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Synergie between molecular imprinted polymer based on solid-phase extraction and quartz crystal microbalance technique for 8-OHdG sensing

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ABSTRACT

Recently, the 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been used as a marker to determine the oxidative stress. There is no any cheap and easy determination method based on chips and sensor systems for the determination of 8-OHdG. In this study, we have proposed imprinting methods for 8-OHdG recognition and determination using methacryloylamidohistidine-platinum(II) [MAH-Pt(II)] as a new metal-chelating monomer. The study includes the solid-phase extraction (SPE) of blood sample by a new 8-OHdG imprinted sorbent and the measurement of binding interaction of 8-OHdG imprinted quartz crystal microbalance (QCM) sensor via ligand interaction. 8-OHdG imprinted sorbent has prepared by bulk polymerization of MAH-Pt(II) and *N*-*N*′-methylenebisacrylamide. 8-OHdG imprinted sensor has prepared on a QCM chip coating the thiol pretreated Au electrode. At the end of these steps, a thin molecular imprinted polymer (MIP) film for the detection of 8-OHdG has developed and analytical performance of QCM sensor which has prepared using MIP was investigated. The affinity constant (K_a) for 8-OHdG using MAH-Pt-based thin film has determined by using the Scatchard method. The average percentage recovery of 8-OHdG from plasma samples was found as 80% by using of 8-OHdG imprinted SPE material. At the last step, 8-OHdG level in several blood plasma has been determined by this improved QCM sensor. The obtained results confirmed that the 8-OHdG level in cancer patient's blood was significantly higher than in general subjects.

1. Introduction

Oxidative damage to DNA in cells by reactive oxygen species (ROS) is a well-documented process (Dizdaroglu, 1992). Living organisms are continuously exposed to ROS, generated as a result of normal biochemical reactions and from various external factors relating to lifestyle and the environment such as tobacco smoking (Loft et al., 1992) and air pollution (Loft et al., 1999). Oxidative damage to DNA has also been implicated in the pathophysiology of a wide variety of human diseases including cancer, atherosclerosis, neurodegenerative disorders, and the aging process (Halliwell and Gutteridge, 1999).

Because the reactive oxidants are not suitable for analysis, oxidized bases like 8-hydroxy-2′-deoxyguanosine (8-OHdG) are used as biomarkers for DNA oxidative damage (Halliwell, 1999; Loft et al., 1993). In 1991, Szatrowski and Nathan (Szatrowski and Nathan, 1991) first reported that some human cancer cell lines can produce large amounts of H_2O_2. Antioxidant enzymes such as superoxide dismutase and catalase also appear to be downregulated in cancer cells (Sun, 1990; Sato et al., 1992; Jaruga et al., 1994). Other studies have concluded that several kinds of human cancer tissues, such as lung carcinomas and renal cell carcinomas, show higher levels of DNA oxidation compared with corresponding normal tissue controls, as determined by measurements of 8-OHdG (Kondo et al., 1999). The amount of 8-OHdG has been also found to increase progressively with age in both nuclear and mitochondrial DNA although the rate of increase with age is much greater in mitochondrial DNA. Nevertheless, oxidized levels of nuclear DNA are even greater in Alzheimer's disease (AD) than in age matched controls (Gabbita et al., 1998). Various analytical methods have been employed for the determination of 8-OHdG. Most of these methods are based on capillary electrophoresis (Arnett et al., 2005), HPLC (Samcová et al., 2004) and immunoaffinity chromatography–monoclonal antibody-based ELISA (Yin et al., 1995). These methods are expensive, and require pretreatment.

The research for highly selective, low cost, stable, sensitive, and foolproof chemical sensors is an attractive field. Quartz crystal microbalance (QCM) is well applicable to sensitive and selective

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detection, and portable in situ measurement. In recent decades, QCM-based piezoelectric immunosensors have found widespread applications in the analysis of clinical targets (Plomer et al., 1992; Prusak-Sochazewski et al., 1990), the monitoring of environmental contaminants, such as pathogen and bacteria (Harteveld et al., 1997; Fung and Wong, 2001) and the detection of biomolecular interaction (Jansoff et al., 2000; Marx, 2003) due to its attractive performance, such as high specificity, low cost, ease of use and rapidness of detection.

Molecular imprinted polymer (MIP) was employed for QCM and solid-phase extraction (SPE) as a selective polymer layer and sorbent material. Molecular imprinting is a method for making selective binding sites in synthetic polymers using molecular template. Target molecules can be used as templates for imprinting crosslinked polymers. After the removal of template, the remaining polymer is more selective. The selectivity of the polymer depends on various factors like the size and shape of the cavity and rebinding interactions. Covalent interactions (Wulff et al., 1977), non-covalent interactions (Arshady and Mosbach, 1981) and metal ion coordination (Kuchen and Shramm, 1988) can be exploited to organize the functional monomers around the template. In terms of strength, specificity and directionality, the metal coordination interaction is more like a covalent interaction than hydrogen bonding or electrostatic interactions in water (Dhal and Arnhold, 1992). These features make metal coordination a promising binding for the preparation of highly specific templated polymers for the recognition of proteins, via the arrangements of metal coordinating ligands on their surface (Özcan et al., 2006).

Recently, the combination of QCM and MIPs have been applied in selective sensing detection. The most application of MIPs in QCM sensor based on biomolecules recognition has been reported (Tai et al., 2005; Stanley et al., 2003; Wu and Syu, 2006) but there is not any MIP–QCM study for 8-OHdG sensing. Also, as reported in the literature (Beltran et al., 2007; Xu et al., 2004; Baggiani et al., 2007), coupling MIPs and SPE is possible to combine the advantages of traditional separation methods. Molecular imprinted solid-phase extraction (MISPE) presents the high specificity, selectivity and sensitivity of the molecular recognition mechanism and the high-resolving power of separation methods.

In this work, 8-OHdG selective memories were formed on QCM electrode surface by using a new metal–chelate interaction based pre-organized monomer and 8-OHdG recognition activity of these molecular memories via molecular imprinting process was investigated. This prepared MIP has the ability of 8-OHdG to chelate Pt(II) ion of methacryloyl histidine monomer to create ligand exchange (LE) assembled monolayer for 8-OHdG recognition, because the Pt(II) primarily interacts with the guanine base of DNA (Macguet and Theophanides, 1976). 8-OHdG level in blood plasma which was extracted with MIP-based sorbent was determined by the prepared QCM sensor.

2. Experimental

2.1. Materials

8-OHdG, *N-N′*-methylenebisacrylamide (*N-N′* MBAA), sodium metabisulfite ($Na_2S_2O_5$), potassium peroxydisulfate ($K_2S_2O_8$) and glycine were obtained from Aldrich (Milwaukee, WI, USA). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed-bed system.

2.2. Apparatus

Microgravimetric measurements were performed by using 9 MHz, AT-cut quartz crystals in a teflon holder and a quartz crystal analyzer, Model QC 922 (Seiko EG&G). The following equation (Sauerbrey's equation) has been established for an AT-cut shear mode QCM:

$$\Delta F = -2F_0^2(\rho_q\mu_q)^{-1/2}\frac{\Delta m}{A} \quad (1)$$

where ΔF is the measured frequency shift due to the added mass in hertz, F_0 is the fundamental oscillation frequency of the dry crystal, Δm is the surface mass loading in grams, ρ_q is the density of quartz ($2.65\,g\,cm^{-3}$), μ_q is the shear modulus ($2.95\times10^{11}\,dyn\,cm^{-2}$), and A is the electrode area ($0.19\pm0.01\,cm^2$). For the 9 MHz quartz crystals used in this work, Eq. (1) predicted that a frequency change of 1 Hz corresponds to a mass increase of $1.03\,ng\,cm^{-2}$ on the electrode (Liu et al., 2007).

Capillary electrophoresis measurements were realized with Prince CEC 760 model capillary electrochromatography system. AFM images were obtained using Q-Scope 250, Quesant Instruments, CA, USA.

2.3. Preparation of metal–chelate monomers and 8-OHdG having pre-organized complexes

Histidine-functional monomer, methacryloylamidohistidine (MAH), was synthesized according to the previously published procedure (Say et al., 2002). Metal–chelate monomer, methacryloylamidohistidine-platinum(II) [MAH-Pt(II)], was synthesized according to the following procedure: 1 mmol of MAH and 1 mmol of Pt(II) chloride in 10 mL of ethanol were mixed for 2 h. Then, the product, brown metal–chelate monomer, was crystallized and purified with ethanol/ethylacetate mixture.

Metal–chelate monomer was pre-organized with 8-OHdG template. For this reason, MAH-Pt(II) (0.25 mmol) and 8-OHdG (0.25 mmol) were dissolved in a vial containing 3.0 mL of ethanol and the solution was stirred for 20 min until precipitation begins. Obtained pre-organized complex was filtered and then dried at room temperature.

2.4. Preparation of MIP-based solid-phase extraction material

MAH-Pt–8-OHdG pre-organized monomer (1.0 mmol) was polymerized by bulk polymerization using $Na_2S_2O_5/K_2S_2O_8$ solution (0.5 mmol) as an initiator and *N-N′* methylenebisacrylamide (0.75 mmol) as a crosslinker. The polymerization mixture was poured in a glass tube and sealed after purging with nitrogen for 2 min. The tube was placed under the UV light and the bulk polymerization was carried out for 24 h. 8-OHdG imprinted (MIP) bulk polymer was ground in a mile after drying and washed several times with ethanol and water to remove any unreacted components completely. For the template removal, the obtained polymer was washed with 50 mL of 0.1 M glycine–HCl (pH 2.2) for 48 h at 60 °C. The non-imprinted polymer (NIP) was also prepared in the same way just using MAH-Pt (II), $Na_2S_2O_5/K_2S_2O_8$ and *N-N′* MBAA.

2.5. Selectivity of MIP-based solid-phase extraction material

The selectivity of MIP particles for 8-OHdG was estimated by using guanine and guanosine which has chemical structure similar to 8-OHdG. Concentration of competitive molecules was $25\,mg\,L^{-1}$ in water. The MIP beads were treated with these competitive molecules. After adsorption equilibrium, the concentration of guanine and guanosine in the remaining solution