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Colorimetric determination of sarcosine in urine samples of prostatic carcinoma by mimic enzyme palladium nanoparticles

Jianming Lan [a,b], Wenming Xu [a], Qiping Wan [a], Xi Zhang [b], Jia Lin [b], Jinghua Chen [b,*], Jianzhong Chen [a,*]

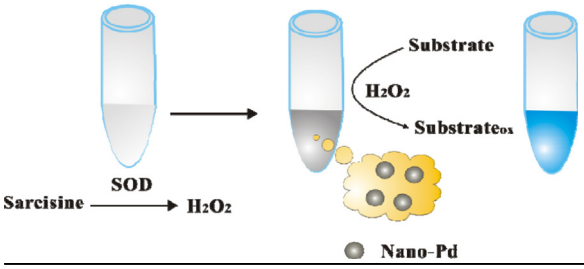
[a] College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou, Fujian 350108, PR China
[b] Faculty of Pharmacy, Fujian Medical University, Fuzhou, Fujian 350108, PR China

HIGHLIGHTS

- The catalytic activity of Pd NPs has been studied as mimic enzyme.
- Pd NPs can be used for the detection of H_2O_2.
- A new method for detecting the content of sarcosine in urine samples of prostatic carcinoma was proposed.

GRAPHICAL ABSTRACT

The sarcosine causes a rapid release of H2O2 by the catalytic action of sarcosine oxidase. As a substitute of the horseradish peroxidase (HRP), the Pd nanoparticles has higher peroxidase activity and can effectively catalyze the H2O2-mediated oxidation of 3,3′,5,5′-tetramethylbenzidine sulfate (TMB), which induces a color change from colorless to blue in solution. Therefore, under certain conditions, this provides a sensing platform for the label-free visual detection of sarcosine with high sensitivity and selectivity.



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ABSTRACT

The proposed palladium nanoparticles (Pd NPs), which with the catalytic activity similar to the horseradish peroxidase (HRP) mimic enzyme, can effectively catalyze the H_2O_2-mediated oxidation of 3,3′,5,5′-tetramethylbenzidine sulfate (TMB) accompanied with a color change from colorless to blue in solution. And as a result, the sensitive detection of sarcosine can be realized by the naked eye observation and ultraviolet spectrophotometry, using Pd NPs as catalyst and TMB as the substrate of the simulation enzyme catalytic reaction. Under the optimal condition, the catalytic system of Pd NPs mimic enzyme can be used for the detection of sarcosine. It has been found that the color change could be clearly observed with the naked eyes, and the absorbance intensity at 653 nm showed a fine linear fitting with the concentration of sarcosine in the range from 0.01 μM to 50 μM, and the detection limit (3σ/S) for sarcosine was calculated to be 5.0 nM. In order to evaluate the feasibility and reliability, the method was also used for analyzing concentrations of sarcosine in human urine samples from diagnosed prostate cancer patients and healthy donors. It is expected to provide a convenient and efficient method for indirect evaluation for the diagnosis of prostatic carcinoma (PCa).

1. Introduction

PCa is occurring in males in the prostatic tissue of malignant tumors, due to the abnormalities of acinar cell of prostate in disordered growth. Currently, early detection methods of PCa usually use rectum digital examination, endorectal

* Corresponding authors. Tel.: +86 59122866232.
E-mail addresses: cjh_huaxue@126.com (J. Chen), jianzhongchen_fzu@163.com (J. Chen).

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ultrasonography (ERUS), prostatic puncture biopsy, CT and MIR [1]. However, the above examination methods involve the disadvantages of complicated operation, expensive equipment and trauma, and may even result in misdiagnosis due to occult lesions and the subjective impression of inspector. More seriously, prostatic puncture biopsy can also cause a variety of complications such as sepsis, rectal bleeding, and hematuria. Therefore, developing a more convenient, accurate, sensitive and humane noninvasive detection of new technology for early clinical diagnosis of PCa will be of great significance. For this purpose, the oncology doctors in clinical diagnosis propose the use of tumor markers detection technology to realize the early diagnosis of PCa [2]. Among them, the most typical case is the prostate specific antigen (PSA) recommended by the American Cancer Society because it is a significant indicator of judging the occurrence and metastasis of PCa [3]. However, this indicator is found to be quite unreliable recently. Although this indicator can be used to diagnose PCa, it can't accurately discriminate whether the prostate tumor is malignant or benign. As a result, the prescribing treatment according to the symptoms is impossible. And using PSA as biomarker can lead to misdiagnosis, causing delay of the treatment or excessive treatments for patients [4]. Therefore, it is of great significance to find a more suitable indicator for the early detection of PCa.

Fortunately, a research published by A. Sreekumar's group showed that sarcosine (*N*-methylglycine, metabolite of glycine) could effectively reflect the invasiveness of PCa and identify the growth behavior of cancer cell [5]. The research discovered that sarcosine was one of the important factors in prostate carcinogenesis, and the accuracy of urinary sarcosine assay for the diagnosis of PCa was higher than that of the clinical serum PSA detection. After a system analysis of cancer tumor cell samples and urine metabolites from the patients with prostate, the researchers discovered that the human muscles and many other biological tissues could produce a common amino acid – sarcosine, which not only increased significantly in patients with malignant prostate cancer cells but also could be easily detected in urine. This behavior made the sarcosine become a significant biological marker for clinical diagnosis of PCa.

Presently, sarcosine detection methods usually include mass spectrometry, isotope internal standard method, time resolved fluorescence method and so on [6–8]. But all of these methods are either hard to operate and time-consuming or expensive and thus, hard to popularize. In order to overcome the above shortcomings, the researchers have proposed the protease-catalyzed method [9,10]. In the case of sarcosine, it can be oxidized to hydrogen peroxide, formaldehyde and glycine by using sarcosine oxidase (SOD) as a catalyst. So it is feasible to carry on indirect quantification of sarcosine by this reaction. For example, an analytical assay for sarcosine includes the sarcosine oxidase-mediated demethylation of sarcosine to hydrogen peroxide, which reacts with 4-aminoantipyrine and phenol to yield quinoneimine dye. However, such colorimetric analyses can be skewed in complex matrices like urine and blood. Therefore, a further study of these methods on clinical analysis is full of significance.

In recent years, lots of researches have shown that the nanoparticles as catalyst exhibit very high catalytic activity and selectivity. Nanoparticles have such features as small size, high specific surface area, different chemical bonding and electronic state between surface and interior, and irregular coordination of surface atoms that it is possible for them to be the potential catalyst. Especially scientists have discovered that some chromogenic substrates can be oxidized by H_2O_2 with the catalysis of nanoparticles and yield the colored products. Thus, the visual and spectrophotometric detection technology of H_2O_2 should be realized. According to this method, the scholars have studied several catalytic systems of mimic enzymes assisted by metal nanoparticles and applied them to detect H_2O_2, glucose and cells, and finally good results have been achieved. And interestingly, recent studies have shown that Pd NPs play an important role in oxygen transport, decomposition of H_2O_2 and electron transfer connecting with oxygen and energy metabolism in vivo [11].

In this work, we developed a method of sensitive detection of H_2O_2 through visual inspection or UV spectrophotometry, using TMB and Pd NPs as substrate and catalyst of catalytic reaction of mimic enzymes respectively. It was based on sarcosine oxidase-catalyzed method that the rapid detection of sarcosine in PCa was established.

2. Experimental details

2.1. Materials and apparatus

The solid Pd NPs were purchased from DK Nano Technology Co., Ltd. (China). HRP (horseradish peroxidase) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (China). Sarcosine oxidase was purchased from Sigma–Aldrich (Saint Louis, MO, USA). Preparation of sarcosine oxidase: a 20 mmol L^{-1} potassium phosphate solution was prepared from ultra-pure water and potassium phosphate monobasic, and the pH was adjusted to 8.3 with 1 mol L^{-1} sodium hydroxide. Others reagents were also purchased from Sigma–Aldrich (Saint Louis, MO, USA). All chemicals were of analytical grade, and sterilized Milli-Q water was used in all experiment. Phosphate buffer solutions (PBS) with different pH were prepared by mixing the stock solutions of 0.05 mol L^{-1} NaCl and 0.1 mol L^{-1} NaH_2PO_4–Na_2HPO_4, and then adjusting the pH with 0.1 mol L^{-1} H_3PO_4 or 0.1 mol L^{-1} NaOH. UV–vis absorption spectra were measured with a UV-lambda 800 spectrophotometer (PerkinElmer, USA) by using a 1.0 cm quartz cell. The photographs were taken with a Nikon digital camera.

2.2. Comparison of activity of HRP and Pd NPs

Reagents were added in 1.5 mL PCR tube as the following steps: 390 μL PBS buffer solution (pH 5.0), 50 μL H_2O_2 solution (250 mM), 50 μL various concentrations of TMB. Finally 10 μL dispersion solutions of Pd NPs were added in the above 500 μL solutions. After reacting under different times, the mixtures were scanned by UV spectrograph.

2.3. Analysis of sarcosine standard solutions

The sarcosine stock solution was added to a 1.5 mL PCR tube to supplement with 75 μL PBS buffer. Then a 25 μL cold enzyme reagent was added to the tube and finally the tube was incubated at 37 °C for 30 min. After incubation, the tube was cooled to room temperature, and 50 μL TMB solution (10 mM), 340 μL PBS buffer solutions (pH 5.0) and 10 μL dispersion solutions of Pd NPs were added in the above 500 μL solutions. After reacting for 5 min, the mixtures were scanned by UV spectrograph.

2.4. Detection of sarcosine

Urine samples were provided by the First Affiliated Hospital of Fujian Medical University. The clean middle urine collected in the morning was centrifugated for 10 min at full speed, and then 1 mL supernatant taken from the resulting solution was diluted with buffer solution whereby 75 μL was inserted into 1.5 mL PCR tube. The subsequent procedure was the same with the analysis of sarcosine standard solutions.