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The electrochemical preparation of FAD/ZnO with hemoglobin film-modified electrodes and their electroanalytical properties

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Abstract

Flavin adenine dinucleotide (FAD)-modified zinc oxide self-assembly films were prepared using repeated cyclic voltammetry. The electrochemical reaction of the hemoglobin with the FAD/ZnO self-assembly film-modified electrodes and their electrocatalytic properties were investigated. This paper describes the successful loading of the electrochemically active molecules of hemoglobin and FAD along with ZnO by electrochemical method. In addition to the cyclic voltammetry, an electrochemical quartz crystal microbalance was used to study the growth mechanism and the properties of the films. The FAD/zinc oxide films exhibited a single redox couple, which corresponded to the FAD redox couple. The electrocatalytic properties of the O_2, H_2O_2, trichloroacetic acid and SO_3^{2-} were studied by the FAD/zinc oxide films in the absence or in the presence of hemoglobin. The electrocatalytic reduction current had been developed from the cathodic peak of the FAD/zinc oxide redox couple. The electrocatalytic process involved an interaction of hemoglobin and FAD/GC film-modified electrode to increase the electrocatalytic reduction current. The electrocatalytic reduction of O_2 using the FAD/zinc oxide films was investigated by cyclic voltammetry and rotating ring-disk electrode methods.

Keywords: Hemoglobin; FAD; Zinc oxide; Film-modified electrodes; Electroanalysis

1. Introduction

Flavin adenine dinucleotide (FAD) is a flavoprotein coenzyme that plays an important biological role in many oxidoreductases and in reversible redox conversions in biochemical reactions. It consists of the nucleotide adenine, the sugar ribose, and two phosphate groups.

FAD and $FADH_2$ have an isoalloxazine ring as the redox-active component that readily accepts and donates electrons. This makes it ideally suited to be an intermediate that is cyclically reduced and then re-oxidized by the metabolic reactions. The adsorption of FAD has been studied on Hg electrode (Birss et al., 1998), on the surface of titanium electrodes (Garjonyte et al., 2003), and its electrochemical properties have been determined (Wang et al., 1997).

Direct electron transfer between the proteins (the redox active group) and the electrode (direct electrochemistry) can serve as a model system to aid the understanding of the electron transfer mechanisms in biological systems. The reversible direct electron transfer between the immobilized heme protein in the film and the electrode (Nassar et al., 1996; Chen et al., 2000; Armstrong et al., 1988) provides a basis for biosensors, biomedical devices, and enzymatic reactors.

The heme protein hemoglobin (Hb) consists of four subunits of polypeptide enzymes and a heme (iron porphyrin) group in each subunit that acts as the active center. The electron transfer between a heme protein hemoglobin in solution and the bare electrode is either not observed or very slow (Taniguchi et al., 1992).

Direct electrochemistry entails the notion of enhancing the electron transfer rate of a heme protein hemoglobin interacted or immobilized on the film-modified solid electrodes. The film-modified electrodes can facilitate and reveal a reversible electron transfer between the heme protein and the solid electrode without mediators. For example, didodecyldimethylammonium

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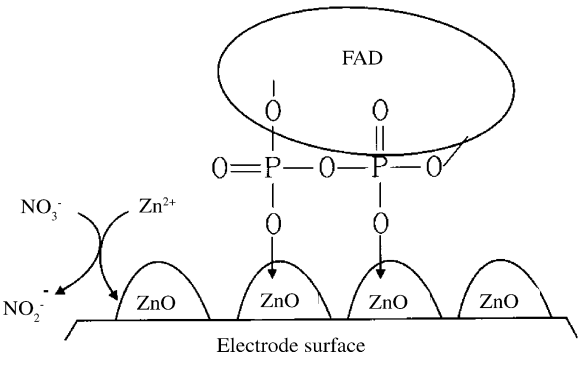
bromide (DDAB) (Ordaz and Bedioui, 1999; Han et al., 2002) and DNA (Nassar et al., 1996; Jin et al., 2003) can form a modified solid electrode to direct and facilitate electron transfer rates.

Nanostructured zinc oxide can have novel applications in optoelectronics, the development of sensors and transducers, and in biomedical science, because it is biosafe. ZnO, a material that has diverse structures, has configurations that are much richer than any known nanomaterials, including carbon nanotubes. Using a solid-state thermal sublimation process and controlling growth kinetics, local growth temperature, and the chemical composition of the source materials, a wide range of ZnO nanohelixes, nanobows, nanopropellers, nanowires, and nanocages have been synthesized (Wang, 2004).

Zinc oxide films are composed of nanosized metal-oxide particles, and have been intensively investigated in recent years for use in self-assembly dye/ZnO thin films (Yoshida et al., 2004; Izaki and Omi, 1996; Karuppuchamy et al., 2001; Yoshida and Minoura, 2000), optoelectronic devices (Petrella et al., 2004; Schlettwein et al., 2000; Keis et al., 2002), and as inorganic acceptor dye-sensitized solar cells (Bahadur and Srivastava, 2003; Rensmo et al., 1997; Van de Walle, 2000).

The zinc oxide almost exhibits n-type conductivity, with the electron in its valance band as charge carriers. Despite years of investigations, the conductivity of ZnO is still in controversy. A recent first report reveals that none of the native defects reveal any characteristics high concentration shallow donors (Farr, 1988). In zinc oxide, zinc is acting as deep acceptor and oxygen in acting as deep donor. In our present study, zinc oxide is acting as a conductive matrix to immobilize organic mediator. During film formation, the FAD is reacting with ZnO by donating its electron presents in phosphate group to zinc in zinc oxide. The interaction between these two materials is given in Scheme 1.

Hydrogen peroxide is a naturally produced purposeful molecule in the body. It functions to aid membrane transport, acts as a hormonal messenger, regulates thermogenesis (heat production), stimulates and regulates immune functions, regulates energy production, and many other important metabolic functions. It is used in the cancer treatment (Tacconi et al., 2003). Reactive oxygen species (ROS), including superoxide ($^{*}O_2^{-}$), hydrogen peroxide (H_2O_2), and hydroxyl anion (OH^{-}), and reactive nitrogen species, such as nitric oxide (NO) and peroxynitrite ($ONOO^{-}$), are biologically important O_2 derivatives that are increasingly recognized to be important in vascular biology through their oxidation/reduction (redox) potential (Touyz and Schiffrin, 2004).



Scheme 1. The FAD/ZnO film formation using cyclic voltammetry in zinc nitrate solution containing FAD.

Chemically modified electrodes are also useful in materials science, in electrochromic devices, and in electroanalysis and electrocatalysis (Hosono et al., 2004; Lin and Bocarsly, 1991; Humphrey et al., 1987; Kulesza et al., 1987; Mortimer, 1995; Chen, 1996; Chen and Lin, 2002; Chen and Hsueh, 2004a,b). They find uses in bioinorganic chemistry as chemicals and biosensors (Bedioui et al., 1995; Chen and Chen, 2003).

This paper reports on the electrochemically induced growth and the electrocatalytic properties of composite films made of ZnO and flavine adenine dinucleotide from an aqueous solution of a mixture of Zn^{2+} and FAD. The electron transfer process and the interaction of Hemoglobin with FAD/ZnO self-assembly film-modified electrodes and their electrocatalytic properties were investigated. The cyclic voltammetry and electrochemical quartz crystal microbalance were used to study the in situ growth mechanism and properties of film.

The FAD/zinc oxide films that electrocatalytically reduced O_2, H_2O_2, trichloroacetic acid, and SO_3^{2-} were studied by the FAD/zinc oxide films and in the absence or in the presence of hemoglobin in neutral aqueous solutions. The electrocatalytic reduction of O_2 using the FAD/zinc oxide films was investigated by employing cyclic voltammetry and rotating ring-disk electrode methods.

2. Experimental

The electrochemistry was performed using a Bioanalytical Systems Model CV-50 W and CH Instruments Model CHI-400 and CHI-750 potentiostats. Cyclic voltammetry was conducted using a three-electrode cell assembly. A BAS glassy carbon electrode, a gold electrode, a platinum electrode, and an indium tin dioxide (ITO) electrode (fabricated by sputtering ITO on a glass substrate) were used as the working electrodes. The glassy carbon electrode was polished with 0.05 μm alumina on Buehler felt pads, and then ultrasonically cleaned for 1 min. The auxiliary compartment contained a platinum wire that was separated by a medium-sized glass frit. All the cell potentials were measured by an Ag|AgCl|KCl (saturated solution) reference electrode.

The working electrode for the EQCM measurements was an 8 MHz AT-cut quartz crystal with gold electrodes. The diameter of the quartz crystal was 13.7 mm, whereas the gold electrode diameter was 5 mm.

UV–visible absorption spectra were measured by a Hitachi (Japan) Model U-3300 spectrophotometer.

The rotating ring-disk electrode (RRDE) experiments were performed by a Pine Instrument Co. electrode in conjunction with a CH Instruments CHI-750 potentiostat connected to a Model AFMSRX analytical rotator. The rotating ring-disk electrode was consisted of a glassy carbon disk electrode and a glassy carbon (or platinum) ring electrode.