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Peroxidase-like catalytic activity of copper ions and its application for highly sensitive detection of glypican-3

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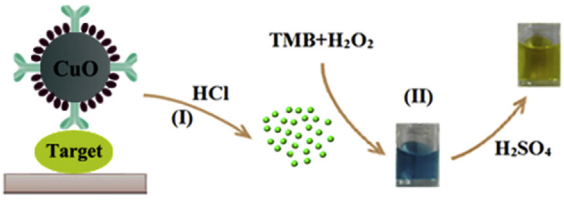
HIGHLIGHTS

- Copper ions possess high and stable peroxidase-like catalytic activity.
- The catalytic activity of copper ions is quite stable, even in the presence of high concentration of proteins.
- Copper ions catalyzed reaction can be used for glypican-3 detection with high sensitivity ans selectivity.
- The high catalytic activity of copper ions and the signal amplification process make this method more simple and effective.

GRAPHICAL ABSTRACT



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ABSTRACT

Glypican-3 (GPC3) might be used as new biomarker of liver cancer for the development of new diagnostic methods. The most commonly used methods for protein detection are based on natural enzymes, which are easily affected by environmental conditions and suffer from the rigorous preparation conditions. Thus, the development of new enzyme mimetics with high and stable catalytic activity is of great significance in diagnostic applications. In this paper, copper ions (Cu^{2+}) was found to possess the peroxidase-like catalytic activity, which can catalyze H_2O_2-mediated oxidation of peroxidase substrate and obtain the oxidation product with color change. This catalytic activity is much more stable than other nanomaterials based peroxidase mimetics, and can significantly increase by increasing the concentration of H_2O_2. It is worth mentioning that the absorbance signal induced by 5 nM Cu^{2+} can be easily detected. This Cu^{2+}-catalyzed reaction can be also applied in the detection of GPC3 by using the anti-GPC3 antibody functionalized CuO NPs, which can release the Cu^{2+} by dissolved in HCl solution. This method permits detection of as low as 0.26 pg mL^{-1} GPC3. This sensitivity is about one or several magnitudes higher than that of ELISA or other peroxidase mimetics based methods. The high catalytic activity of Cu^{2+} and the signal amplification process of CuO NPs into high amount of Cu^{2+} also make this method more simple and effective.

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1. Introduction

Liver cancer is one of the most common malignancies worldwide, which also ranks as the third leading cause of cancer-related death [1,2]. The majority of liver cancers are hepatocellular carcinoma (HCC), which account for 90% of all liver cancers [3]. HCC exhibits a high degree of malignancy and poor prognosis, which often has no obvious symptoms until the later stages. Thus, the development of sensitive and specific biomarkers for the early diagnosis of HCC is very important to improve the survival time of patients. Alpha fetoprotein (AFP) has been long time used as diagnostic biomarker for HCC in clinics. However, it has significant limitations that the sensitivity is relatively poor [4,5]. There is an urgent need to find new diagnostic biomarkers of HCC for the development of new diagnostic methods. Glypican-3 (GPC3) belongs to the glypican family of heparan sulfate proteoglycan, which plays an important role in cellular growth, cell differentiation and cell migration. It has been suggested that GPC3 might be a useful diagnostic biomarker for HCC, because that GPC3 is specifically highly expressed in HCC but less expressed or not expressed in normal liver tissue [6–8]. Meanwhile, GPC3 has good sensitivity and specificity for HCC, which can further increase the sensitivity of early diagnosis when combined with AFP.

Up to now, many approaches have been developed for the detection of proteins. Antibody is the most commonly used affinity ligand for protein recognition through the interaction between antibody and antigen [9,10]. In these enzyme immunoassays, one of the antibodies was always conjugated with natural enzymes, which can be used for the indirect detection of protein through the enzyme-catalyzed color reaction. Due to the high substrate specificity and high catalytic activity of natural enzymes, these methods have attracted wide attention in recent years. However, the catalytic activity of these natural enzymes can be easily affected by environmental conditions, such as pH, temperature and the inhibitors. Furthermore, the high costs and the rigorous conditions of preparation, purification and storage also limit their applications. Therefore, recent attentions have been paid to the construction of enzyme mimetics for the practical applications [11–13].

In 2007, Yan and co-workers reported that Fe_3O_4 nanoparticles possess the peroxidase-like catalytic activity, which can catalyze the oxidation of peroxidase substrate in the presence of H_2O_2 [14]. Subsequently, magnetic nanomaterials [15,16], carbon nanomaterials [17,18], CeO_2 nanoparticles [19,20], and some other nanomaterials [21–23] have also been reported to have the peroxidase-like catalytic activities. Comparing to natural enzymes, they have significant advantages, such as simple synthesis, good stability and low cost. Due to their high catalytic activities, these peroxidase mimetics can be used in various biosensing applications. When combined with enzyme immunoassays, these peroxidase mimetics are always conjugated with specific antibody and then blocked with other nonspecific proteins. In this way, their catalytic activity would be significantly reduced due to the block of the catalytic sites on nanomaterials surface, which further lead to the reduced sensitivity of these peroxidase mimetics based protein sensors. Therefore, the development of new enzyme mimetics with high and stable catalytic activity is of great significance in practical applications.

It is well known that copper (II) ion (Cu^{2+}) is able to catalyze the decomposition of H_2O_2. In this paper, we found that Cu^{2+} can catalyze the oxidation of 3, 3′, 5, 5′-tetramethylbenzidine (TMB) and obtain the oxidation product with color change in the presence of H_2O_2. This catalytic activity is not inhibited by the concentration of the substrate, which can be significantly increased by increasing the concentration of H_2O_2. Meanwhile, the catalytic activity of the Cu^{2+} is quite stable even in the presence of high concentration of proteins. This Cu^{2+}-catalyzed reaction can be also applied in the detection of protein by using the antibody functionalized CuO NPs (Scheme 1), which can release the Cu^{2+} by dissolved in HCl solution [24,25]. The proposed protein assay is more simple and effective than the previous reported peroxidase mimetics based methods.

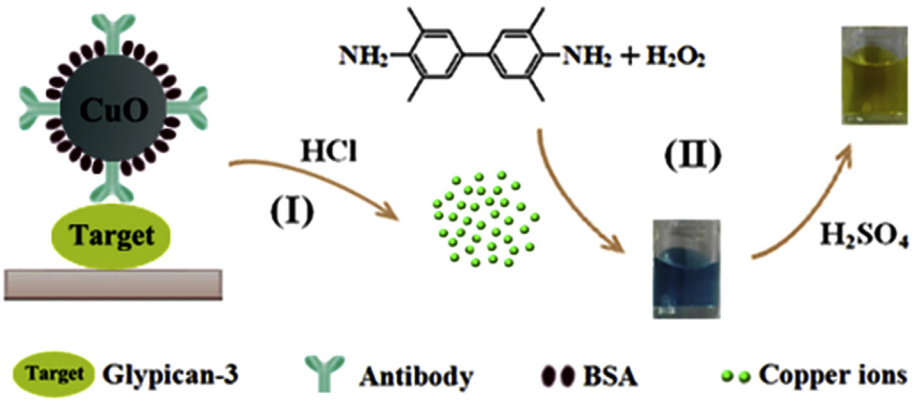
2. Materials and methods

2.1. Reagents and apparatus

HRP, 3, 3′, 5, 5′-tetramethylbenzidine (TMB), copper (II) oxide (nanopowder, <50 nm particle size), bovine serum albumin (BSA), Human serum albumin (HSA), hemoglobin (Hb), egg albumin (EA) and Tween-20 were purchased from Sigma-Aldrich Chemical Co. (USA). 96 microplates were purchased from NEST Biotechnology Co. Ltd (China). Glypican-3 was purchased from Sino biological Inc. (China). Monoclonal anti-GPC3 antibody (1G12) was purchased from Santa Cruz Biotechnology, Inc. Phosphate buffer solution (6.7 mM PBS, pH 7.4) using for protein dilution and coating was supplied by Hyclone Co. (USA). All other reagents were of analytical grade and obtained from Sinopharm Chemical Reagent co., Ltd. (China). Ultrapure water obtained from a Millipore water purification system (Milli-Q) was used throughout all the experiments. The absorbance signals of the system were all measured by microplate reader (Spectra Max M5, Molecular Devices).

2.2. Preparation of antibody functionalized CuO NPs

1 mg of copper (II) oxide (nanopowder, <50 nm particle size) was added into 1 mL of PBS buffer, and then dispersed by



Scheme 1. Schematic presentation of highly-efficient peroxidase-like catalytic activity of copper ions and its application for protein detection.