DNA interstrand cross-links from modified nucleotides: mechanism and application

Marc M. Greenberg
Johns Hopkins University, Department of Chemistry, 3400 N. Charles St., Baltimore, MD 21218, USA

ABSTRACT

Interstrand DNA cross-links are believed to be the source of cytotoxicity of antitumor agents such as mitomycin C and nitrogen mustards. We observed the first example in which DNA-DNA cross-links result from the reaction of a DNA radical, 5-(2’-Deoxuryridinyl)methyl radical (1) is produced during γ-irradiation of DNA and other methods of oxidative stress. Independent generation of this reactive intermediate in duplex DNA results in significant levels of interstrand cross-links. Cross-link formation does not require O2 and involves reaction between the nucleotide where the radical is originally generated and the opposing deoxyadenosine. Mechanistic studies have led to the identification of other molecules that produce interstrand DNA cross-links via 1. In addition, other methods for producing interstrand DNA cross-links via the radical precursor have been discovered that may be therapeutically useful.

INTRODUCTION

Interstrand DNA cross-links exert significant biological effects by preventing replication and transcription.(1) DNA cross-links are believed to be the source of the cytotoxicity of the antitumor agents mitomycin C and nitrogen mustards.(2,3) Recently, antitumor agents that damage DNA through radical processes (e.g., C-1027, neocarzinostatin) have also been observed to produce ISC’s.(4) In each instance DNA ISC formation is mediated by a molecule, which forms a covalent bond to each strand. Modified nucleotides have been incorporated into DNA that produce ISC’s by directly reacting as electrophiles with nucleotides on the opposing strand.(5,6) We describe efficient DNA ISC formation by the radical resulting from formal hydrogen atom abstraction from the thymine methyl group, 5-(2’-deoxuryridinyl)methyl radical (1). This is the first example in which formation of a DNA radical results in an interstrand cross-link directly.

RESULTS AND DISCUSSION

Product studies indicate that 5-(2’-deoxuryridinyl)methyl radical (1) is produced during γ-irradiation of DNA and other methods of oxidative stress.(7,8) Previous studies of 1 were carried out on the monomer and in single stranded oligonucleotides by independently generating it from photochemical precursors.(9,10) The analogous radical derived from 5-methyl-2’-deoxycytidine has also been studied.(11) These radicals form tandem lesions (interstrand cross-links) via addition to adjacent guanines. We were interested in studying the reactivity of 1 in duplex DNA and chose to use phenyl selenide 2 as a photochemical (350 nm) precursor.(12)

\[
\text{5-d(AGA TGG AC2 CAG GTA C)}
\]
\[
\text{3-d(TCT ACC TGA GTC CAT G)}
\]

3

The phenyl selenide was introduced at defined sites in oligonucleotides using standard oligonucleotide synthesis methods. To our surprise, photolysis (350 nm) of 5’-3’P-3 produced cross-linked material as the sole product detectable by denaturing gel electrophoresis (Figure 1).(13)

\[
\begin{align*}
\text{hv} & \quad + \quad + \\ 
\text{O}_2 & \quad + \quad - \quad + \\
& \quad \text{Cross-link}
\end{align*}
\]

\[
\text{16mer}
\]

Figure 1. Phosphorimage autoradiogram of denaturing PAGE analysis of the decomposition of 5’-3’P-3 (20 nM). Photolysis: (350 nm, 20 min)

Cross-link formation was independent of molecular oxygen, which is also surprising considering that a radical is believed to be responsible for cross-link formation. ESI-
MS analysis of the cross-linked duplex confirmed that O_2 was unnecessary. The cross-linked material consists of the elements resulting from 3 minus PhSeH (m/z = 9760.4). Hydroxyl radical cleavage revealed that the cross-link was between the nucleotide at which 1 was produced and the opposing dA. Phosphodiesterase degradation of the cross-linked material and analysis of the isolated material by ESI-MS, NMR, and UV absorption spectroscopy revealed that 4 was the ultimate product formed. However, molecular modeling suggested that 1 initially added to N1 of the opposing dA.

Subsequent studies on polymers containing 2 and other precursors for 1 have confirmed the radical nature of this process. Furthermore, mechanistic studies on 3 have brought to light biologically relevant methods by which 2 can be used as a tool for producing DNA interstrand cross-links.

CONCLUSION

Independent generation of a nucleotide radical in DNA has provided the first example of interstrand cross-linking in DNA via a radical process that does not involve any exogenous reagents. The process is independent of O_2, which suggests it could be useful in hypoxic tumor cells.

ACKNOWLEDGEMENTS

We are grateful for financial support from the National Institutes of Health (GM-054996).

REFERENCES