RBC-DETERMINING BIOSENSORS IN ATHLETES' URINE

BIOSSENSORES DETERMINANTES DE HEMÁCIAS NA URINA DE ATLETAS

BIOSENSORES DETERMINANTES DE GLÓBULOS ROJOS EN LA ORINA DE DEPORTISTAS

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ABSTRACT

Introduction: There is a lack of electrochemical biosensors that allow finding hemoglobin (Hb), a protein found within red blood cells, available in athletes' urine samples. Objective: This work is focused on the production of dsDNA immobilized on an Au-modified glassy carbon electrode (dsDNA/Au/GCE) and its use as a sensor for the presence of urinary hemoglobin. Methods: The elements were deposited in spherical form and tested as a porosity electrode surface for DNA immobilization according to the surface scan of the functionalized dsDNA/Au/GCE using SEM analysis. DPV and amperometry were used to conduct electrochemical studies. Results: Amperometric analyses showed that Hb determination on dsDNA/Au/GCE showed better stability and sensitivity. In the existence of multiple interfering species and clinical urine samples produced, the selectivity and the actual ability of dsDNA/Au/GCE for hemoglobin determination were investigated. Conclusion: The results showed that dsDNA/Au/GCE is effective, reliable, and selective as an electrochemical sensor of Hb. ***Level of evidence II; Therapeutic studies - investigation of treatment outcomes.***

Keywords: Athletes; Hemoglobins; Biosensing Techniques; Urine.

RESUMO

Introdução: Há uma carência de biossensores eletroquímicos que permitam encontrar a hemoglobina (Hb), uma proteína encontrada dentro dos glóbulos vermelhos do sangue, disponível em amostras de urina dos atletas. Objetivo: Este trabalho é focado na produção de dsDNA imobilizado em um eletrodo de carbono vítreo Au-modificado (dsDNA/Au/GCE) e seu uso como sensor para a presença de hemoglobina urinária. Métodos: Os elementos foram depositados em forma esférica e testados como superfície de eletrodo de porosidade para imobilização do DNA, de acordo com o exame de superfície do dsDNA/Au/GCE funcionalizado, utilizando análise SEM. DPV e amperometria foram usados para conduzir estudos eletroquímicos. Resultados: As análises amperométricas demonstraram que a determinação de Hb em dsDNA/Au/GCE apresentou um melhor grau de estabilidade e sensibilidade. Na existência de múltiplas espécies interferentes e amostras clínicas de urina produzidas, a seletividade e capacidade real do dsDNA/Au/GCE para a determinação da hemoglobina foram investigadas. Conclusão: Os resultados mostraram que o dsDNA/Au/GCE é efetivo, confiável e seletivo como sensor eletroquímico de Hb. ***Nível de evidência II; Estudos terapêuticos - investigação dos resultados do tratamento.***

Descritores: *Atletas; Hemoglobinas; Técnicas Biossensoriais; Urina.*

RESUMEN

Introducción: Se carece de biosensores electroquímicos que permitan encontrar la hemoglobina (Hb), una proteína que se encuentra dentro de los glóbulos rojos, disponible en las muestras de orina de los deportistas. Objetivo: Este trabajo se centra en la producción de dsDNA inmovilizado en un electrodo de carbono vítreo modificado con Au (dsDNA/Au/GCE) y su uso como sensor de la presencia de hemoglobina urinaria. Métodos: Los elementos fueron depositados en forma esférica y probados como una superficie de electrodo porosa para la inmovilización de ADN, según el escaneo de la superficie del dsDNA/Au/GCE funcionalizado, utilizando el análisis SEM. Se utilizó la DPV y la amperometría para realizar estudios electroquímicos. Resultados: Los análisis amperométricos demostraron que la determinación de Hb en dsDNA/Au/GCE mostraba un mayor grado de estabilidad y sensibilidad. En la existencia de múltiples especies interferentes y muestras clínicas de orina producidas, se investigó la selectividad y la capacidad real del dsDNA/Au/GCE para la determinación de Hb. Conclusión: Los resultados mostraron que el dsDNA/Au/GCE es eficaz, fiable y selectivo como sensor electroquímico de Hb. ***Nivel de evidencia II; Estudios terapéuticos - investigación de los resultados del tratamiento.***

Descriptores: *Atletas; Hemoglobinas; Técnicas Biosensibles; Orina.*

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INTRODUCTION

Numerous by-products of cellular metabolism into human bodies, many of which are nitrogen-rich species including creatinine, uric, and, urea must be removed from the plasma.[1] Urine's hue, smell, and volume can already reveal whether a problem exists.[2] 95 percent of urine is water, and it also contains a variety of inorganic ions, including sodium, chlorides, phosphates, and ammonia, as well as organic substances, including urea, uric, proteins, hormones, and metabolites.[3] The major method for determining expelled water-soluble compounds from the body is the analysis of the by-products produced during urine.[4]

Microscopy, photoelectricity, biuret, photometry, microcolorimetry, spectrophotometry, liquid chromatography/electrospray-mass

spectrometry and electrochemical approaches have all been demonstrated to be effective urine test methodologies.[5-13] The approaches now in use, however, have been found to fall short in terms of accuracy, sensitivity, selectivity, and ease. These flaws were brought about by efforts to create and enhance the detection process. Electrochemical methods are one of these approaches that are easy, inexpensive, and quick, and they have the capacity to change surface of electrodes with several nanostructured material to enhance sensing properties.[14]

On the gold, platinum, and silver electrodes, several electrochemical investigations of urine tests have been carried out.[15-19] As a result, locate and synthesize low-cost, stable materials so that they can be used to modify urine electrochemical measurement electrodes as needed. Therefore, the development and usage of gHb-DNA/MWCNTs modified Electrode as a biosensor of hemoglobin as a protein within red blood cells for urine samples at various degrees of exercise training were the main focuses of this investigation.

METHODS

Experimental details

The GCE surfaces was polished with alumina slurry on just a polished surface for 15 minutes before to the electrodeposition procedure. The electrode was then ultrasonically cleaned for 10 minutes in an equal parts mixture of ethanol and deionized water, and drying under nitrogen flow. In an electrochemical cell containing an equal volume ratio of 2M $HAuCl_4 \cdot 3H_2O$ as the electrodeposition electrolyte, electrodeposition was carried out using a potentiostat and the cyclic voltammetry (CV) method at potential ranges between 1.4 and 1.4 V at scan rates of 20 mV/s for 20 cycles.[20] The working, reference, and counter electrodes employed were the GCE, Ag/AgCl, and Pt wire. The Au/GCE was then placed in a 0.2M native calves thymus doubled stranded DNA (dsDNA) mixture for 35 minutes after the dsDNA had been dissolved in a 0.2M phosphate buffer (PBS). The soaking of Au/GCE was maintained for an additional 10 minutes in order to immobilize dsDNA.

On an Autolab potentiostat/galvanostat with only a three-electrode cell that contained a preduced electrode as the working-electrode, Ag/AgCl as the reference-electrode, and Pt plate as the counter-electrode, electrochemical properties utilizing differential pulse voltammetry (DPV) as well as amperometry were carried out. The electrolyte for the electrochemical experiments was produced from 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 and contained the following concentrations: 5 mM $K_4[Fe(CN)_6]$ and 0.2 M PBS.

Urine samples at various degrees of exercise were composed and placed right away in a refrigerator. 20 mL of specimens were centrifuged for 20 minutes at 2000 rpm to obtain the supernatant, which was then filtered. The solution was then poured into a glass beaker (15 mL) and diluted to the proper concentration using 0.2 M PBS. Different concentrations of hemoglobin were added into the diluted sample. Amperometric analysis and a urine dipstick test were used to examine the samples (UDT). The surface morphology of Au/GCE was studied using scanning electron microscopy (SEM).

In this research only urine sample was used in the analysis and the work was conducted based on the Declaration of Helsinki principle. The participants signed the Free and Informed Consent Form (EHIC).

RESULTS AND DISCUSSION

The surface morphology of AuNPs on the GCE surface is depicted in Figure 1a. As seen, the AuNPs were successfully deposited with an average diameter of 130nm, a very uniform covering, and a spherical shape. The X-ray diffraction pattern of nanocrystalline Au NPs powder is displayed in Figure 1b. Also, the (111),(200),(220),and (311) planes, respectively, are assigned to the diffraction pattern at 38.98°, 44.52°, 64.83°, and 78.04°, which indicate that they are reflections of the face-centered cubic (fcc) form of the AuNPs.

The DPV graphs of GCE, Au/GCE, and dsDNA/Au/GCE at 20 mV/s scan rate into 0.2M PBS containing 5mg/ml Hb are shown in Figure 2. Figures 2 shows that while there is a very faint anodic peak for Au/GCE at 0.65 V, neither bare GCE nor the presence of Hb exhibit any notable anodic peaks. But Figure 2 shows the considerable anodic peaks at 0.62 V because Hb has accumulated on the surface by contact with immobilized DNA onto Au/GCE, which raises the oxide peak.[21,22] The immobilization of the dsDNA on the surface of the electrode, which reacts with Hb and boosts the signal of the electrochemical reaction, is the cause of the differing peak current and potential values for Au/GCEand dsDNA/Au/GCE.[23] Additionally, a biomaterial DNA immobilizing platform that served as an electron transfer mediator and catalyzed the oxidation reaction of Hb was provided by the electrodeposited large porosity layer of Au NPs with high electron permeability and large surface area.[24, 25]

Figure 3 displays the gHb-DsDNA/Au/amperometric GCE's reactions to incremental addition of 1µg/mL Hb into 0.2M PBS at potential 0.25V. When can be seen, as the Hb concentration rises, so does the amperometric current. The calibration curve for electrocatalytic current vs. hemoglobin concentration can be seen in Figure 3, which shows that oxidation current and hemoglobin concentration have a linear relationship. Additionally, for Hb concentrations greater than 50 g/mL, the calibration graph begins to depart from linear behavior. It might be connected to a common restriction in biosensors with nanomaterials surface membrane, which makes it easier for the enzyme to convert and attain steady state and can cause some buildup of the substrates at inner membrane boundaries.[26] This unfavorable component can saturate the biosensor's active site. For the determination of Hb, the detection limits and sensitivity were found to be 0.005ng/mL and 7.4263µA/µg mL^{-1}, respectively.

The gHb-DsDNA/Au/analytical GCE's sensing capabilities are contrasted with those of other Hb sensors that have been published. Because of MWCNT's excellent stability, quick reaction, and simple operation, the analysis results for gHb-DsDNA/Au/GCE are satisfactory, and the

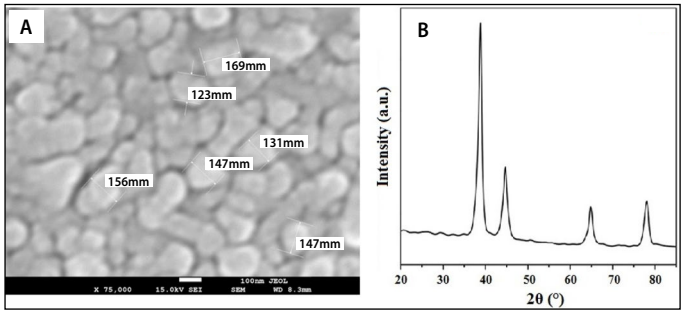

Figure 1. (A) Surface morphology of AuNPs onto GCE and (B) X-ray diffraction pattern of electrodeposited AuNPs.

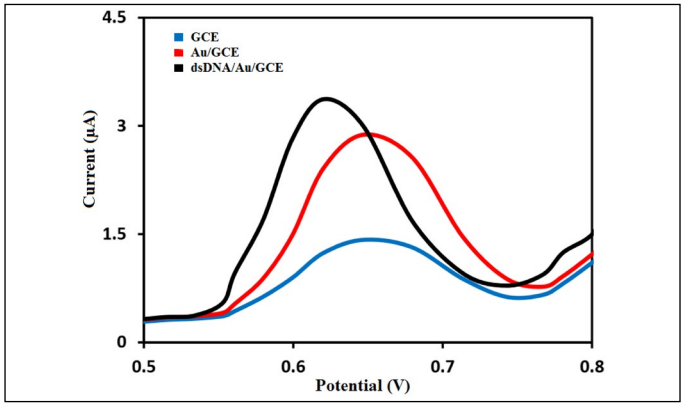

Figure 2. DPVs of GCE,Au/GCEand dsDNA/Au/GCE at 20mV/s scan rate into 0.2M PBS including 10mg/ml Hb.

linear-range is greater than those of other techniques and electrochemical sensors. This electrochemical biosensor built on CNTs can also function as a biocatalyst and bioaffinity sensors.[27]

Investigations were made into the electrochemical interference effects of the ions Ca^{2+} and Pb^{+2} on the measurement of Hb. For the consecutive addition of 1g/mL Hb and 10mg/mL of interferents, Table 1 shows the amperometric results of gHb-DsDNA/Au/GCE into 0.2M PBS at 0.25V. As can be observed, adding Hb solution to the proposed electrode causes a noticeable response, however adding other interferents at 0.25V causes no discernible reaction.

Table 2 displays the findings from the identification of two species into actual samples using amperometric and UDT analysis. These findings showed that there was good accordance by SI units aimed at clinical lab data as well as appropriate agreements between both the UDT and amperometric findings. Table 2 displays excellent relative standard deviation (RSD) and recovery. The findings show that for the determination of Hb, the observed recovery values were greater than 98.50% and less than 4.11%, respectively. These findings demonstrate the gHb-DsDNA/Au/dependable GCE's and accurate performance as electrochemical Hb sensing.

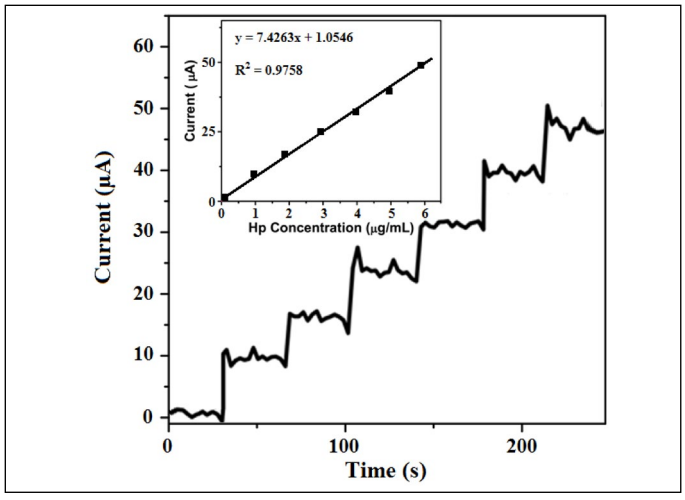

Figure 3. (A) Amperometric measurements of gHb-DsDNA/Au/GCE to successive adding 1μg/mL Hb into 0.2 M PBS at 0.25 V potential; (B) Inset figure shows the calibration curve.

Table 1. The amperometric results of gHb-DsDNA/Au/GCE into 0.2M PBS at 0.25V for successive adding 1μg/mL Hb, and 10μg/mL interferent.

Substances	Added(μg/mL)	Current response(μA) at 0.25V	RSD(%)
Hemoglobin	1	7.426	±0.212
Dopamine	10	0.254	±0.009
Nitrite	10	0.261	±0.012
Ascorbic acid	10	0.232	±0.004
Glucose	10	0.359	±0.006
Serotonin	10	0.162	±0.006
Ca^{2+}	10	0.113	±0.003
Pb^{2+}	10	0.077	±0.004

Table 2. Analytical results of detection for clinical urine samples using UDT and amperometric methods.

Amperometry							UDT	
Found (ng/mL)		Added (ng/mL)	Measured (μg/mL)	RSD (%)	Recovery (%)	Content in sample (ng/mL)	Content in sample (ng/mL)	RSD (%)
Hb	5.2	10.0	14.8	2.06	98.50	0.0059	5.5	2.12
		20.0	24.7	2.82	99.00			
		30.0	35.1	3.20	99.86			
		40.0	44.7	4.11	99.75			

CONCLUSIONS

This research focused on the production of gHb-DsDNA/Au/GCE and its use as a hemoglobin sensor in urine samples at various degrees of exercise training. The electrochemical results showed that, compared to Au/GCE and gHb-DNA/GCE, gHb-DsDNA/Au/GCE exhibits simultaneous detection of Hb with a greater stability and sensitivity signal. The linear--range, detection limit, and sensitivity for determining hemoglobin were determined to be 1 to 50 μg/mL, 0.005ng/mL, and 7.4263μA/μg mL^{-1}, respectively, according to the amperometric studies of gHb-DsDNA/Au/GCE. In the presence of various interferents and produced clinical urine samples, the selectivity and actual capacity of gHb-DsDNA/Au/GCE to determine Hb were studied. The findings demonstrated that gHb-DsDNA/Au/GCE electrochemical Hb sensor responded in a selective, dependable, and precise manner.

All authors declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: The work is conceived by Feng Xu, its knowledge content and execution is completed by Hongkwan Cho. The manuscript is drafted by GuiBin Su. Each authors contributed in execution and writing of this manuscript.

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