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SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONS) MODIFIED WITH SARCOSINE OXIDASE - ENZYMATIC ACTIVITY ANALYSIS BY SDS-PAGE

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Abstract

Sarcosine oxidase (SOX) is an enzyme, that catalyzes the oxidative demethylation of sarcosine with the glycine as a product and is physiologically active in human body and other mammals. However, prostate cancer cells have a high expression of sarcosine. In this study the superparamagnetic iron oxide nanoparticles (SPIONs) were prepared and their surface was modified with gold nanoparticles (AuNPs). These AuNPs were modified with chitosan (CS) and SOX. The subsequent obtained AuNPs were characterized by physicochemical methods, such as dynamic light scattering and spectrophotometry, where the pseudo-peroxidase activity of the AuNPs was used. Hydrogen peroxide decomposes because of the pseudo-peroxidase activity with the appearance of a blue coloration of 3,3',5,5'-tetramethylbenzidine (TMB). For the analysis of AuNPs enzymatic activity the SDS-PAGE with silver staining has been used. Gels (7.5%) were prepared using acrylamide stock solution 30% (m / V) with bisacrylamide 1% (m / V). Separating gel contained: acrylamide 7.5% (m / V), bisacrylamide 0.5% (m / V), 0.4 M Tris/HCl, 0.1% (m / V) sodium dodecyl sulfate (SDS), pH 8.8. Stacking gel contained: 4.5% acrylamide (m / V), 0.15% bisacrylamide (m / V), 0.1% SDS (m / V) and 0.1M Tris/HCl at pH 6.8. Nanoconstructs were diluted 2:1 with a loading buffer (PLB Max). Each well contained 15 µl of the diluted solutions. Electrophoretic measuring conditions were: 120 V, 1.5 hours in a running buffer (24mM Tris, 0.2M glycine and 3mM SDS). After measurement the gel was stained with silver, scanned and evaluated by Colortest in the laboratory system Qinslab. The SPIONs or AuNPs cannot be detected themselves alone using SDS-PAGE, therefore this method served as a confirmation, that all parts of the nanoconstruct are connected and we were able to analyze them and use them for other research or possible diagnostic purposes.

Keywords: superparamagnetic iron oxide nanoparticles, gold nanoparticles, sarcosine oxidase, chitosan, enzymatic activity, SDS-PAGE

1. INTRODUCTION

Sarcosine is an amino acid that is formed by methylation of glycine and is present in trace amounts in the body. Increased sarcosine concentrations in blood plasma and urine are manifested in sarcosinemia and in some other diseases such as prostate cancer[1-3]. For this purpose, sarcosine detection using the nanomedicine approach was proposed. In this study, we have prepared superparamagnetic iron oxide nanoparticles (SPIONs) with different modified surface area. Gold nanoparticles (AuNPs) were modified by chitosan (CS), and sarcosine oxidase (SOX). SPIONs without any modification were taken as controls.

2. MATERIAL AND METHODS

All chemicals were purchased from Merck unless otherwise stated. All reagents were of analytical grade and used without any further purification. Ninhydrin, hydrindatin were purchased from Ingos, (Czech Republic). Aqueous solutions for size analysis were prepared using PURELAB® Ultra (Elga, High Wycombe, United Kingdom) resistivity 18 MΩ-cm. For other purposes deionized water was used. The obtained NPs were characterized by physicochemical methods. Structure of NPs was characterized by scanning electron microscopy (SEM). For documentation of the NPs structure, the MIRA3 LMU (Tescan, Brno, Czech Republic) was used. Absorbance scan was carried out in the range from 300–850 nm by 2 nm steps. All measurements were performed at 22°C (V UV-3100PC, VWR, Germany). The absorbance or spectra in plate (Brand, Germany) were recorded by using a reader Infinite M200 (Tecan, Männedorf, Switzerland).The size distribution (i.e. the hydrodynamic diameter, DH) was determined by dynamic light scattering (DLS) using the Zetasizer Nano ZS ZEN3600 (Malvern Instruments, Malvern, UK) with the detection angle of 173° in optically homogeneous square polystyrene cells. For the analysis of AuNPs enzymatic activity the SDS-PAGE with silver staining were used. Gels (7.5%) were prepared using acrylamide stock solution 30% (m / V) with bisacrylamide 1% (m / V). Separating gel contained: acrylamide 7.5% (m / V), bisacrylamide 0.5% (m / V), 0.4 M Tris/HCl, 0.1% (m / V) sodium dodecyl sulfate (SDS), pH 8.8. Stacking gel contained: 4.5% acrylamide (m / V), 0.15% bisacrylamide (m / V), 0.1% SDS (m / V) and 0.1M Tris/HCl at pH 6.8. Nanoconstructs were diluted 2:1 with a loading buffer (PLB Max). Each vial contained 15 µL of the diluted solutions. Electrophoretic measuring conditions were: 120 V, 1.5 hours in a running buffer (24mM Tris, 0.2M glycine and 3mM SDS). After measurement the gel was stained with silver, scanned and evaluated by Colortest in the laboratory system Qinslab.

RESULTS

The obtained NPs were characterized by physicochemical methods. The size of the NPs was determined by the dynamic light scattering method which was described as follows: SPIONs/Au/NPs (100-300 nm), SPIONs/Au/CS/NPs (300-700 nm), and SPIONs/Au/CS/SOX/NPs (600-1500 nm). The SPIONs surface was modified with CS. The amount of CS deposited on the NP surface was found to be 48 mg/mL and 39 mg/mL for SPIONs/Au/CS/NPs and SPIONs/Au/CS/SOX/NPs, respectively, and it repeatability varied around 10%. Pseudo-peroxidase activity of NPs was verified using sarcosine, horseradish peroxidase (HRP) and 3,3',5,5'-tetramethylbenzidine (TMB) substrate (Figure 1).

Figure 1: Pseudo-peroxidase activity of SPIONs/Au/NPs, SPIONs/Au/CS/NPs and SPIONs/Au/CS/SOX/NPs. Photographs (camera Canon PowerShot SX610 HS, 20.2 Mpx CMOS sensor, ultra wide-angle lens with 18x superzoom) showing (A) a typical appearance of individual used types of SPIONs in a range of concentrations from 0 to 40 mg/mL in 50 mM phosphate buffer (pH 8.0) and (B) a typical color course of pseudo-peroxidase reaction with SPIONs/Au/NPs in a range of concentrations from 0 to 40 mg/mL; representative image; (C) Time course of pseudo-peroxidase reaction (0 to 30 min) for SPIONs/Au/NPs in a range of concentrations from 0 to 10 mg/mL; SPIONs/Au/CS/Nos and SPIONs/Au/CS/SOX/NPs (0–40 mg/mL); (D) Signal intensity of SPIONs/Au/NPs in dependence on pseudo-peroxidase reaction signal in a range of concentrations from 0 to 40 mg/mL. Composition of reaction mixture for the measurement of pseudo-peroxidase activity was as follows: 5 mM TMB, hydrogen peroxide 30%, 0.5 M acetate buffer pH 4. The measurement was carried out in the Brand plate after washing with 18 MΩ water, at 650 nm in 1 min reading interval, each value being the average of 5 repeated measurements.

We used the following particle types for gel electrophoresis: SPIONs, chitosan modified SPIONs, SPIONs with the enzyme sarcosine oxidase, SPIONs with the enzyme sarcosine oxidase and horseradish peroxidase (HRP), SPIONs with the enzyme sarcosine oxidase, HRP and dithiotreitol, SPIONs with the enzyme sarcosin oxidase, HRP and zinc. The last type of nanoparticles were SPION nanoparticles with the enzyme sarcosine oxidase, HRP, dithiothreitol and zinc. In gel electrophoresis, sarcosin oxidase was shown as tetramer, therefore we followed 4 bands on a gel electrophoresis, which we evaluated individually. Figure 2 shows a calibration curve of sarcosine oxidase at a concentration range up to 10 µg / mL. The calibration curve is evaluated using Colortest in Qinslab laboratory system. This is the dependence of the density (expressed by LOG (Io / I)) and the concentration of the enzyme.

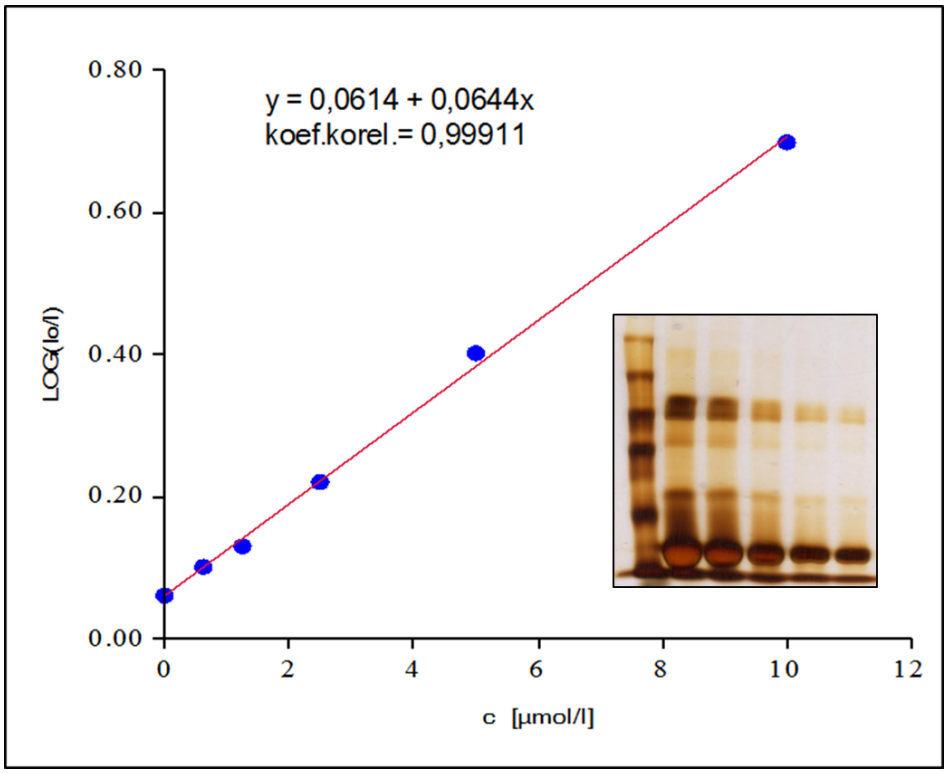

Figure 2 Calibration curve of sarcosine oxidase at a concentrations of 0, 0.62, 1.25, 2.50, 5.00 and 10.00 µg / mL. The curve was evaluated using the Colortest in the Qinslab laboratory system. This is the dependence of the density (expressed by LOG (Io / I)) and the concentration of the enzyme. The inset shows different concentrations of sarcosine oxidase on gel electrophoresis from the highest concentration to the lowest.

SPIONs and SPIONs modified chitosan alone was used as negative control, when no band appeared on the gel. For the other nanoparticles, 4 bands of sarcosine oxidase appeared, the intensity of which was evaluated by Colortest. If dithiothreitol and zinc are coupled with the enzymes, the band intensity is low. Conversely, if we added FAD or magnesium chloride, the band intensity improved.

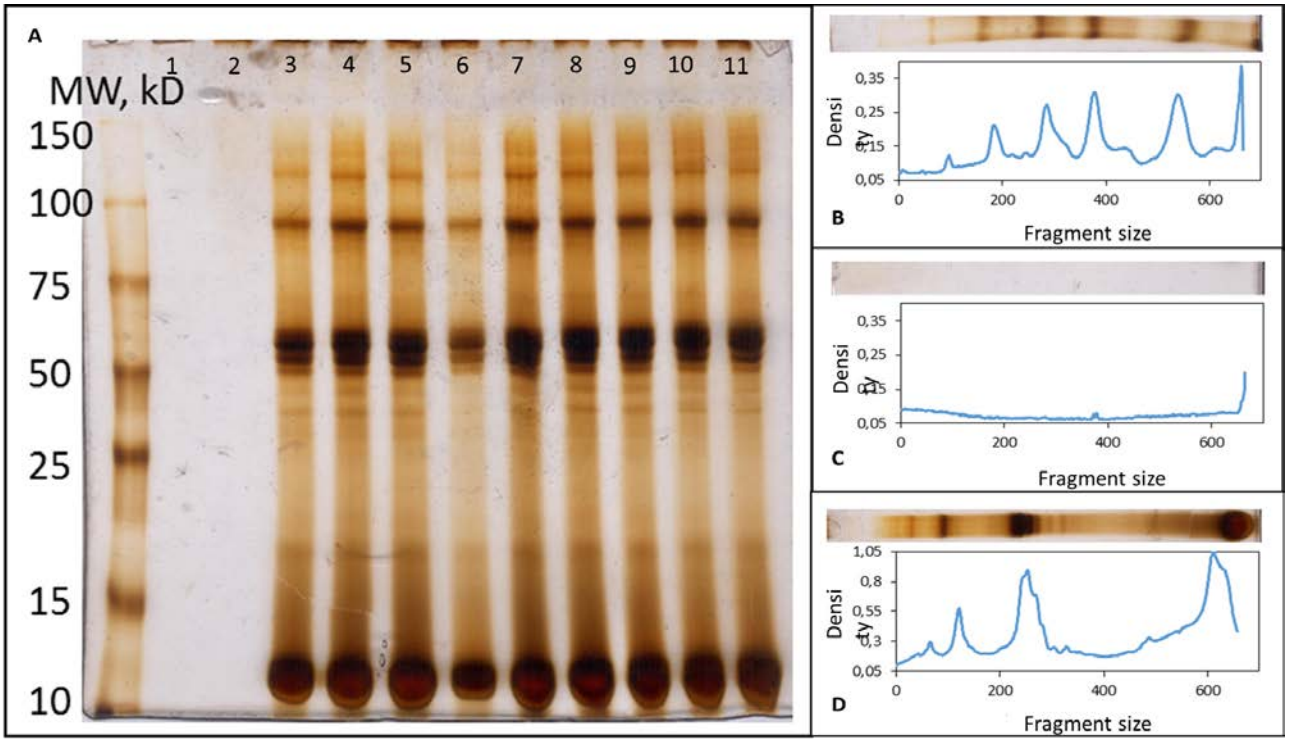

Figure 3: A) On gel electrophoresis, the order of samples behind the protein ladder is shown: 1- SPIONs without modification; 2 - chitosan modified SPIONs; 3 - SPIONs modified with chitosan and sarcosine oxidase; 4 - SPIONs modified with chitosan, sarcosine oxidase and 1mM DTT; 5-Spions modified with chitosan, sarcosine oxidase, 1 mM DTT and 0.2 mg Zn; 6 - SPIONs modified with chitosan, sarcosine oxidase and 1 mg Zn; 7 - SPIONs modified with chitosan, sarcosine oxidase and 0.5 mg Zn; 8 - SPIONs modified with chitosan, sarcosine oxidase and 0.2 mg Zn; 9-SPIONs modified with chitosan, the enzyme sarcosine oxidase and 0.1 mg Zn; 10-SPIONs modified with chitosan, the enzyme sarcosine oxidase and 0.05 mg of Zn; 11 - SPIONs modified with chitosan, enzyme sarcosine oxidase and 0.025 mg Zn B) Evaluation of ladder by Colortest. The fragments are displayed by size. C) Evaluation of the first sample (SPION nanoparticles) using Colortest. In the picture it can be seen that no band was created. D) Evaluation of SPION specimen modified with chitosan, sarcosin oxidase enzyme and 1mM DTT again using Colortest. The individual peaks are beautifully visible on the graph, representing bands on the gel.

3. CONCLUSION

The proposed detection system allows to analyse sarcosine as a potential prostate cancer marker at micromolar concentrations and to monitor changes in its levels. The whole system is suitable for low-cost miniaturization and point-of-care testing technology/diagnostic systems. This system is simple, inexpensive, convenient for screening tests and telemedicine applications.

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