Applied Surface Science 329 (2015) 262–268

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Activity of formaldehyde dehydrogenase on titanium dioxide films with different crystallinities

Hitomi Nakamura, Katsuya Kato*, Yoshitake Masuda, Kazumi Kato

National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan

ARTICLE INFO

Article history:
Received 22 July 2014
Received in revised form
18 November 2014
Accepted 4 December 2014
Available online 25 December 2014

Keywords:
TiO_2 thin films
Formaldehyde dehydrogenase
Enzyme immobilization
Enzyme activity
Surface material crystallinity

ABSTRACT

Many biosensors have been developed and used in recent years, and to enhance the sensitivity and stability of enzyme biosensors, immobilization of the enzymes on material surfaces is a necessary and important step. Therefore, there has been considerable interest in understanding how material interfaces affect enzyme adsorption. In this study, the influence of the crystallinity of titanium dioxide (TiO_2) films on the quantity and activity of the immobilized enzyme, i.e., formaldehyde dehydrogenase (FDH), was investigated. It was found that TiO_2 films with high crystallinity, which were annealed at 550 °C, showed higher enzyme immobilization and activity compared with the non-annealed TiO_2 film. These results suggest that the activity of enzymes could be affected by the crystallinity of surface materials.

1. Introduction

Many biosensors using biological molecules (e.g., enzymes, antibodies, and nucleic acids) have attracted considerable attention because of their many advantages such as their high sensitivity, specific recognition, fast response, and extensive applications. To date, for instance, various biosensors have been developed for the detection of glucose [1–4], lactic acid [5], and cholesterol [6]. A biosensor system consists of four components: (1) a recognition section that contains the biological molecules, (2) a solid-phase support to immobilize the biological agent, (3) a selective membrane or mediator, and (4) a transducer. The solid-phase support is essential for anchoring the biological molecules on the transducer. Thus, to enhance the sensitivity and stability of enzyme biosensors, enzymatic binding to a desired solid-phase support surface with retention of activity are very important. Therefore, enzyme immobilization methods, such as adsorption, covalent binding, and cross-linking, play a key role [7].

Formaldehyde is a highly toxic and carcinogenic compound, which can induce respiratory diseases. However, it has been used in broad applications, such as preservatives and adhesives for building materials and as a sterilizing agent for medicine [8,9]. Note that an extremely low concentration of formaldehyde is permissible for use in various applications; thus, many attempts to develop biosensors for formaldehyde have been reported [9–11].

Formaldehyde dehydrogenase (FDH), a member of the zinc-containing medium-chain alcohol dehydrogenase family, is present in a wide variety of organisms ranging from bacteria to animals [8]. FDH requires NAD^+ as an electron acceptor and catalyzes the irreversible oxidation of formaldehyde to formate. The FDH from most organisms is glutathione-dependent in the catalysis of formaldehyde oxidation, while FDH from *Pseudomonas putida* is the only glutathione-independent example known to date [12–15]. FDH is known to react to formaldehyde with high selectively and sensitivity. Therefore, here we are working on the development of formaldehyde biosensor using FDH.

Titanium dioxide (TiO_2) is a low-cost, nontoxic, and highly stable nanocrystalline material that is important for a wide range of industrial applications. For instance, TiO_2 particles have been used in sunscreens [16], and food additives [17], whereas TiO_2 thin films have been extensively used as microelectronics [18], highly efficient catalysts [19], and microorganism photolysis [20]. Moreover, TiO_2 finds use in gas sensors and biosensors. TiO_2 gas sensors designed for the detection of hydrogen, SO_2, and ammonia [21–23], as well as TiO_2 biosensors designed for the detection of glucose and hydrogen peroxide [24–26], have been reported. These TiO_2 gas sensors show high sensitivity and a rapid response, and they utilize the properties of TiO_2 as a metal oxide semiconductor, which involves the adsorption of the gas molecules on the surface of the TiO_2, and charge redistribution between the surfaces and the

* Corresponding author. Tel.: +81 52 736 7551; fax: +81 52 736 7405.
E-mail address: katsuya-kato@aist.go.jp (K. Kato).

http://dx.doi.org/10.1016/j.apsusc.2014.12.027
0169-4332/

adsorbed molecules. In addition, TiO_2 is used as a matrix for the immobilization of enzymes in biosensors because of its chemical inertness, rigidity, and thermal stability [27].

Because immobilization of enzymes on material surfaces is a necessary and important step in the design of biosensors, there has been considerable interest in understanding how material interfaces affect enzyme adsorption. However, to the best of our knowledge, there have been very few reports on the relationship between the crystallinity of biosensor surface materials and the activity of immobilized enzymes. Therefore, in this study, we investigated the influence of the crystallinity of TiO_2 films on the activity of immobilized FDH.

2. Materials and methods

2.1. Materials

Formaldehyde dehydrogenase (FDH) from *Pseudomonas* sp. (EC 1.2.1.46; 1.0 U/mg) and nicotinamide adenine dinucleotide were purchased from TOYOBO ENZYME (Osaka, Japan) and Sigma–Aldrich (St. Louis, MO), respectively. The formaldehyde solution (assay (HCHO): 36.0–38.0% (mass/mass)) and phosphate buffer powder (1/15 mol/L, pH 7.4) used in the study were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Note that all chemicals were used without further purification.

The TiO_2 thin films were prepared as described previously [28] with some modifications. Briefly, glass substrates coated with F-doped SnO_2 transparent conductive films were cut into square-shaped pieces having dimension of 13 mm × 13 mm and exposed to vacuum ultraviolet light (VUV; low-pressure mercury lamp PL16-110, air flow, 100 V, 200 W, SEN Lights Co., Osaka, Japan) for 20 min in air. The substrates were then immersed perpendicularly in the middle of a solution containing 50 mM ammonium hexafluorotitanate and 150 mM boric acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The solution was maintained at 50 °C with no stirring for 24 h. The obtained TiO_2 thin films were heated in an FP100 muffle furnace (Yamato, Tokyo, Japan) to 550 °C (rate: 1.5 °C/min) in air and annealed at this temperature for 4 h.

2.2. Characterization

The topography of the surfaces was studied using a dynamic force microscope (DFM, Nanonavi SPA400-DFM, SII Nanotechnology, Tokyo, Japan). A silicon aluminum-coated probe cantilever with a resonance frequency of 131 kHz and a spring constant of 13 N m^{-1} was used, and DFM imaging was performed in air at room temperature. The contact angle was measured using an image processing method (DropMaster DM300, Kyowa Interface Surface, Saitama, Japan). The morphology of the TiO_2 thin films was observed using a field-emission scanning electron microscope (FE-SEM; JSM-6335FM, JEOL Ltd., Tokyo, Japan) operated at 20 kV and a field-emission transmission electron microscope (FE-TEM; HF-2000, Hitachi, Tokyo, Japan) operated at 200 kV. The crystal phase was evaluated using an X-ray diffractometer (XRD; RINT-2100 V, Rigaku, Tokyo, Japan) with Cu Kα radiation (40 kV, 30 mA). The zeta potential of the FDH was determined using an ELSZ-2 zeta-potential and particle size analyzer (Otsuka Electronics, Osaka, Japan).

2.3. Immobilization of the enzyme on the TiO_2 thin films

The TiO_2 thin films were exposed to vacuum ultraviolet light (low-pressure mercury lamp PL16-110, air flow, 100 V, 200 W, SEN Lights Co., Osaka, Japan) for 20 min to generate superhydrophilic surfaces. Next, an FDH solution (10 μL, 5 mg/mL) was dropped on the superhydrophilic surfaces, which were stored first at room temperature for 1 h and then at 4 °C overnight. The amount of immobilized FDH on the TiO_2 thin films was determined using a FluoroProfile® Protein Quantification Kit (Sigma–Aldrich, St. Louis, MO), and the qualitative detection of the immobilized FDH on the 550 °C-annealed film was performed using X-ray photoelectron spectroscopy (XPS; Kratos analytical, ESCA-3400, Shimadzu, Kyoto, Japan).

2.4. Enzyme activity assay

The enzyme activity assay was performed as follows. The TiO_2 thin film was placed in a well of a 12-well MultiDish (2 mL/well, Nunk, Denmark). A reaction solution (1 mL) containing 100 μL of an 85.2 mM formaldehyde solution as the substrate, 100 μL of a 4 mg/mL NAD^+ solution, and 800 μL of a 50 mM phosphate buffer (pH 7.4) was added to the TiO_2 thin film. The enzyme reaction was allowed to proceed for 10 min at 30 °C with gentle shaking using a Mini-Shaker 3D (Biosan, Riga, Latvia), and then the absorbance of the reaction solution was measured at 340 nm using a DU 800 spectrophotometer (Beckman Coulter, Brea, CA) to determine the amount of generated NADH as an indicator of extent of the reaction mediated by FDH, as shown in Eq. (1).

$$HCHO + NAD^+ + H_2O \rightarrow HCOOH + NADH + H^+ \quad (1)$$

After the absorbance was measured, the TiO_2 thin film was washed with 800 μL of a 50 mM phosphate buffer (pH 7.4) for 5 min with gentle shaking, and then fresh reaction solution was added to the TiO_2 thin film to determine its reusability. The activity of MultiDish-immobilized FDH was measured as a positive control (Free), and the enzyme activity was confirmed as the relative activity. Error bars represent the standard deviations (SD) for triplicate determinations.

3. Results and discussion

3.1. Characterization of the non-annealed and 550 °C-annealed TiO_2 thin films

The surface topography of the non-annealed and 550 °C-annealed TiO_2 thin films was evaluated using DFM. Each TiO_2 film surface was scanned over a 2 μm × 2 μm region, and the DFM images of the surface roughness are shown in Fig. 1. The analysis revealed no significant difference in the surface roughness of the non-annealed and 550 °C-annealed films (average roughness (Ra) of 17.1 ± 4.0 nm and 24.5 ± 1.8 nm, respectively).

The hydrophilicity of these films was also analyzed based on their water-contact angles. The water-contact angle of the non-annealed films was approximately 86°; however, after 20 min of UV irradiation, these films exhibited superhydrophilicity with water-contact angles of <1°. TiO_2 is generally considered to be hydrophilic and superhydrophilic particularly after UV irradiation [29]. In contrast, the water-contact angles for the 550 °C-annealed films were <1° both before and after UV irradiation. Thus, the 550 °C-annealed films exhibited superhydrophilicity without UV irradiation.

Next, the morphologies, microstructures, and crystal phases of the films were examined using FE-SEM and TEM. As shown in Fig. 2a and f, acicular TiO_2 crystals were observed by FE-SEM. Fig. 2b and g shows TEM images of the cross-sections of these acicular TiO_2 crystals, in which they can be seen growing perpendicular to the substrate. These results suggested that the morphology and microstructure of the non-annealed and 550 °C-annealed TiO_2 thin films were similar. Fig. 2c and h shows magnified areas of Fig. 2b and g, respectively; moreover, the electron diffraction patterns of both non-annealed and 550 °C-annealed TiO_2 films indicated that they were each composed of a single phase of anatase (Fig. 2d