SHORT COMMUNICATION

Preferential targeting of oxidative base damage to internucleosomal DNA

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The structure of nuclear chromatin may limit the accessibility of carcinogenic agents to DNA. In the case of oxidative DNA strand cleavage mediated by the physiologically relevant iron chelate, iron-ADP, histone-associated nucleosomal DNA is protected while internucleosomal DNA is susceptible to damage. We now find that the distribution of iron-ADP-generated 8-hydroxydeoxyguanosine, a potentially mutagenic oxidative base change, shows relative targeting to internucleosomal sites (3.5-fold increased oxidative modification of internucleosomal compared with nucleosomal DNA as the minimal degree of enrichment). In contrast, iron-EDTA, which generates hydroxyl radical in the 'fluid phase', does not target internucleosomal DNA. Thus, physiologic iron chelates may promote site-specific damage and thereby be relevant to mechanisms of iron-dependent oxidative mutagenesis and carcinogenesis.

Iron-mediated oxygen radical damage to isolated DNA may manifest as strand cleavage (single- or double-strand breaks) and/or oxidation of DNA bases (1-3). In the nucleus, however, DNA is associated with chromatin protein in a conformation that limits its accessibility to nucleases (4,5) and perhaps to other damaging agents including carcinogens (6-8). It is likely that the heterogeneity of damage to nuclear DNA with targeting of specific, accessible sites is important in the development of malignancy and that both the site specificity and the nature of such DNA lesions determine their carcinogenic potential (9,10).

The hypothesis that nucleosome structure modulates oxidative damage to DNA in chromatin form is supported by our previous observation of internucleosomal targeting of iron-ADP-mediated double-strand breaks (11), with nucleosomal DNA associated with histone protein protected compared to the random, indiscriminate fragmentation observed for protein-free DNA. The interaction of different chelates of iron with DNA in chromatin form is not uniform, however, since iron-EDTA, which is unable to bind the DNA polyanion (12,13) and thus generates damaging oxygen radicals in the 'fluid phase', cleaves polynucleosomes indiscriminately.

Oxidative base change, e.g. the conversion of deoxyguanosine to 8-hydroxydeoxyguanosine (8-OHdG*), is probably more relevant to mutagenesis than strand cleavage (10,14). Using micrococcal nuclease (which digests chromatin to mononucleosomes by degradation of internucleosomal DNA) as a probe for chromatin structure (15,16) we explored the preferential susceptibility of internucleosomal, linker DNA to generation of 8-OHdG by the physiologic chelate iron-ADP (17).

The generation of 8-OHdG from deoxyguanosine in nucleosomal and internucleosomal DNA was compared for polynucleosomes exposed either to iron-ADP or to iron-EDTA (Figure 1). Briefly, polynucleosomes were exposed either to ferrous iron-ADP (500 μM) or iron-EDTA (100 μM) (1:2 molar ratio of iron:ADP or iron:EDTA) in the presence of H2O2 (500 μM) and ascorbate (500 μM), the reaction being stopped by the addition of a molar excess of desferrioxamine. Following oxidant exposure, polynucleosomes were extensively dialyzed into digestion buffer (5 mM Tris, pH 7.5, 80 mM NaCl, 1 mM CaCl2, 200 mM sucrose) for micrococcal nuclease digestion (250 U/mg DNA, 45 min at 37°C). Digestion was terminated with 5 mM EDTA. Polynucleosomes exposed to oxidant but not subsequently enzyme digested were handled under precisely the same conditions. Following exposure to oxidant with or without subsequent micrococcal nuclease digestion, the amounts of 8-OHdG generated were measured using HPLC. For this, DNA was extracted and extensively digested with the following enzymes as previously described: DNase I, endonuclease Neurospora crassa, phosphodiesterase snake venom Crotalus duriss., and alkaline phosphatase. The amounts of deoxyguanosine and 8-OHdG in the resulting deoxyribonucleotide mixtures were measured using HPLC with in-line UV and electrochemical detectors, respectively (18,19).

The DNA extraction process itself did not increase the amount of 8-OHdG detected in control or in oxidatively damaged DNA (data not shown). Polynucleosomes were the commercially available chromatin preparation from Worthington Biochemical Corporation (Freehold, NJ). The preservation of chromatin nucleosomal structure in each preparation was confirmed by micrococcal nuclease digestion, which resulted consistently in generation of monomer-sized fragments.

Both iron-ADP and iron-EDTA resulted in the generation of 8-OHdG in polynucleosomal DNA. To investigate the site of oxidative base change, polynucleosomes were exposed to the oxygen radical generating systems (iron-ADP or iron-EDTA) and then compared for 8-OHdG content with versus without digestion by the linker-specific micrococcal nuclease. When polynucleosomes damaged by iron-ADP were subjected to micrococcal nuclease digestion, a significant decrease in detectable 8-OHdG was observed (Figure 1). Assuming a nucleosome size of 200 bp (comprising 146 core base pairs and 54 linker base pairs) and assuming complete micrococcal nuclease digestion, 27% of the 8-OHdG should be digested and 73% should remain in the undigested core if oxidative base changes are randomly distributed. The observed decrease in 8-OHdG after micrococcal nuclease digestion indicates that oxidative modification by iron-ADP is concentrated in linker DNA (at least 3.5-fold increased damage to linker compared with nucleosomal core DNA). Taking the values obtained for the control samples (not exposed to iron-ADP: 9.16 8-OHdG/106 Dg and 12.04 8-OHdG/106 Dg for micrococcal-nuclease digested and undigested samples respectively) into account, digestion of iron-ADP-exposed

*Abbreviation: 8-OHdG, 8-hydroxydeoxyguanosine.
polynucleosomes decreased the 8-OHdG:dG ratio from 35.81 8-OHdG/10^5 dG to 21.54 8-OHdG/10^5 dG (mean values), consistent with excessive oxidative damage to internucleosomal linker DNA. To understand the magnitude of the difference, we must compare the concentration of base damage in the linker to that in the nucleosomal core. To determine the percentage of total changed bases which reside in the core:

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\frac{(21.54 \times 8\text{-OHdG/10}^5 \text{ dG}) \times 146 \text{ bases/core}}{(35.81 \times 8\text{-OHdG/10}^5 \text{ dG}) \times 200 \text{ bases/core + linker}} = 43.9\%
\]

Thus, the core contains 43.9% of total 8-OHdG, while the linker contains 56.1%. To determine the concentration of 8-OHdG in linker compared with core nucleosomal DNA:

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\frac{56.1\%/54 \text{ bases}}{43.9\%/146 \text{ bases}} = 3.5 \text{ times as much 8-OHdG in internucleosomal compared with core DNA}
\]

This represents a minimum degree of enrichment.

Such calculations are, of course, dependent on specific and complete digestion of internucleosomal DNA by micrococcal nuclease (15). They may, in fact, underestimate the preferential modification of internucleosomal DNA to the extent that micrococcal nuclease digestion under these experimental conditions may be incomplete. Incomplete digestion did, in fact, occur since both dimer and monomer nucleosome forms are present following enzyme treatment (Figure 1). Thus, 3.5-fold enrichment represents the minimal degree of targeting.

In contrast, in polynucleosomes damaged by iron–EDTA, 8-OHdG is equally distributed in nucleosomal and internucleosomal DNA (Figure 1). Thus, the interaction of different chelates of iron with DNA in chromatin is not uniform, and physiologic chelates of iron such as iron–ADP may allow iron binding to accessible sites in DNA, which may in turn determine the specific sites of damage. An alternative explanation for the preferential modification of guanine residues of linker DNA might be simply that internucleosomal DNA has a GC-rich base composition. This is not the case, however, as neither micrococcal nuclease-sensitive DNA nor chicken erythrocyte linker DNA is enriched for GC compared with nucleosomal DNA (20,21). In addition, the polynucleosomes used for these experiments did not show linker enrichment for deoxyguanosine (measured deoxyguanosine concentration for polynucleosomal and for core DNA 0.665 ± 0.017 and 0.775 ± 0.028 nmol/μg DNA respectively). Furthermore, such an explanation would require a similar distribution of iron–EDTA mediated base change. Iron–ADP damage to chromatin DNA may not be totally specific to the accessible internucleosomal area since micrococcal nuclease did not digest 8-OHdG to baseline levels. Alternatively, as discussed above, micrococcal nuclease digestion may be incomplete.

This demonstration that nucleosomal DNA is protected against the oxidative conversion of deoxyguanosine to 8-OHdG has potential importance in the pathogenesis of malignancy since the presence of 8-OHdG may be directly mutagenic, leading to misreading at the altered base or at adjacent bases during subsequent DNA replication (10,14,22). In addition to measurement of this base change in isolated DNA exposed to various oxidative damaging agents (3,18,23), 8-
References


