Comprehensive Analysis of High Performing Electrochemical Biosensors and Their Applications: II, System Update

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ABSTRACT

We have mounted 5 different biocatalysts on a sensor platform to examine the performance of this electrochemical sensing system for the detection of different biomolecules/metabolites and environmental important molecules, with such we also compared how this sensing system fares with literature results of similar measurements.

The biocatalysts are LDH (lactate dehydrogenase), GDH (glutamate dehydrogenase), human IgG (human immunoglobulin G), Hb (hemoglobin), and PSA (prostate-specific antigen). The sensor platform constitutes of a layer of biocomposite mounted on different electrodes made out of Au, Ag, Pt, and glass carbon; the biocomposite is fabricated with polymers and sol-gel Au nanoparticles with or without an extra layer of biomolecules. The targeting species for measurements include NH_4^+, NO_3^-, CN^-, H_2O_2, and the biomolecules specific to the biomolecules/antigens as mentioned above coated on the surface of the biocomposites.

In this report, we will provide a systematic update of analyses of this sensing system, including the unique identification potentials and sensitivities. This novel sensing system can be a valuable tool in biomedical diagnosis and environmental forensics; in particular the sensor platform, any biomedical diagnosis can be conducted with extremely high sensitivity as long as the biomolecules and their antigens are known.

Keywords: metabolites, diagnosis, nanoparticles, biosensor, environmental monitoring.

1 INTRODUCTION

For more than two decades, research into the uses, functionality, and fabrication of immunosensors have received considerable interest from the biochemical and biomedical communities. Different methods of fabrication and combination of reagents are being employed to obtain efficient biosensors for a variety of purposes. In particular, focus has been concentrated on biosensors for the early detection of diseases in the human body such as cancers and congenital disorders. With such, the need for a highly sensitive and selective biosensor is therefore the key to unlock this avenue of research.

Biosensors are generally composed of immobilized layers of biomolecules such as proteins that are attached to support materials, these biomolecules are selectively coupled with the targeted substrates, thus define the selectivity of the sensors. Sensitivity of the biosensors often depends on the efficacy of the availability of the biomolecules that will bind with the targeted substrates, thus modification of how the biomolecules are linked to the supporting materials and their (distribution) configurations can improve sensitivity of the biosensors. For example, implanting of nanogold particles between the supporting materials and biomolecules enhances the binding surface availability for the substrates, thus increases the sensitivity of the biosensors [1, 2, 3, 4, 5].

In this study, we updated the results of our findings of several different enzymes that we used for developing an electrochemical sensor platform that we can be used for varieties of biomedical applications and environmental monitoring. The resulting biosensors have superior detecting sensitivity and in general are orders of magnitude better than those found in literature reports. More importantly, all these sensors were based on one sensor platform that can be used for many applications as long as the enzyme conjugate pairs are known.

2. MATERIALS AND METHODS

2.1 Materials

The electrodes used in this study were highly modified and the surface of the electrodes were Gold (Au), Platinum (Pt) and Glassy carbon (GCE), they were purchased from Tianjin Aida Heng Sheng Co, Tianjin, China. The electrodes had a diameter of 0.2 cm. The platinum counter electrode had diameter of 0.1 cm and length of 0.5 cm. The biocatalysts LDH (lactate dehydrogenase), GDH (glutamate dehydrogenase), human IgG (human immunoglobulin G), Hb (hemoglobin), glial fibrillary acidic protein (GFAP) and anti-GFAP, and PSA (prostate-specific antigen), and other chemicals such as cysteamine, melamine, bovine serum albumen (BSA), $AuCl_3HCl{\cdot}4H_2O$ (Au %> 48 %), ethanolamine (EA), and sodium citrate were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA. Potassium cyanide was obtained from Fisher Scientific, all the other chemicals used were of analytical grade. All experiments were carried out in a 1 eoxygenated 0.1M phosphate buffer solution at pH 7.0.

2.2 Methods

The preparation of electrodes were the same as previously reported [2, 3], except that the biocomposite layers were comprised of the said enzymes as mentioned previously depending on applications. To enhance the performance of the sensors, BSA or EA was coated as a final surface layer to eliminate all the possible sites that might be competitive with the reactive bindings of the enzymes.

The electrochemical measurements were carried out on the Gamry 600 potentiostat.

3 RESULTS AND DISCUSSIONS

Table 1 summarize the results of the 5 enzymes that we used for the analyzes of different metabolites and environmental pollutants. The lowest detection limits for the sensors are in general lower than $1x10^{-18}$ M which allowed these sensors to be used for many applications that were not considered in biomedical diagnosis and environmental monitoring, as well as homeland security.

Thus far, the best anchoring material for the electrode sensor is Pt, however, GCE may be preferred in certain applications.

Table 1: Performance summary of Au, GCE, and Pt electrode and the associated enzymes.

Anchoring Material	Target species	Enzyme	Characteristic Peak (V)	Other Peaks (V)	Current Magnitude*, µA
Platinum	α-ketoglutarate /NH_4^+	GDH	0.75 (O)	0 (R)	12.6 (O)
Gold			0.75 (O)	-	7.27 (O)
Glassy Carbon			0.75 (O)	-	3.0(O)
Platinum	Lactate	LDH	0(R)	0.6 (O)	0.4 (R)
Gold			0.6 (O)	0.5 (R)	0.15 (O)
Glassy Carbon			0.5 (R)	0.6 (O)	0.37 (R)
Platinum	H_2O_2	Hemoglobin	0 (R)	0.75 (0)	4.33 (R)
Gold			0.485 (R)	1.1 (O)	2.04 (O)
Glassy Carbon			0.45 (R)	0.6 (O), 1.1 (O)	5.5 (O)
Platinum	NO_2^-	Hemoglobin	0 (R)	0.8 (O), 0.5 (R)	3.71 (O)
Gold			0.5 (R)	0.8 (O)	4.20 (O)
Glassy Carbon			0.45 (R)	0.8 (O)	2.24 (O)
Platinum	HigG	Anti-HigG	0.04 (R)	0.6 (O)	4.55 (R)
Gold			0.5 (R)	0.3 (O)	0.073 (R)
Glassy Carbon			1.1 (O)	0.4 (R)	1.95 (O)
Platinum	PSA	Anti-PSA	0.04 (R)	-	2.05 (R)
Gold			-0.2 (R)	-	0.032 (R)
Glassy Carbon			0.5 (R)	-	0.17 (R)
Platinum	CN^-	Anti-HigG	0.025	-	4.8 (R)
Gold			0.025	-	2.6 (R)
Gold	GFAP	Anti-GFAP	0.46	-	6.0 (R)

(O) = Oxidative peak
(R) = Reductive peak
*Concentration dependent of enzymes used.

The most significant development within our research group recently was the development of GFAP sensor that is directly related to head trauma. As shown in Figure 1, the sensor was able to detect the release of GFAP at extremely low concentrations beyond $1x10^{-18}$ M, that has the likely potential to develop into a fast responding medical instrument for head trauma detection.

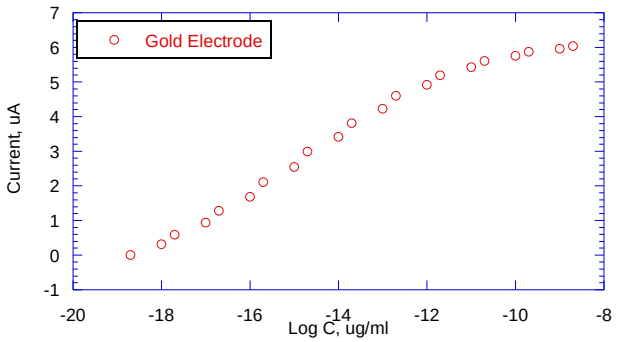

Figure 1: Measurements of GFAP with a Au electrode coated with biocomposite. The measurement takes less than 5 minutes.

4 CONCLUSIONS

Our research group has developed some of the most sensitive biosensors ever reported. These biosensors are selective and durable, that allow researchers and practitioners to explore applications in medical diagnosis and monitoring that were not possible in the past. All the measurements were based on one sensor platform with different conjugate enzyme pairs as shown in Table 1 above, applications for these biosensors are yet to be explored.

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