Biomedical Applications of Modified Carbon Glassy Electrode Sensor with Nanoparticles and Dendrimers

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ABSTRACT

In our previous reports, we studied the development of biosensor platform that are capable of measuring biometabolites and environmental sensitive species, such as peroxide and nitrate/nitrate, to concentration in the order of ppb (parts per billion) or lower.

In our more recent development, we modified our platform with dendrimers to enhance its performance. Zero and second generation of dendrimers were coated on the surface of a carbon glassy platform electrode modified with GDH (glutamate dehydrogenase) and it was used to measured ammonium, a common biometabolite, at near neutral pH that is common for normal bioactivities.

The resulting electrode was tested with ammonium concentrations ranged from 0.002 to 0.3 μM with satisfactory results. Measurements at lower concentrations had better resolution than at higher concentrations and it is believed that the lower concentration limit can be better than the tested lower limit at 0.002 μM. Performance of the modified carbon glassy electrode was compared with other glassy electrodes that were modified differently, and the results will be reported.

The biosensor platform thus far was proven to be versatile and can be used in many biomedical and environmental applications.

Key words: biosensor, nanoparticle, GDH, ammonium, electrode, PAMAM

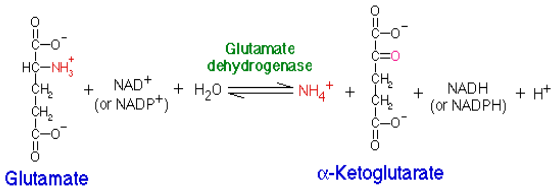
1 INTRODUCTION

In the past several years, our research group has been developing a platform for biosensor construction that are capable of detecting target species that are in nano and subnanomolar levels [1, 2].

In this study, we were developing a biosensor that can measure ammonia at submicromolar levels that cannot be detected by commercial ammonia electrodes or wet chemistry method easily.

1.1 Theory

As shown in the following equation, glutamate and NAD^+ can be hydrolyzed to form α-keto glutarate, NADH, and ammonium ion with the enzyme, glutamate dehydrogenase. The equilibrium constant is in favor of the formation of glutamate and thus the reverse reaction is faster kinetically.



For many biological reactions, the end product or metabolite is ammonium, accumulation of high concentration of ammonium is toxic to the body. Environmentally, ammonium is a byproduct of many industrial processes, uncontrolled discharges of ammonium will lead to harmful consequence to the environment. Our goal here was to develop a sensor device that can measure ammonium ion at the lowest concentrations possible, its utilities can be in biomedical or environmental applications. Hence, it is the reverse of the reaction as shown above (glutamate formation) that we utilized.

2 MATERIALS AND METHODS

2.1 Materials

L-glutamic dehydrogenase (from bovine liver, solution in 50% glycerol) (GDH) was purchased from Sigma-Aldrich (St. Louis, MO, USA), the concentration of GDH is 28 mg protein/ml, and 46 units/mg. Cysteamine, polyamidoamine dendrimer generation 0 (PAMAM_0), polyamidoamine dendrimer generation 2nd (PAMAM_2nd), and α-keto glutarate, $AuCl_3HCl{\cdot}4H_2O$ (Au %> 48%) and Na_3citrate were purchased from Sigma. All the other chemicals were of analytical grade or highest grade available. All the experiments were carried out under deoxygenated condition in 0.1 M phosphate buffer solution.

2.2 Electrode Preparation

The cleaned glassy carbon electrode was first immersed in 0.1 M cysteamine solution in darkness. The resulting monolayer-modified electrode was rinsed thoroughly with twice-distilled water and soaked in water. Then, it was dipped into the colloidal gold. The gold colloid–cysteamine-modified electrode was dipped into the l-glutamate dehydrogenase (GDH) solution (pH 7.4) (or GDH solution containing NADH). In such a way, a GDH (/NADH) gold colloid–cysteamine-modified glassy carbon electrode was obtained.

In case that both cysteamine and PAMAM were coated onto electrodes, the cleaned glassy carbon electrode was first immersed in cysteamine solution, the resulting electrode was dipped into the PAMAM solution. The gold colloid–cysteamine/PAMAM-modified electrode was dipped into the GDH solution (pH 7.4) (or GDH solution containing NADH). In such a way, a GDH (/NADH) gold colloid–cysteamine/PAMAM-modified glassy carbon electrode was obtained.

2.3 Nanoparticles Solution Preparations

Nanoparticles Au was prepared by reacting $HAuCl_4$ with citric acid [2].

2.4 Detections

UV-VIS spectrophotometry was carried out by an Agilent diodearray spectrophotometer; cyclic voltammetry was conducted by using a Gamry 600 Potentiostat. Voltammetric potential was measured against a saturated chloride electrode (SCE).

3 RESULTS AND DISCUSSION

3.1 Stability of Electrodes

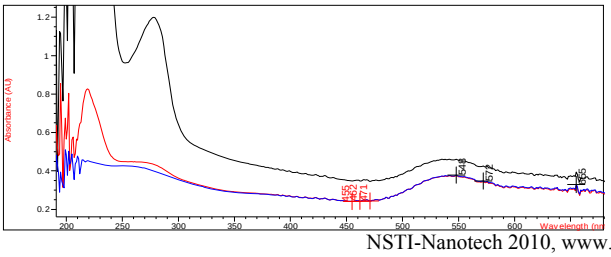


Figure 1. Cysteamine-PAMAM-Au-GDH coated on plastic UV-VIS cell with permanent absorption peak at 278 nm. Various lines indicated concentration differences.

As shown in Figure 1, PAMAMs can be attached to cysteamine to enhance the available sites for the electrons transfer between reactions, in this case, ammonium and α-keto glutarate, therefore increase the detection lower limit.

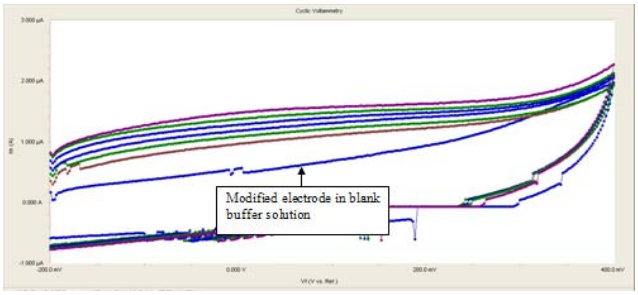


Figure 2. Cyclic voltammograms (i/v) of gold colloid-cysteamine/PAMAM_2ND-modified glassy carbon electrode measured from-0.2 to 0.4 V for the measurement of NH_4^+, the lowest concentration was 2 nM.

Figure 2 shows the cyclic voltammograms of various ammonium concentrations measured by the modified glassy carbon electrode, the lowest concentration was 2 nM. As indicated in the voltammogram in the reductive curves (upper lines), there was a big gap (current difference) between the blank (of buffer solution) and the first reductive curve (2 nM). Therefore, it is possible that the modified electrode can measure ammonium concentrations down to subnanomolar levels.

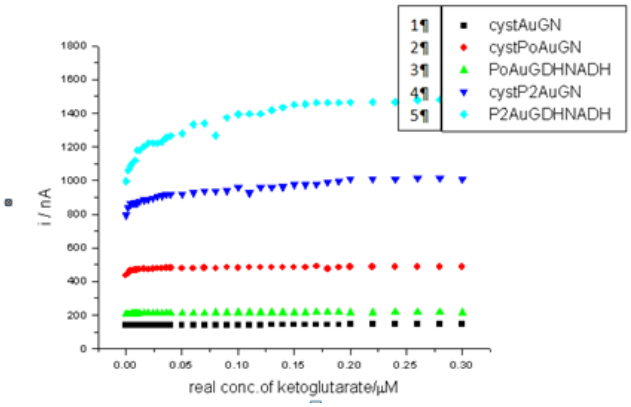


Figure 3. Performance differences of the glassy carbon electrode modified by different materials: 1. Modified with cysteamine-Au-GDH/NADH; 2. Cysteamine /PAMAM_0-Au-GDH/NADH; 3. PAMAM_0-Au-GDH/NADH; 4. Cysteamine /PAMAM_2nd-Au-GDH/NADH; 5. PAMAM_2nd -Au-GDH/NADH.

Figure 3 shows that there were distinctive current differences for the reaction of ammonium and α-keto glutarate when the electrode was modified by different

materials. From the stand point of detection, within the five modifications, sensor coated with second generation of PAMAM would have the highest sensitivity (current vs. concentration). The results were conceivably due to the higher branching property of PAMAM_2nd.

4 CONCLUSIONS

We successfully modified our glassy carbon electrode biosensor platform [2] for ammonium detection; we further modified the sensor platform with PAMAMs and the detection lower limit was enhanced. This highly modified electrode can detect ammonium concentrations down to 2 nM, or lower. The low detection limit of this biosensor is far more superior to most available methods, more research is needed for noise reduction and the stability of the electrode.

5 ACKNOWLEDGMENT

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REFERENCES

[1] S. W. Leung, Y. Wang, H. Gu, and J.C.K. Lai, "Characterization and Applications of Modified Gold Electrode Sensor with Nanoparticles", in Technical Proceedings of the 2009 Nanotechnology Conference and Trade Show, Volume 2: Chapter 4: Biosensors and Diagnostics, pp. 233-235.

[2] J.C.K. Lai, Y. Wang, W. Gao, H. Gu, and S. W. Leung, "Performance Comparisons of Nanoparticle Modified Sensor Electrodes for the Detection of Nitrite and Peroxide", in Technical Proceedings of the 2009 Nanotechnology Conference and Trade Show, Volume 2: Chapter 4: Biosensors and Diagnostics, pp. 233-235.