Sensors and Actuators B 161 (2012) 334–340

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

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

Development of microplate-based photoelectrochemical DNA biosensor array for high throughput detection of DNA damage

Yang Liu, Suping Jia, Liang-Hong Guo*

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, P.O Box 2871, Beijing 100085, China

ARTICLE INFO

Article history:
Received 22 August 2011
Received in revised form 7 October 2011
Accepted 17 October 2011
Available online 20 October 2011

Keywords:
Microplate
Electrode array
Tin oxide
Photoelectrochemistry
DNA damage

ABSTRACT

Many chemicals have been found to induce DNA damages which may lead to gene mutation and tumor generation. In this report, a microplate-based photoelectrochemical DNA sensor array was developed for the rapid and high throughput screening of DNA damaging chemicals. A 96-well plate with built-in electrodes was fabricated on a plastic substrate by the standard electronics industry processes. The working electrode in each well was deposited with SnO_2 nanoparticles, and the resulting film was sintered at low temperatures tolerable for the plastic substrate. The film was characterized by scanning electron microscopy, X-ray diffraction and X-ray photoelectron spectroscopy. On the plates sintered at 150 °C, a significant amount of photocurrent was obtained in a $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) solution. To construct a DNA sensor, poly-(diallydimethyl ammonium chloride) and double-stranded DNA were sequentially assembled on the SnO_2 electrode by electrostatic interaction, and a DNA intercalator, $Ru(bpy)_2(dppz)^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine) was used as the photoelectrochemical signal indicator. After the DNA film was exposed to tetrafluoro-1,4-benzoquinone (TFBQ) or TFBQ/H_2O_2, the photocurrent dropped by 38% and 73% respectively. The photocurrent reduction can be attributed to less binding of $Ru(bpy)_2(dppz)^{2+}$ to the electrode after DNA damage. Photocurrent measurement of the entire 96-well plate was completed within 22 min automatically.

1. Introduction

According to official statistics, currently there are around 100,000 chemicals on the world market. In addition, about 4000 new chemicals are produced each year. The sad truth is that safety data for the large majority of these chemicals are very scarce. This lack of information poses potentially very serious threat to human health and ecosystems [1]. Among the tested chemicals, 107 are carcinogenic to humans (Group 1), and 326 chemicals are probably or possibly carcinogenic to humans (Group 2) [2]. Some of these carcinogens are geno-toxic, which act directly on genes and induce DNA damages. If unrepaired, damaged DNA can lead to gene mutation, and may eventually develop into cancer.

It has been generally recognized that there are two common types of chemically induced DNA damages, DNA oxidation by reactive oxygen species (ROS), and DNA adduct formation with exogenous chemicals and their *in vivo* metabolites. Some transition metal ions such as Fe^{2+} and Cu^{2+} have been found to react with H_2O_2 to produce extremely reactive hydroxyl radicals by the so-called Fenton reaction. The radical can induce several classes of oxidative DNA damage, including single-strand break, double-strand break, abasic sites, and base oxidation [3]. Polycyclic aromatic hydrocarbons (PAHs) are an important class of air pollutants generated from incomplete burning of organic matters such as automobile exhausts and cigarette smoking. Benzo-[α]-pyrene, one member of the PAH family with five fused benzene rings, is a confirmed carcinogen. It is converted *in vivo* by metabolizing enzymes to a reactive intermediate, benzo-[α]-pyrene diol epoxide, which is capable of forming covalent adduct with the amine groups on DNA bases [4].

To test for the genetic toxicity of an unknown chemical, it is recommended that at least three cell-based assays should be performed, including gene mutation assay in bacteria, gene mutation assay in mammalian cells, and chromosomal aberration test in mammalian cells [5]. Although these assays have relatively high sensitivity and specificity for genetic toxicity screening, the drawbacks are obvious, such as low throughput, long duration, and high complexity. There are also a few established analytical methods for the identification and quantification of DNA damage products which can be used as molecular markers for genetic toxicity [6]. 8-Oxo-guanine (8-HO-dG) is a common product of oxidative DNA damage, and can be identified using HPLC separation followed by online electrochemical detection [7]. This method is very specific, but it requires relatively large and expensive equipments. The throughput is also very low. ^{32}P post-labeling is the most widely

* Corresponding author. Tel.: +86 10 62849685; fax: +86 10 62849685.
E-mail address: LHGuo@rcees.ac.cn (L.-H. Guo).

0925-4005/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2011.10.041

used method for the identification and quantification of DNA adducts [8,9]. The procedure, however, is very time-consuming, including enzymatic digestion of DNA sample, purification, labeling with ^{32}P, and TLC or PAGE analysis.

To overcome the difficulties associated with the above methods and techniques, toxicity sensors have been proposed and developed as a rapid screening tool for the identification of hazardous chemicals. For instance, DNA damage was investigated with electrochemical and electrochemiluminescent sensors and sensor arrays by a number of research groups [10–15]. We also developed a photoelectrochemical DNA sensor for the rapid detection of chemical-induced DNA damage [16–19]. The sensor was fabricated by assembling a double-stranded DNA film on SnO_2 electrode surface. A DNA intercalator, $Ru(bpy)_2(dppz)^{2+}$, was allowed to bind to the DNA film, and produced photocurrent upon light irradiation. If the DNA film was exposed to a DNA-damaging chemical, photocurrent would drop due to the disruption of DNA double helical structure and consequently less binding of $Ru(bpy)_2(dppz)^{2+}$. Our results showed that the sensor was able to detect both oxidative DNA damage induced by Fe^{2+}/H_2O_2 and DNA adducts formed with styrene oxide.

In principle, this photoelectrochemistry DNA sensor is very sensitive, as the detected signal (electrical current) is separated from the signal generation source (light) [20,21]. To cite a few examples, Tokudome et al. constructed a DNA sensor by immobilizing a ss-DNA on TO_2 electrode and analyzing the complementary ss-DNA in solution. This sensor can quantitatively detect target DNA at lower than nanomolar concentrations [22]. Liu et al. employed the $Ru(bpy)_2(dppz)^{2+}$ metallointercalator and tin oxide nanoparticle electrode for the detection of ds-DNA in solution. The detection limit of calf thymus ds-DNA reached 1.8×10^{-10} M [23]. Gao and Tansil employed a threading DNA bis-intercalator as the photoelectrochemical reporter and achieved a dynamic range of 50 fM to 1.0 nM and a detection limit of 20 fM [24]. In addition, the instrumentation for electric detection should be simpler and of lower cost than optical methods. Thus, it is a promising method for the rapid and high-throughput assessment of DNA damage. However, due to the lack of appropriate platforms (sensor array and array detector), the potential of high throughput has not been demonstrated. It is the aim of the current work to develop microplate-based photoelectrochemical DNA sensors for the rapid and high throughput detection of DNA damage.

2. Material and methods

2.1. Materials

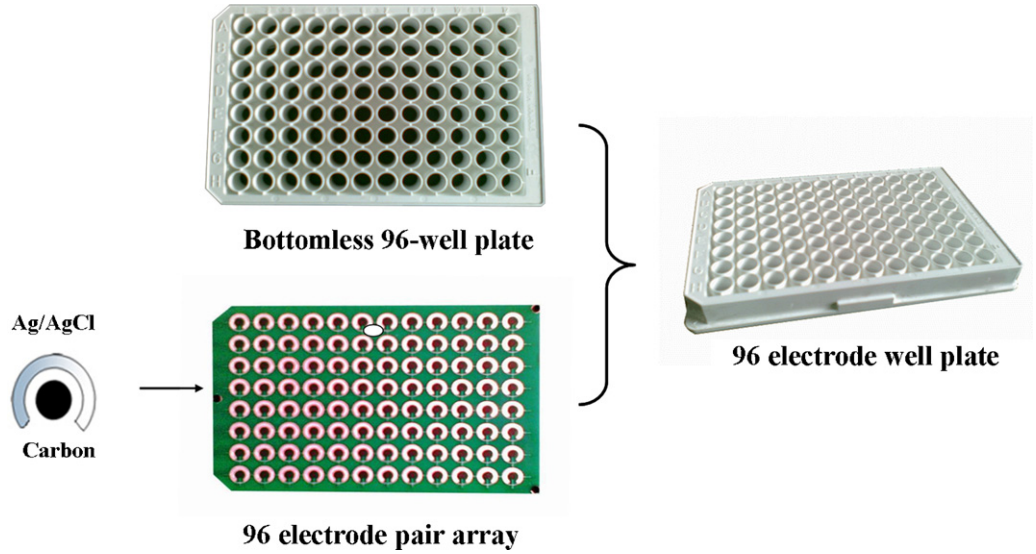
Fifteen percent tin (IV) oxide, as a colloidal dispersion of 15 nm particles, and hydrogen peroxide were both purchased from Alfa Aesar (Ward Hill, MA). Double-stranded calf thymus DNA (ds-DNA, approximately 13 K base pairs) was purchased from Merck (San Diego, CA). Poly-(diallydimethyl ammonium chloride) (PDDA) and tetrafluoro-1,4-benzoquinone (TFBQ) were purchased from Sigma–Aldrich (St. Louis, MO). $Ru(bpy)_2(dppz)(BF4)_2$ was synthesized according to the published procedure [25,26]. Indium tin oxide (ITO) conductive glass was supplied by Weiguang Corp (Shenzhen, Guangdong Province, China). All solutions were prepared in high purity water from a Millipore Milli-Q (Biocel) (Billerica, MA) water purification system.

2.2. Micro-plate fabrication and sensor assembly

Plastic substrates with an 8×12 electrode pair array were fabricated by a local manufacturer using the standard industrial procedures for the manufacturing of printed circuit boards. The electrode pair consists of a 2 mm diameter carbon disc electrode surrounded by an Ag/AgCl circular band (I.D. 4 mm, O.D. 7 mm) electrode. The footprint of the circular band matches that of a standard 96-well plate (Scheme 1). All the disc electrodes in the same line were electrically connected by a metal band on the backside of the substrate. So were the circular band electrodes in the same row.

To prepare a SnO_2 semiconductor electrode, typically the original colloidal dispersion of 15 nm SnO_2 particles was diluted to 3.75% with de-ionized water, and 0.1% Triton X-100 was added into the solution to facilitate the colloid spreading. A total of 0.25 μL diluted SnO_2 colloidal solution was then spread over the entire surface of each disc electrode. After the solution got dried naturally at room temperature, the film on the plastic substrate was sintered at 150 °C in an oven for 2 h. Then, the substrate was attached to a bottomless 96-well plate (Greiner Bio-One GmbH, Germany) with a double-sided adhesive tape. The substrate and the plate were aligned carefully so that all the electrode pairs were exposed at the bottom of the 96 wells.

The layer-by-layer self-assembly method was employed to fabricate DNA sensor films on the SnO_2 electrode inside the



Scheme 1. Fabrication of a 96 electrode well plate. Conduction lines are fabricated on a plastic substrate by the standard printed circuit board manufacturing process. An array of 96 electrode pairs (carbon and Ag/AgCl) is processed by screen-printing. The board is then attached to a bottomless 96-well plate to finish the final assembly.