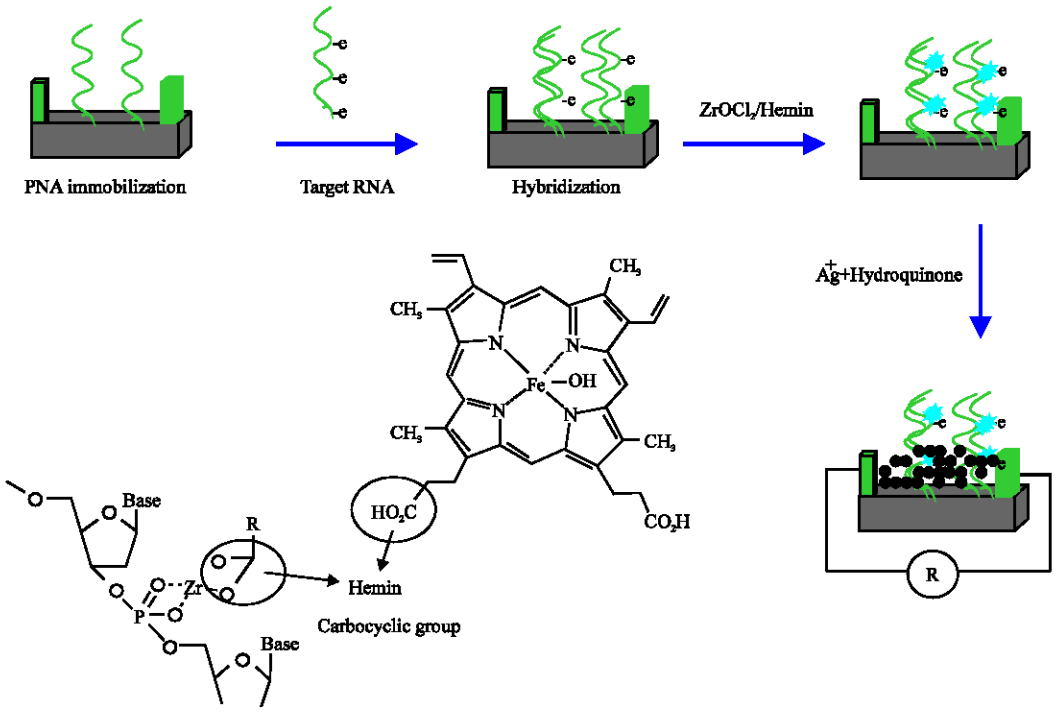

Fig. 1: Descriptive plan of determine mechanism

RESULTS AND DISCUSSION

Alternative of Ag ion concentration: Of numerous factors which influence the conductance between the pair electrodes in a sub-microgapped sensor, one of the important factors is the Ag concentration. So, the consequence of Ag concentration used in the Ag reduction step by hemin was investigated. In this trial, hemin concentration and RNA concentration as well as the reaction time are fixed at 250 μM in DMF, 10-12 M in buffer and 4 min correspondingly; the single alteration is the concentration of Ag acetate. Figure 2 shows that at low Ag ion concentrations, 12 mM and below, conductance of both control and sample groups were small, showing little difference. Between Ag ion 13 and 14 mM the conductance of the sample groups was much larger than the control groups. While above 14 mM, the conductance of control groups amplified leading to an augment in background noise and a diminish in sympathy. Based on these results, it was concluded that 14 mM of Ag acetate would be the most suitable Ag concentration and it was accept for the following experiment.

Alternative of hemin concentration: The effect of hemin on the conductance consequences of the sub-microgapped sensor was investigated. In this experiment, 14 mM Ag acetate solution and 10-12 M RNA sample were used and the reaction time is 5 min. It can be seen from Fig. 3 that the conductance reached a plateau above the hemin concentration of 300 μM, indicating that the response of RNA to hemin saturated at this concentration which was so used for all the following experiments.

Calibration curve: The conductance of a couple of sub-microgap electrodes on a chip primarily depends on the numeral of Ag nanoparticles Formed along gap. This numeral is in turn dependent virtual on the quantity of hemin bound to the objective RNA strands in the gaps. Provided the saturation concentration of hemin is used, this is unswervingly proportional to the amount of objective RNA molecules hybridized. The hybridization of PNA and objective RNA is in the ratio of 1:1, thus the amount of the PNA imprisons probes powerless in the gaps and the hybridization competence will eventually conclude the number Ag nanoparticles shaped in the gaps of the biosensor.

Based on earlier experimental results (Schoning and Poghossian, 2002), the finest circumstances for analysis were indomitable to be: Ag acetate 14 mM, hemin concentration 300 μM, reaction time 5 min. Under such circumstances, sub-microgapped biosensors were extravagance with RNA samples, their concentration

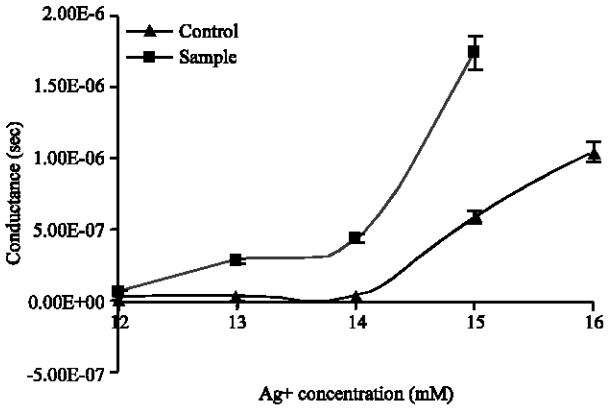

Fig. 2: Consequence of Ag ion concentration on conductance. Experimental condition are: the hemin concentration 250 μM in DMF and RNA concentration 10-12 M, reaction time 5 min

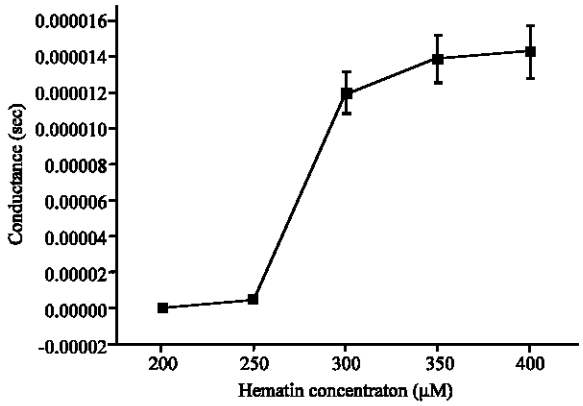

Fig. 3: Effect of hemin concentration on conductance. Experimental condition Ag ion concentration 14 mM and RNA 10-12 M, reaction time 5 min

variety from 10-11 to 10-15M with 20 measurements at each concentration. The acquired calibration curve of the regular of the conductance versus the RNA concentration is shown in Fig. 4. As predictable there is a linear affiliation among RNA concentration and conductance with a regression coefficient of 0.993, which shows that a good quality linear affiliation between RNA concentration and conductance survive, establish that the number of the goal RNA molecules hybridized in the sub-microgaps is unswervingly related to the conductance. These consequences established that hemin certainly act as a catalyst catalysing the formation of Ag nanoparticles, which is alike to the study of Mller *et al.* (2005) and Rosi and Mirkin (2005) by using of the HRP for catalytic metal deposition from a source of metal ions in on an interdigited microelectrode sensor. The detection limit is

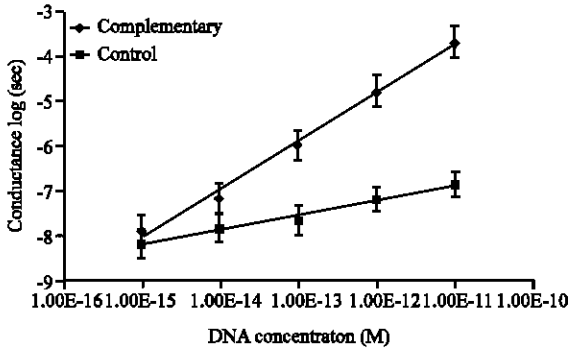

Fig. 4: Calibration curve for complementary RNA and control sample. Experimental Condition: Ag ion concentration 14 mM, hemin concentration 300 M in DMF, reaction time 5 min

near 1 fM, 2 orders of magnitude better than the one attain using Ag-enhanced gold nanoparticle labeling technique for detection of nucleic acids (Park *et al*., 2002; Ray, 2007).

CONCLUSIONS

Detection of RNA on a sub-microgapped biosensor has been established in this paper. For the first time, hemin was used to catalyze the Ag nanoparticle creation in a submicrogapped sensor for RNA detection, which has deeply abridged the background noise and cut down the detection process. It can be predictable that a thinner gap thickness will give the possible for senior sensitivity. For the meantime, since the metallic Ag shaped in the sub-microgapped sensor is steady, it can be uncovered to the exterior surroundings for longer time, which would be an benefit for mounting multiplexed detection of different RNA samples.

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