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An electrochemiluminescence biosensor for 8-oxo-7,8-dihydro-2′-deoxyguanosine quantification and DNA repair enzyme activity analysis using a novel bifunctional probe

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A new electrochemiluminescence (ECL) sensor was developed for 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) quantification and *Escherichia coli* formamidopyrimidine-DNA glycosylase (FPG) activity assay. The sensor employed a novel spermine conjugated ruthenium tris-(bipyridine) derivative (spermine-Ru) which binds specifically with 8-oxodGuo through a one-step reaction and also acts as an ECL signal reporter. In the sensor, an 8-oxodGuo-containing ds-DNA film was first immobilized on a gold electrode by self-assembly. The DNA film was then incubated with spermine-Ru under oxidative condition for 8-oxodGuo labeling. The ECL intensity was found to correlate with the amount of 8-oxodGuo on the surface and the detection limit was estimated to be about 1 lesion in 500 DNA bases. Addition of FPG resulted in some loss of the signal due to the excision of 8-oxodGuo by the enzyme. An inverse relationship between ECL intensity and FPG concentration was observed in a range from 0 to 4.0 U/μL, demonstrating that this sensor could be used for FPG activity assay. A number of metal ions were screened by the sensor for their inhibition effect on FPG activity. Among them, Hg^{2+} and methyl Hg(II) shown very potent inhibition, with IC_{50} values of 4.04 μM and 4.34 nM respectively. The result may suggest that interference on the DNA repair system could be another mechanism for the high toxicity of MeHg.

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1. Introduction

Oxidative DNA damage is an inevitable consequence of cellular metabolism, with a tendency of increasing levels under the invasion of toxic exogenous substances (Jackson and Loeb, 2001; Cooke et al., 2003; Halliwell, 1991; Valko et al., 2004). In numerous oxidatively damaged DNA products, 8-oxodGuo has received the most extensive study. Accumulation of 8-oxodGuo in bacterial and mammalian cells may lead to G:C to A:T transversion mutations and even cancerous tumors (Cheng et al., 1992; Andrew et al., 1996). In medical diagnosis, the level of urinary 8-oxodGuo has been established as a crucial biomarker for indicating cancer risk and other oxidative stress-related diseases (Kasai, 1997; Wu et al., 2004; Valavanidis et al., 2009).

Living organisms have developed a number of repairing pathways to maintain the integrity of genome. The main strategy for correcting DNA base lesions is base excision repair (BER), which is accurately controlled by certain glycosylases (Sancar, 1996; Lindahl et al., 1997; Krokan et al., 1997). For example, formamidopyrimidine-DNA glycosylase (FPG) operates the excision of 8-oxodGuo in bacterial species (Perlow-Poehnelt et al., 2004; Hamm et al., 2007). The enzyme combines the function of a glycosylase and an endonuclease, thus converting its substrates into single-strand breaks (Tchou et al., 1991; Boiteux et al., 1992). It is well documented that aberrant expression of glycosylases has profound implication in many human diseases (Wilson Iii and Bohr, 2007). Thus, glycosylases have become important biomarkers and potential therapeutic targets for related diseases (Helleday et al., 2008).

Some metal ions have been shown to be carcinogenic to humans and/or experimental animals, but the mechanisms involved in tumor formation have been not resolved yet (Crespo-López et al., 2009; Hartwig, 2013). One frequently discussed mechanism

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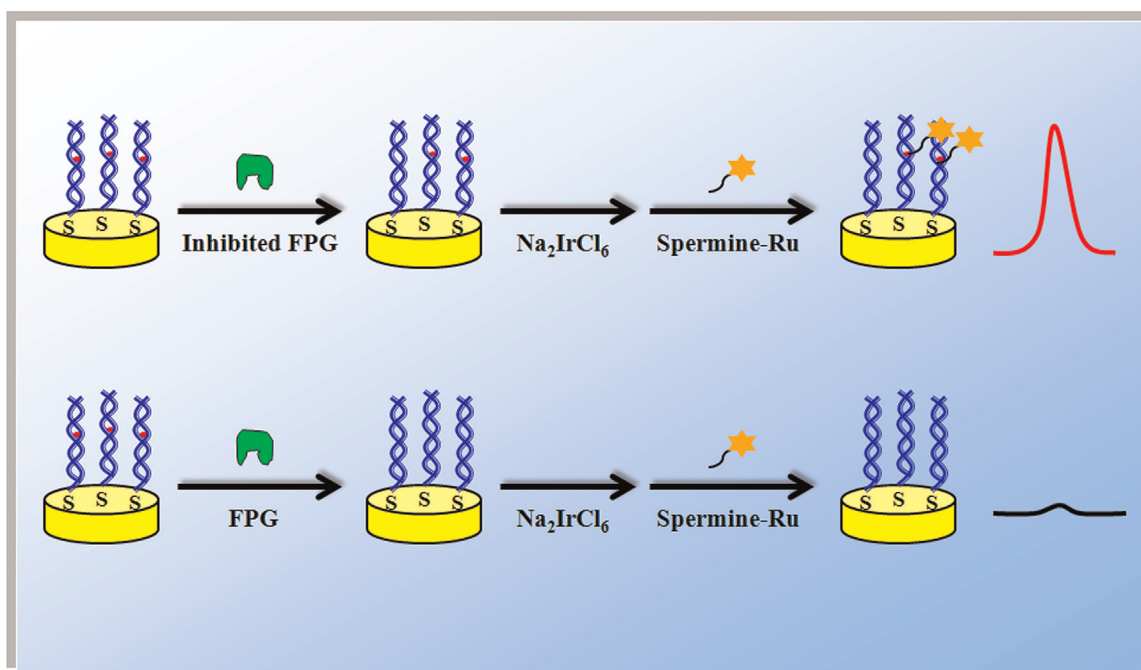

Scheme 1. Schematic diagram of the electrochemiluminescence biosensor for DNA repair enzyme activity analysis.

in metal induced geno-toxicity is the formation of oxidatively damaged DNA products such as 8-oxodGuo and DNA strand breaks (Lloyd and Phillips, 1999). Another mechanism, which has attracted more attention recently, is the inactivation of DNA repair system by the metal ions (Hartwig, 1998; Hartwig et al., 2002). An investigation on human individuals found a correlation between heavy metal exposure and DNA single strand break induction (Hengstler et al., 2003). Inhibition of some isolated DNA repair proteins on their base excision activity by a number of heavy metals were observed (Asmuss et al., 2000a,b; Wang et al., 2006), which was attributed to metal binding with the proteins.

As such, selective determination of 8-oxodGuo, assessment of DNA repair protein activity and evaluation of exogenous chemicals on protein activity are all import issues in molecular carcinogenesis. Over the past decade, a number of analytical methods have been developed for selective quantification of 8-oxodGuo, such as HPLC-MS, HPLC-EC and enzyme-linked immunosorbent assay (ELISA) (Rebelo et al., 2004; Harri et al., 2007; Cooke et al., 2009; Nakagawa et al., 2007). The chromatography-based methods require tedious DNA fragmentation and expensive instrumentations, whereas ELISA suffers from low specificity. Electrochemical sensors offer some distinctive advantages in terms of simplicity, low cost, fast response and miniaturization. Electrochemical detection of several DNA damage products has been studied extensively (Paleček and Fojta, 2001; Rusling et al., 2007; Paleček and Bartošík, 2012; Wu and Guo, 2014). In particular, voltammetric and electrochemiluminescence (ECL) sensors for the detection of 8-oxodGuo were developed by relying on the electrocatalytic reaction between $Os(bpy)_2Cl^+$-polyvinylpyridine and 8-oxodGuo (Dennany et al., 2004; Mugweru et al., 2004). Compared to DNA damage detection, the number of reports on the electrochemical detection of DNA repair is very limited. Pyrimidine dimmers and apurinic sites in DNA were converted to DNA single strand breaks by T4 endonuclease V and exonuclease III respectively, which were then detected electrochemically (Lao et al., 2005; Havran et al., 2008). In principle, this approach can be used for DNA repair detection. In our previous work, the photoelectrochemical sensor was used briefly for the detection of 8-oxodGuo excision by FPG (Zhang et al., 2012). More recently, real-time electrochemical monitoring of 8-oxodGuo and uracil repair by DNA glycosylases were achieved (McWilliams et al., 2014).

With these in mind, in the present work we developed a DNA biosensor for the detection of 8-oxodGuo lesion repair by FPG enzyme. The sensor was fabricated using a surface-immobilized, 8-oxodGuo containing oligonucleotide monolayer and a bifunctional chemical label for both 8-oxodGuo recognition and electrochemiluminescence signal generation. The developed sensor was then employed in the assessment of metal compounds for their inhibition effect on FPG repair activity so as to explore possible mechanisms of their geno-toxicity (Scheme 1).

2. Experimental section

2.1. Chemicals and materials

Escherichia coli formamidopyrimidine-DNA glycosylase (FPG) and 1 × NEBuffer 1 (10 mM Bis-Tris–Propane–HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0) were purchased from New England Biolabs (Ipswich, MA, USA). 6-Mercapto-1-hexanol (MCH), hexaammine ruthenium (III) chloride ($Ru(NH_3)_6Cl_3$), tripropylamine (TPA), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 4-(4′-methyl-[2,2′]bipyridinyl-4-yl)-butyric acid and spermine were from Sigma-Aldrich (St. Louis, MO, USA). Cis-bis (2,2′-bipyridine) dichlororuthenium ($Ru(bpy)_2Cl_2$) was purchased from Alfa Aesar (Ward Hill, MA, USA). Oligonucleotides with 25 bases containing one 8-oxodGuo were purchased from Takara Bio Inc. (Otsu, Japan). The sequence was 5′-GGA CTG /8-oxodGuo/GA GGA GAT GGG GGA GGA G-3′ (target DNA). The control and complementary oligonucleotides were purchased from Sangon Inc. (Shanghai, China). The sequences were 5′-GGA CTG GGA GGA GAT GGG GGA GGA G-3' (control DNA, no lesion) and 5′-SH-C6-CTC CTC CCC CAT CTC CTC CCA GTC C-3′ (complementary DNA thiolated at 5' terminus with a C6 spacer). All solutions were prepared with high-purity water from a Millipore Milli-Q (Biocel, Merck, U.S.A.). Buffers used in this work were as follow: DNA hybridization solution (2 × SSC buffer), 0.3 M sodium chloride, 30 mM sodium citrate, pH 7.0; DNA immobilization buffer (I-buffer), 10 mM Tris–HCl, 1 mM EDTA, 1.0 M

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