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Mini-review

Oxidatively generated complex DNA damage: Tandem and clustered lesions

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ABSTRACT

There is an increasing interest for oxidatively generated complex lesions that are potentially more detrimental than single oxidized nucleobases. In this survey, the recently available information on the formation and processing of several classes of complex DNA damage formed upon one radical hit including mostly hydroxyl radical and one-electron oxidants is critically reviewed. The modifications include tandem base lesions, DNA-protein cross-links and intrastrand (purine 5′,8-cyclonucleosides, adjacent base cross-links) and interstrand cross-links. Information is also provided on clustered lesions produced essentially by exposure of cells to ionizing radiation and high energetic heavy ions through the involvement of multiple radical events that induce several lesions DNA in a close spatial vicinity. These consist mainly of double strand breaks (DSBs) and non-DSB clustered lesions that are referred as to oxidatively generated clustered DNA lesions (OCDLs).

1. Introduction

Oxidative stress results from the imbalance between endogenous generation of reactive oxygen species (ROS) and anti-oxidant defence systems [1] that involve scavenging of low reactive ROS such as superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), the precursors of highly damaging hydroxyl radical ($^{\cdot}OH$) [2]. These oxygen species may be triggered by inflammation reactions [3,4] that also lead to the exalted production of nitric oxide [5,6], one of the main nitrogen reactive species (NOS). Oxidative stress have been shown to be associated with physical exercise [7], metal toxicities [8], aging [9,10] and several pathologies including mellitus diabetis [11], cardiovascular diseases [12,13], neurological disorders [14,15] and cancers [16–20]. Exacerbated generation of ROS and other oxidizing processes such as one-electron oxidation of biomolecules are also provided by ionizing radiation [21,22] and the UVA component of solar light [23,24], two well established physical carcinogens. DNA is the main critical cellular target to oxidatively generated damage that may participate in the initiation and/or propagation processes leading to carcinogenesis through mutagenesis [17–20,25]. An abundant literature is now available on the mechanisms of oxidative degradation of nucleobases [26–29] and 2-deoxyribose [30] in isolated DNA and model compounds that are mediated by $^{\cdot}OH$, singlet oxygen ($^1O_2$) and one-electron oxidants, the three main identified reactive oxidizing species and agents. The measurement of oxidized bases in cellular DNA has been hampered until the end of the 1990s by the use of inappropriate methods that has led to overestimation of the levels of oxidized bases up to three orders of magnitude [31]. Accurate data are now available on the formation of several oxidatively generated base lesions including ubiquitous 8-oxo-7,8-dihydroguanine (8-oxoGua) and thirteen single oxidized purine and pyrimidine bases in cellular DNA. This may be achieved for electrochemically active lesions including 8-oxoGua, 8-oxo-7,8-dihydroadenine (8-oxoAde) and 5-hydroxycytosine using high-performance liquid chromatography coupled to electrochemical detection (HPLC-ECD) as the analytical method. However, the method of choice appears to be HPLC associated with electrospray ionization tandem mass spectrometry (ESI-MS/MS) as a versatile and accurate method [31]. Information on the mutagenic features of several single base lesions has been gained from shuttle vector experiments [32,33] and polymerase-mediated incorporation into DNA of oxidized precursors present in the nucleotide pools [34]. Major efforts have been also devoted to the determination of substrate specificity and removal mechanisms of repair enzymes that mostly operate for single lesions through the base excision repair pathways [35,36]. The radiation-induced formation of locally multiply damaged sites, also referred as to oxidatively generated clustered lesions (ODCLs) [37,38] was suggested more than 25 years ago in addition to

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previously identified double strand breaks (DSBs) [39,40]. The formation of these complex lesions that may include DSBs, single strand breaks (SSBs), oxidized bases and abasic sites, is accounted for by the occurrence of at least two radical hits within one or two helix turns [41,42]. Following the pioneering contributions of Box and collaborators in the 1990s [43] it was found that tandem base modifications can be generated in DNA consecutively to the initial formation of peroxyl pyrimidine radicals that subsequently react with vicinal bases [44]. Other types of complex damage whose formation involves a single initial radical hit include DNA-protein cross-links, intra- and interstrand DNA cross-links [28]. In this short review article emphasis is placed on recent aspects of the formation of non-single oxidatively generated damage in cellular DNA that receive increasing attention due to their deleterious biological potential [45].

2. Hydroxyl radical and one-electron oxidants as inducers of complex DNA damage

As already briefly mentioned, the oxidation reactions indentified so far in DNA may be mostly explained in terms of initial involvement of ·OH, one-electron oxidant or $^1O_2$. It is now well documented that $^1O_2$ reacts specifically with guanine producing 8-oxoGua in cellular DNA at the exclusion of rearrangement products [23,27,46]. As a result, the lack of formation of the reactive quinonoid intermediate that is observed in free 2′-deoxyguanosine upon [4 + 2] cycloaddition of $^1O_2$ to the purine base [47] would prevent the formation of DNA-protein cross-links. So far only ·OH and one-electron oxidants have been shown to generate organic radicals that are able in a second step to react either as radical cations, carbon centred radical or peroxyl radical with other DNA constituents or proteins. Typically ·OH may be produced in cells as the result of Fenton reactions that involve the reduction of H_2O_2 by ferrous ions and with a lower efficiency and specificity by copper ions [48]. Radiolysis of water molecules through the indirect effects of ionizing radiation is another convenient source of ·OH [21]. However as a relatively minor process, direct interaction of ionizing radiation with either the 2-deoxyribose moieties or any of the four bases of DNA leads in cells to the generation of the corresponding radical cations [21]. A suitable way to produce purine and pyrimidine base radical cations in DNA consists in exposing cells to high intensity nanosecond 266 nm laser pulses [49]. Recently it was found that 6-thioguanine (TG), once metabolized and inserted into cellular DNA is able under absorption of UVA light to specifically oxidize proximal guanine bases by one-electron abstraction [50]. One may also mention that one-electron oxidation of guanine may occur in cells during inflammation processes that give rise to $O_2^{\cdot-}$ and ·NO [48]. Recombination of the two later species give rise to peroxynitrite anion ($ONOO^-$) that is converted upon reaction with CO_2 into nitrosoperoxycarbonate, the precursor of carbonate anion radical ($CO_3^{\cdot-}$), a strong one-electron oxidant [51,52].

3. One radical hit-mediated intra- and inter-strand DNA damage

3.1. Tandem base modifications

Two main types of tandem base modifications have been shown to be generated by one radical hit that may involve ·OH and/or one-electron oxidants. Subsequently, either pyrimidine centered radicals or related pyrimidine peroxyl radicals thus generated are able to react with the adjacent base. The efficiency of the intramolecular reaction is higher when the target base is located on the 5′-side with respect to the reactive pyrimidine radicals. This may be rationalized in terms of shorter distances between the pyrimidine radical and the vicinal purine base involved in the addition or oxidation reactions.

3.1.1. Addition of pyrimidine carbon centered radicals to adjacent guanine

Several model studies have shown that 5-(2-deoxyuridylyl) methyl and 6-hydroxy-5,6-dihydro-2′-deoxycytid-5-yl radicals are able to bind to the carbon 8 of the 5′-adjacent purine bases giving rise to tandem base lesions [53–56]. However a limiting factor to the intramolecular reaction is the presence of O_2 that efficiently reacts with carbon centered radicals. This explains why only the predominant G[8-5m]T and G[8-5]C lesions (Fig. 1) whose formation involves the generation of a covalent bond between either the methyl group of thymine or the C5 carbon of cytosine and 5′-guanine has been detected in cellular DNA upon exposure to H_2O_2, the likely precursor of highly reactive ·OH through Fenton type reactions [55,56]. The measurement of G[8-5m]T and G[8-5]C that are generated in very low yields, typically 0.050 and 0.037 lesions per 10^9 normal nucleosides in about 30–50 μg extracted DNA has required the use of an accurate and highly sensitive HPLC/MS^3 assay in order to prevent artefactual detection of erratic ions. Relevant information on the biochemical processing of both G[8-5m]T and G[8-5]C intrastrand cross-links [57] has been gained from *in vitro* replication studies [55,58–60] and shuttle vector experiments combined with HPLC-MS/MS analysis [56]. Both tandem lesions are able to block high-fidelity DNA polymerases [55,58–60] while replication of G[8-5]C in AB1157 *Escherichia coli* cells led to an elevated level of G → T and G → C mutations [56]. It was recently shown that in *E. coli* strains, polymerase IV (pol IV) and polymerase V (pol V) would allow bypassing G[8-5m]T cross-link that may be error prone. In that respect pol IV and pol V are implicated in the observed T deletions and most G to T transversions respectively [61]. It was also reported that yeast and human DNA η polymerases are able to bypass G[8-5]C lesion with however a lower efficiency and a reduced fidelity in nucleotide incorporation with respect to control [55,62,63]. Evidence has been provided for the implication of the nucleotide excision repair (NER) pathway in the removal G[8-5m]T and G[8-5]C at least in bacterial cells as shown by the recognition and incision of the tandem base lesions inserted into site-specific oligonucleotides by UvrABC nuclease [64,65].

3.1.2. Implication of pyrimidine peroxyl radicals in addition reactions with vicinal bases

Earlier observations have shown that X-irradiation of dinucleoside monophosphates and d(CpGpTpA) in aerated aqueous solutions gave rise to tandem base lesions consisting of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) and N-(2-deoxy-β-D-*erythro*-pentofuranosyl)formylamine (dF) as the result of one initial ·OH hit on either thymine or cytosine [66,67]. It may be remembered that dF is one of the main ·OH degradation products of thymidine [68] or 2′-deoxycytidine [29] in aerated aqueous solutions. As observed for the formation of pyrimidine-guanine intrastrand cross-links there is strong sequence dependence on the ·OH-mediated formation of the latter vicinal base lesions in isolated DNA since the yield of 8-oxodGuo/dF (Fig. 2) is about 20-fold higher than that of dF/8-oxodGuo [69]. The mechanism of formation of 8-oxodGuo/dF and dF/8-oxodGuo that was inferred from [^{18}O]-labeling experiments was rationalized in terms of addition of 5-(6)-hydroxy-6-(5)-peroxy-5,6-pyrimidyl radicals to C8 of guanine with subsequent cleavage of the peroxide bond thus generated and further rearrangement [44]. It was recently estimated that about 50% of 8-oxodGuo and 8-oxo-7,8-dihydro-2′-deoxyadenosine formed in aerated aqueous solutions of isolated DNA upon exposure to ·OH were part of tandem base lesions as the result of initial addition of peroxyl pyrimidine radicals at C8 of