Insights into Nucleic Acids Oxidation by Electrochemistry/Liquid Chromatography/Mass Spectrometry

Robert Erb[1], Sabine Plattner[1], Martin Eysberg[2], Jean-Pierre Chervet[2], Herbert Oberacher[1]

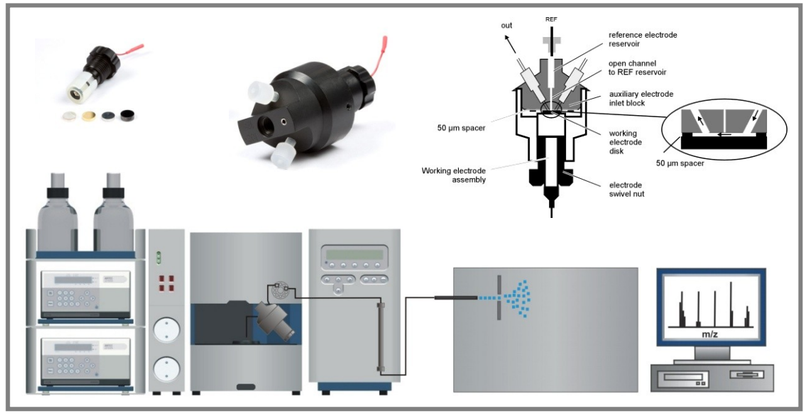
[1]Institute of Legal Medicine, Innsbruck Medical University, Muellerstrasse 44, 6020 Innsbruck, Austria; [2]Antec, Industrieweg 12, 2382 NV Zoeterwoude, The Netherlands.

Background

DNA damage has emerged as a major culprit in cancer and many diseases related to aging. Particularly, oxidative stress can disrupt the integrity of genetic material. Accordingly, major efforts have been put into the elucidation of mechanisms involved.
Electrochemistry (EC) hyphenated to liquid chromatography/mass spectrometry (LC/MS) represents a very powerful tool for studying mechanisms of biomolecule oxidation. Electrochemistry is used to mimic oxidation reaction occurring in biological systems. LC/MS allows separation and characterization of the lesions produced. Established fields of application of this technique include drug metabolism, environmental degradation of pollutants and proteomics. Herein, the applicability of the assay for studying nucleic acids oxidation is presented.

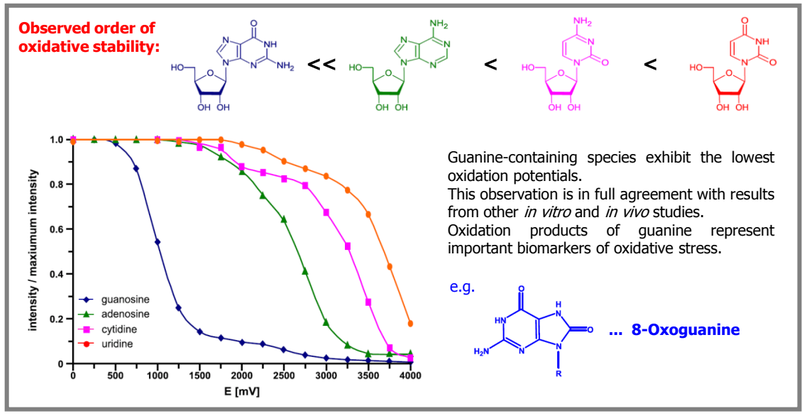
Experimental setup

For studying nucleic acids oxidation commercially available instrumentation was used (ROXY, Antec, Zoeterwoude, The Netherlands). EC was performed in an electrochemical thin-layer cell. A conductive diamond electrode was used as working electrode material. LC was performed on a column (200×0.2 mm) packed with Eurospher 100-5 C18 particles (Knauer, Berlin, Germany). Chromatographic separations were accomplished using gradients of acetonitrile in 10 mM ammonium formate (pH 7.3). The flow rate was set to 3.0 µl/min. The column outlet was directly coupled to the mass spectrometer (Qstar XL, AB Sciex, Foster City, CA, USA). MS and MS/MS experiments were used to detect and to elucidate the structures of the formed reaction products.



Mechanistic details of nucleic acids oxidation

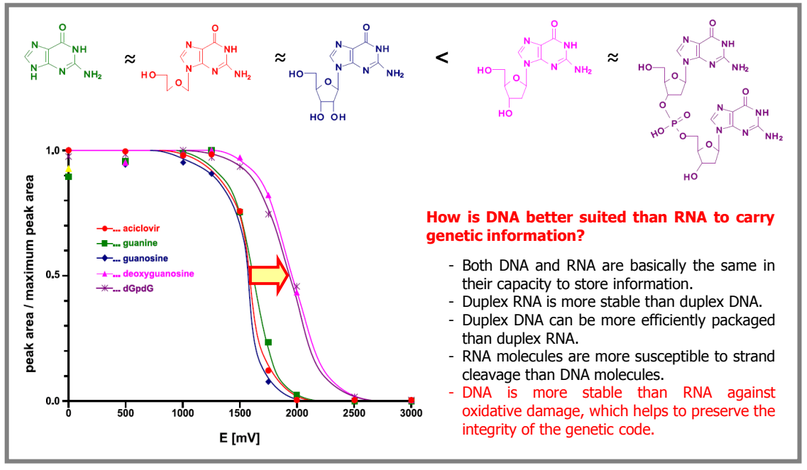
(1) Impact of nucleobase on electrochemical stability



Guanine-containing species exhibit the lowest oxidation potentials.
This observation is in full agreement with results from other *in vitro* and *in vivo* studies.
Oxidation products of guanine represent important biomarkers of oxidative stress.

e.g. ... 8-Oxoguanine

(2) Impact of "backbone" on electrochemical stability

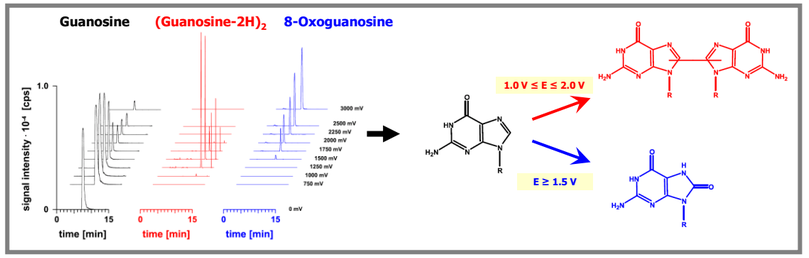


How is DNA better suited than RNA to carry genetic information?

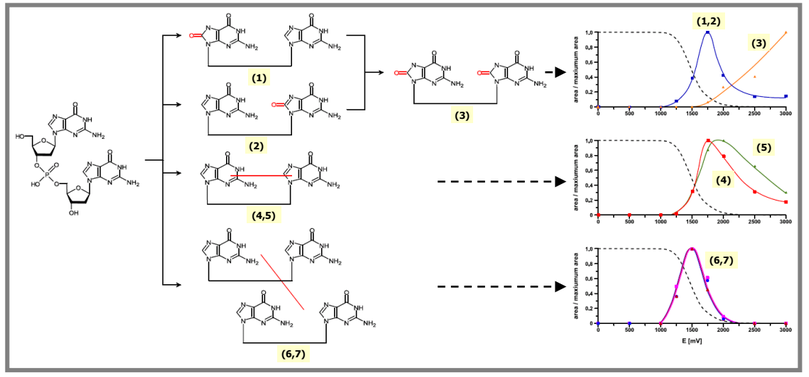
- Both DNA and RNA are basically the same in their capacity to store information.
- Duplex RNA is more stable than duplex DNA.
- Duplex DNA can be more efficiently packaged than duplex RNA.
- RNA molecules are more susceptible to strand cleavage than DNA molecules.
- DNA is more stable than RNA against oxidative damage, which helps to preserve the integrity of the genetic code.

Oxidation of guanine-containing species

(1) Primary products of guanosine oxidation



(2) Primary products of dGpdG oxidation



Conclusions

EC/LC/MS represents a convenient in vitro method to study oxidation reactions involving nucleic acids.
Insights into mechanisms leading to DNA damage can be retrieved.

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