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Sensitive and selective real-time electrochemical monitoring of DNA repair

Marc A. McWilliams [a], Fadwa H. Anka [b], Kenneth J. Balkus [b], Jason D. Slinker [a,*]

[a] Department of Physics, The University of Texas at Dallas, 800 W. Campbell Road, EC 36, Richardson, TX 75080, USA
[b] Department of Chemistry, The University of Texas at Dallas, 800 W. Campbell Road, BE 26, Richardson, TX 75080, USA

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ABSTRACT

Unrepaired DNA damage can lead to mutation, cancer, and death of cells or organisms. However, due to the subtlety of DNA damage, it is difficult to sense the presence of damage repair with high selectivity and sensitivity. We have shown sensitive and selective electrochemical sensing of 8-oxoguanine and uracil repair glycosylase activity within DNA monolayers on gold by multiplexed analysis with silicon chips and low-cost electrospun nanofibers. Our approach compared the electrochemical signal of electroactive, probe-modified DNA monolayers containing a base defect versus the rational control of defect-free monolayers. We found damage-specific sensitivity thresholds on the order of femtomoles of proteins and dynamic ranges of over two orders of magnitude for each target. Temperature-dependent kinetics were extracted, showing exponential signal loss with time constants of seconds. Damage specific detection in a mixture of enzymes and in response to environmental oxidative damage was also demonstrated. Nanofibers were shown to behave similarly to conventional gold-on-silicon devices, showing the potential of these low-cost devices for sensing applications. This device approach achieves a sensitive, selective, and rapid assay of repair protein activity, enabling a biological interrogation of DNA damage repair.

1. Introduction

DNA is constantly subject to damage from endogenous and exogenous factors that threaten the vitality of cells and the integrity of the genome. Left unchecked, this damage can give rise to unregulated cell division leading to cancerous tumors (David et al., 2007; Kunkel and Erie, 2005; Sancar et al., 2004). From a chemical standpoint, DNA damage is very subtle. For example, 8-oxoguanine, the most prevalent oxidative DNA damage product, involves the addition of a single oxo-bond, yet this moiety has drastic consequences for DNA replication and genomic stability (Obtułowicz et al., 2010; Maehara et al., 2008). To address damage such as this, cells are equipped with an intricate repair pathway to counteract the devastating proliferative effects of DNA damage (David et al., 2007; Kunkel and Erie, 2005; Sancar et al., 2004). For the first step in this pathway, various DNA glycosylases recognize and excise damage products. Study of this crucial step should have great consequences in cancer research, but to date, the connection of deficiencies in repair proteins to cancer has remained elusive, in part due to overlapping functionalities of repair glycosylases (Paz-Elizur et al., 2008). A biological assay that could follow repair at the level of the subtle DNA damage site would greatly assist the understanding of damage repair and potentially lead to new insight into the connection of DNA repair pathways and cancer.

The challenge in detecting DNA repair is tied to the difficulty of selectively distinguishing removal of subtle base defects. Achieving this selectivity in a stand-alone device for convenient assay and low-cost clinical detection is highly advantageous. 8-oxoguanine itself has been distinguished by GC–MS and HPLC-coupled electrochemical detection, but these techniques require digestion of the DNA and are not compatible with real-time quantitative analysis of protein activity (Abalea et al., 1999; Ivandini et al., 2007; Ravanat et al., 1998; Rebelo et al., 2004). Electrochemical devices have been used to indirectly study DNA damage. Cahová-Kuchaříková et al. (2005) detected UV-induced pyrimidine dimer formation in supercoiled DNA by treating with T4 endonuclease and directly detecting the end-termini of resulting single strand breaks and release of single stranded DNA. Havran et al. (2008) detected DNA damage products similarly extracted from supercoiled DNA with an osmium marker specific to single-stranded DNA. Wang et al. (1997) followed the formation of 8-oxoguanine on DNA-modified carbon electrodes by following the loss of undamaged guanine signal. Similarly, the electrochemically-induced generation of 8-oxoguanine and 8-oxoadinine was studied by Harfield et al. (2011) on nickel-modified boron-doped diamond

* Corresponding author. Tel.: +1 972 883 6513.
E-mail address: slinker@utdallas.edu (J.D. Slinker).

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electrodes. The group of Rusling has demonstrated a highly sensitive electrogenerated chemiluminescence 8-oxoguanine sensor (Dennany et al., 2004; Mugweru et al., 2004). Recently, an effective photoelectrochemical device was created for measuring 8-oxoguanine presence with good sensitivity and measure of post-repair changes in concentration (Zhang et al., 2012). Nanopore devices for location-specific oxidative damage reporting have been developed (Schibel et al., 2010). These techniques, however, have not offered real-time quantitative measure of repair activity.

Alternatively, to follow damage repair real-time in a stand-alone device, a recognition element capable of selectively distinguishing subtle damage products and an appropriate transduction pathway is needed. DNA is the natural recognition element for not only the binding of repair proteins but also their repair activity, and DNA can be synthesized with or without damage/lesion sites to establish controls. Furthermore, DNA can additionally serve as an electrical transducing element when modified with a redox-active probe and self-assembled on a working electrode (Paleček and Bartošík (2012)). Previous experiments have shown that DNA electrochemistry can be used to sense subtle DNA lesions and DNA-binding protein activity (Boal and Barton, 2005; Boon et al., 2000, 2002a; Zuo et al., 2009). Transduction of sensing involves a change in the efficiency of the oxidation or reduction of the redox probe due to a change in charge transport efficiency of the DNA bridge. DNA monolayers may be prepared with probes that interact directly with the electrode surface (Cash et al., 2009; Ricci et al., 2009; White and Plaxco, 2010; Zhang et al., 2012; Zuo et al., 2009) or involve charge propagation through the DNA bridge (Boal and Barton, 2005; Boal et al., 2005; Boon et al., 2000, 2002a, 2002b; Gorodetsky et al., 2006, 2008; Mui et al., 2011; Slinker et al., 2010). For the latter, charge transport through the DNA base pair π-stack enables sensitivity to single-base defects (Boal and Barton, 2005; Boon et al., 2000; Slinker et al., 2011). Along these lines, DNA electrochemistry has been used to study glycosylases, in large part to sense their presence through DNA-mediated electrochemical reduction of the 4Fe–4S cluster (Boal et al., 2005; Boon et al., 2002a; Boon et al., 2002b; Gorodetsky et al., 2008; Mui et al., 2011). However, the sensitivity, selectivity, and kinetics of DNA electrochemistry to repair protein activity have yet to be fully clarified. In particular, the quantitative real-time electrical detection of the repair of 8-oxoguanine, one of the most common oxidative damage products and a key factor in aging and cancer, has yet to be shown.

Here, we investigate the concentration and kinetics of DNA 8-oxoguanine and uracil repair glycosylase activity using selective DNA modified electrodes with rational control. This is accomplished through DNA electrochemistry with multiplexed electrodes on silicon chips and also with unconventional devices utilizing low-cost electrospun nanofibers. Using multiplexed analysis, we demonstrate the selectivity of our devices to damaged products against defect-free controls and observe the temperature-dependent rate of repair. We also investigate the use of electrospun fibers as low-cost alternative sensors.

2. Materials and methods

2.1. DNA preparation

The double stranded DNA monolayers utilized were from the 17mer sequence 3′-CTCTATATTTCGTGCGT$_{NB}$-5′ and the fully complementary sequence 5′-(C6 thiol)–GAGATATAAAGCACGCA-3′, where the underlined bases note the positions of an inserted uracil (T) or 8-oxoguanine (G), and T_{NB} notes the position of a Nile Blue modified thymine. The thiolated undamaged and uracil-containing DNA strands were purchased from Integrated DNA Technologies (IDT, USA) and was received cleaved from solid support and deprotected. The other strands were purchased from TriLink (USA). For the 8-oxoguanine strand, the 8-oxo-dG-CE phosphoramidite from Glen Research (product 10-1028-xx, USA) was incorporated at the specified position. This strand was deprotected in ammonium hydroxide with 0.25 M 2-mercaptoethanol 17 h at 55 °C to avoid degradation of 8-oxoguanine. For the Nile Blue modified strand, a 5-[3-acrylate NHS ester]-2'-deoxyUridine phosphoramidite (Glen Research) was incorporated at the 5' terminus by TriLink using ultramild synthesis conditions. The DNA on solid support was then dried and reacted with a 10 mg/mL solution of Nile Blue perchlorate (Acros Organics, USA) in 9:1 dichloromethane/N,N-diisopropylethylamine solution for approximately 24 h. Excess reagents were then removed by washing with dichloromethane, methanol, and acetonitrile. These Nile Blue modified DNA strands were then cleaved from the support and deprotected according to ultramild conditions with 0.05 M potassium carbonate in methanol at ambient temperature for 8 h.

Oligonucleotides were purified by high performance liquid chromatography (HPLC). Following HPLC purification of the products, the oligonucleotides were treated to remove the dimethoxytrityl (DMT) protecting group. For the thiolated oligonucleotides, the disulfide of the thiolated linker was cleaved with an excess of dithiothreitol in concentrated ammonium hydroxide for 2 h to yield the free thiol. The DMT was removed from the Nile Blue DNA strands by treating with an 80% solution of glacial acetic acid for 20 min, followed by quenching of the reaction with an excess of ethanol. All the oligonucleotides were dried and purified with a second round of HPLC. The products were characterized by HPLC, matrix assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry, and UV-visible (UV–vis) spectrophotometry. The oligonucleotides were subsequently desalted and quantified by UV–vis spectrophotometry according to their extinction coefficients (IDT Oligo Analyzer). Duplexes were formed by thermally annealing equimolar amounts of oligonucleotides at 90 °C for 5 min in deoxygenated phosphate buffer (5 mM NaPhos, 50 mM NaCl, pH 7.0) followed by slow cooling to ambient temperature.

2.2. Silicon chip preparation

One millimeter thick Si wafers with a 10,000 Å thick oxide layer were purchased from Silicon Quest. Wafers were cleaned thoroughly in 1165 Remover (Microchem, USA) and vapor primed with hexamethyldisilizane (HMDS). SPR 220 3.0 photoresist (Microchem) was spin-cast at 4000 rpm and baked. The photoresist was patterned with a Karl Suss MA6 contact aligner and a chrome photomask. Following post exposure baking, wafers were developed in AZ 300 MIF (Microchem) developer for 1 min and rinsed thoroughly with deionized water. A 15 Å Ti adhesion layer and a 1000 Å Au layer were deposited on the chips with an electron beam evaporator. Wafers were then immersed in 1165 Remover overnight and sonicated as needed to complete metal lift-off. The wafers were thoroughly baked and cleaned by UV ozone treatment. Subsequently, SU-8 2002 (Microchem) was spin-cast at 3000 rpm, baked, and photopatterned as above. Wafers were developed in SU-8 Developer (Microchem) for 1 min and baked for a permanent set of the photoresist. The wafers were subsequently diced into 1-in. by 1-in. chips by hand with a diamond scribe and stored under vacuum until use.

2.3. Electrospun fiber preparation

Polyacrylonitrile (150,000 MW, PAN) was purchased from Pfaltz and Bauer (USA). Dimethylformamide (DMF), hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) and gold(III) chloride hydrate