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Recent progress in electrochemical sensors and assays for DNA damage and repair

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ABSTRACT

DNA in cells is permanently exposed to endogenous or exogenous chemical or physical agents which may cause chemical changes in the DNA structure collectively called "DNA damage", which may have severe impacts on human health. Therefore, efficient techniques to detect DNA damage are sought, among which electroanalytical methods hold an important position. This review is focused on recent progress in the field of electrochemical sensors and assays for DNA damage, particularly on typical applications of advanced electrochemical techniques in detecting oxidative DNA damage, abasic lesions, base mismatches, non-covalent interactions with drugs and other substances. Special attention is paid to combinations of electrochemical detection with biochemical tools such as enzymatic systems mimicking metabolic activation of xenobiotics, or DNA repair enzymes converting specific nucleobase lesions to species offering more facile electrochemical detection. These applications create conditions for application of electroanalytical approaches in biological research, including studies of DNA repair processes.

Contents

1. Introduction

1.1. DNA damage and repair

Preservation of genetic information, its transfer to progeny and precisely controlled expression are the key functions of DNA featuring

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the genetic material of most of the living organisms. Albeit the DNA is a chemically relatively stable substance, it is permanently challenged by a number of chemical or physical agents, occurring in the environment or being generated as (by)products of cellular metabolism, that may cause chemical changes in the DNA molecules [1]. These changes may involve interruption of chemical bonds in the DNA sugar phosphate backbone, resulting in formation of single- or double-strand breaks (SSB or DSB, respectively) or of N-glycosidic bonds linking nucleobases to the sugar (giving rise to abasic sites), as well as chemical modification of nucleobase residues (e.g., their oxidation, alkylation at nitrogen atoms, photochemical reactions resulting in dimerization of pyrimidines, formation of adducts with metal complexes etc.; see Fig. 1). These entities can collectively be named "DNA damage", meaning any irreversible covalent modification of the DNA molecule. In a more general sense, the term "DNA damage" refers also to changes in the DNA structure caused by interactions with substances that bind to the DNA non-covalently (e.g., via intercalation of planar aromatic molecules between base pairs in the duplex DNA, or via binding into its minor or major grooves).

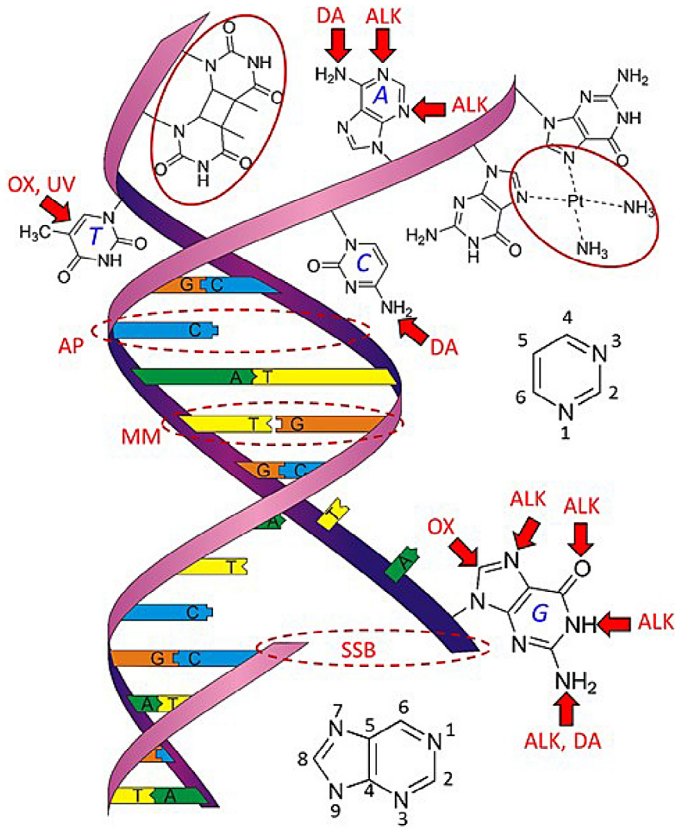

Fig. 1. Scheme of typical products of DNA damage and/or related structural perturbations in DNA. An interruption of the DNA sugar-phosphate backbone represents a single strand break (SSB), release of a base by breakage of N-glycosidic bond gives rise to an abasic (apurinic or apyridiminic, AP) site. Base mismatches (MM) may arise from DNA damage events and/or from imprecise DNA replication. Red arrows indicate sites in nucleobases susceptible to chemical modifications those induced by UV light (UV – namely 5,6 C = C double bond in pyrimidines), oxidation agents (OX – the same site as above and C8 of purines) and alkylation agents (ALK – endocyclic nitrogen atoms with N7 of guanine representing the most reactive site, exocyclic substituents of guanine), as well as amino groups prone to deamination (DA). Particularly alkylation at N1 of purines and N3 of pyrimidines, O-alkylation of guanine and deamination of cytosine in position 4 or of adenine in position 6 (for numbering see pyrimidine and purine rings inset in the figure) can have severe biological impacts because these modifications affect directly the Watson-Crick hydrogen bonding system. As examples of bulky adducts strongly affecting DNA structure, pyrimidine cyclobutane dimers (products of DNA damage by UV light), and an intrastrand crosslink of two neighboring guanines by cancer therapeutics cisplatin, are shown.

Selective non-covalent binding of a substance to DNA may also precede a covalent change and facilitate it (which is often utilized when a covalent attack to the DNA is desirable, such as in the case of chemical nucleases [2] or DNA-targeted therapeutics [3]).

Both covalent and non-covalent modifications of the DNA structure can affect crucial processes, such as DNA replication or transcription, and give rise to hereditable, permanent changes in the genetic information – mutations. In this context, it should be stressed that the term "DNA damage" should not be confused with the term "mutation". The former refers to a chemical and/or structural alteration(s) in the DNA molecule (these entities are commonly called "lesions" and often involve nucleobase mispairing, "base mismatches"), which are detectable and repairable by one of DNA repair mechanisms, that normally counteract nearly all DNA damage events in living cells [1]. On the other hand, mutations commonly arise from damaged DNA replication over lesions, which have escaped the repair machinery, and comprise exchange, insertion or deletion of a complete base pair (or more base pairs). Thus mutated nucleotide sequences do not contain base mismatches or other lesions and look like "normal" DNA from the points of view of both the DNA repair mechanism and analytical techniques developed for detection of the DNA lesions.

Accidental or systematic (e.g., in chemical industry, uranium mining, radiomedicine etc.) exposure of healthy humans to various genotoxic agents may cause undesired damage to their genomes, accumulation of mutations and development of severe diseases (such as cancer). On the other hand, in specific cases, such as radio- or chemotherapy in oncology, accumulation of specific DNA lesions (DSB, nucleobase adducts) is desirable to induce lethal effects on the cancerous cell populations. Both these aspects create a need for efficient analytical tools suitable for the detection of DNA lesions, changes in DNA structure induced by covalent DNA damage and/or the non-covalent interactions, identification of various DNA adducts, intermediates of DNA repair, as well as detection of the genotoxic agents themselves (whilst utilizing their specific effects on DNA as the natural basis of selectivity). A variety of analytical approaches has been applied for this purpose [4]. Among them, electrochemical approaches and their applications in sensors or assays for DNA damage have attracted attention of (bio)analytical chemist all over the world due to convenient parameters of the electroanalytical techniques (such as selectivity, sensitivity, low cost etc.), and particularly their usefulness in nucleic acid research [5,6].

1.2. Electrochemical techniques applied to detect DNA damage

It has been well established that nucleic acids possess their intrinsic electrochemical activity [6]. Particularly, nucleobases adenine, cytosine and guanine can be reduced at mercury-based electrodes (such as the hanging mercury drop electrode (HMDE); or some types of silver solid amalgam electrodes (AgSAE)); using the same electrodes, it is also possible to measure analytically useful tensammetric signals originating from adsorption/desorption processes of DNA chains at the negatively charged electrode surface [5,6]. Notably, voltammetric DNA signals measured at the HMDE [7,8] or AgSAE [9] are strongly sensitive to changes in DNA structure, making it possible to detect sensitively some types of DNA lesions, including strand breaks and/or single-stranded DNA (ssDNA) regions in double-stranded DNA (dsDNA) molecules without DNA labeling or using additional reagents (Fig. 2). At carbon electrodes (such as pyrolytic graphite (PGE); glassy carbon (GCE); pencil graphite (PeGE); various carbon paste; screen-printed (SPE); and boron-doped diamond electrodes), all DNA bases can be electrochemically oxidized [10], and particularly signal related to oxidation of guanine (peak G^{ox}, Fig. 2B) has found broad analytical use. Compared to those observed at the mercury-based electrodes, DNA signals at the carbon electrodes are less sensitive to changes in DNA structure [5,6].