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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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A Radical View of Genomic Damage: DNA Peroxidation

by

Michael J. Hitchler

Department of Radiation Oncology
Department of Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

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ABREVIATIONS

6-TOOH	*cis*-5,6-dihydroperoxy-5-hydroxythymine
DNA	Deoxyribonucleic acid
DNA-OOH	DNA peroxide
GPx	Glutathione peroxidase
GSH	Glutathione
$HO^{\bullet}$	Hydroxyl radical
LC/MS	Liquid chromatography mass spectrometry
PHGPx	Phospholipid hydroperoxide glutathione peroxidase
ROOH	Hydroperoxide
RT	Retention time
T-OOH	Thymine peroxide

OUTLINE

i. ABSTRACT

Free radicals can damage an organism's genetic information causing mutations. DNA hydroperoxides represent one type of free radical damage cells face after exposure to biological stresses such as X-irradiation. Their presence in the genome, or nucleotide pool is readily detectable using both biological and analytical techniques. Understanding the chemistry behind how these damages occur, can be detected, and repaired is the focus of this review.

1 INTRODUCTION

1.1 Overview of genomic distress

Genome maintenance is essential for an organism's survival. The world is a dangerous place in which our cells are exposed to a wide variety of genotoxic stresses. Theses stresses can arise from the environment, or be generated during normal cell processes. If left unchecked, they lead to the accumulation of deleterious mutations at genetic and epigenetic levels that could eventually lead to neoplastic transformation, or cell death. Lucky for us evolution has fostered the development of mechanisms capable of detecting and repairing damages that are the consequence of being exposed to such stresses.

1.2 Peroxidation of genetic information, DNA and chromatin take center stage

Whether during normal metabolism or outside sources such as X-rays, free radical production commonly leads to the generation of hydroperoxides (R-OOH) though reactive hydroxyl radicals ($HO^{\bullet}$) [1]. The direct hydroperoxidation of DNA nucleotide bases is the most prominent example of damage to genetic information. However, in recent years it has become increasingly apparent that modifications to proteins composing the histone core octomer can influence local chromatin structure and hence DNA function. Also since DNA and histone proteins are in very close proximity, any hydroperoxide formed on the histones will likely initiate the oxidation of DNA. These chemical changes to the DNA can form sequence mutations that can be passed on to progeny [2, 3]. Understanding the chemistry behind how these damages occur, can be detected, and repaired is the focus of this review.

2 DETECTION AND MEASUREMENT

2.1 Quantifying biological effect

One of the simplest ways to quantify the effect peroxidation has on DNA is to determine the rate

at which it causes mutations. A simple and yet elegant experiment put forth by Thomas and collaborators in 1976 effectively accomplishes this [4]. This experiment works by inducing deleterious mutations to the DNA sequence that encodes a selectable marker, such as streptomycin. These mutations then negate the function of the resistance gene and hence cell growth in the presence of the selecting agent. Mutations can then be quantified by transforming treated DNA into bacteria and counting the change in the number of colonies appearing on selectable media. For the purposes of detecting what effects peroxidation might have on DNA, *cis*-5,6-dihydroperoxy-5-hydroxythymine (6-TOOH) is mixed with DNA harboring a wild type streptomycin resistance gene. Mutational frequency can then be determined by dividing the number of colonies present on plates from treated samples, by that of the untreated control. Later the overall results can be visualized by generating a plot of mutation frequency versus treatment time at different 6-TOOH concentrations similar to that shown in **Figure 2.1** [4].

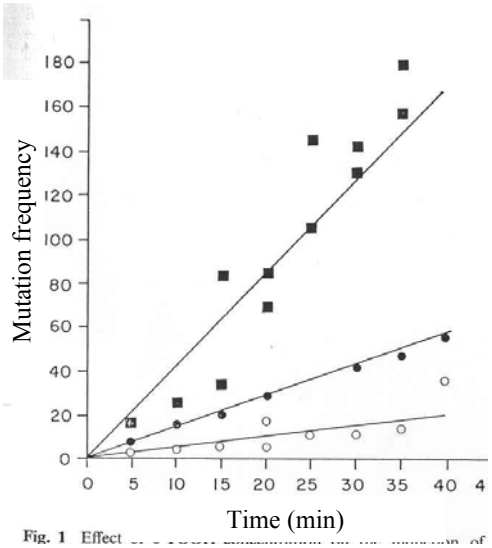

Figure 2.1: Determining the effect of 6-TOOH concentration on the introduction of mutations in DNA. Experimental reactions contained approximately 10 μg of DNA treated for varying times with one of three concentrations of 6-TOOH (o, 11.3 mM; •, 22.6 mM; ■, 45 mM). The mutational frequency was calculated as plotted versus treatment time. [4]

2.2 Analytical techniques

An analytical method to directly detect and quantify peroxidation of DNA is through the use of liquid chromatography/mass spectrometry (LC/MS) [1]. Isolated DNA from experiments can be enzymatically cleaved to form single nucleotides. Initial separation of the nucleotide mixture is achieved by liquid chromatography, a process that separates molecules based on their affinity for

a column matrix. In simple liquid chromatography, eluted compounds can be detected by a UV-Visible light detector after their elution from the column. Using the retention time of synthetic standards the identity of the compounds can then be determined. However, in LC/MS the UV-Visible detector is replaced with a mass spectrometer. Quadrupole mass spectrometers are suitable for this application based on their compact size, and ability to accommodate flow injection form LC. This is a great improvement because it allows for the identification of compounds based on two properties, retention time (RT) and mass charge ratio (m/z^+). A representative elution profile is shown in **Figure 2.2** [1]. Notice quantification based on mass spectrometry 'counts" on the ordinate, and the retention time given on the abscissa, both giving a unique fingerprint to identify the modified bases present. Quantification of specific base damages in a sample can be determined using standard addition to the unknown sample and generating a subsequent standard addition curve.

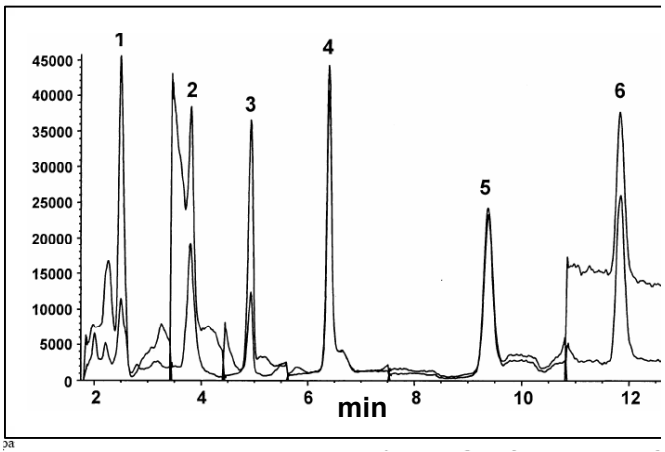

Figure 2.2: Separation and identification of six modified DNA nucleosides by LC/MS. Signals represent the ion-current profiles of the typical ions of six modified nucleosides and their stable isotope-labeled analogues. **Peak 1**: m/z 271 [MH^+ of (5'R)-8,5'-cdGuo-15N5] and m/z 266 [MH^+ of (5'R)-8,5'-cdGuo]; **Peak 2**: m/z 250 [MH^+ of (5'R)-8,5'-cdAdo] and m/z 265 [MH^+ of (5'R)-8,5'-cdAdo-13C10-15N5]; **Peak 3**: m/z 271 [MH^+ of (5'S)-8,5'-cdGuo-15N5] and m/z 266 [MH^+ of (5'S)-8,5'-cdGuo]; **Peak 4**: m/z 168 ($BH2^+$ of 8-OH-dGuo) and m/z 170 ($BH2^+$ of 8-OH-dGuo-18O); **Peak 5**: m/z 250 [MH+ of (5'S)-8,5'-cdAdo] and m/z 265 [MH^+ of (5'S)-8,5'-cdAdo-13C10-15N5]; **Peak 6**: m/z 152 ($BH2^+$ of 8-OH-dAdo) and m/z 162 ($BH2^+$ of 8-OH-dAdo-13C10-15N5). [1]

3 DNA PEROXIDE CHEMISTRY

3.1 Formation of DNA-OOH

Most DNA peroxidation is the result of DNA being exposed to ionizing radiation. Irradiation

with X-rays generates $HO^{\bullet}$, which can directly attack bases like thymine. Most commonly these chemical modifications are found on pyrimidine bases and result in the compounds shown in **Figure 3.1** [4, 5, 6, 7]. The addition of peroxide can generate a *cis* or *trans* configuration with respect to the methyl branch already present on the ring structure. These four modified forms of thymine can be further characterized by their unique ability to react with iodine. **Table 3.1** shows the rate constants of each compound reacting with iodine as determined by Scholes and Weiss in 1960 [7]. Clearly this chemical difference is attributed to the structural placement of the peroxide modification. Work by Christophersen in 1969 demonstrated that X-rays induced DNA damage is reversible by a glutathione utilizing enzyme [5]. They also reported that the reactions to reverse DNA peroxidation damage occur at an intrinsically low rate.

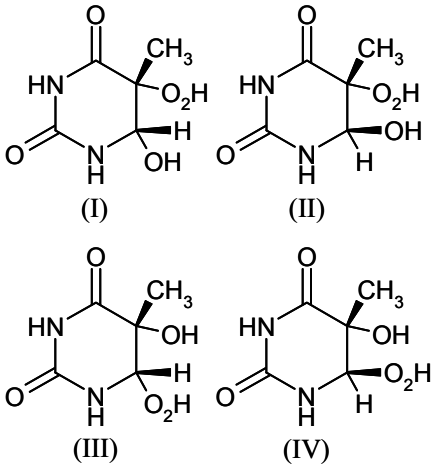

Figure 3.1: Hydroperoxide products generated from thymine after attack from $HO^{\bullet}$. 4-Hydroxy, 5-hydroperoxy thymine (I and II) 4-Hydroperoxy, 5-hydroxy thymine (III and IV) [7]

3.2 Position effect

A report by Wang and Parkhill demonstrated that thymine hydroperoxides (T-OOH) could react with normal nucleotide bases while in a DNA polymer [6]. Adenine and uracil both appeared to be unreactive towards T-OOH, while cytosine, guanine and thymine in the DNA backbone were highly reactive resulting in the formation of several unique modifications [6]. Thus, this illustrates a position effect of hydroperoxide damage to DNA, where neighboring bases can be modified after the formation of peroxides. Thomas and

Table 3.1: Rate constants for the conversation of 4-Hydroxy, 5-hydroperoxy thymine and 4-Hydroperoxy, 5-hydroxy thymine by Iodine. [7]

Compound	Rate constant (M^{-1} S^{-1})
Hydroperoxide by radiation of thymine	1.59
4-hydroxy,5-hydroperoxy thymine (I, II)	1.60
4-hydroperoxy,5-hydroxy thymine (III, IV)	7.53
Hydrogen peroxide	1.13×10^{-2}

others also demonstrated this principle by showing T-OOH could induce mutations in *H. influenzae*. They showed that the reaction was both T-OOH concentration dependent, and required the presence of a metal for it to convert a neighboring nucleotide [4]. Their work also demonstrates that hydroperoxidated nucleotides in the dNTP pool can induce mutations even if they are not subsequently incorporated into the genome.

3.3 Histone protein changes

Histone octomers form the protein core of nucleosomes, which allow for efficient packaging of DNA in eukaryotic nuclei. Two of each of the major histone proteins (H2A, H2B, H3 and H4) and one H1 histone make up this structure which resides in close contact with the DNA they package [2, 3]. Work from the Michael Davies group at the Heart Research Institute has demonstrated that histone protein peroxides can chemically modify nucleotide bases [2, 3]. They studied the effect hydroperoxides of H1 and the other histone core proteins have on DNA. This was accomplished by measuring how readily the common DNA oxidation product 8-oxo-guanosine was formed when either DNA or 2-deoxy-guanosine were mixed with the various histone hydroperoxides. Moreover they also demonstrated a position effect. DNA mixed with the histone hydroperoxides yielded a higher production of 8-oxo-guanosine formation over free 2-deoxy-guanosine [2, 3]. They speculate that this difference can be attributed to DNA strands being attracted to the histones and therefore increase the local concentration of protein and DNA. Alternatively the free nucleotide is not tightly associated with the histones and results in slower reaction kinetics between 2-deoxy-guanosine and the protein peroxides.

4 BIOCHEMICAL ASPECTS

4.1 Repair by glutathione peroxidases

The peroxidation of DNA is not a terminal event. Early work by Christophersen demonstrated that thymine hydroperoxides could be reversed by a glutathione peroxidase. His experiments reveled that a glutathione (GSH) utilizing enzyme could reverse hydroperoxide damage of DNA in solution after X-irradiation [5]. Although he referred to the enzyme as glutathione peroxidase (GPx), he could not be sure which form of the enzyme was responsible for this repair. However, he could determine that two molar equivalents of GSH are required to reverse one mol of thymine hydroperoxide [5]. Later studies have demonstrated that multiple GPx enzymes have specificity for DNA-OOH. About 30 years after Christophersen, Boa and coworkers demonstrated that phospholipid hydroperoxide glutathione peroxidase (PHGPx), has the ability to reduce thymine hydroperoxide to 5-hydroxymethyluracil (**Figure 4.1**). Most useful from their study is the activity series they determined that demonstrates the differences in activity among GPx's and glutathione transferases (**Table 4.1**). Clearly, DNA hydroperoxides are the substrate of many different detoxification enzymes.

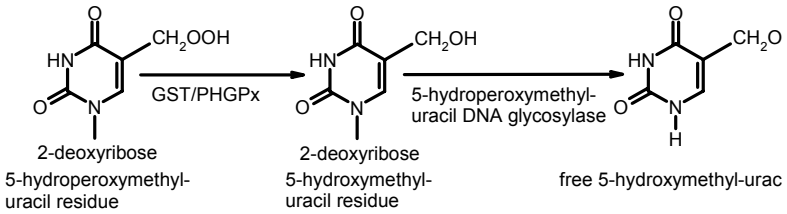

Figure 4.1: Catalytic reactions carried out by glutathione peroxidase and glutathione transferase during repair of DNA hydroperoxides. [9]

Table 4.1: Glutatione peroxidase activities of GPx, GST's and PHGPx on T-OOH. [9]

Enzyme	Specific Activity[a]
Se-PHGPx	461 ± 24
Se-GPx	n.d.
hGST A1-1	0.007 ± 0.001
hGST A2-2	0.008 ± 0.003
hGST A4-4	n.d.
hGST M1-1	n.d.
hGST P1-1	0.011 ± 0.001
hGST T1-1	0.009 ± 0.003
rGST T2-2	0.120 ± 0.022

[a.] μmol min-1 per mg protein

4.2 Glutathione transferase repair

It has been demonstrated that glutathione transferases have some peroxidase activity. Kettereer and Meyer showed a nuclear located GSH transferase can detoxify thymine hydroperoxides [8]. This would be crucial for DNA repair following genotoxic stress. Even more recently the

aforementioned study by Boa and coworkers showed good evidence to support this. During their study they measured the specific activity of purified GPx's and GSH transferase enzymes to reduce T-OOH (**Table 4.1**) [9]. These studies demonstrate that DNA peroxides are the target of several repair pathways. This is expected since maintenance of the genome would be of paramount importance to cell survival.

5 SUMMARY

This review has described the chemistry of DNA-OOH formation and how it is repaired by cellular mechanisms. It is commonly believed that DNA damage by free radicals can lead to the accumulation of mutations, and eventually neoplastic transformation of cells. DNA peroxides seem to be a prime candidate for this given the simple nature of chemistry required to generate them. We have seen though the discussion of DNA-OOH detection techniques that studies to investigate their mutagenic role can be readily carried out. Moreover I have discussed that they are a target for repair by several mechanisms. This would in turn allow us to measure the required enzymatic activity of glutathione peroxidases and transferases needed to delay their progression into deleterious mutations. Although DNA peroxides have not received much recent attention in the literature, it remains clear that they exert an influence on genomic stability and thus creates a topic of further study.

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