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Close encounters for the first time: Helicase interactions with DNA damage

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ABSTRACT

DNA helicases are molecular motors that harness the energy of nucleoside triphosphate hydrolysis to unwinding structured DNA molecules that must be resolved during cellular replication, DNA repair, recombination, and transcription. In vivo, DNA helicases are expected to encounter a wide spectrum of covalent DNA modifications to the sugar phosphate backbone or the nitrogenous bases; these modifications can be induced by endogenous biochemical processes or exposure to environmental agents. The frequency of lesion abundance can vary depending on the lesion type. Certain adducts such as oxidative base modifications can be quite numerous, and their effects can be helix-distorting or subtle perturbations to DNA structure. Helicase encounters with specific DNA lesions and more novel forms of DNA damage will be discussed. We will also review the battery of assays that have been used to characterize helicase-catalyzed unwinding of damaged DNA substrates. Characterization of the effects of specific DNA adducts on unwinding by various DNA repair and replication helicases has proven to be insightful for understanding mechanistic and biological aspects of helicase function in cellular DNA metabolism.

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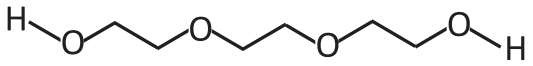
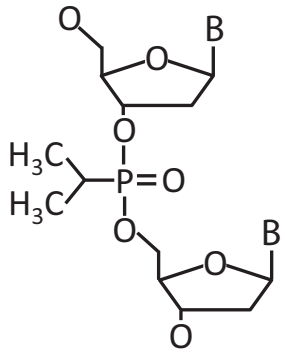
1. Introduction

Helicases involved in cellular replication, DNA repair, and transcription are likely to be among the first proteins to encounter a DNA lesion; moreover, helicase-dependent mechanisms are vital to how cells cope with endogenous or exogenous stress. Therefore, understanding how modifications to the base, sugar, or phosphate backbone of the DNA double helix affect helicase-catalyzed unwinding of the complementary strands will be insightful from both biochemical and biological viewpoints. In this review we will provide the readers a sense of the recent developments in understanding the consequences of helicase encounters with damaged DNA from an experimental perspective. An emphasis is placed on laboratory approaches that have been used for in vitro helicase studies with damaged DNA substrates so that the reader can appreciate what has been learned and some novel aspects of helicase interactions with unique forms of DNA damage that remain understudied. Although the focus of the published work has largely been on DNA unwinding measurements, future studies will likely address the effects of DNA adducts on translocation, branch-migration, and even strand annealing catalyzed by certain helicases or helicase-like proteins. With new discoveries pertaining to structural and biophysical properties of DNA helicases and their mechanisms of DNA unwinding, it seems probable that a comprehensive understanding of how helicases tolerate DNA damage will provide insights for how this specialized class of molecular motors behave in biological situations that evoke cellular responses to DNA damage and replication stress.

2. DNA Lesions and Their Effects on Helicases

Over the years, a number of laboratories including ours have investigated the effects of DNA damage on catalytic DNA unwinding performed by various purified recombinant DNA repair and replication helicases. These experiments assessed the ability of DNA helicase proteins to unwind a variety of site- and strand-specific DNA lesions located at defined positions in partial duplex DNA substrates. Such lesions are broadly categorized into two classes: 1) base modifications; 2) backbone modifications. This general classification of DNA lesions can be useful for investigating the importance of helicase contacts with the DNA substrate during single-stranded DNA translocation or duplex DNA unwinding. As discussed recently in a review from the Keck laboratory [1], crystal structures of DNA helicases from the conserved Superfamily (SF) 1 and SF2 along with mechanistic studies of helicase proteins have provided important insights to how they bind and unwind DNA. This has enabled researchers to conceptually visualize how structural elements of the helicase domain and other protein domains make dynamic contacts with the bases and sugar phosphate backbone to enact base-pair (bp) separation in a manner dependent on nucleoside triphosphate hydrolysis. Studying DNA modifications that affect the bases versus the backbone of DNA can be a useful approach for probing mechanistic aspects of helicase function. Moreover, a number of the DNA lesions tested for their effects on unwinding by helicases are biologically relevant as they potentially interfere with normal maintenance of chromosomal stability and genome homeostasis. In addition, recent developments in the area of helicase-catalyzed protein-DNA remodeling are likely to be relevant to events that occur in the metabolism of DNA-protein covalent complexes (DPC) or interstrand cross-links (ICL). In the following sections, we will highlight some insights gained in understanding the effects of DNA damage on helicase function, placing an emphasis on mechanistic aspects and biological pathways characterized by recent developments within the past several years. For a more historical perspective of earlier discoveries describing effects of DNA damage on helicase function, the reader is referred to several comprehensive review articles [2–4].

Table 1
Representative backbone modifications.

Name	Structure
Triethylene Glycol	
Alkyl Phosphotriester	

3. Backbone modifications

The large majority of biochemical studies with helicases and damaged nucleic acid substrates characterized by sugar phosphate backbone modifications have focused exclusively on synthetic non-natural types of linkages such as peptide-nucleic acid [5], polyvinyl [6,7], or polyglycol [8,9] insertions, or a methylphosphonate modification [10]. However, an emerging area of study is the biochemical and biological effects of backbone alterations arising from chemical genotoxins that can be found in the environment or are used in chemotherapy. Phosphotriester (PTE) adducts are recognized as a prominent class of such DNA lesions [11]. Thus from not only a mechanistic standpoint but also a health perspective, it is important to evaluate how helicases and other DNA metabolizing proteins are affected by chemical alterations to the DNA backbone.

3.1. Polyglycol phosphotriester linkage

A sugar phosphate backbone modification that has been particularly useful for probing the structural elements of a DNA substrate important for efficient helicase unwinding is the polyglycol linkage that can be synthetically incorporated into the backbone. A typical polyglycol linkage which we have used in our helicase studies is an 18-atom triethylene glycol linker that spans a distance of 3 bp in duplex DNA and 6 nucleotides (nt) in single-stranded DNA (Table 1). The polyglycol linkage neutralizes the negative charge characteristic of the phosphate groups within the backbone; there-