Nano-enzyme colorimetric biosensor and its application

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Abstract. Nano-enzymatic colorimetric biosensors have emerged as a promising technology for identifying and measuring analytes. These biosensors utilize nano-enzymes, which are nanomaterials with enzyme-like properties, to catalyze reactions that cause color changes. The simplicity, cost-effectiveness, and ability to visually interpret results without complex instrumentation make this colorimetric sensing technique advantageous. The interaction between the analyte and nanomaterial is crucial in the mechanism of these biosensors, where binding of the analyte to the nano-enzyme's surface triggers a catalytic process resulting in a noticeable color change. Gold nanoparticles (Au NPs) and transition metal nanoparticles are explored for their unique characteristics and potential applications in colorimetric biosensors. Graphene, a carbon substance, is also discussed for its potential in biosensing applications. The use of nano-enzyme colorimetric biosensors in medical diagnosis, environmental protection, and antibacterial applications is expanding rapidly. The article highlights the development of biosensors for identifying oxidase enzyme substrates in food and biological samples, utilizing immobilized enzymes on magnetic nanoparticles and the CUPRAC colorimetric method for detection. The potential of nano-enzyme colorimetric biosensors in medical diagnosis is emphasized, offering a quick, accurate, and practical method for identifying biomolecules and disorders, with the potential for improving patient outcomes and advancing healthcare through further research and development.

Keywords: Nano-enzyme, biosensing, mechanism, application.

1. Introduction

Biosensors have spots where biomolecules including enzymes, DNA, and antibodies can be recognized. For the purpose of diagnosing various diseases, they can offer sensitive and selective detection of important biomolecules. Recently, high surface area nanoscale sensors with exceptional catalytic characteristics have been created. Enzymes among other biorecognition components provide a special selectivity to the tested analyte. Due to their low specificity and turnover rates, recently discovered nano-enzymes frequently find it difficult to compete with enzymes. Despite their efficiency, enzymes are unstable, which makes them difficult to employ and expensive to separate for in vitro research. However, immobilization, which entails affixing enzymes to sturdy supports, aids in getting over these restrictions. Another difficulty is separating immobilized enzymes from the reaction medium, especially when working with nanoparticles (NPs) because of their small size. In this situation, conventional techniques like centrifugation can have problems. As a solid support alternative, magnetic nanoparticles (MNPs) stand out because they are simple to separate using an external magnetic field. MNPs also have other benefits, such as a large specific surface area and the capacity to electrostatically bind probes [1].

2. Overview of Nano-Enzymatic Colorimetric Biosensors

2.1. Mechanism

Colorimetric sensing technology is mainly based on color changes, and qualitatively determines the presence and concentration changes of analytes by observing them with the naked eye. Colorimetric sensing based on nanase is mainly based on the fact that hydrogen peroxide (H_2O_2) can catalyze the oxidation reaction of some color developing substrates, thus making the color developing substrates change color and achieving the purpose of visual detection. H_2O_2 can also be detected

without the presence of nano-enzymes, but there are shortcomings such as longer time and general selectivity. Therefore, colorimetric biosensors based on nano-enzymes are recognized by more and more people. Colorimetric method mainly adopts two methods: visual method and spectrophotometer method. The visual method is to observe the color of the solution directly through the naked eye. By measuring the change of absorbance value of a substance, spectrophotometer can not only identify the measured substance qualitatively, but also analyze and detect it quantitatively, which is fast and convenient, but also has good accuracy. At present, the color developing substrate detected is generally 3,3',5,5' -tetramethylbenzidine (TMB), and there are other color developing substrates such as 2, 2' -diazo-di-3-ethylbenzothiazolin-6-sulfonic acid (ABTS) or o-phenylenediamine (OPD). Taking the color rendering substrate TMB as an example, the color rendering process of the colorimetric biosensor based on nanase is mainly that the active site of the nanase first adsorbs TMB and provides certain lone electron pairs to the surface of the nanase, resulting in an increase in the electron density and mobility of the nanase. Due to the strong interaction on the interface of the system at this time, the active site will get electrons and be reduced. At the same time, electrons are transferred to H_2O_2, where the active site is oxidized, thus accelerating the electron transfer process between the nanase and TMB, and greatly shortening the detection time [2].

2.2. Several Device Construction Forms of Colorimetric Biosensor Based on Nanase

2.2.1. Paper-based bioassay

First of all, the Whatman filter paper is perforated into a small disk of the same size, and the nanofase is used as the basis, and the nanofase is loaded on the paper to obtain a one-time high-sensitivity paper bioanalyzer, and then the colorimetric signal on the paper device after adding the substance to be tested is monitored in RGB mode through a smartphone app, and the fitting is carried out. The functional relationship between the color of the strip and the concentration of the substance to be measured was obtained. The paper-based detection method is mainly that the qualitative filter paper is first immersed in a solution containing TMB for a period of time, and after drying at room temperature, the solution containing nano-enzymes and substances to be detected is added to observe the color change of the paper, and then the software is further used for analysis.

2.2.2. Portable hydrogel kit testing

Firstly, the hydrogel device of nano-enzyme and TMB is prepared, and then the substance to be tested is added and waited for a period of time, so that the substance to be tested can better diffuse into the hydrogel device. The detection results are recorded by digital camera, and then quantified by software and analyzed by data. The linear histogram of the concentration of the substance to be measured and the color depth of the hydrogel was obtained by image processing. Portable hydrogel kits have the advantages of simple operation, good selectivity and low cost, which can meet the needs of frequent screening and diagnostic follow-up.

2.2.3. Agarose gel reagent strip detection

First, agarose reagent strips were prepared, and then the nanase and TMB were fixed in agarose gel for colorimetric detection of the substance to be measured. After a period of time, the color change of agarose gel reagent strips could be observed, and the relationship curve between the absorbance of the reagent strips and the concentration of the substance to be measured was obtained by further analysis with UV-vis analyzer. Agarose reagent strips have high sensitivity and are expected to be used for gas detection [2].

2.3. Characterization and Classification of Nano-Enzymes

A class of synthetic enzymes known as nano-enzymes was developed at the nanoscale size to replicate the catalytic functions of natural enzymes. They have special qualities that make them extremely beneficial in a variety of applications. Due to their small size, which typically ranges from 1 to 100 nanometers, nano-enzymes can interact with molecules at the nanoscale. They are remarkably stable and can tolerate a variety of pH, temperature, and chemical conditions. Because

they are adaptable, nano-enzymes can be created to catalyze a wide variety of processes. They have high catalytic efficiency, which enables them to speed up chemical reactions more quickly than naturally occurring enzymes. Based on their structure and composition, nano-enzymes can be divided into various groups. Researchers are actively researching novel materials and designs to create more effective and adaptable nano-enzymes for a variety of applications, which is driving the area of nano-enzymes forward.

2.3.1. Colorimetric biosensor based on noble metal nanomases

Noble metal nanomaterials have been widely used in some fields because of their unique physical and chemical properties, good active sites and local surface plasmon resonance properties. At present, some precious metals have been found to have a good simulation of enzyme catalytic activity, such as gold, silver, platinum and so on. In which gold nanoparticles (Au NPs) with enzymatic capabilities have several potentials in biomedical applications. Numerous studies have noted that Au NPs have the capacity to imitate a variety of enzymes, including glucose oxidase (GOD) and peroxidase. Au nanoparticles' GOD-like behavior can deplete glucose levels and produce H2O2, which can devour glucose nutrients and cause cell starvation in tumor tissues. For instance, Gao et al. created the inorganic nano-enzyme platform (DMSN-Au-Fe3O4-PEG) that contains Au. At the same time, there have been more and more reports on metal Pt in recent years. Nanase with catalase-like activity can advance oxygenated tumor therapy technique based on the action of catalase. Platinum-based nanomaterials are frequently employed for tumor therapy, including PDT and radiotherapy, to treat tumors, reduce tumor hypoxia, and break down endogenous H_2O_2 into oxygen [3].

2.3.2. Colorimetric biosensor based on transition metal nanomases

Transition metals usually refer to the elemental elements composed of metallic elements in the d region of the periodic table, and these metals have strong electrochemical properties and promote electron transfer properties. Transition metal nanomaterials have excellent electrical conductivity, optical and chemical stability, a large number of active catalytic sites, adjustable crystal structure, and band gap is closely related to material thickness. The above advantages stimulate more and more researchers to study and pay attention to transition metal nanomaterials. After a certain modification, transition metal materials will show physical properties incomparable to other materials, and at the same time, with a large specific surface area and good electrical conductivity, it has become a new material that can detect chemical substances or biological substances.

For instance, although manganese can exist in oxidation states from 0 to 7, only oxidation levels +2 to +4 are important for catalysis [4]. Mn^{2+} and Mn^{4+} are the only Mn ions that are stable in the aquatic environment, which is necessary for bio applications. The simple interconversion between Mn^{2+} and Mn^{4+} via Mn^{3+} enables the speedy and efficient Fenton-like activation of H_2O_2 by Mn. All Mn ions normally occur as oxide polymorphs, and they interact well with H_2O_2 when "doped" or included in NPs [5]. However, there are drawbacks associated with the variety of oxidation states for Mn in that physical form because chemical concentration and composition can result in the production of a number of ROS, including the extremely deadly HO and superoxide [6].

2.3.3. Colorimetric biosensor based on carbon-based nanase

The majority of carbon-based nanomaterials (CNMs) display superoxide dismutase and peroxidase-like activities. These CNMs are more stable and economical than real enzymes because of their superior physical and chemical properties. Long carbon chains can only be created through the polymerization of carbon at the atomic level, which is only possible because carbon has the potential to make single, double, or triple bonds with other elements utilizing its four outer layer electrons. This quality contributes to the unique qualities of CNMs. Additionally, due to their stability under adverse conditions, CNMs can be used as metal-free catalysts [7].

As one of the most representative materials of carbon materials, graphene is a kind of carbon atoms formed by sp2 hybridization, in which carbon atoms are tightly packed to form a two-dimensional honeycomb structure of the material, the σ bond in each lattice of graphene is very stable, and at the

same time, it contributes an electron in the p orbital to form a large π bond, because π electrons can move freely. So, graphene has good electrical conductivity [8].

3. Application of Nano-Enzyme Colorimetric Biosensor in Medical Diagnosis

The broad use of nano-enzymes expanded at a rapid rate to several disciplines, including environmental protection, anti-bacterial treatment, cancer therapy, cytoprotection, biosensing, and more, thanks to the rapid technological advancements linked with them. Different methodological approaches, such as optical and electrochemical detection techniques, were used to advance the field. Due to the growing demand for reliable, affordable catalytic instruments for use in clinical and basic research, the use of nano-enzymes in biosensing has garnered particular attention.

3.1. Environmental Monitoring

Toxic ion pollution is a pervasive environmental issue that has emerged as a result of the global manufacturing sector's explosive growth, over-exploitation of minerals and groundwater, and the release of industrial wastewater. Heavy metal ions, including those of mercury, cadmium, lead, chromium, arsenic, and other metals that may be harmful to living things, are the main concern here. Only natural or biological processes can change these ions into various molecules; they cannot be eliminated. They consequently pose a serious risk to both the ecology and public health. The use of nano-enzymes in various methods for detecting these dangerous ions is now being researched. Many of these sensors are designed to be portable and compact, making them suitable for point-of-care testing (POCT).

By coating glass carbon electrodes (GCE) with HS-rGO modified gold nanoparticles (Au NPs), Wang et al. created a sensing platform. To trap Hg^{2+} ions, they employed a signal enhancer made of Au Pd-modified zirconium metal organic skeleton (AuPd@UiO-67). By boosting the amount of modified Hg2+, which increased the amount of Apt2-AuPd@UiO-67, the detection of Hg^{2+} was made possible. The linear range of the electrochemical sensor was broad (1.0 nmol/L-1.0 mmol/L) and the detection limit was low (0.16 nmol/L). When functionalized with L-cysteine, a graphene oxide nanosheet (CGO) demonstrated high peroxidase-like activity, which is a standard method for detecting Hg^{2+}. More S and N species were added, which improved the carbon's peroxidase-like characteristics and allowed for the micro detection of Hg^{2+}. In order to measure As (V) by colorimetry, Zhong et al. exploited the peroxidase-like activity of iron hydroxide (FeOOH) nanorods. The oxidation product showed a green color with a maximum absorption peak at 418 nm. The catalytic substrate was ABST. TMB was used as a photothermal and colorimetric double-readout sensor, and it was also used to detect Ag+. The oxidation of TMB to oxTMB was mediated by MnO2 nanosheets (NSs), however GSH-induced reduction of MnO2 NSs decreased their catalytic activity. By inhibiting this reduction process, Ag+ and GSH together made it possible to measure Ag+ concentration using temperature and color cues. Point-of-care testing (POCT) for environmental detection was appropriate for this technique [9].

3.2. Medical Usage

3.2.1. Ion detection

Metal ions, particularly heavy metals like mercury, pose a significant threat to human health and the environment. Over time, these metal ions have a propensity to build up in organs and tissues, causing tissue damage and raising the risk of contracting numerous diseases. Therefore, precise metal ion detection is essential for both therapeutic interventions and environmental cleanup operations.

Particularly toxic metals like mercury have been linked to a number of diseases, including Alzheimer's disease, cardiovascular illness, and Minamata disease. Mercury exposure has been linked to damage to the lungs, kidneys, and brain. For determining the degree of contamination and putting effective remediation plans in place, mercury ions in the environment or tissues must be found.

Researchers have developed various nano-enzyme-based approaches for the detection of mercury ions. One method is to use oligo-ethylene glycol (OEG) to functionalize gold nanoparticles (Au NPs). The creation of an Au-Hg amalgamation is improved by this functionalization, according to research by Cao and colleagues, making it possible to identify pollutants in water samples at incredibly low concentrations—as low as 10 parts per billion (ppb)—in very small quantities. This technique offers an accurate and sensitive way to find mercury ions in environmental samples.

Mercury ions can also be detected using platinum (Pt) nano-enzymes. Pt nano-enzymes have peroxidase-like activity that can be suppressed in a luminol system by Hg2+ ions. Zhao et al. showed that Pt nanoparticles can accelerate the luminol system's chemiluminescence (CL). They were able to detect mercury ions down to a low-end detection limit of 8.6 nanomolar (nM) by taking use of this catalytic process. For the detection of mercury ions, this method offers good sensitivity and specificity.

In addition to gold and platinum nanoparticles, researchers have explored the use of Au@AgPt nanoparticles with surface-enhanced Raman scattering (SERS) and active peroxidase activity for mercury ion detection. These nanoparticles were created by Wang and his colleagues and are capable of detecting signal molecules via SERS or colorimetric signals. Through colorimetric examination, the Au@AgPt nanoparticles had detection limits of 0.52 micromolar (M), and through SERS assay, they had detection limits of 0.28 nM. The accuracy of the detection of mercury ions is made possible by this dual-mode detection method, which combines the ease of colorimetric analysis with the high sensitivity of SERS.

Furthermore, metal oxide nanoparticles can also be employed for the detection of mercury ions, expanding the range of materials used in nano-enzyme-based detection methods. These nanoparticles have distinctive qualities and can be customized for certain detecting needs.

The examples given here emphasize how crucial it is to reliably identify metal ions, such as mercury, in environmental and therapeutic situations. Sensitive and effective ways for detecting metal ions are provided by nano-enzyme-based systems, allowing for improved environmental monitoring and illness detection. The development of efficient detection methods for diverse metal ions will be made possible by continued study in this area, enabling better environmental management and medical procedures [6].

3.2.2. Cancer cell detection

Cancer has had a significant impact on human health in the 21st century. The majority of the cancer diagnosis and screening techniques used today are CT and MRI, although every type of detection technique has certain negative health effects. According to reports, cancer can be detected early on and treated by measuring the H2O2 level of cancer cells, which is much higher than that of healthy human cells. Zhang et al. effectively completed colorimetric detection of H_2O_2 in breast cancer cells (MCF-7) by in-situ synthesizing porous platinum nanoparticles on the surface of graphene oxide. These nanoparticles had strong peroxidase activity. By using polyvinylpyrrolidone (PVP) and cetyltrimethyl ammonium bromide (CTAB) as various template agents in 2021, Lian et al. created a bimetallic sulfide NiCo2S4 (PVP) and NiCo2S4 (CTAB). It was shown that NiCo2S4 (PVP) NP had a great deal of selectivity and outstanding peroxidase activity. High Ni/Co ratio and strong electrostatic driving synergy between the cations of TMB and NiCo2S4 (PVP) NP anions are likely responsible for NiCo2S4 (PVP) NP's good catalytic activity and high specificity, respectively. Its specificity can be improved to some extent by detection under intense electrostatic driving. The creation of bimetallic sulfides helped to some extent increase the NP stability of NiCo2S4 (PVP). Highly sensitive and focused H_2O_2 detection can be achieved using NiCo2S4 (PVP) NP-based nanomases as the sensing platform, and H_2O_2 generated in human breast cancer (MDA-MB-231) cells can be detected with good biocompatibility and low biotoxicity. This offers a fresh approach and concept for the development of high specificity nanase [2,10].

3.2.3. Proteins detection

Proteins are not only essential for life but also serve as the primary carriers of life processes. They serve as the structural backbone of cells and take part in almost every aspect of life. Proteins are essential to life as we know it. These amazing organic macromolecules are made up of amino acids, which are connected in certain ways to create distinctive protein structures.

For cellular function to remain normal, protein expression, modification, and folding must be properly regulated. However, proteins can cause a variety of disorders when they are mis regulated or mis expressed. As a result, alterations to proteins after translation or even the way proteins fold might act as biomarkers or indicators of specific diseases or stages of disease. Understanding these changes and keeping an eye out for them can help with disease diagnosis and therapy.

Researchers have made great strides in the creation of nano-enzyme-based methods for ultrasensitive biomarker detection in recent years. These methods rely on tiny enzymes, like Au/Co@HNCF bimetallic nanoparticles on a hollow nanopore carbon framework, to detect particular biomarkers with extraordinary sensitivity. For instance, the Au/Co@HNCF system was created by Wang and colleagues expressly for the purpose of detecting uric acid, which is linked to diseases like gout, tumor lysis syndrome, and Type 2 diabetes. The amazing 0.023 M limit of detection for uric acid was attained using this method.

Another class of nanoparticles that shows great promise in biomarker detection is metal-organic frameworks (MOFs). These nanomaterials have special qualities like well-defined structures, the capacity to change the surface, and tunability. In order to identify thrombin, an essential serine protease involved in coagulation-related events, Li et al. used a photoactive ion-based MOF material named Fe-MIL-88A. With a low limit of detection of 10 nM, thrombin could be colorimetrically detected and quantified thanks to Fe-MIL-88A's catalytic activity towards TMB. The use of matched aptamers allows this approach to be modified to detect additional target proteins, offering adaptability and flexibility in protein detection.

The creation of quick and easy techniques for producing nano-enzymes and identifying different proteins has significant benefits for clinical diagnosis and other sectors. These techniques can improve illness diagnosis's precision and effectiveness, allowing for early detection and intervention. Nano-enzymes' capacity to concurrently detect several proteins also creates new opportunities for thorough biomarker profiling and individualized treatment. It is crucial to remember that using nano-enzymes to detect different proteins is a challenging and extensive undertaking that calls for additional study and the development of other approaches. Unquestionably, continued work in this field will increase our knowledge of protein biology and help diagnose and treat diseases[6,11].

4. Summary

In conclusion, nano-enzymatic colorimetric biosensors have become a promising technology for the identification and measurement of analytes. These biosensors catalyze reactions that lead to color changes using nano-enzymes, which are nanomaterials having enzyme-like characteristics. The simplicity, cost-effectiveness, and ability to visually view the results without the need for complicated instrumentation are just a few benefits of this colorimetric sensing technique.

The interaction between the analyte and the nanomaterial plays a role in the mechanism of colorimetric biosensors based on nano-enzymes. When the analyte binds to the nano-enzyme's surface, the electron density and mobility change. This contact sets off a catalytic process that results in an obvious change in color. Usually, spectrophotometry or ocular inspection are used to spot the color change.

The use of gold nanoparticles (Au NPs), in particular, in colorimetric biosensors is further examined in this article about noble metal nanomaterials. These nanomaterials have distinct physical and chemical characteristics, have good active sites, and exhibit local surface plasmon resonance features. Au NPs can mimic a variety of enzymes, according to numerous studies, making them appropriate for biomedical applications.

The application of transition metal nanoparticles in colorimetric biosensors is also covered in the paper. These metals are advantageous for sensor design because they exhibit potent electrochemical characteristics and encourage electron transport. Due to its special features, graphene, a typical carbon substance, is also discussed for its potential in biosensing applications.

The application of nano-enzyme colorimetric biosensors in medical diagnosis has expanded rapidly in various disciplines, including environmental protection and antibacterial applications. The development of colorimetric biosensors for the identification of oxidase enzyme substrates in food and biological samples is highlighted in the article. The enzymes are immobilized on magnetic nanoparticles in the proposed method, and the CUPRAC colorimetric method is used for detection.

The article's main focus is on the potential of nano-enzyme colorimetric biosensors for medical diagnosis, which provide a quick, accurate, and practical method for identifying biomolecules and identifying disorders. Improvements in patient outcomes and breakthroughs in healthcare may result from additional study and development in this area.

Despite the fact that nanomases outperform conventional biocatalyst techniques, there are still certain difficulties to be solved. There are currently much too few different forms of nanase, which are mostly found in the hydrolase and REDOX enzyme families. Most nano-enzymes have substantially lower catalytic activity than normal enzymes. Therefore, methods for improving its catalytic activity must be continually investigated. More research is required in order to increase the catalytic activity and specificity of nanase while lowering the potential toxicity in light of these difficulties. Due to the quick advancement of nanotechnology and the potential of these materials to replicate the functions of peroxidase, oxidase, peroxidase, phosphatase, and peroxidase dismutase, more nanomaterials are currently being produced for use as biosensors. The extension of the type and scope of nanase targets is another topic of study that can be investigated. The mechanism by which nanase activity is inhibited is also a key subject of research. Overall, there is a lot of potential for nanase research and its use in biosensing. There is hope that we can accomplish a variety of biosensing objectives with a growing emphasis on building nano-enzymes with improved selectivity and lower toxicity.

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