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UV-ozone dry-cleaning process for indium oxide electrodes for protein electrochemistry

Masato Tominaga *, Norifumi Hirata, Isao Taniguchi *

Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan

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Abstract

The present study reports, for the first time, on electrochemical responses of cytochrome *c* at a UV-ozone treated indium oxide electrode. Results from surface tension measurements indicate that UV-ozone treatment is an efficient cleaning procedure to remove organic species contamination on surfaces. Well-defined redox responses for cytochrome *c* were observed at a UV-ozone treated fully hydrophilic indium oxide electrode. Electrochemical parameters, including the diffusion coefficient, the heterogeneous electron transfer rate constant and the redox potential, were in good agreement with those previously reported. However, decrease in peak current for cytochrome *c* and $[Fe(CN)_6]^{4-}$ were observed at a UV-ozone treated electrode. From XPS results, this behavior would be understood to indicate a decrease in homogeneous active electrode surface area by a decrease in conductivity of the indium oxide surface by UV-ozone treatment. Simple and effective UV-ozone treatment methods are useful for surface contamination sensitive electrochemistry.

Keywords: UV-ozone; Indium oxide; Electrochemistry; Protein; Cytochrome *c*

1. Introduction

Indium oxide electrodes have been used extensively for protein electrochemistry [1], because they are highly conductive and transparent in the visible region due to high carrier densities ($\sim 10^{21}$ cm^{-3}) and wide band gaps (3.5–4.3 eV) [2]. It is well known, however, that electron transfer reaction of protein at indium oxide electrode surfaces is strongly inhibited by contamination on electrode surfaces [1,3]. Therefore, prior to electrochemical measurements, polishing or detergent cleaning procedures are necessary to remove contamination adsorbed onto indium oxide electrode surfaces [1,3]. However, electrode surfaces may be contaminated upon exposure to impurities contained in polishing materials and detergents during cleaning processes. Cleaning procedures that utilize UV-ozone would safeguard against contamination which may arise from wet cleaning processes with detergents and polishing materials, because UV-ozone processes are dry-cleaning processes [4–7]. UV-ozone radiation changes organic compounds adsorbed onto electrode surfaces into volatile species [4–10], which seems to be an efficient cleaning procedure for indium oxide electrodes without introducing additional contaminants during wet-cleaning processes. In fact, a UV-ozone cleaning treatment significantly improved the work function and eliminated indium oxide contamination [11] and X-ray photoemission spectroscopic studies on UV-ozone treated indium oxide electrodes have been investigated [12,13].

To our best knowledge, however, effects of UV-ozone cleaning on indium oxide electrodes used for protein electrochemistry have not been reported. The present study reports, for the first time, on electrochemical responses of cytochrome *c* at a UV-ozone treated indium oxide electrode. Results from surface tension measurements indicated that UV-ozone treatment is an efficient cleaning procedure to remove organic contaminants on electrode

* Corresponding author. Tel./fax: +81 96 342 3656.
E-mail address: masato@gpo.kumamoto-u.ac.jp (M. Tominaga).

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surfaces. Well-defined redox responses for cytochrome *c* were observed at a fully hydrophilic, UV-ozone treated, indium oxide electrode (72 dyn cm^{-1} at 25 °C).

2. Experiment

2.1. Cytochrome c

Cytochrome *c* (from horse heart, Sigma) was purified chromatographically using a HPLC system (Bio CAD 700E-KT, Applied Biosystems) [14]. The purity was certified by the ratio of its absorbances at 280 and 550 nm for the oxidized and reduced forms, respectively (A_{280}/A_{550}; the purity index being = 1.25) [15]. The concentration of cytochrome *c* was estimated spectroscopically for the reduced form in a phosphate buffer solution (pH 7.0) using a molar absorptivity coefficient of 29,500 M^{-1} cm^{-1} at 550 nm [16]. All other reagents used in this study were of analytical grade. Milli-Q water (Millipore Corp.) (>18 MΩ cm) was used.

2.2. Indium oxide electrode and UV-ozone treatment

A vacuum evaporated indium oxide electrode (ca. 30 nm) doped with tin (Kinoene Optics. Corp., Japan) was used as a working electrode with a sheet resistance of ca. 10 Ω cm^{-2} and an area of 0.25(±0.02) cm^{-1} as the electrode surface. The electrode was used as received without any cleaning process prior to UV-ozone treatment. The composition ratio of indium and tin was evaluated to be indium (94%) and tin (6%) by X-ray photoemission spectroscopy (XPS) measurements.

A UV-ozone treatment system (Model OC-2503, Eye Graphics Co., Japan) was used in this study. UV light had main emissions at 185 and 254 nm. The density at 230 – 280 nm (sensitivity peak: ca. 255 nm) was evaluated as 11 mW cm^{-2} by an ultraviolet ray integration illuminance meter (UVPF-A1, Eye Graphics Co., Japan). The concentration of ozone was ~100 ppm. To protect the electrode from contamination, after UV-ozone cleaning, the treated electrode was quickly immersed into a pure water solution and kept there until further use.

The mechanism by which UV-ozone destroys organic species is well established and involves a complex set of photosensitized oxidation processes. A simple description of the overall electrode cleaning process is as follows [4–13]: ozone and atomic oxygen are generated by decomposition of ambient oxygen by UV irradiation at wavelengths below 245.5 nm (optimally at 185 nm). Simultaneously, UV irradiation at 254 nm exists and/or dissociates organic species on electrode surfaces, thereby producing activated species, such as ions, free radicals, and excited molecules. Eventually, activated organic species are readily attacked by atomic oxygen and ozone synergistically to form simpler volatile molecules, such as CO_2, H_2O, and N_2, which are simply eliminated by rinsing with water.

2.3. Cyclic voltammetric, surface tension and XPS measurements

Electrochemical measurements were carried out in a three-electrode cell. Cyclic voltammetric measurements were performed in a phosphate buffer solution (pH 7.0) using an electrochemical analyzer (Bioanalytical Systems, BAS 100B/W). An Ag/AgCl (saturated KCl) electrode and a platinum electrode were used as reference and counter electrodes, respectively. All potentials are reported with respect to the Ag/AgCl (saturated KCl) electrode. Prior to cyclic voltammetric measurements, the buffer solution was deaerated with high purity nitrogen, and a positive pressure of nitrogen was kept over the solution during electrochemical experiments.

Simulations of cyclic voltammograms were performed with a cyclic voltammetric simulation, DigiSim 2.0, Bioanalytical Systems [17].

The hydrophilicity of the indium oxide electrode surface was measured in purified water at 28(±3) °C, using a Shimadzu ST-1 surface tensiometer by the Wilhelmy method [3]. For surface tension measurements, the indium oxide electrode was prepared by vacuum evaporated indium oxide films (ca. 30 nm) on both sides of a thin glass plate (0.3 × 10 × 5 mm).

XPS measurements were carried out using a Thermo VG Scientific, Sigma Probe HA6000II. The instrument uses a focused monochromatic Al Kα X-ray (1486.68 eV) source for excitation and a spherical section analyzer, and a 6-element multichannel detection system. The X-ray beam was incident normal to the sample and the detector was 37° away from the normal. The percentage of individual elements detected was determined from the relative composition analysis of peak band areas.

3. Results and discussion

3.1. Surface tension measurements for the UV-ozone treated electrode surface

Fig. 1 shows the surface tension evaluated by the Wilhelmy method after UV-ozone treatment for various times.

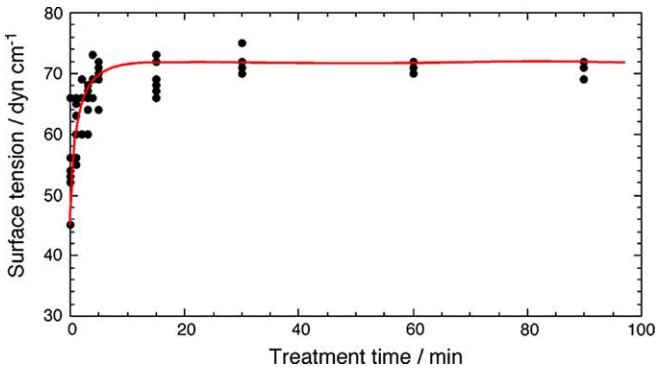

Fig. 1. Changes in surface tension as a function of UV-ozone treatment time.