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Probing for DNA damage with β-hairpins: Similarities in incision efficiencies of bulky DNA adducts by prokaryotic and human nucleotide excision repair systems *in vitro*

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ABSTRACT

Nucleotide excision repair (NER) is an important prokaryotic and eukaryotic defense mechanism that removes a large variety of structurally distinct lesions in cellular DNA. While the proteins involved are completely different, the mode of action of these two repair systems is similar, involving a cut-and-patch mechanism in which an oligonucleotide sequence containing the lesion is excised. The prokaryotic and eukaryotic NER damage-recognition factors have common structural features of β-hairpin intrusion between the two DNA strands at the site of the lesion. In the present study, we explored the hypothesis that this common β-hairpin intrusion motif is mirrored in parallel NER incision efficiencies in the two systems. We have utilized human HeLa cell extracts and the prokaryotic UvrABC proteins to determine their relative NER incision efficiencies. We report here comparisons of relative NER efficiencies with a set of stereoisomeric DNA lesions derived from metabolites of benzo[*a*]pyrene and equine estrogens in different sequence contexts, utilizing 21 samples. We found a general qualitative trend toward similar relative NER incision efficiencies for ∼65% of these substrates; the other cases deviate mostly by ∼30% or less from a perfect correlation, although several more distant outliers are also evident. This resemblance is consistent with the hypothesis that lesion recognition through β-hairpin insertion, a common feature of the two systems, is facilitated by local thermodynamic destabilization induced by the lesions in both cases. In the case of the UvrABC system, varying the nature of the UvrC endonuclease, while maintaining the same UvrA/B proteins, can markedly affect the relative incision efficiencies. These observations suggest that, in addition to recognition involving the initial modified duplexes, downstream events involving UvrC can also play a role in distinguishing and processing different lesions in prokaryotic NER.

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1. Introduction

1.1. Overview

Nucleotide excision repair (NER) is important for recognition and removal of a large variety of structurally and chemically distinct DNA lesions in prokaryotic [1] and eukaryotic [2] organisms. While the proteins involved share little sequence homology, the mode of action of this repair system in all branches of life is similar in many respects. Since a large range of substrates are removed by both NER systems, it is widely accepted that the recognition process involves the sensing of the structural distortions/destabilizations in the DNA duplex caused by bulky lesions, rather than the lesions themselves. While NER can remove a large variety of DNA lesions, the DNA repair capacity, or efficiency, can vary by two orders of

Abbreviations: NER, nucleotide excision repair; BP, benzo[*a*]pyrene; 4-OHEN, 4-hydroxyequilenin; dG, 2′-deoxyguanosine; dA, 2′-deoxyadenosine; CPD, cyclobutane pyrimidine dimer; (+)-*anti*-BPDE, (+)-7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; *cis*-BP-dG, (+)-(7*R*,8*S*,9*R*,10*R*)-N^2-[10-(7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-2′-deoxyguanosine; *trans*-BP-dG, (+)-(7*R*,8*S*,9*R*,10*S*)-N^2-[10-(7,8,9,10-tetrahydrobenzo[*a*]pyrenyl)]-2′-deoxyguanosine.

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magnitude or more, depending on the lesion [2–5]. The chemical structures of the DNA lesions, the base sequence contexts in which the lesions are embedded, as well as their stereochemical properties can affect the DNA repair efficiencies catalyzed by prokaryotic [6–12] and eukaryotic [4,5,13–16] repair systems. While the importance of these factors on NER efficiencies is well documented, the exact molecular origins underlying these differences are still not well understood [17].

Recently, crystal structures of several prokaryotic and eukaryotic NER proteins that recognize and bind to DNA lesions have provided important new insights into the structural features that have an impact on lesion-removal by the two NER systems [16,18–26] (see also review by Fuss and Tainer in this issue of *DNA Repair*). Furthermore, we have accumulated a substantial library of NER substrates consisting of structurally defined DNA lesions positioned in similar or different oligonucleotide sequence contexts. These substrates provide unique opportunities for investigating the relationships between the chemical structures of the lesions and the impact of base sequence context effects on their recognition and processing by eukaryotic and prokaryotic NER systems.

These two NER systems (e.g., [1,2,27,28]) share common overall features that include: (1) the initial steps involve the recognition of the local distortions/destabilizations caused by the lesions, rather than the lesions themselves, thus allowing for the processing of a large range of structurally unrelated DNA lesions; (2) the damaged strand is incised by endonucleases on both sides of the lesions, thus removing entire oligonucleotide sequences containing the damage rather than just the lesions; (3) recently published crystal structures suggest that one of the recognition steps in prokaryotic [20,25,28] and eukaryotic [23,25,28] NER systems involves the insertion of a β-hairpin between the two DNA strands in the immediate vicinity of the lesion. These structural findings are particularly intriguing because they suggest that thermodynamic destabilization of the native B-DNA structure can play an important role [29,30] in the recognition of DNA lesions in both NER systems, a concept that had been previously proposed [4,31–33].

In recent years, we have accumulated a body of results on the relationships between the structural properties of DNA lesions and incision efficiencies of prokaryotic and eukaryotic NER systems [9,17,34,35]. Here, we examine the similarities and differences in the processing of bulky DNA lesions by prokaryotic UvrABC NER proteins and the human NER apparatus *in vitro*. In both cases, the formation of excision products is a result of a complex series of steps that include the critical initial recognition step. Our hypothesis is that the ease of insertion of a β-hairpin into the duplex in the vicinity of a lesion plays an important role in the recognition step in both eukaryotic and prokaryotic NER.

In this article, we first consider some of the key features of these NER systems on which this hypothesis is based. We then compare the relative efficiencies of incision of a series of DNA substrates catalyzed by prokaryotic UvrABC proteins or by the eukaryotic NER system in HeLa cell extracts *in vitro*. Our studies are focused on two different types of bulky stereoisomeric DNA lesions that are under study in our laboratories. The first type is derived from the reactions of a diol epoxide derivative of benzo[*a*]pyrene (BP) to DNA [14,36], and the second is derived from the binding of 4-hydroxyequilenin (4-OHEN), a reactive metabolite of the equine estrogens equilin and equilenin. Both equine estrogens are important components of the hormone replacement therapy preparation Premarin© [37]. The catechol 4-OHEN forms a variety of pre-mutagenic DNA lesions that are suspected to contribute to human cancers by genotoxic pathways [38]. Because of the potential importance of these equine estrogen DNA lesions, we have been interested in their structural properties [39,40], as well as their response to nucleotide excision repair systems [41,42].

1.2. Functional and structural characteristics of prokaryotic and eukaryotic NER: similarities and differences

1.2.1. Overview of NER lesion-recognition

It is generally accepted that the recognition and subsequent processing of DNA lesions by NER occur via a two-step process. The first is the recognition of the lesion, and the second is a verification step that ensures that a lesion is actually present (see also review by Naegeli and Sugasawa in this issue of *DNA Repair*). Remarkable insights into the molecular bases of these recognition phenomena have been obtained from the crystal structures of the key prokaryotic UvrA [20,43,44], UvrB [25], UvrC [21,45], and eukaryotic [23,24] DNA damage-sensing proteins that initiate NER by binding to the sites of the DNA lesions.

In prokaryotic cells, three proteins, UvrA, UvrB, and UvrC are needed to generate the dual incisions on the two sides of the lesion that generate the 12–13-mer excision products. The distortions/destabilizations caused by the lesion are first recognized by a dimer of UvrA that binds to the damaged site in the form of a $UvrA_2UvrB_2$ complex [46]. In an ATP-driven mechanism, the UvrA dimer then dissociates and the UvrB–DNA complex is stabilized by the insertion of a β-hairpin between the two strands in the vicinity of the lesion (Fig. 1A). In this damage verification step [28], the local destabilization of the duplex at the site of the damage most likely facilitates the insertion of the UvrB β-hairpin (see also Section 1.2.2). If the latter fails to insert between the two strands, as might be the case in the absence of a lesion, the UvrA–UvrB dissociates from the DNA [6,33]. The stable binding of UvrB to damaged DNA stimulates the activity of its ATPase, which is essential for the recruitment of the endonuclease UvrC by this pre-incision complex [28]. The hydrolysis of one phosphodiester bond on the 3′-side and another on the 5′-side of the lesion results in the 12–13-mer dual incision products.

In the case of the mammalian NER pathway, the presence of the helix-distorting/destabilizing lesion is sensed by the XPC-RAD23B heteroprotein complex that opens a ~6-base pair sequence, as shown with duplexes containing a cisplatin [47] or a bulky BP diol epoxide-N^2-dG adduct [36]. This complex initiates the recruitment of other NER factors to the site of the lesion, starting with the multi-protein TFIIH complex which contains the helicases XPB [18] and XPD [19,22,26]; the latter induce the unwinding of a 20–25 nucleotide-long region around the lesion [48,49].

1.2.2. Crystal structures: shared feature of β-hairpin insertion

A crystal structure of a complex of the *Bacillus caldotenax* (*Bca*) UvrB bound to double-stranded damaged DNA has been solved [25]. In this structure, one DNA strand, containing a 3′ overhang, threads behind a β-hairpin motif of UvrB, indicating that this hairpin is inserted between the strands of the double helix, while the nucleotides directly behind the β-hairpin are flipped out and inserted into a small, highly conserved pocket in UvrB. Molecular modeling and molecular dynamics simulations have been utilized to complete the DNA structure and to include a BP-derived lesion (Fig. 1A) [35].

Although a crystal structure of an XPC-RAD23B complex containing damaged DNA is not yet available, the X-ray crystallographic structure of a truncated form of the yeast *S. cerevisiae* Rad4/Rad23 homologue of the mammalian XPC-RAD23B in a complex with an oligonucleotide containing a cyclobutane pyrimidine dimer (CPD) lesion has been determined [23]. There are three β-hairpin domains in the Rad4 protein (BHD1, BHD2, and BHD3). While BHD1 binds non-specifically to an unmodified 11-mer sequence on the 3′-side of the CPD lesion, BHD2 and BHD3 are in contact with the DNA in the vicinity of the lesion, although not in direct contact with the CPD. The BHD3 β-hairpin is inserted into the DNA helix, thus separating the lesion from the unmodified strand.

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