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Detection of DNA hybridization on indium tin oxide surfaces

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Abstract

Indium tin oxide (ITO) surfaces were modified with ssDNA by coupling oligonucleotides to a monolayer of 12-phosphonododecanoic acid (12-PDA) on ITO surfaces. This coupling involved the formation of an amide bond between the carboxylic acid moiety of 12-PDA to the amine group of a 5′-aminopropyl-labeled single strand of DNA. The self-assembled monolayer of 12-PDA and surface-attached oligonucleotides were characterized by X-ray photoelectron and reflectance FTIR spectroscopy. Detection of selective surface DNA hybridization was achieved by labeling the target ssDNA with gold nanoparticles. The presence of gold nanoparticles was probed using X-ray photoelectron spectroscopy, stripping voltammetry, atomic force microscopy, thermography, photoelectrochemistry (chronoamperometry) and cyclic voltammetry (CV). CV was used to successfully detect DNA hybridization for nanoparticle concentrations as low as 10 pM when using the gold nanoparticles bound to an ITO electrode as catalysts for the electrochemical oxidation of $FeCl_2$. The studies described here provided the basis for surface attachment methodology for various electrochemical and thermographic sensing methods that use ITO thin films as a substrate.

Keywords: Thermography; Electrochemistry; Fourier-transform infrared; Nanoparticle detection; Stripping voltammetry

1. Introduction

Indium tin oxide (ITO) is a versatile material that has shown promise in novel detection strategies for biomolecules. ITO has been shown to have favorable properties for electrochemical [1], photothermal electrochemical [2], thermographic [3], and surface plasmon resonance [4–6] detection methods. ITO electrodes can support a wide range of potentials. ITO is an infrared reflective material [4,5,7–10], which is advantageous for thermographic [3] and infrared sensing technologies [11]. Recently, it has been shown that surface plasmons can be excited on ITO [5]. Surface plasmon resonance is a method widely used on gold surfaces that gained widespread acceptance during the last decade [12–14]. However, the application to conducting metal oxides provides opportunities for new surface chemistry [5,11,15,16], a wide range of simultaneous electrochemistry [2,17–23] applications and novel effects because of the fact that the plasmon itself is in the near infrared [4,6,8,24]. The application of ITO for any of these detection strategies requires a robust attachment strategy. ITO is much less studied than substrates such as gold [25] and glass [26,27], which have a demonstrated ability to support self-assembled monolayers on these surfaces with thiol or chloro- or alkoxysilane containing molecules [28], respectively. These systems have shown the detection of selective DNA hybridization through a variety of detection techniques including fluorescence [29–32], surface plasmon resonance (SPR) [33,34], electrochemistry [35–37], FTIR spectroscopy [38], X-ray photoelectron spectroscopy (XPS) [25] and through labeling with nanoparticles [39–41].

In this study, indium tin oxide thin films were investigated to develop a surface DNA modification procedure to be used for the subsequent detection of DNA hybridization. The electronic and optical properties of ITO have spurred interest in the formation of adlayers on ITO [11,15,42–45] and electrochemical methods have been developed for the detection of DNA or PNA on ITO [1,22,23]. The detection strategies employed

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in this study involve the detection of gold-nanoparticle-labeled target single-stranded DNA (ssDNA) [2]. The formation of self-assembled monolayers and the coupling of ssDNA to those monolayers on ITO thin films were confirmed by XPS, reflectance FTIR spectroscopy and chronocoulometry while the gold nanoparticle/target ssDNA conjugates were detected on the ITO surface by XPS, stripping voltammetry, atomic force microscopy (AFM), thermography, photoelectrochemistry (chronoamperometry) and cyclic voltammetry (CV).

2. Materials and methods

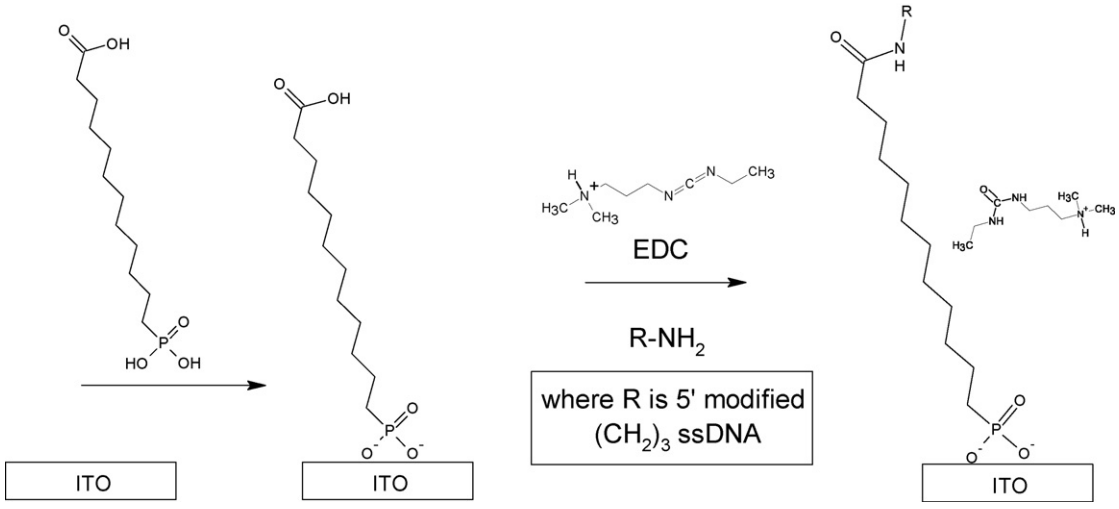
2.1.1. ssDNA Modification of ITO

Scheme 1 outlines the strategy employed in the modification of indium tin oxide (Delta Technologies, Ltd.) surfaces with ssDNA. The ITO thin films were comprised of 90% indium oxide and 10% tin oxide, had a nominal thickness of 1500 Å and a sheet resistance of 8–12 Ω. Initially, a monolayer of 12-phosphonododecanoic acid (12-PDA) (Xanthon, Inc.) (10 mM in 50/50 DMSO/18 MΩ cm H_2O for 16 h) was formed on the ITO surface (cleaned 20 min with UV/O_3 (UVO-cleaner (UVO-60), model number 42, Jelight Company, Inc.)). The carboxylic acid functional group of 12-PDA was activated by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (Sigma–Aldrich) and reacted with the primary amine of a 5′ modified H_2N–$(CH_2)_3$ ssDNA (5′H_2N–$(CH_2)_3$ modified 5′-GTGAGCGGATAATCCTGGTT-3′) (Applied Biosystems, Inc.) to form an amide bond between the 12-PDA and the 5′ modified H_2N–$(CH_2)_3$ ssDNA. The coupling conditions were 1 μM 5′ modified H_2N–$(CH_2)_3$ ssDNA and 200 mM EDC for 4 h in a 0.1 M MES (2-(N-morpholino)ethane sulfonic acid) buffer at pH 5 with 0.25 M NaCl. The surface was then rinsed with 18 MΩ cm H_2O (BARNSTEAD E-PURE) and dried with N_2 gas. The characterization of ssDNA modified ITO substrates is discussed in Supporting Information.

2.1.2. Preparation of the gold nanoparticle/oligonucleotide conjugates

The gold nanoparticle conjugates were synthesized by the attachment of 5′ thiol modified ssDNA. The complementary ssDNA target strand used in the hybridization experiments was 5′-HS$(CH_2)_6$ modified 5′-AACCAGGATTATCCGCTCAC-3′ while the non-complementary ssDNA target strand was 5′-HS$(CH_2)_6$ modified 5′-GTGAGCGGATAATCCTGGTT-3′. Gold nanoparticles (10 nm diameter) coated with bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) were synthesized by a literature procedure [46]. Briefly, 10 nm citrate coated Au colloids (Ted Pella) were stirred with an excess of BSPP at room temperature overnight. The particles were aggregated slightly by progressive addition of a 1 M NaCl solution until the NaCl concentration was >100 mM. As the NaCl concentration increased, the color of the colloidal suspension changed from burgundy to a slightly purplish color. The sample was centrifuged to pellet the particles and the supernatant containing citrate solution was removed. Afterwards, the pellet was re-suspended in a 250 mg/L solution of BSPP. Methanol was added dropwise until the color of the particles turned from a burgundy red to a purplish color again. The colloidal solution was centrifuged and the supernatant was discarded. The gold nanoparticles were finally re-dissolved in the BSPP solution and stored at 4 °C.

For the preparation of the gold nanoparticle/ssDNA conjugates, a 1:100 ratio of gold nanoparticles to ssDNA was stirred at room temperature overnight. In order to remove non-reacted ssDNA, the solution was centrifuged at 14,000 rpm for 30 min and the precipitate was re-suspended into 1× SSC buffer, defined as 150 mM NaCl and 15 mM sodium citrate at a pH of 7. This procedure was performed twice and resulted in approximately 5–6 ssDNA strands per gold nanoparticle determined by fluorescence (data not shown). The final concentration conjugate solution had a final concentration of 3.7 nM gold nanoparticles as determined from the absorbance of the plasmon band



Scheme 1. Modification of ITO with single-stranded DNA through the formation of an amide bond between the carboxylic acid functional group of a monolayer of 12-phosphonododecanoic acid with the primary amine of 5′ modified $H_2N(CH_2)_3$ ssDNA.