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Efficient cleavage of single and clustered AP site lesions within mono-nucleosome templates by CHO-K1 nuclear extract contrasts with retardation of incision by purified APE1

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ABSTRACT

Clustered DNA damage is a unique characteristic of radiation-induced DNA damage and the formation of these sites poses a serious challenge to the cell's repair machinery. Within a cell DNA is compacted, with nucleosomes being the first order of higher level structure. However, few data are reported on the efficiency of clustered-lesion processing within nucleosomal DNA templates. Here, we show retardation of cleavage of a single AP site by purified APE1 when contained in nucleosomal DNA, compared to cleavage of an AP site in non-nucleosomal DNA. This retardation seen in nucleosomal DNA was alleviated by incubation with CHO-K1 nuclear extract. When clustered DNA damage sites containing bistranded AP sites were present in nucleosomal DNA, efficient cleavage of the AP sites was observed after treatment with nuclear extract. The resultant DSB formation led to DNA dissociating from the histone core and nucleosomal dispersion. Clustered damaged sites containing bistranded AP site/8-oxoG residues showed no retardation of cleavage of the AP site but retardation of 8-oxoG excision, compared to isolated lesions, thus DSB formation was not seen. An increased understanding of processing of clustered DNA damage in a nucleosomal environment may lead to new strategies to enhance the cytotoxic effects of radiotherapeutics.

1. Introduction

Many thousands of DNA modifications are induced daily in each cell through reactive oxygen species (ROS), formed as a by-product of aerobic metabolism, in contrast to the much lower levels of DNA damage induced at low doses of ionizing radiation. In contrast, the formation of clustered DNA damage sites, defined as two or more lesions formed within one or two helical turns of the DNA double helix by a single radiation track, is a characteristic of exposure to ionizing radiation when compared with their rarer occurrence when induced endogenously [1,2]. The type of lesions produced via exposure to ionizing radiation is thought to be chemically identical to those formed by ROS [3,4]. The formation of clustered DNA damage has been experimentally verified in both isolated DNA and mammalian cells [5–11]. The yield of bistranded non-DSB clusters is at least 4–8 times that of prompt DSB damage [7,9,10], with ~10% of the total yield of non-DSB clustered damage converted into DSB at early times following γ-irradiation in xrs5 cells [12]. As such, it is the formation of these clustered damage sites, including DSB with lesions downstream of the DSB ends, that ultimately determines the severity of the biological consequences of exposure to ionizing radiation.

Within the cell, the base excision repair (BER) pathway is predominantly used for repair of base lesions, AP sites and SSB, irrespective of whether they are formed endogenously or through exposure to ionizing radiation [13,14]. Numerous studies using oligonucleotides containing synthetic clustered damage sites have verified the hypothesis [15] that clustered DNA damage sites are more difficult to repair than single lesions, as a result of the reduced efficiency of BER (reviewed in [16–18]). Complementary investigations using plasmid-based bacterial or mammalian reporter systems confirmed the retardation of BER during the repair of clustered damage sites (reviewed in [16–20]) and as a consequence an increase in mutation frequency was seen resulting from the lifetime extension of lesions within clusters.

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Cellular DNA must be compacted in order to be contained within the nucleus and DNA compaction is highly conserved throughout eukaryotes [21], with the first level of DNA higher order structure being the nucleosome. The nucleosome structure consists of a dimer of H3-H4 histones heterodimers with two individual heterodimers of H2A–H2B that bind to form a histone octamer. Approximately 147 base pairs of DNA associate with the histone octamer; the central 60 base pairs bound by the H3–H4 octamer whilst the H2A–H2B dimers contact 30 base pairs either side of this central region [22]. The superhelical turns of the DNA do not remain constant throughout the nucleosome, with a more relaxed structure observed towards the outer regions than at the central dyad [23]. Although DNA compaction is essential, the presence of the histone octamer has been shown to interfere with cellular processes, particularly DNA repair, as binding to the histone proteins causes DNA distortion and may also provide a physical block to interactions of other proteins. This is evident in a number of studies where reduced digestion with restriction enzymes was observed in nucleosomal templates in comparison to non-nucleosomal ("free") DNA [24–27]. Despite this level of protection offered by histone-association, the DNA is still susceptible to damaging agents.

Only a few studies have reported on the levels of DNA damage processing by BER within nucleosomes and have focused on the level of retardation of repair of DNA damage seen within nucleosomal DNA compared with free DNA, using purified proteins. Despite some variability in the extent of retardation, attributed to different DNA sequences used with varying positioning affinity, the data unequivocally show that repair occurs less efficiently within nucleosomal templates as opposed to free DNA [28–34]. Importantly, treatment of reconstituted nucleosomes with repair proteins does not lead to disruption of the structure, with the exception of the ligaseIII/XRCC1 complex [34]. Efficiency of enzymatic processing may differ between damage located close to the dyad rather than towards the outer regions of the nucleosome [26,27,30] in that a higher level of retardation was observed when the lesion is placed closer to the dyad. Further, the efficiency of processing is dependent upon whether a lesion was oriented "in" i.e., facing the histone octamer, or "out" i.e., facing solution, with higher levels of retardation observed with lesions facing inwards [26–30,34–37] and recently reviewed in ref [38]. Addition of the chromatin remodelling factor SWI/SNF leads to an increase in the rate of excision of 8-oxoG by OGG1 from native nucleosomes [31]. The activity of polymerase (pol) β within nucleosomal templates was demonstrated to be minimal [28,29,31] leading to a stall in the BER pathway. Despite a reduction in the level of ligation achieved within nucleosomes [33,39,40], this appeared to be the most efficient step during repair by BER of lesions contained within nucleosomal DNA. However, a more recent study has shown contrasting results to these earlier studies in that pol β could process its substrate in nucleosomal bound DNA but ligation could only occur if the nucleosome had been disrupted [34]. The retardation of DNA repair is not limited to the BER proteins; reports of similar levels of protein retardation have been shown in the nucleotide excision repair, mismatch repair and non-homologous end joining pathways [25,41–43]. Few studies have been undertaken on bistranded clusters in a nucleosome environment. However, two recent studies on bistranded clustered damage showed that the efficiency of glycosylases or APE1 to cause SSB from excision of a lesion within the clusters is even more reduced when in a nucleosome environment and importantly cause suppression of BER-generated DSB [44,45] than previously reported in 'free' DNA [16–18].

Building on ours and others studies of the efficiency of clustered DNA damage processing by both mammalian nuclear extract and purified proteins, within short sequence oligonucleotides (reviewed in [16]), we have now extended this present study to nucleosomal DNA containing clustered damage sites, to assess the rates of cleavage of the lesions by CHO-K1 nuclear extract, in comparison with that of purified AP endonuclease (APE1). To date, studies investigating the processing of DNA lesions within nucleosomes have generally focused on repair of single lesions. We have designed DNA sequences containing a single AP site or clustered sites containing two lesions, either bistranded AP sites or an AP site opposing an 8-oxoG residue (see Table 1). The mono-nucleosome constructs contain these DNA damage sites close to the nucleosome dyad. Through treatment of these DNA substrates with either purified APE1 or OGG1 and CHO-K1 nuclear extract, we aim to gain more insight into the processing of clustered DNA damage within the added complexity of a higher order DNA environment. Using clustered DNA damage, we identified the formation of DSB through cleavage of opposing AP sites which leads to nucleosome disruption. In contrast, an AP site opposing 8-oxoG does not lead to DSB but retardation of cleavage of the 8-oxoG lesion was observed. Significant retardation of cleavage of the single AP site contained within nucleosomes by APE1 was noted, in comparison to free DNA, which was alleviated when DNA substrates were incubated with CHO-K1 nuclear extract.

2. Materials and methods

2.1. Substrate oligonucleotides

The oligonucleotide sequences, as depicted in Table 1, were purchased PAGE purified with 5′ phosphorylated termini from Eurogentec. Strand 1 contains either uracil (Y) or 8-oxoG (X) at variable positions. Strand 2 contains a single uracil (Y) at a fixed position. The control oligonucleotides contain a single uracil (Y), tetrahydrofuran (Z) or 8-oxoG (X) present in strand 2 and no lesion in strand 1. Based on the previous nomenclature [46] lesions situated on strand 1, 3′ to the single uracil found on strand 2 within the cluster are given a negative number and 5′ lesions a positive number, the number relating to the base separation of the lesions.

2.2. Preparation of lesion-containing DNA for nucleosome reconstitution

The plasmid pGEM3Z-601 contains the strong rotational positioning 601 DNA sequence [47,48]. In order to incorporate restriction site sequences at each termini of the 601 sequence, plasmid DNA was used as a PCR template using the following primers: forward 5′ – CTCGGAATTCTATCCGACTGGCACCGGCAAG

Table 1
Oligonucleotide sequences.

Cluster	Sequence	Strand
AP control	5′ TTGGTGCGTTTAAGCCGTGC 3′	1
	3′ CGCAACCAC**Y**CAAATTCGGC 5′	2
THF control	5′ TTGGTGCGTTTAAGCCGTGC 3′	1
	3′ CGCAACCAC**Z**CAAATTCGGC 5′	2
8-oxoG control	5′ TTGGTGCGTTTAAGCCGTGC 3′	1
	3′ CGCAACCAC**X**CAAATTCGGC 5′	2
AP/AP + 1	5′ TTGGTGC**Y**TTTAAGCCGTGC 3′	1
	3′ CGCAACCAC**Y**AAAATTCGGC 5′	2
AP/AP − 3	5′ TTG**Y**TGCGTTTAAGCCGTGC 3′	1
	3′ CGCAACAAC**Y**CAAATTCGGC 5′	2
AP/8-oxoG + 1	5′ TTGGTGC**X**TTTAAGCCGTGC 3′	1
	3′ CGCAACCAC**Y**CAAATTCGGC 5′	2

Oligonucleotide sequences: Y represents a uracil residue converted to an AP site before use and X represents 8-oxoG. The lesions on strand 1 have been given a number relating their positions to the AP site on the complementary strand 2. The number denotes the base separation. A positive number is given if the lesion on strand 1 is in the 5′ direction to that on strand 2 and a negative number is given if the lesion on strand 1 is in the 3′ direction to that on strand 2. The AP, THF and 8-oxoG control oligonucleotides consists of a single lesion on strand 2 with no lesion on strand 1.