Electrochemical Simulation of Oxidation Processes Involving Nucleic Acids Online Monitored with Electrospray Ionization-Mass Spectrometry

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Summary

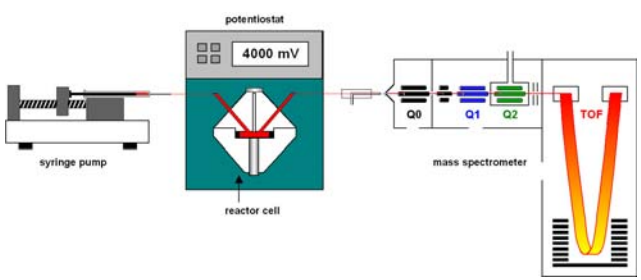
EC/ESI-MS is introduced as efficient and fast method to mimic oxidative modification of nucleic acids (nucleosides, nucleotides and small oligonucleotides) occurring in biological systems.
EC/ESI-MS represents a valuable tool for studying the fundamental principles of nucleobase oxidation and to find new biomarkers of oxidative stress.
EC/ESI-MS can be used to specifically initiate and monitor adduct formation reactions between nucleic acids and hazardous chemicals. Identified nucleotide alterations can represent putative biomarkers for studying the impact of chemicals in in vivo assays as well as in epidemiological studies.

Background

Nucleic acids present within living systems are continuously exposed to reactive chemicals. Reactive oxygen species represent one class of reactive chemicals that give rise to nucleic acids modification. The formation of covalent adducts between nucleic acids and small molecules represents another mechanism involved in nucleic acids alteration. Modified purine and pyrimidine bases are potential substrates for repair enzymes or polymerases, or they can block these activities, triggering biological responses including mutation, cell death, malignancy, and aging.

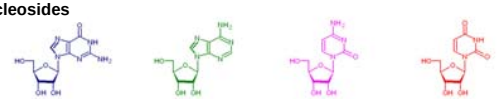
Experimental Setup

We have applied online EC/ESI-MS to study oxidative processes involving nucleic acids. Oxidation reactions were performed in an electrochemical thin-layer cell (ReactorCell, Antec Leyden). A conductive diamond electrode (Magic Diamond, Antec Leyden) was used as working electrode allowing voltages up to 4.0 V. Potentials (0.0 - 4.0 V) were applied using a dedicated potentiostat (ROXY Potentiostat, Antec Leyden). The cell was continuously flushed with sample solutions at a flow rate of 3.0 µl/min. The reaction mixture was online monitored with ESI-MS. Tandem mass spectrometric experiments (in-source fragmentation, MS/MS, and combinations thereof) were used to elucidate the structures of the oxidation products. All mass spectrometric experiments were performed on a QqTOF instrument (Qstar XL, Applied Biosystems, Foster City, CA, USA) offering mass accuracy in the low ppm range.

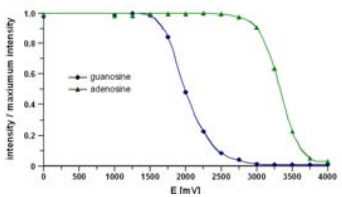


Extracted Ion Voltammograms of Nucleosides

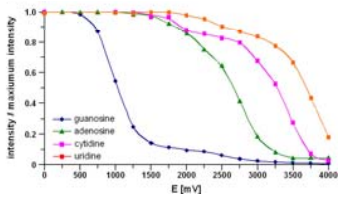
(a) nucleosides



(b) positive ion mode

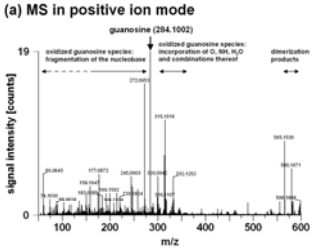


(c) negative ion mode

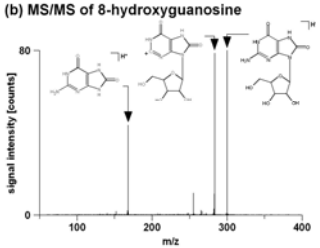


EC/ESI-MS of Guanosine

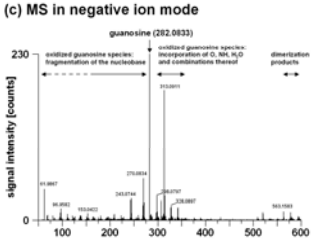
(a) MS in positive ion mode



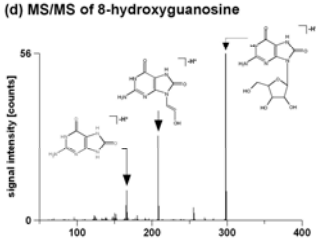
(b) MS/MS of 8-hydroxyguanosine



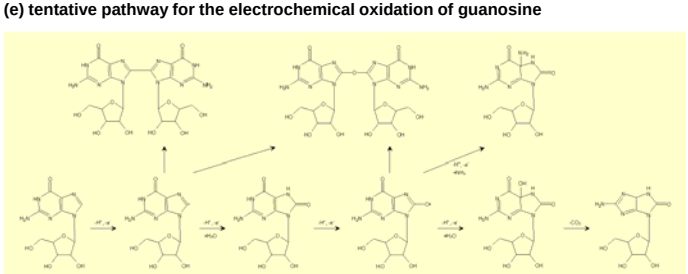
(c) MS in negative ion mode



(d) MS/MS of 8-hydroxyguanosine

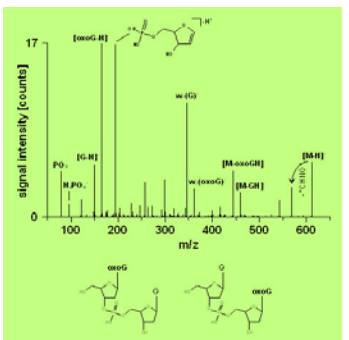


(e) tentative pathway for the electrochemical oxidation of guanosine

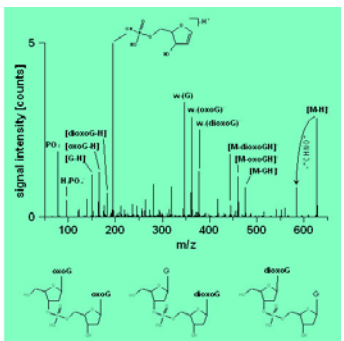


Oxidation of dGpdG

(a) singly oxidized form



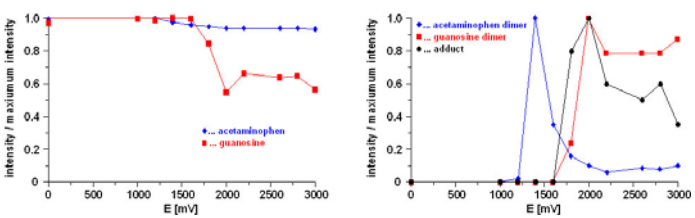
(b) doubly oxidized form



Formation of Drug-DNA Adducts

Acetaminophen (paracetamol, N-acetyl-p-aminophenol, CAS No. 103-90-2) is a widely used over-the-counter analgesic and antipyretic, and is therefore a major ingredient in numerous cold and flu remedies. Several studies on cell cultures and rodents have demonstrated that acetaminophen can covalently bind to nucleic acids after metabolic activation.

(a) extracted ion voltammograms



(b) observed oxidation reactions