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RADIOBIOLOGY SPECIAL FEATURE: REVIEW ARTICLE

Radiation-mediated formation of complex damage to DNA: a chemical aspect overview

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ABSTRACT

During the last three decades, a considerable amount of work has been undertaken to determine the nature, the mechanism of formation and the biological consequences of radiation-induced DNA lesions. Most of the information was obtained via the development of chemical approaches, including theoretical, analytical and organic synthesis methods. Since it is not possible to present all the results obtained in this review article, we will focus on recent data dealing with the formation of complex DNA lesions produced by a single oxidation event, as these lesions may play a significant role in cellular responses to ionizing radiation and also to other sources of oxidative stress. Through the description of specific results, the contribution of different chemical disciplines in the assessment of the structure, the identification of the mechanism of formation and the biological impacts in terms of repair and mutagenicity of these complex radiation-induced DNA lesions will be highlighted.

The term radiation chemistry¹ deals with the chemical effects induced by radiation. A considerable amount of work has been performed to study, at the molecular level, the effects of radiation on DNA. For simplification, initial studies were performed with isolated nucleosides, the monomeric units of DNA, to identify the undergoing decomposition reactions. Analytical methods involving mostly high-performance liquid chromatography (HPLC) separation were used to isolate the radiation-induced nucleosidic modifications, which were then identified and characterized thanks to a combination of physicochemical approaches, including, among others, nuclear magnetic resonance (NMR) and mass spectrometry (MS). To elucidate the mechanism of formation of the identified DNA lesions, works have been done to identify the reactive oxygen species involved, to determine the role of molecular oxygen and to use time-resolved spectroscopy to obtain information about the short-lived transient radical intermediates. In this short survey, all identified reactions involved in DNA decomposition will not be described, as detailed information can be obtained from recent review articles.^{2–5} It should be summarised that the main pathway of radiation-induced DNA decomposition involves the transient formation of hydroxyl radicals (HO[•]) produced by water radiolysis (the so-called

indirect effect of ionizing radiation). Reaction of HO[•] with the sugar or phosphate moiety of DNA gives rise to strand breaks through an H-abstraction reaction. Reactivity with DNA bases is mediated mostly through addition at the double bonds of both purine and pyrimidine bases or through the H-abstraction occurring mostly on the methyl group of thymine.⁶ The so-called direct effect of radiation, involving a one-electron oxidation reaction, produces mostly guanine modifications as a consequence of the lower ionization potential of the guanine base compared with other DNA constituents and an efficient electron transfer reaction in double-stranded DNA (dsDNA).^{7,8} Nowadays, an almost complete description of the radiation-induced decomposition pathways of the four DNA bases is proposed, thanks to the identification of about 70 major decomposition products.^{5,9} Recent results have, however, highlighted the fact that the decomposition of initially produced radicals in a dsDNA is partly different than that observed at the nucleoside level. This notably concerns, for example, complex DNA lesions that represent a considerable challenge to the DNA repair machinery.

Analytical chemistry is of major importance for the study of radiation-induced DNA lesions. The most popular

approach that has been developed for measuring 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) is HPLC coupled to electrochemical detection^{10,11} that has received numerous applications. Tandem MS (MS/MS) coupled through electrospray ionization to HPLC^{12,13} is nowadays more versatile and sensitive. However, the key steps for acquiring accurate results remain DNA extraction and digestion, which should be optimized to prevent spurious oxidation from occurring.¹⁴ This explains why large variations in background levels of oxidatively produced DNA lesions have been reported in the literature, mostly concerning 8-oxodGuo.^{15–18} The gas chromatography-MS (GC-MS) methodology perfectly illustrates the problem of the impact of sample preparation on the quantification of oxidized bases. This method, used for many years, was found to induce significant artefacts¹⁹ owing to oxidation reactions occurring during the work-up procedure. Optimized protocols are now available for DNA extraction,¹⁴ and using HPLC-MS/MS low levels of well-defined DNA lesions can be measured and accurately quantified by using isotopically labelled internal standards prepared by chemical synthesis.²⁰ As a general observation, we should highlight the fact that the measurement of radiation-induced DNA lesions is a challenging task for two major reasons. First, the yield of formation of lesions per dose unit (Gray, Gy) is very low, <1 modification per 100 million normal bases per Gray. In addition, the identified lesions produced by ionizing radiation are similar to those generated by endogenous oxidative stress. Thus, even in the absence of radiation, oxidative DNA lesions are detected in cells at levels around one modification per million DNA bases for 8-oxodGuo. This indicates that about 50 Gy is required to double the yield above the background level of these DNA lesions. However, in contrast to endogenous oxidative stress, a fraction of radiation-induced DNA lesions, particularly for high linear energy transfer (LET) particles, consists of several lesions generated in clusters, creating the so-called locally multiple damage sites (MDSs).²¹ Well-known examples are double strand breaks (DSBs), but the importance of non-DSB clustered lesions, also named oxidative clustered DNA lesions involving a combination of single strand breaks and oxidized base lesions,²² should not be neglected. The harmful effect of ionizing radiations could be attributed mostly to these MDSs.²³ Interestingly, increasing the LET of radiation does not increase the number of oxidative DNA lesions²⁴ produced but increases the complexity of the cluster damage sites. In addition, recent works have highlighted the fact that, following a single oxidation event, complex DNA lesions involving more than one modification could be produced also in dsDNA. These modifications are thus not specific of ionizing radiations but could be produced also by other sources of oxidative stress.

COMPLEX DNA LESIONS INDUCED BY A SINGLE OXIDATION EVENT

HPLC-MS/MS was found to be highly sensitive for measuring low levels of well-characterized DNA lesions. In addition, this approach has been successfully used to search for new radiation-induced lesions directly in irradiated dsDNA. Using the so-called neutral loss scan mode, four new lesions have been detected in γ -irradiated DNA.²⁵ One of the four lesions has also been measured in cellular DNA, and the identification of its mechanism of formation²⁶ was one of the first examples of a complex DNA lesion generated by a single oxidation event (Figure 1). Indeed, the identified cytosine adduct named dCyd341 (that could exist in an open or a closed ring form) was found to be produced by the reaction of a cytosine base with a reactive keto-aldehyde **2** arising from the 2-deoxyribose decomposition as a consequence of the H-abstraction at C4' producing C4' radical **1**. The formation of the conjugated keto-aldehyde **2** is accompanied by the generation of a strand break, thus the lesion represents a challenge for the DNA repair machinery. The formation of other DNA adducts arising from the initial formation of aldehydic derivatives subsequent to 2-deoxyribose oxidation has been reported.^{27–29}

A second illustration of the powerfulness of HPLC-MS/MS is illustrated by the identification of the mechanisms of formation of well-known lesions 8-oxodGuo. Until recently, 8-oxodGuo formation was attributed to the addition of a HO $\cdot$ at C8 of guanine.¹¹ However, a recent study has demonstrated that only 5% of 8-oxodGuo is generated by this mechanism in dsDNA exposed to low LET radiation. Most of the hydroxylated purine bases were found to be produced by a peroxidation reaction involving the addition of a peroxyl pyrimidine radical **3** onto the C8 of an adjacent purine base³⁰ producing the so-called tandem lesions involving two adjacent modifications,³¹ such as 8-oxodGuo and formylamine **5** (Figure 2).³² Interestingly, the possible formation of such tandem lesions was also studied *in silico*,³³ confirming the possible formation of endoperoxide **4** and providing a possible explanation for the sequence preference observed experimentally.³²

Similar theoretical approaches have been used^{34,35} to study the formation of other tandem lesions that are generated in the absence of oxygen. Under these conditions, intrastrand cross-links can be produced between a pyrimidine and a purine base **7** as initially reported by Box *et al*,^{36,37} and a similar sequence effect has been observed.³⁸ It should be highlighted that the formation of such tandem damage has been studied also thanks to the chemical synthesis of photochemical precursors of DNA radicals. Indeed, a thiophenyl derivative of thymidine when

Figure 1. The mechanism of formation of dCyd341 arising from the C4'-radical **1** through reaction of 2'-deoxycytidine (dCyd) with transiently produced keto-aldehyde **2**.

Figure 2. Mechanisms of formation of tandem DNA lesions involving, in the absence of oxygen, formation of intrastrand cross-links **7** or, in the presence of oxygen, formation of two adjacent modified DNA bases **5** through decomposition of endoperoxide **4** generated by the addition of a pyrimidine peroxy radical **3** onto C8 of guanine.

incorporated by a solid phase synthesis into oligonucleotides³⁹ was found to give rise, following ultraviolet A (UVA) irradiation, to tandem lesions through the transient formation of radical **6** (Figure 2). The possibility to incorporate photo-precursors of specific radicals into synthetic oligonucleotides is a powerful approach to study the decomposition of a given radical in the dsDNA environment.⁴⁰ The use of a photochemical precursor of C4' oxidized abasic site was found to be particularly relevant to demonstrate that the formation of dCyd341 involves reaction of a keto-aldehyde moiety with a cytosine base located on the opposite strand,⁴¹ thus creating an interstrand cross-link. This

also allowed for a study of the decomposition of initially generated reactive aldehyde in a nucleosome core particle that mimics the structure of a DNA in the nucleus. Interestingly, under these conditions, the possibility to generate DNA–protein cross-links has been highlighted through the reaction of nucleophilic amino acids with the reactive aldehyde **2**.⁴² The nucleophilic addition of a lysine residue has also been shown to occur at the C8 of guanine radical cation,⁴³ producing a chemical cross-link **9** between a DNA base and an amino acid (Figure 3). Additional efforts should be made to evaluate at the cellular level the importance of these radiation-induced DNA–protein

Figure 3. The formation of complex lesions **9** and **10** generated through the transient formation of guanine radical cation **8** produced by a one-electron oxidation reaction.

cross-links, lesions that have unfortunately been little studied to date.⁴⁴ Interestingly, the addition of thymine at C8 of guanine radical cation (or its deprotonated form that should predominate at neutral pH) could also give rise to a complex lesion **10**⁴⁵ that was recently detected in cells⁴⁶ again by HPLC-MS/MS.

Although the development of highly sensitive and specific analytical methods has allowed for an improvement of the measurement of DNA lesions at the cellular level, we also have to mention that some controversies still persist. This is the case for 5',8-cyclopurines, complex DNA lesions involving formation of an additional bond between the base and the sugar moiety, following the initial formation of a C5' radical.⁴⁷ In our group, we found that these lesions are produced in a very low yield in cells exposed to ionizing radiations,⁴⁸ and the background level of these lesions in untreated cells is lower than our limit of detection. Meanwhile, another group has used these modifications as biomarkers of oxidative stress^{49,50} and has reported background levels of these lesions about two orders of magnitude higher than those measured in our laboratory. Thus, additional work has to be performed to resolve these discrepancies. Another important issue is the measurement of clustered lesions that could not be quantified using methods (such as HPLC-MS/MS) requiring DNA digestion. Assays based on the use of repair enzymes have been proposed,⁵¹ but the risk of overestimation has been questioned.⁵²

BIOLOGICAL CONSEQUENCES OF DNA LESIONS, REPAIR AND MUTAGENICITY

Radiation initiates a wide range of biomolecular and cellular events, including DNA repair, cell cycle dysregulation or apoptosis. Chemistry and its derived tools are commonly used to understand some of these biological consequences. Indeed, analytical chemistry can be used to study the specificity of substrate of DNA repair enzymes simply by measuring the remaining level of modified bases in irradiated dsDNA incubated in the presence of, for instance, glycosylases. Such an approach has been used to demonstrate that 8-oxodGuo involved in tandem lesion is partly refractory to repair by base excision repair glycosylases that are able to excise single lesions very efficiently.³¹ In addition, the kinetics of repair can be also obtained in cells by delaying the measurement of the lesion after an acute exposure, as reported for dCyd341.²⁶ Another powerful approach to evaluate the substrate specificity of DNA repair enzymes is to use oligonucleotides containing a defined DNA lesion. Thus, during the last two decades, tremendous work has been made by organic chemists to increase the number of possible lesions that could be incorporated into oligonucleotides by solid phase synthesis.^{53–55} Biochemical kinetic parameters can be obtained from the use of these oligonucleotides through electrophoretic assays. In addition, MS such as Maldi-ToF (matrix-assisted laser desorption/ionization–time of flight) can provide very precise information on the action mechanisms of enzymes.⁵⁶ A lot of information has thus been accumulated for different glycosylases and several possible substrates.^{53,57} In addition, a similar strategy could be used to determine the mutagenicity of a given lesion, simply by allowing a polymerase to extend a DNA fragment using a complementary strand containing a lesion.^{58,59} However, a specific characteristic of ionizing radiation is that it mostly leads to the formation of MDSs, containing several DNA lesions within one or two helix turns of duplex DNA. Thus, to

study the biological consequences of an MDS is a challenging task that has been partly resolved by the power of organic chemistry. The solid phase strategy that has been developed is suitable with the incorporation of several DNA lesions in a given sequence. Thus, oligonucleotides containing more than one lesion, including oxidized bases or abasic sites, that could be located either on one strand or on the two opposite strands could be synthesized. Such clustered damages can be considered analogous to the MDS produced by ionizing radiation.^{60,61} This allows us to determine how these complex lesions can be processed by either isolated enzymes or cell extracts.^{60–63} In addition, these lesions could then be introduced into plasmids and transfected into cells to evaluate their repair fidelity and mutagenicity.⁶⁴

Oligonucleotides containing DNA lesions could also be used to evaluate the repair capacity of cell extracts. This can be done in solution using ³²P labelled oligonucleotides, but a fluorescent multiplexed approach on biochips has been recently developed that has several advantages. This allows the simultaneous monitoring of the repair of several DNA lesions.⁶⁵ Recent applications highlight the power of such an approach^{66,67} for measuring small changes in cellular DNA repair capacities. Immobilized modified DNA fragments containing DNA lesions also may be used to trap specific proteins involved in the so-called DNA damage interactome.⁶⁸ This strategy has been applied to identify proteins that recognize bulky lesions⁶⁹ or epigenetic DNA modifications,⁷⁰ and work is in progress to search for proteins that could bind to radiation-induced complex DNA lesions.

CONCLUSION

Chemical approaches have been used to identify, at the molecular level, the radiation-induced DNA lesions and to provide information on the mechanism of their formation. Although a considerable amount of work has been performed, recent data suggest that the decomposition of the initially produced radical could be influenced by its environment. This explains why new radiation-induced DNA lesions have recently been identified and detected in dsDNA through the development of modern analytical approaches. Thus, additional work should be done to determine, first in isolated dsDNA and then at a cellular level, the contribution of the decomposition reactions to the overall radical-induced DNA damage. In addition, efforts should be pursued to improve the sensitivity and versatility of the developed analytical methods to increase the number of lesions that could be measured in cells and also to identify transiently produced radicals.⁷¹ Organic chemistry through the synthesis of oligonucleotide containing lesions or through the incorporation of photochemical precursors of specific radicals has still a unique place in this field of investigation. Thus, there is still a need to involve chemists in radiation biology, and one of the main goals would be to identify a DNA lesion that is specifically produced by ionizing radiation and not by endogenous oxidative stress. One could imagine that such lesions possibly may be generated through the reaction between two initially produced radicals. That lesion would be a potential biomarker of exposure to ionizing radiation.

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