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A highly sensitive peptide-based biosensor using $NiCo_2O_4$ nanosheets and g-C_3N_4 nanocomposite to construct amplified strategy for trypsin detection

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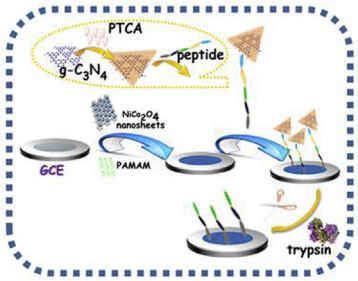
HIGHLIGHTS

- A peptide-based biosensor was constructed based on superstructure to detect trypsin.
- This is the first time $NiCo_2O_4$ superstructure was used in designing a peptide-based biosensor.
- The proposed peptide-based biosensor shows extraordinary performance in low detection limit and wide linear range.

GRAPHICAL ABSTRACT



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ABSTRACT

Here, a simple electrochemical biosensor was proposed based on the specific recognition between trypsin and peptide. Initially, $NiCo_2O_4$-PAMAM nanocomposite was casted on the bare electrode to achieve the electrochemical signal amplification in 0.1 mM $[Ru(NH_3)_6]^{3+}$ solution owing to the great electronic conductivity and high electrochemical activity induced by the special structure of $NiCo_2O_4$ nanosheets (Ni^{3+} cations in octachedral sites of the Co_3O_4). Subsequently, a declined electrochemical signal was obtained when g-C_3N_4 labeled peptide composites were anchored on the electrode. However, after trypsin was added into solution and incubated with the biosensor, the electrochemical signal was re-promoted. Therefore, the as-synthesized biosensor could realize the sensitive detection of trypsin by virtue of the specific recognition between trypsin and peptide. As a result, the developed peptide-based exhibited a linear range from 10^{-10} to 10^{-4} mg mL^{-1} with an ultralow detection limit of 10^{-10} mg mL^{-1}, providing sensitive analytical performance and acceptable application potential in clinical test and disease diagnosis due to its high stability, excellent selectivity, acceptable reproducibility and accurate signal output.

1. Introduction

Trypsin, a kind of serine protease produced by the pancreas, has been widely studied in recent years. Trypsin is of vital importance

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in controlling the function of pancreatic exocrine due to the active form of trypsin could lead to the transformation of other pancreatic proenzymes into active forms [1]. Therefore, as it was reported, trypsin has been widely used in biochemical applications, identifying protein in peptide sequencing techniques and diagnostic tests of pancreatic function, because it could specifically and effectively self-cleave proteins on the C-terminal side of arginine or lysine residues [1]. Besides, it is known that the level of trypsin also could serve as a reliable and specific biomarker for some diseases, such as cystic fibrosis, chronic pancreatic and even cancer [2]. Accordingly, it is significant to detect the trypsin activities in the fields of the physiological and pathological diagnosis [3]. Nowadays, a variety of methods have been developed to detect trypsin, such as radioimmunoassay [4], enzyme-linked immunosorbent assay (ELISA) [5], gelatin-based film technique [6] and colorimetric assay [7]. Nevertheless, these traditional methods require not only multifarious and time-consuming preparations of samples, but also specialized instruments and trained personnel. Thus designing a simple and quick biosensor for the sensitive determination of trypsin is especially important.

Several biosensors based on different methods, such as Quartz Crystal Microbalance (QCM) [8], fluorescent [9] and electrochemical [10] methods have been developed to detect trypsin. However, these methods still could not meet the requirement of low detection limit and satisfying linear range. For example, M. Stoytcheva et al. reported a sensitive trypsin activity evaluation applying a nanostructured QCM-sensors but its detection limit can be further improved [1]. In addition, although an optical sensitive nanoprobe (Mn: ZnSe d-dots-Arg_6) for trypsin determination and its inhibitor response was fabricated based on the fluorescence quenching by Xue Gao et al., its liner range was only from 0.1 to 12.0 $\mu g\ mL^{-1}$ [11]. Recent years, using short binding peptides as substrates has been considered as a promising way to detect enzyme because of their unique merits of stable, reliable, cost-effective, resistant to harsh environments and more amendable to engineering at the molecular level [12]. However, considering rare reports of peptide-based biosensor for trypsin detection have been developed till now, in this work, a peptide-based platform designed by electrochemical approach was fabricated to achieve sensitive detection for trypsin.

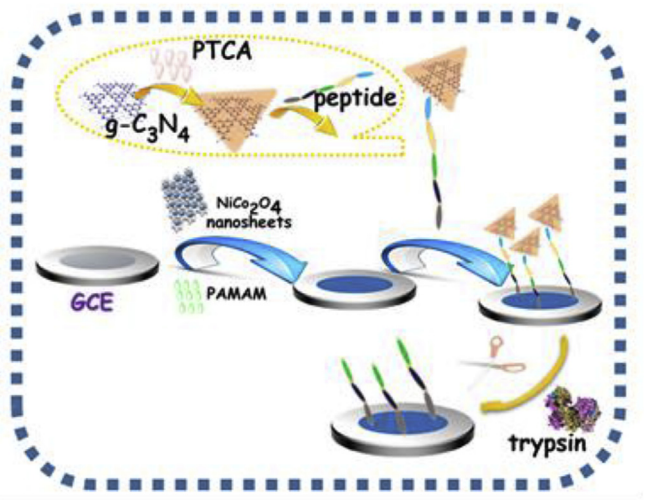
In the electrochemical peptide-based sensing system, selecting proper materials to realize the immobilization of peptides and the amplification of electrochemical signal is the first thing should be considered. In the last few years, nanomaterials have attracted considerable attention due to its charming properties of biocompatibility, nontoxicity, environmental-friendly, especially the excellent electrochemical catalytic activity, which was significant in designing signal amplification strategies. Among them, the particular properties of transition metal-based nanomaterials make them the potential candidates to be chosen. $NiCo_2O_4$, a kind of mixed transition metal oxide with spinel structure has drawn considerable attention in many fields due to its much better electronic conductivity and higher electrochemical activity than single nickel oxides or cobalt oxides [12,13]. Besides, other advantages of better electrochemical catalytic activity, biocompatibility, nontoxicity, and environmental-friendly [13] made the structure of $NiCo_2O_4$ be further investigated. Compared with other structures like $NiCo_2O_4$ nanoparticle, nanotubes and $NiCo_2O_4$ cluster, $NiCo_2O_4$ superstructure shows much better performance because its larger surface area could effectively contact the electrolyte and electrode materials and its better conductivity could accelerate transport rate of ions/electrons in the electrode and the interface of electrode/electrolyte [14]. Even so, the superstructure of $NiCo_2O_4$ has not been applied in designing electrochemical biosensor. Herein, $NiCo_2O_4$ superstructure was synthesized by a similar way with Hejun Li group [15] and employed as sensing matrix to amplify electrochemical signals. Additionally, to further promote the performance of $NiCo_2O_4$, PAMAM was introduced to realize the functionalization of $NiCo_2O_4$. The space structures of PAMAM dendrimers are highly branched three-dimensional macromolecules with massive functional amino-groups (-NH_2), which enable $NiCo_2O_4$-PAMAM nanocomposites film to capture more peptides. Considering the assemble ability between peptides and 3,4,9,10-perylene tetracarboxylic acid (PTCA) and the π-π conjugation between the PTCA and g-C_3N_4 [17], PTCA was employed to connect peptides and g-C_3N_4 which could quench the electrochemical signal [18].

In this work, a sensitive electrochemical peptide-based sensor was developed based on the amplified strategy of $NiCo_2O_4$ nanosheets. The fabricated process of the biosensor was presented in Scheme 1. First of all, $NiCo_2O_4$-PAMAM nanocomposite was modified on the bare glassy carbon electrode, which could effectively promote the electrochemical signal owing to the excellent conductivity of $NiCo_2O_4$. Moreover, the large surface area of $NiCo_2O_4$ [19] and the highly branched 3D structure of PAMAM [16] provided more active sites for the anchor of peptides to further expand the range of assay. Subsequently, the peptides were labeled with PTCA functionalized g-C_3N_4 via the π-π stacking between the PTCA and g-C_3N_4 and then linked to $NiCo_2O_4$-PAMAM nanocomposite, leading to a distinctly declined electrochemical response due to the quench effect of g-C_3N_4. Nevertheless, after the incubation between trypsin and peptides, a remarkably raised electrochemical signal was realized, providing an effective electrochemical strategy for the sensitive determination of trypsin. The reason could be explained by the specific recognization between the peptide and trypsin, which triggered the releasing of g-C_3N_4 from the electrode and thus resulted in the electrochemical signal recovered proportionally to the concentration of trypsin. In a word, the peptide-based biosensor was first proposed on the basis of $NiCo_2O_4$ superstructure as the electrochemical signal amplification matrix to realize the fast, sensitive, and accurate detection of trypsin activity in real samples, showing promising application in clinical test and disease diagnosis.

2. Experimental

2.1. Materials and reagents

Peptide (CAGRAAADAD) was purchased from Biodee



Scheme 1. Schematic illustration the process of fabrication of peptide-based biosensor for sensing trypsin.

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