OXIDATIVE LESIONS

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HOT SPOTS FOR RADICAL ATTACK
BASE PRODUCTS OF OXIDATIVE DAMAGE

5-Hydroxy-6-hydrothymine
Thymine glycol (cis and trans)
5,6-Dihydrothymine

Cytosine glycol
5-Hydroxycytosine
5,6-hydroxy uracil
5-hydroxy-5-methylhydantoin
8-Hydroxyadenine
8-Hydroxyguanine
5-hydroxyhydantoin
5-hydroxymethyluracil
5-hydroxy-6-hydouracil
5-hydroxyuracil
4,6-Diamino-5-formamidopyrimidine
2,6-Diamino-4-hydroxy-5-formamidopyrimidine
8,5'-Cyclo-2'-deoxyadenosine
8,5'-Cyclo-2'-deoxyguanosine
8-oxo-dG

oxazolone

4-OH-8-oxo-dG

8-oxo-dA
OXIDATIVE SPECIES

\( O_2^+ + HOCl \rightarrow HO^+ + Cl^- + O_2 \) \hspace{1cm} (1)

\( O_2^+ + \cdot NO + H^+ \rightarrow ONOOH \rightarrow \cdot NO_2 + HO^+ \) \hspace{1cm} (2)

\[ \text{Fe(III) + Red}^n \rightarrow \text{Fe(II) + Red}^{(n+1)} \]

\[ O_2^+ + HO_2^+ + H^+ \rightarrow H_2O_2 + O_2 \]

\[ \text{Fe(II) + H}_2O_2 \rightarrow HO^+ + \text{Fe(III)} + OH^- \]
METHODS FOR DETECTION OF OXIDATION OF DNA

1. GC/MS (3)
   a. Advantage: detects the majority of products of oxidative damage
   b. Disadvantage: requires derivatization before analysis which can artifactually inflate the amount of oxidative damage observed; requires expensive instrumentation

2. HPLC-EC (4)
   a. Advantage: very sensitive and accurate technique; requires less expensive equipment than GC/MS
   b. Disadvantage: can only detect electrochemically active compounds (8-oxodG, 5-OHdCyd, 5-OHdUrd, 8-oxodA)

3. Immunoaffinity Isolation with Detection by ELISA or HPLC-EC (5)
   a. Advantage: can be used for concentration of oxidized bases from dilute solutions (urine, culture medium) for quantitation
   b. Disadvantage: confines analysis to only one modified base at a time
4. Postlabelling (6)

\[^{3}\text{H}]\text{acetic anhydride, enzymatic }^{32}\text{P, dansyl chloride}\]

a. Advantage: requires very little DNA and has at least an order of magnitude more sensitivity than some of the other techniques

b. Disadvantage: presence of significant background from cellular processes and analytical work-up

5. DNA Strand Breaks

Nick translation, alkaline elution, alkaline unwinding, supercoiled gel mobility shift, comet assay

a. Advantage: can be very sensitive

b. Disadvantage: when viewing in whole cells, not always specific for oxidative lesions; may indicate DNA repair

6. DNA Repair Assays

Formamidopyrimidine glycosylase, endonuclease followed by strand break assay

a. Advantage: sensitive measure of specific types of damage

b. Disadvantage: limited to the types of damage for which repair enzymes have been identified
PITFALLS OF ASSAY TECHNIQUES

1. Isolation of DNA
   a. Phenol extraction: breakdown products of phenol can introduce oxidative damage to DNA as it is isolated
   b. Contamination by metals (Fe)
      Photochemical reduction leads to DNA oxidation

2. Derivatization of DNA for GC/MS
   a. Isolation of DNA
   b. Acid hydrolysis
   c. Silylation

REPAIR OF OXIDATIVE DAMAGE
Reviewed in (7)

1. DNA glycosylases
   a. Simple glycosylases: hydrolyze N-glycosylic bonds between damaged or inappropriate base and deoxyribose sugar to release the base and produce an unmodified AP site
   b. Not only hydrolyzes N-glycosylic bonds, but also contains lyase activity that cleaves resulting AP sites, producing 3’-terminal , 3-unsaturated aldehyde, which requires processing by enzymes that remove the blocking group

2. AP endonucleases
   a. Class I
   b. Class II
3. **Endonuclease III**
   Acts as glycosylase, removing modified base, and AP endonuclease, leaving 3'-termini, requiring processing by exonuclease III or endonuclease IV

4. **Formamidopyrimidine-DNA glycosylase (FAPy glycosylase)**
   Releases damaged bases (ruptured purine rings, 8-oxodG, 8-oxodA) and, like endonuclease III, incises AP sites by $\exists$-elimination

5. **Exonuclease III (3' 5')**
   Cleaves 5' to AP site

6. **Endonuclease IV**
   Removes 3' blocking damage and attacks AP sites

7. **Nucleotide excision repair**
   Removal of a larger section of DNA surrounding the damaged base, followed by repair synthesis and ligation

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**MUTAGENESIS**

1. **Base substitutions** *(8-10)*

2. **Deletions** *(10-13)*
REFERENCES


