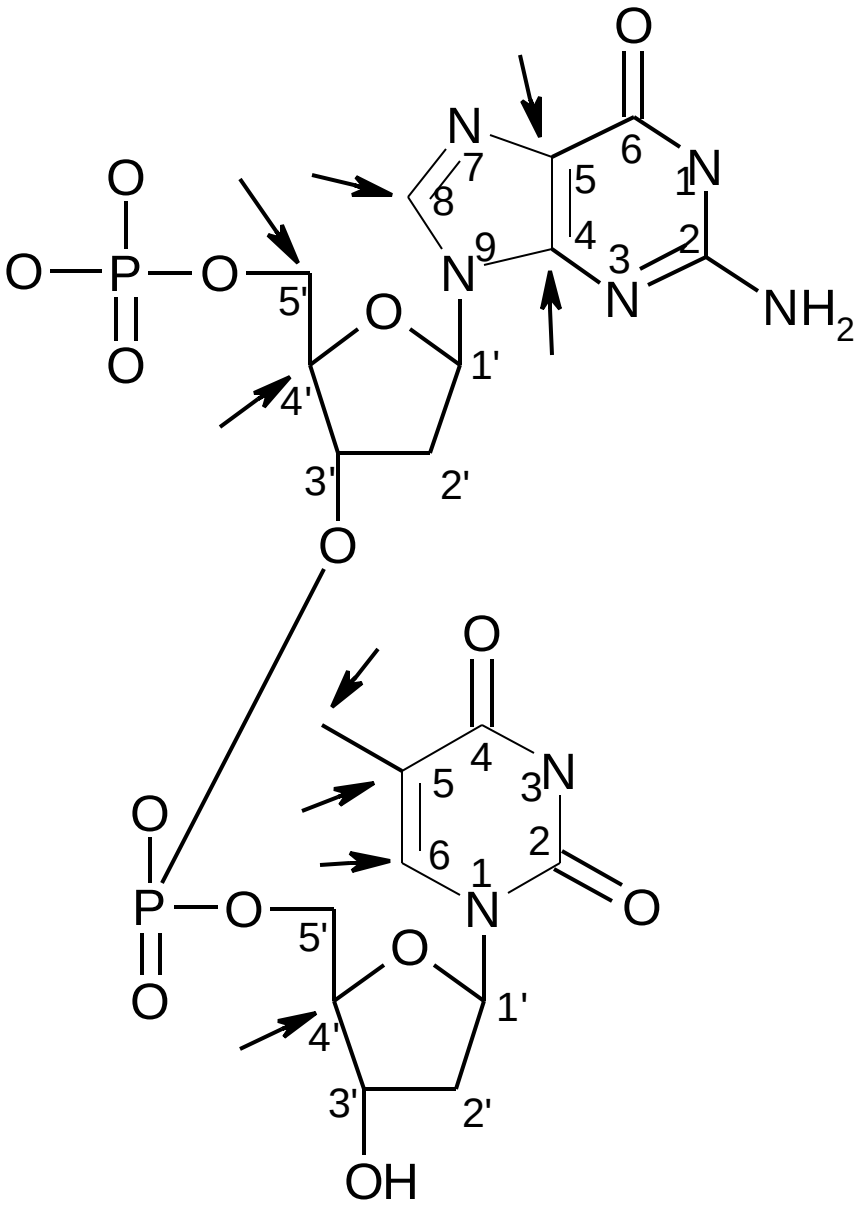
OXIDATIVE LESIONS

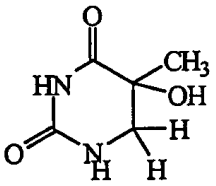
Ann E. Aust, Ph. D.

Department of Chemistry and Biochemistry
Utah State University
Logan, UT 84322-0300
Tel: 435/797-1629
FAX: 435/797-3390
Email: AAUST@cc.usu.edu

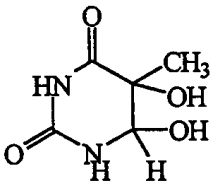
HOT SPOTS FOR RADICAL ATTACK



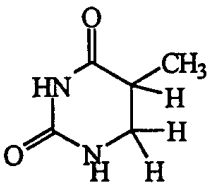
BASE PRODUCTS OF OXIDATIVE DAMAGE



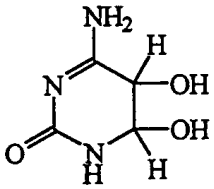
5-Hydroxy-6-hydrothymine



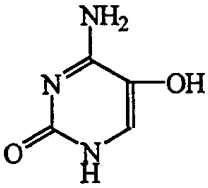
Thymine glycol (cis and trans)



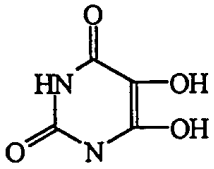
5,6-Dihydrothymine



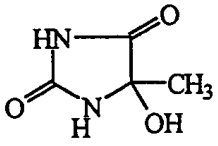
Cytosine glycol



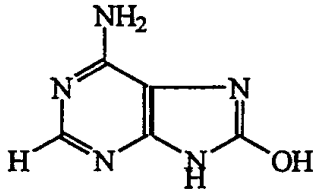
5-Hydroxycytosine



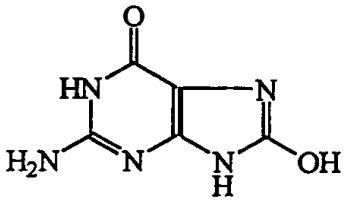
5,6-hydroxy uracil



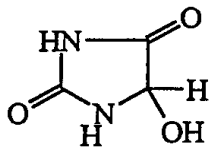
5-hydroxy-5-methylhydantoin



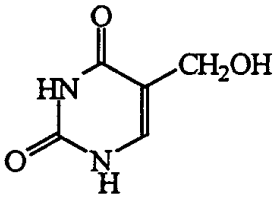
8-Hydroxyadenine



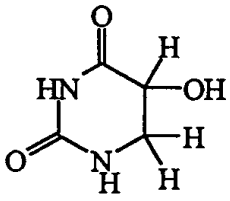
8-Hydroxyguanine



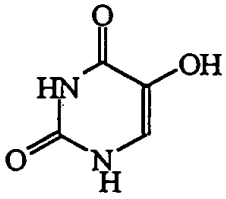
5-hydroxy hydantoin



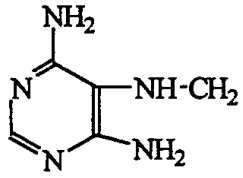
5-hydroxymethyluracil



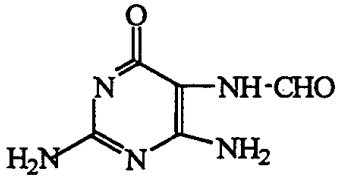
5-hydroxy-6-hydrouracil



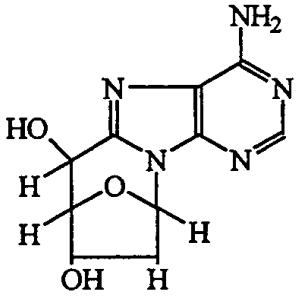
5-hydroxyuracil



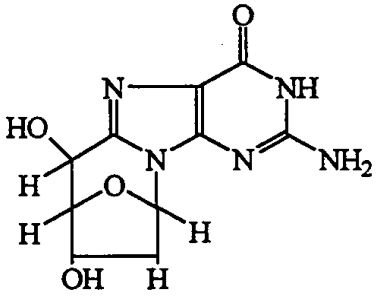
4,6-Diamino-5-formamidopyrimidine



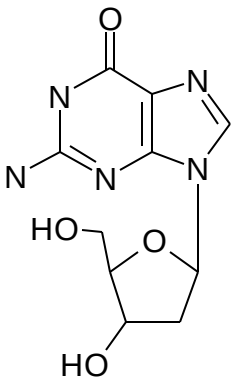
2,6-Diamino-4-hydroxy-5-formamidopyrimidine



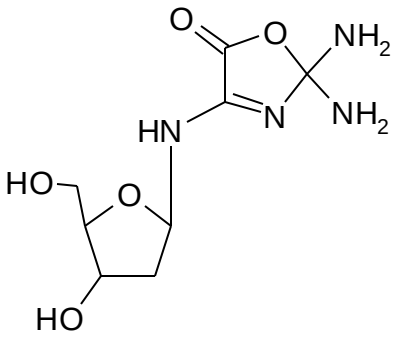
8,5'-Cyclo-2'-deoxyadenosine



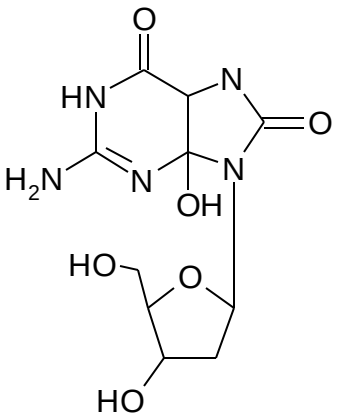
8,5'-Cyclo-2'-deoxyguanosine



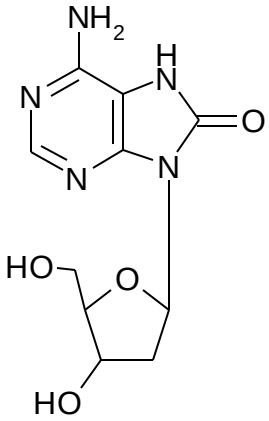
8-oxo-dG



oxazolone



4-OH-8-oxo-dG



8-oxo-dA

OXIDATIVE SPECIES

$$O_2^{\bullet -} + HOCl \rightarrow \mathbf{HO^{\bullet}} + Cl^- + O_2 \quad (1)$$

$$O_2^{\bullet -} + {}^{\bullet}NO + H^+ \rightarrow ONOOH \rightarrow \text{“}{}^{\bullet}NO_2 + \mathbf{HO^{\bullet}}\text{ “} \quad (2)$$

$$Fe(III) + Red^n \rightarrow Fe(II) + Red^{(n+1)}$$

$$O_2^{\bullet -} + HO_2^{\bullet -} + H^+ \rightarrow H_2O_2 + O_2$$

$$Fe(II) + H_2O_2 \rightarrow \mathbf{HO^{\bullet}} + Fe(III) + OH^-$$

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}H]acetic anhydride, enzymatic ^{32}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

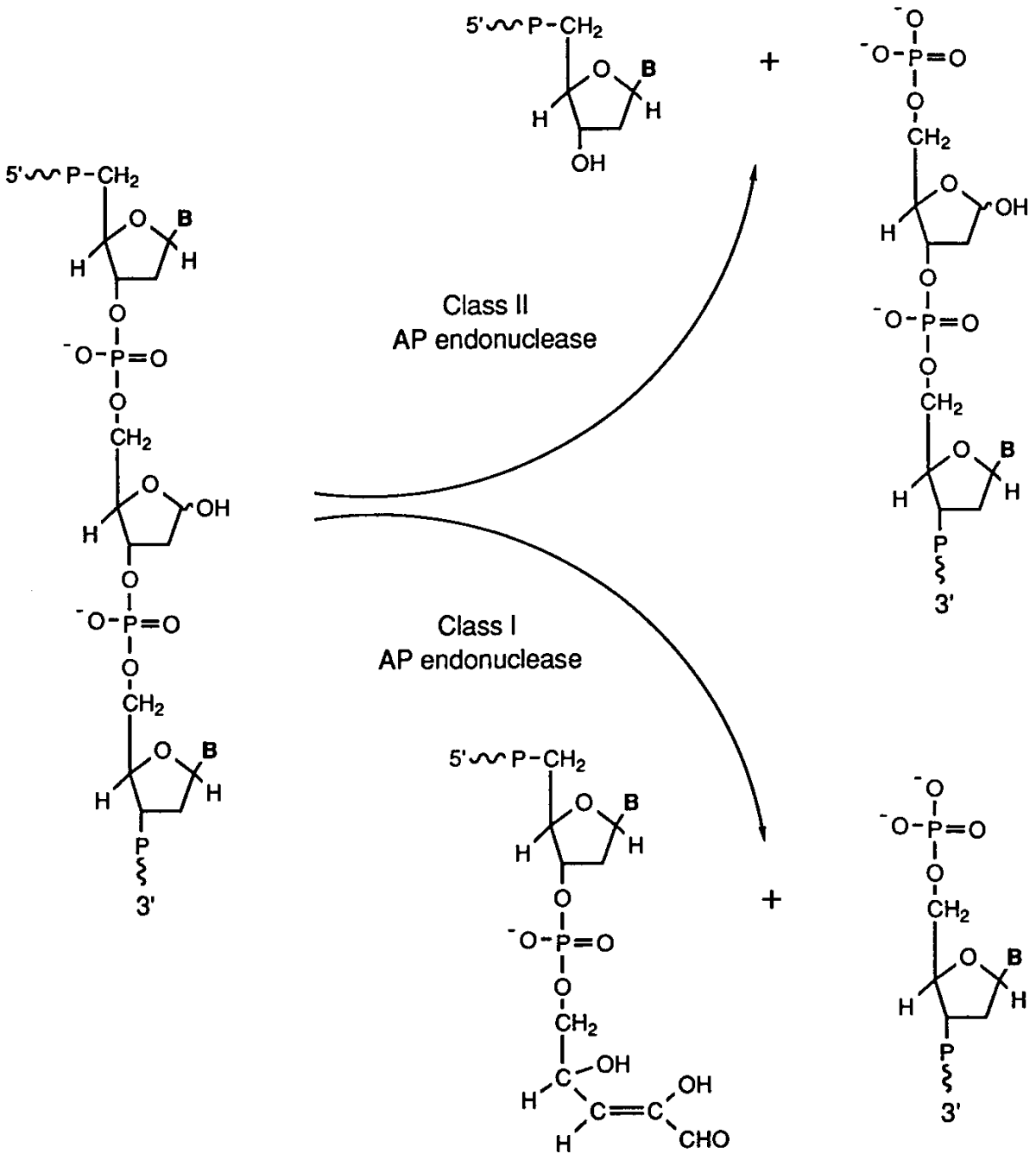
2. Derivatization of DNA for GC/MS
   a. Isolation of DNA
   b. Acid hydrolysis
   c. Silylation

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 , ∃-unsaturated aldehyde, which requires procesing by enzymes that remove the blocking group

2. AP endonucleases
   a. Class I
   b. Class II


Class II
AP endonuclease
Class I
AP endonuclease

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 ∃-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

MUTAGENESIS

1. Base substitutions (*8-10*)

2. Deletions (*10-13*)

REFERENCES

1. Candeias, L. P., Patel, K. B., Stratford, M. R. L., and Wardman, P. (1992) Free hydroxyl radicals are formed in reaction between the neutrophil-derived species superoxide anion and hypochlorus acid. *FEBS Lett.* **1**, 151-153.
2. Beckman, J. S., Beckman, T. W., Chen, J., and Marshall, P. A. (1990) Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA* **87**, 1620-1624.
3. Diadaroglu, M. (1991) Chemical determination of free radical-induced damage to DNA. *Free Rad. Biol. Med.* **10**, 225-242.
4. Floyd, R. A., Watson, J. J., Wong, P. K., altmiller, D. H., and Rickard, R. C. (1986) Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanism of formation. *Free Rad. Res. Commun.* **1**, 163-172.
5. Park, E.-M., Shigenaga, M. K., Degan, P., Korn, T. S., Kitzler, J. W., Wehr, C. M., Kolachana, P., and Ames, B. N. (1992) Assay of excised oxidative DNA lesions: Isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc. Natl. Acad. Sci. USA* **89**.
6. Cadet, J., Odin, F., Mouret, J.-F., Polverelli, M., Audic, A., Giacomoni, P., Favier, A., and Richard, M.-J. (1992) Chemical and biochemical postlabeling methods for singling out specific oxidative DNA lesions. *Mutat. Res.* **275**, 343-354.
7. Ramotar, D., and Demple, B. (1993) Enzymes that repair oxidative damage to DNA. In *DNA and Free Radicals* (Halliwell, B., and Aruoma, O. I., Eds.) pp 165-191, Ellis Horwood Limited, Chichester, England.
8. Wood, M. L., Dizdaroglu, M., Gajewski, E., and Essigmann, J. M. (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hdroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry* **29**, 7024-7032.
9. Reid, T. M., and Loeb, L. A. (1992) Mutagenic specificity of oxygen radicals produced by human leukemia cells. *Cancer Res.* **52**, 1082-1086.
10. Juedes, M. J., and Wogan, G. N. (1996) Peroxynitrite-induced mutation spectra of pSP189 following replication in bacteria and in human cells. *Mutat. Res.* **349**, 51-61.
11. Klein, C. B., Su, L., Rossman, T. G., and Snow, E. T. (1994) Transgenic *gpt*$^+$ V79 cell lines differ in their mutagenic response to clastogens. *Mutat. Res.* **304**, 217-228.
12. Hei, T. K., Piao, C. Q., He, Z. Y., Vannais, D., and Waldren, C. A. (1992) Chrysotile fiber is a strong mutagen in mammalian cells. *Cancer Res.* **52**, 6305-6309.
13. Park, S.-H., and Aust, A. E. (1998) Participation of iron and nitric oxide in the mutagenicity of asbestos in *hgprt*$^-$, *gpt*$^+$ chinese hamster V79 cells. *Cancer Res.* **58**, 1144-1148.