ELECTROCHEMICAL DETECTION OF *IN SITU* OXIDATIVE DAMAGE TO DNA

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In a health preventing perspective, detection of *in vivo* oxidative damage to DNA can be very useful for screening and evaluating the effect caused to DNA by health hazardous compounds and oxidising substances in general, for which voltammetric methods are an inexpensive and faster detection procedure.

Electrochemical voltammetric *in situ* detection of DNA oxidative damage caused by reduced adriamycin, an antibiotic of the family of anthracyclines, intercalated into DNA was possible using a DNA-electrochemical biosensor. The results indicate that adriamycin intercalated in double helix DNA can undergo oxidation or reduction and cause oxidative damage to DNA by reacting specifically with the guanine moiety in guanine-rich regions at CpG homologous sequences, and thence to the formation of mutagenic 8-oxoG residues, the main product of guanine oxidation.

Adriamycin reduction occurs inside the dsDNA layer and clearly conditions the adriamycin-dsDNA interaction as the radical formed can interact with DNA *in situ* and the products of DNA damage are retained in the DNA layer and detected by electrochemistry. Since the experiments were carried out in buffer, the peaks recorded for the reduction or oxidation of adriamycin can only be attributed to the reaction of adriamycin molecules that are inside the film of dsDNA on the modified glassy carbon electrode.

The *in situ* generation of the adriamycin semiquinone radical at -0.60 V permits a detailed study of this interaction mechanism. A mechanism is proposed for the generation of 8-oxoG when a negative conditioning potential is applied, via intercalation of adriamycin into the double helix DNA. In the mechanism both adriamycin electroactive functional groups (the oxidisable hydroquinone group and the reducible quinone function) are intercalated between the base pairs in the DNA-adriamycin complex in close interaction with the GC base pair. The reducible quinone group protrudes slightly into the major groove, and this enables *in situ* (*in helix*) generation of an adriamycin radical within the double helix and involves the simultaneous oxidation of one neighbouring guanine residue.

In this way electron transfer from the guanine moiety to the quinone without hydrogen abstraction is likely to be the predominant reaction leading to the formation of the guanine radical cation. Due to the fast hydrolysis of the radical cation, the semiquinone undergoes further reduction to the fully reduced adriamycin and the formation of 8-oxoG occurs. So the hydrolysis step is followed by a second electron transfer and leads to the ultimate formation of 8-oxoG and reduced adriamycin. Therefore, a redox reaction between adriamycin and guanine residues inside the double helix of DNA can be considered, in order to explain the experimental data.

Contrary to the oxidation of free guanine in solution, in the biopolymer deprotonation of the guanine radical cation is partially prevented by base-stacking interactions with cytosine, and the hydrolysis reaction is most favourable to occur in double helix DNA. The transitory generation of the guanine radical cation close to the adriamycin semiquinone radical may result in an adriamycin-base adduct formation that competes with the hydrolysis step. Intercalation may cause sufficient distortion of the dsDNA to expose the radical cation to hydrolysis. The π-stacked base pairs in double helix DNA might serve as a pathway for charge transport mediating the redox reaction between adriamycin radicals, generated during reduction, and guanine residues in the double helix. The trapping by water of the generated radical cation leads to *in situ* formation of the oxidative DNA damage product 8-oxoG, and further oxidation of 8-oxoG could lead to hydrolysis of the glycosidic bond generating an abasic site. This could disrupt the double helix causing strand break, exposing 8-oxoG residues and other purines to the electrode surface that can then be oxidised.

This model for the electrochemically observed *in situ* oxidative damage to DNA may be used to explain the levels of 8-oxoG found when cells are treated with adriamycin as well as its well know free radical activity. This is very relevant because the mechanism of interaction of DNA-adriamycin at charged interfaces mimics better the *in vivo* DNA-adriamycin complex situation, where it is expected that DNA is in close contact with charged phospholipid membranes and proteins, rather than when the interaction is in solution.

The potential use of the DNA film-modified GCE for the understanding of DNA interactions with molecules or ions explores, in a promising way, the use of voltammetric techniques for *in situ* generation of reactive intermediates and is a complementary tool for the study of biomolecular interaction mechanisms.