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REVIEW

Recent Advances in the Dehydrogenase Biosensors Based on Carbon Nanotube Modified Electrodes

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Abstract: Nicotinamide adenine dinucleotide (NADH) is involved as a cofactor in over 300 enzymatic reaction of NAD^+/NADH dependent dehydrogenases. The application of amperometric NADH sensors has evolved to provide a promising measurement technique for detection of substrate or enzymatic activity. However, the direct oxidation of NADH at ordinary electrodes often requires high overpotential and suffers from low sensitivity and the fouling of the electrode surface by its oxidation products. In recent years, carbon nanotubes (CNT) are attracting growing attention in decreasing the high overpotential for NADH oxidation and minimizing the surface fouling. This paper introduces the research progress of the NADH electrochemical sensors based on CNT-modified electrodes, and foretells its application prospect.

Key Words: Carbon nanotube; Nicotinamide adenine dinucleotide; Electrochemical detection; Dehydrogenase biosensor; Review

1 Introduction

Nicotinamide adenine dinucleotide (NADH), which is also known as reduced coenzyme I, plays important roles in organisms as energy metabolism, expression and modulation of genes, oxidative stress and antioxidation effects, immunization and anticarcinogenesis etc. Among many oxidoreductases, over 300 kinds of dehydrogenases dependent Nicotinamide coenzymes ($NAD(P)^+$/NAD(P)H) as the coenzyme. By measuring the amount of NADH, a variety of substrates and enzymes' activity can be indirectly detected, which has a very broad prospect of application in biosensing area. There are many techniques developed for the detection of NADH, e.g. UV-spectrometric[1], chromatographic[2] and enzymatic[3], etc. Although these methdos had higher analytical sensitivity, the drawbacks such as complicated sampling processes, time-costing detection periods, unacceptable testing expenses limited there applications in large scale measurements of industrial and scientific targets. Electrochemical technique exhibits many more advantages in NADH detection due to its high sensitivity, short response time, excellent reproducibility, facile operation and low cost etc. On the other hands, due to the effects of low electron transfer rate, dissatisfactory kinetic features, interferences from the oxidation of such constituents as ascorbic acid or dopamine in samples under comparatively higher detecting potential, non-emzymatically active dimer will be generated and which may lead to passivation of electrodes[4]. So far these are often thorny problems that can be hardly solved. Other examples are the porous structure of graphite can bring wider and low reproducible background current to electrodes. Orderlessly arranged long carbon fibers of glassy carbon electrode (GCE) reduce its electrocatalytic capacity Conventional metallic electrodes like that of platinum, gold and silver presented very low electrocatalytic activity for NADH owing to their electrochemical oxidation potentials > 1 V in common cases[5], which make the direct detection of NADH very difficult[6].

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It is a breakthrough to coupling nano-materials in the electrochemical sensing of NADH, especially in the construction of dehydrogenase biosensors. Nowadays, the major nano-materials applied in the electrochemical detection of NADH are nanoparticles of metal[7,8], nanoparticles of metallic/non-metallic oxides[9], carbonaceous nano-materials etc. The discovery and application of carbon nanotubes (CNT) provided the electrochemical measurement of NADH and the construction of dehydrogenase biosensors with whole new spaces of development both in theoretical and practical aspects[10], which can be attribute to their excellent electric conductivity, high electrocatalytic activity for biomolecules, robust capacity of adsorption and ideal biocompatibility. In this work, we made a summary on the advances in the applications of carbon nanotube modified electrodes in the construction of dehydrogenase biosensors as well as the electrochemical detection of NADH, and their prospect were also outlined.

2 Applications of carbon nanotubes in dehydrogenase biosensors

2.1 Electrochemically modified electrodes based on carbon nanotubes

As researches demonstrated, CNT showed favorable electrocatalytic activity for NADH commonly by significantly reducing its oxidation overpotential, prompting electron transfer rate, anti-interference ability, anti-fouling ability and the signal-to-noise of electrodes. It is suggested that the electrocatalytic nature of CNT can be attribute to its active sites reside in the nanotubes' open ends as which can also be found in edge plane pyrolytic graphite and the defect sites reside along in the side walls of CNT molecules[11]. Multi-walled carbon nanotubes (MWCNT) and single-walled carbon nanotubes (SWCNT) are the most common forms of CNT in current applications. Even though they have similar physical and chemical properties, SWCNT possess less defect sites along the wall[12] and therefore the majority of CNT family applied in the current researches are MWCNT.

The electrochemical responsivity of NADH on electrodes can be improved to some extent via merely coupling with CNT. Musameh *et al*[13] achieved comparatively ideal current response by dropping the dispersions of MWCNT and SWCNT, which were pretreated with the acid onto the clean GCE surfaces for detecting free NADH. Comparing with conventional electrodes, the oxidation overpotential of NADH was reduced to about 490 mV. Zhang *et al*[14] constructed formaldehyde dehydrogenase (FDH) biosensor with carboxylated MWCNT (MWCNT-COOH) by which formaldehyde in samples was indirectly measured when the presence of the cofactor NADH. The method required drops of dispersed MWCNT-COOH solution on the functioning area of screen-printed (SPE) electrode to obtain the detector interface. This enzyme electrode was allowed to test at least 0.2 μM of its substrate with great anti-interference capacity and high sensitivity.

In addition, owing to the highly delocalized pi-bonds, NAD^+ can be adsorbed on the surface of CNT through π-π stacking interaction between the heterocyclic structure on it and CNT molecules. Other than accomplishing the immobilization of NAD^+ cofactor, CNT can also be well dispersed. Zhou *et al*[15] prepared dehydrogenase modified electrode successfully by using this mechanism. Also Tominaga *et al*[6] provided experimental evidences and more elaborate explanation with electrochemistry and spectroscopy.

2.2 Electrochemically modified electrodes based on carbon nanotube composite materials

CNT could improve the electrocatalytic activity of electrodes to some extent, but it is limited. The common position of NADH oxidation peak on GCE occurs at about 500 mV. However at this position, some composites that can be oxidized at low potential may cause interferences and the immobilization efficiency of enzymes which is not high enough. Therefore in reported studies, only few made use of plain CNT for NADH detecting and dehydrogenase biosensor construction, in contrast the other majority researchers focused on developing electrodes modified by composite materials based on CNT. CNT's special properties allow it to make good use of many physical and chemical interactions as π-π stacking interaction, electrostatic interaction, hydrophobic interaction, van de Waals interaction, and covalent linkages etc. for better immobilization of enzymes[16]. Also, excellent properties of a variety of non-carbonaceous composites could be exerted by coupling with CNT, and thus can be used as a new tool for the immobilization of enzymes and their cofactor NAD^+. In this way, a rapid development has been achieved in studying and developing reagentless dehydrogenase biosensors in recent years. In addition, owing to the powerful functions, the applications of CNT were also expended in separation technologies[17]. The most common kinds of studied CNT composite materials used in modified electrodes include modified electrodes based on electron mediator/CNT composites, modified electrodes based on polymer/CNT composites, modified electrodes based on inorganic nanoparticle/CNT composites, modified electrodes based on ionic liquid/CNT composites and modified electrodes based on other CNT composites. Each composite is reviewed in detail below.

2.2.1 Modified electrodes based on electron mediator/CNT composites

Coenzyme Q, which structurally contained a anthraquinone