POLITECNICO DI MILANO

Department CMIC

Lecture 12 – FR12





Free-Radicals: Chemistry and Biology

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http://iscamap.chem.polimi.it/citterio/education/free-radical-chemistry/

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Radicals involving DNA and Proteins

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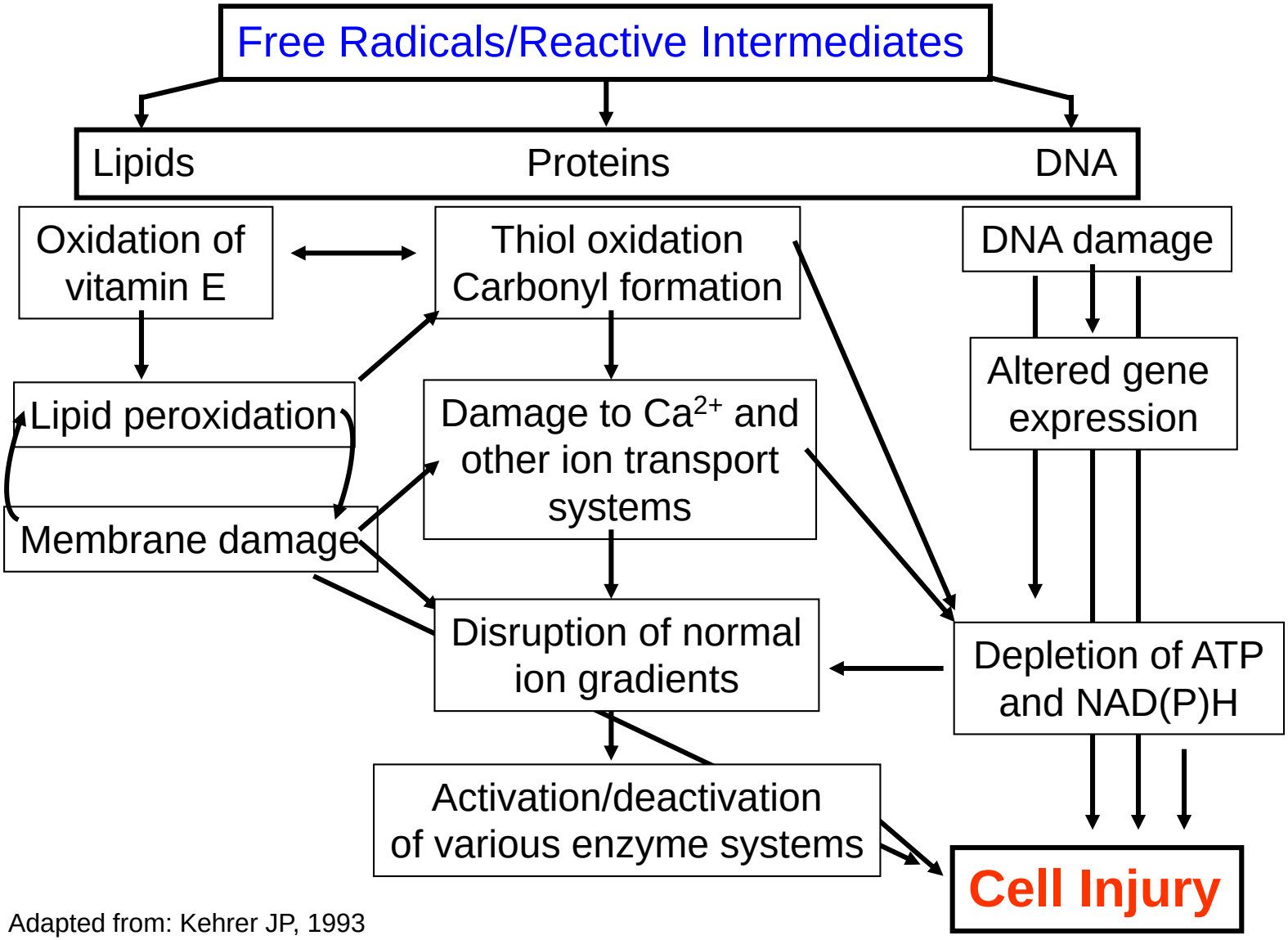
From Radicals to Cell Injury



Adapted from: Kehrer JP, 1993

Why Focus on Proteins ?

1. **Concentration of target**

   - **Proteins are major components of most biological systems:**
   - **Organ level (liver, per kg wet weight): 146 g protein, 2.6 g DNA, 49 g total lipid, 3.9 g cholesterol.**
   - **Cellular level (per 1012 leukocytes): 100 g protein, 6.9 g DNA, 8.2 g RNA, 15.6 g total lipid, 2 g cholesterol.**
   - **Plasma (per dm^3):**

     73 g protein, 0.4 g free amino acids, 0.5 g total lipid, 1 g carbohydrates, 1.5 - 2.5 g cholesterol.
   - **Low-density lipoproteins (molecules per particle):**

     1 protein (4535 amino acids), 1600 cholesterol esters, 700 phospholipids, 600 free cholesterol, 26 free fatty acids, 9 tocopherol.

Why Focus on Proteins ?

2. Rate constants for reaction

Rate constants for reaction of $HO^{\bullet}$ with macromolecules:

DNA	8×10^{8} $dm^3 \cdot mol^{-1} \cdot s^{-1}$
RNA	1×10^{9} $dm^3 \cdot mol^{-1} \cdot s^{-1}$
Hyaluronan	7×10^{8} $dm^3 \cdot mol^{-1} \cdot s^{-1}$
Linoleic acid	9×10^{9} $dm^3 \cdot mol^{-1} \cdot s^{-1}$
Collagen	4×10^{11} $dm^3 \cdot mol^{-1} \cdot s^{-1}$
Albumin	8×10^{10} $dm^3 \cdot mol^{-1} \cdot s^{-1}$

Antioxidants:

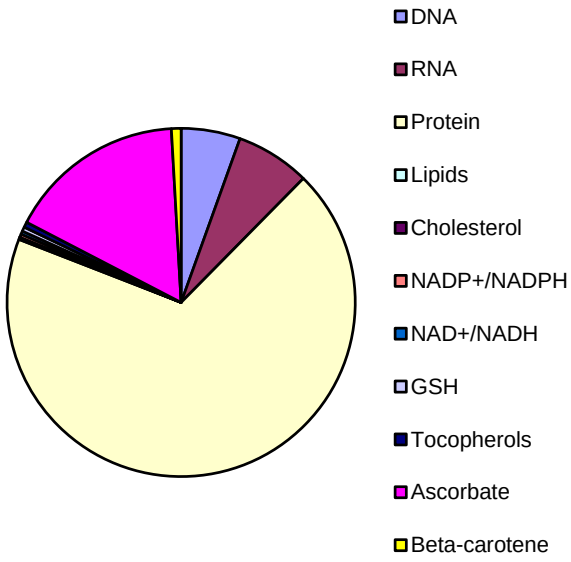
Ascorbate	1×10^{10} $dm^3 \cdot mol^{-1} \cdot s^{-1}$,
GSH	1.4×10^{10} $dm^3 \cdot mol^{-1} \cdot s^{-1}$,
Trolox C	6.9×10^{9} $dm^3 \cdot mol^{-1} \cdot s^{-1}$

Why Concentrate on Proteins ?

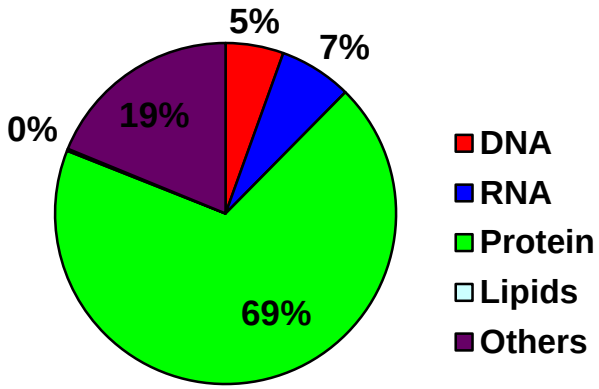
Kinetic and abundance data can be used to predict sites of damage.

For leukocytes:

Singlet oxygen



$HO^{\bullet}$



Such data needs to be treated with great caution !

Protein versus DNA versus Lipid Oxidation

- **Medline / Pubmed searches**
  - **DNA oxidation - 8894**
  - **Lipid peroxidation - 26041**
  - **Protein oxidation - 5463**

- **Oxidation of proteins studied to a much lesser extent than other targets**

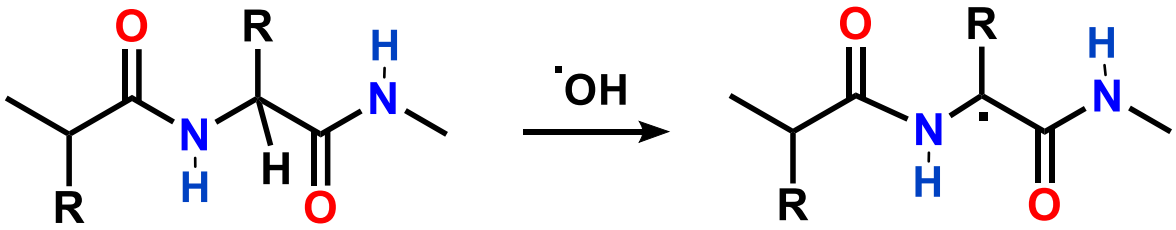
First volume of J. Biol. Chem.

Dakin, H.D. (1906) "The oxidation of amino-acids with the production of substances of biological importance" J. Biol. Chem., 1, 171-176.

Follow-up papers in 1908, occupied approximately half of the total page-count of J. Biol. Chem. for the entire year.

Sites of Oxidant Damage on Proteins

- **Backbone - primarily hydrogen atom abstraction at alpha carbon**



- can result in backbone fragmentation

- **Side-chains - 20 different types (excluding unusual amino acids and any post-translational modifications).**
  - **hydrogen abstraction - primarily with aliphatic**
  - **addition - primarily with aromatic**

- usually results in the formation of altered side-chains

Chem Rev, 1987, 87, 381-398; Free Rad Biol Med, 1990, 9, 315-325; J Biol Chem, 1987, 262, 9895-9920

Selectivity of Damage by Different Oxidants

- **The most reactive radicals tend to be the least selective**

  e.g. $HO^{\bullet}$ - difference in rate constants is relatively small.

  Most reactive: Trp, Tyr, His, Met, Cys, Phe, Arg: $k \approx 10^{10}\ dm^3 \cdot mol^{-1} \cdot s^{-1}$

  Least reactive: Ala, Asp, Asn: $k \approx 10^7 - 10^8\ dm^3 \cdot mol^{-1} \cdot s^{-1}$

  Result - most side-chains are oxidised

  Reaction with backbone sites $k \approx 10^9\ dm^3 \cdot mol^{-1} \cdot s^{-1}$

  Significant backbone fragmentation as well as side-chain oxidation

- **Less reactive radicals tend to be more selective**

  e.g. $CCl_3OO^{\bullet}$ - difference in value of rate constants between most reactive side-chain (Trp $k \approx 9 \times 10^7\ dm^3 \cdot mol^{-1} \cdot s^{-1}$) and least (aliphatic side-chains - no measurable reaction) very large.

- **Many radicals are electron-deficient (electrophilic) and hence react most rapidly with electron-rich side-chains (Trp, Tyr, His, Met, Cys, Phe). Few nucleophilic oxidants (e^-, $Ph^{\bullet}$, $CO_2^{\bullet -}$).**

Sources of Kinetic and Associated Data

Compilations of kinetic data:

$HO^{\bullet}$ and $H^{\bullet}$	**J. Phys. Chem. Ref. Data, 1988, 17, 513-886**
$HOO^{\bullet}$ / $O_2^{\bullet-}$	**J. Phys. Chem. Ref. Data, 1988, 17, 1027-1284**
Inorganic radicals (*e.g.* $NO_2^{\bullet}$, $CO_2^{\bullet-}$, $CO_3^{\bullet-}$, $Br_2^{\bullet-}$, $N_3^{\bullet}$)	**J. Phys. Chem. Ref. Data, , 1990, 19, 1027-1284**
$ROO^{\bullet}$	**J. Phys. Chem. Ref. Data, 1990, 19, 413-513**
$^1O_2$	**J. Phys. Chem. Ref. Data, 1995, 24, 663-1021**
HOCl	**Chem. Res. Toxicol, 2001, 14, 1453-1464**
	Chem. Res. Toxicol, 2003, 16, 439-449

Website: NDRL/NIST Solution Kinetics Database - 14,000 rate constants

http://www.rcdc.nd.edu/RCDC/RadChemHomePage.html

Reduction potentials for one-electron reactions involving radicals

J. Phys. Chem. Ref. Data, 1989, 18, 1637-1755.

http://www.rcdc.nd.edu/RCDC/RadChemHomePage.html

General Types of Protein Oxidative Modification

- Sulfur oxidation (Cys disulfides, S-thiolation; Met sulfoxide)
- Protein carbonyls (side chain aldehydes, ketones)
- Tyrosine crosslinks, chlorination, nitrosation, hydroxylation
- Tryptophanyl modifications
- Hydro(pero)xy derivatives of aliphatic amino acids
- Chloramines, deamination
- Amino acid interconversions (*e.g.*, His to Asn; Pro to OH-Pro)
- Lipid peroxidation adducts (MDA, HNE, acrolein)
- Amino acid oxidation adducts (*e.g.*, *p*-hydroxyphenyl-acetaldehyde)
- Glycoxidation adducts (*e.g.*, carboxymethyllysine)
- Cross-links, aggregation, peptide bond cleavage

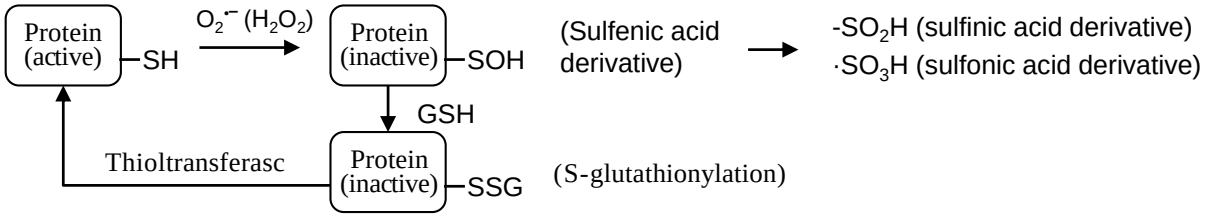
Amino Acids Most Susceptible to Oxidation and Their Main Reaction Products

Amino Acid	Physiological oxidation products
Cysteine	Disulfides, mixed disulfides (*e.g.*, glutathiolation), HNE-Cys
Methionine	Methionine sulfoxide
Tyrosine	Dityrosine, nitrotyrosine, chlorotyrosines, dopa
Tryptophan	Hydroxy- and nitro-tryptophans, kynurenines
Phenylalanine	Hydroxyphenylalanines
Valine, Leucine	Hydro(pero)xides
Histidine	2-Oxohistidine, asparagine, aspartate, HNE-His
Glutamyl	Oxalic acid, pyruvic acid
Proline	Hydroxyproline, pyrrolidone, glutamic semialdehyde
Threonine	2-Amino-3-ketobutyric acid
Arginine	Glutamic semialdehyde, chloramines
Lysine	α-Aminoadipic semialdehyde, chloramines, MDA-Lys, HNE-Lys, acrolein-Lys, carboxymethyllysine, pHA-Lys

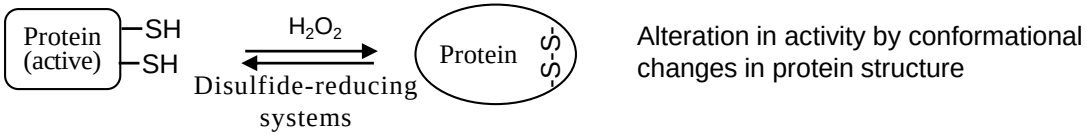
JBC(1997) 272; 19095-19102

Oxidation of Proteins

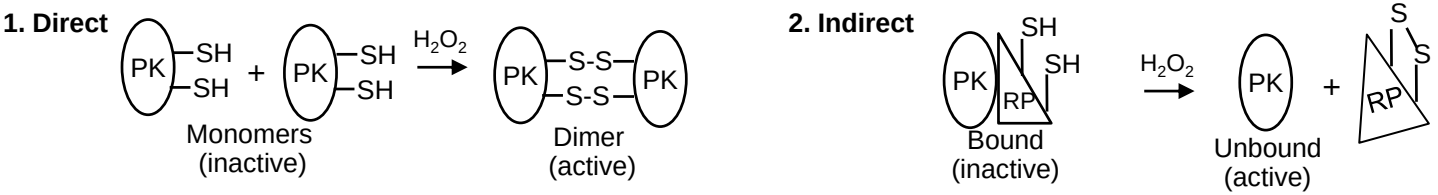
A MODIFICATION OF PROTEINS OY OXIDATION OF CYSTEINE RESIDUES



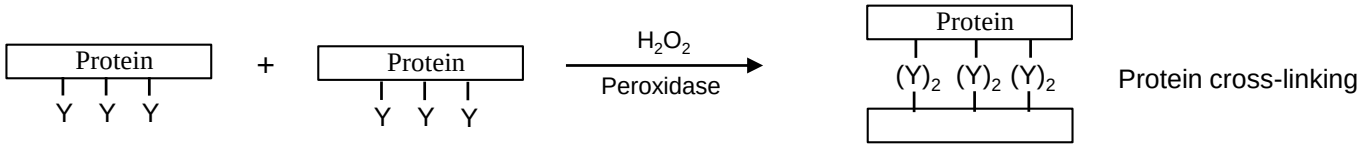
B FORMATION OF INTRA-MOLECULAR DISULFIDE LINKAGES



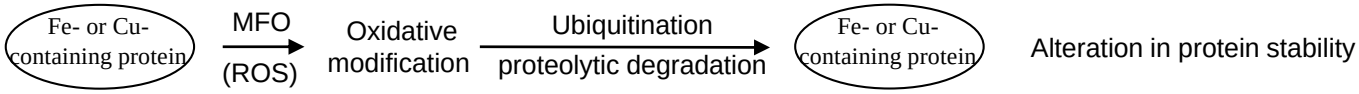
C PROTEIN DIMERIZATION BY INTER-MOLECULAR DIFULFIDE LINKAGES



D DITYROSINE FORMATION BY H_2O_2 PEROXIDASE-DEPENDENT REACTIONS



E METAL-CATALYZED OXIDATION OF PROTEINS IIY "FENTON-LIKE" CHEMISTRY



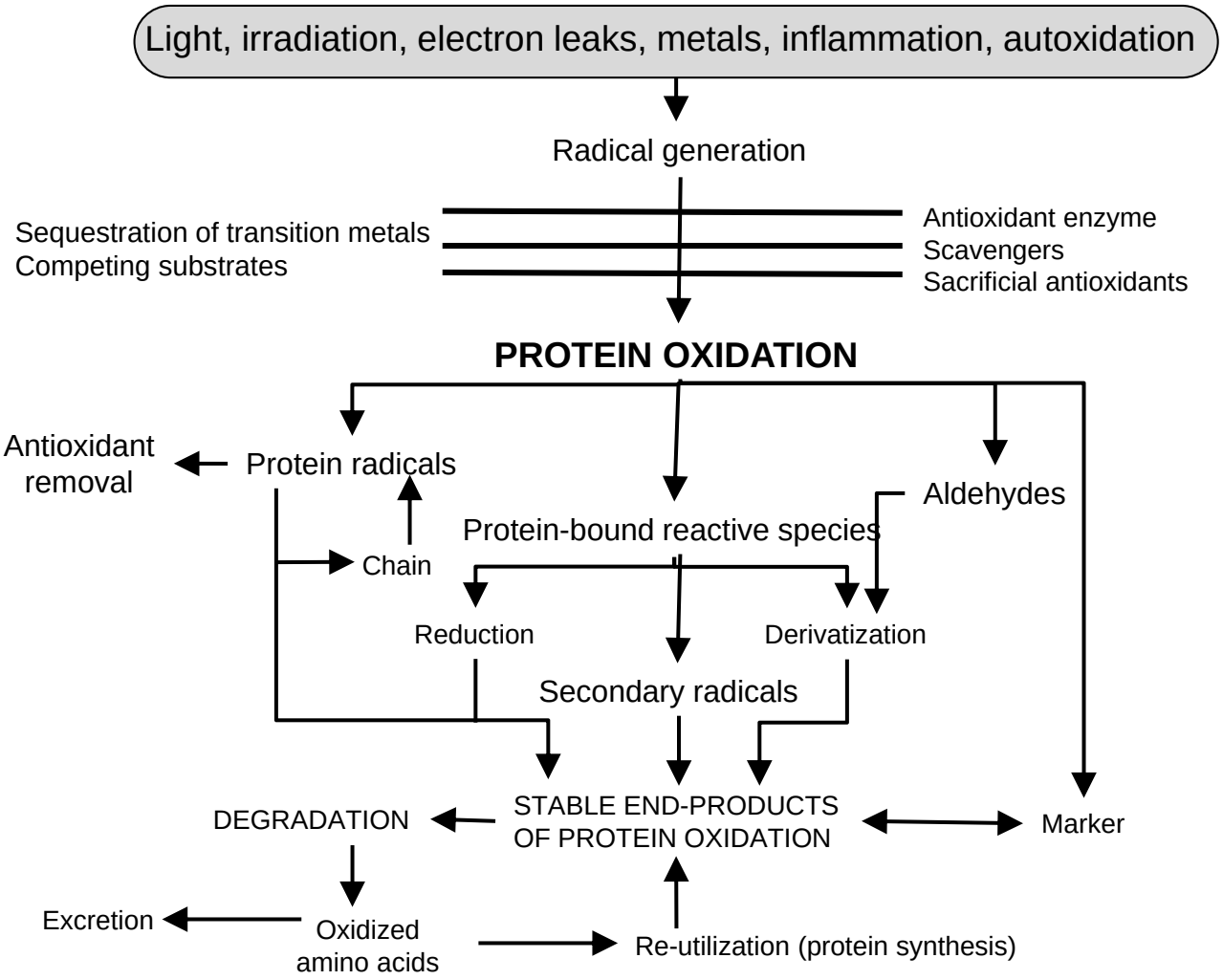
Selectivity of Damage Between Different Sites by a Single Oxidant

Kinetic data does not usually yield information on selectivity of damage at different sites, unless specific absorptions are monitored - usually only possible for aromatic and sulfur-containing residues.

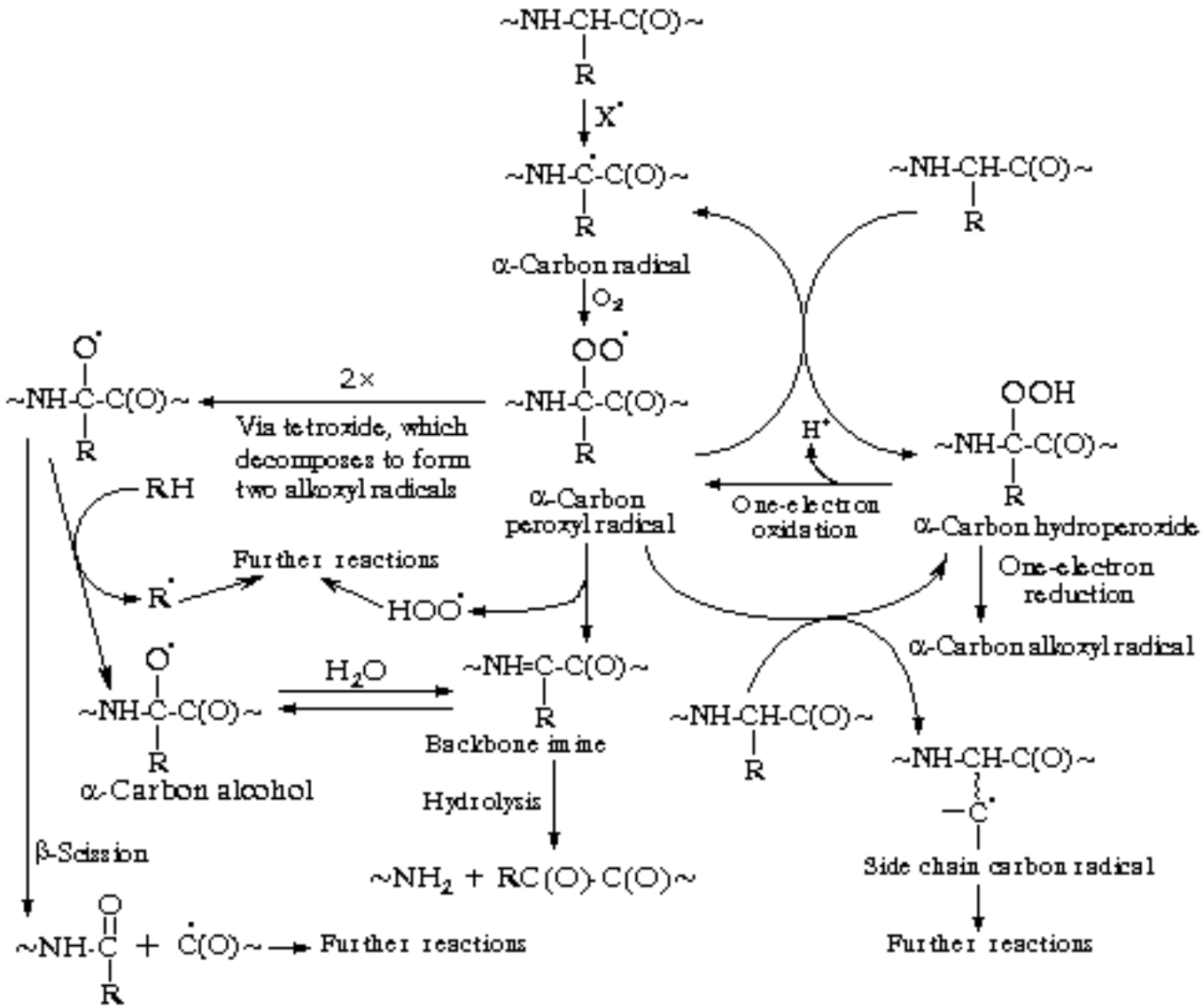
Number of factors influence which sites are most favored

- Stability of incipient radical
  - **tertiary > secondary > primary; delocalisation on to other atoms**
- Statistics
  - **number of available C-H bonds / sites of addition**
- Accessibility
  - **buried versus exposed; steric and charge interactions**

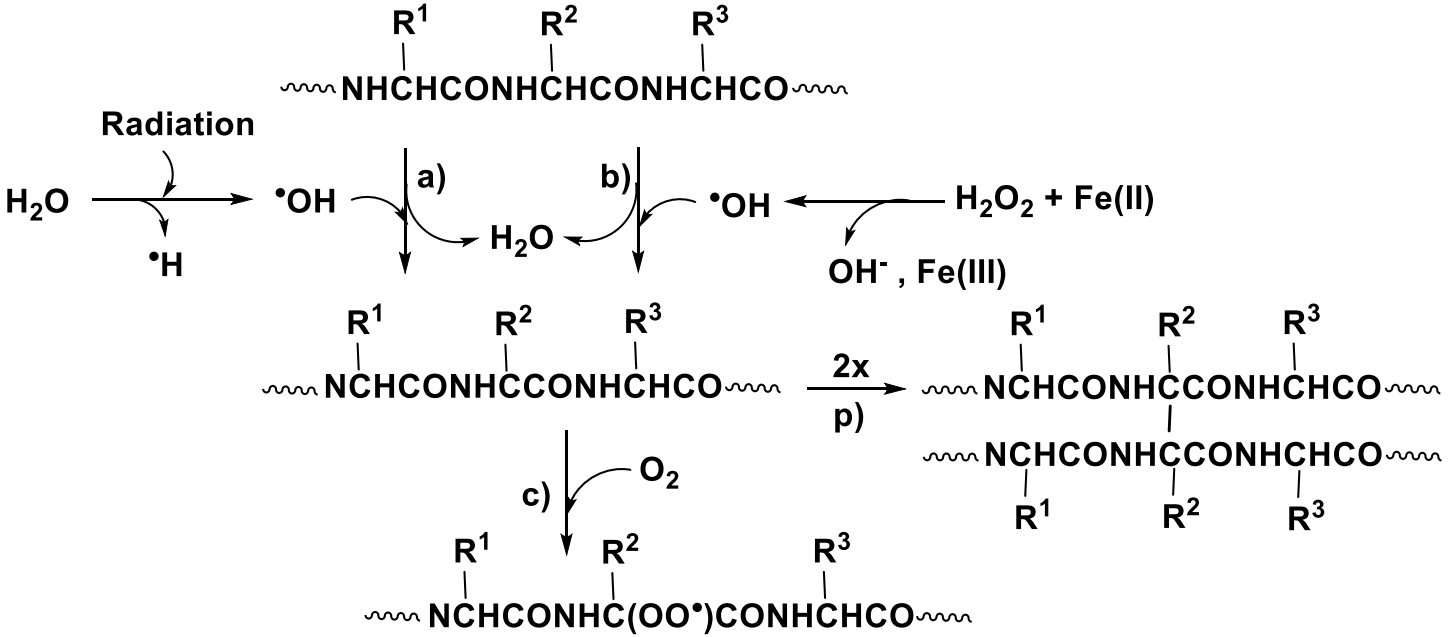
Protein Oxidation *in vivo* (Summary)



Major Reactions of Backbone Radicals formed during Oxidation in the Presence of Oxygen



Peptide Bond Cleavage Due to Reaction with Hydroxyl Radical



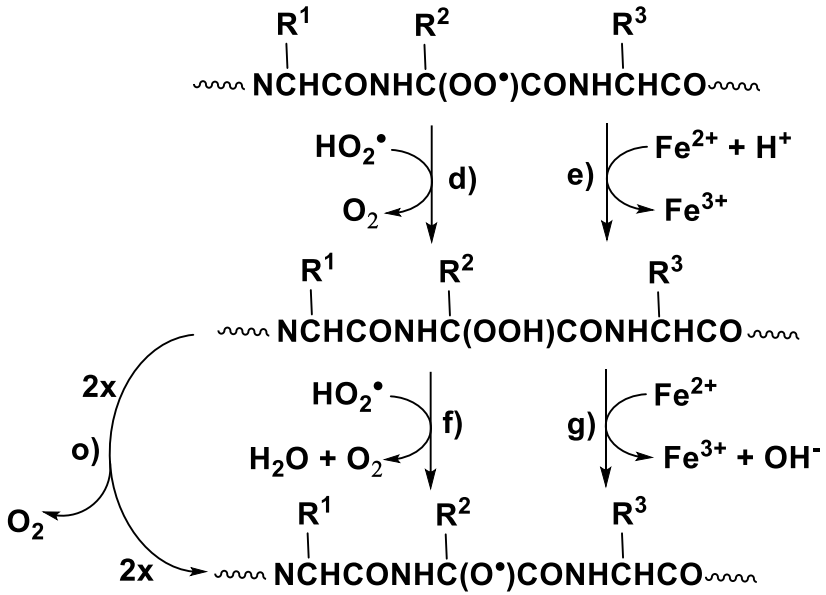
Peptide Bond Cleavage.

OH, generated by either radiolysis of water or the metal-catalyzed cleavage of H_2O_2 can abstract hydrogen atoms from the -CH(R)- group of the polypeptide backbone (reactions a, b).

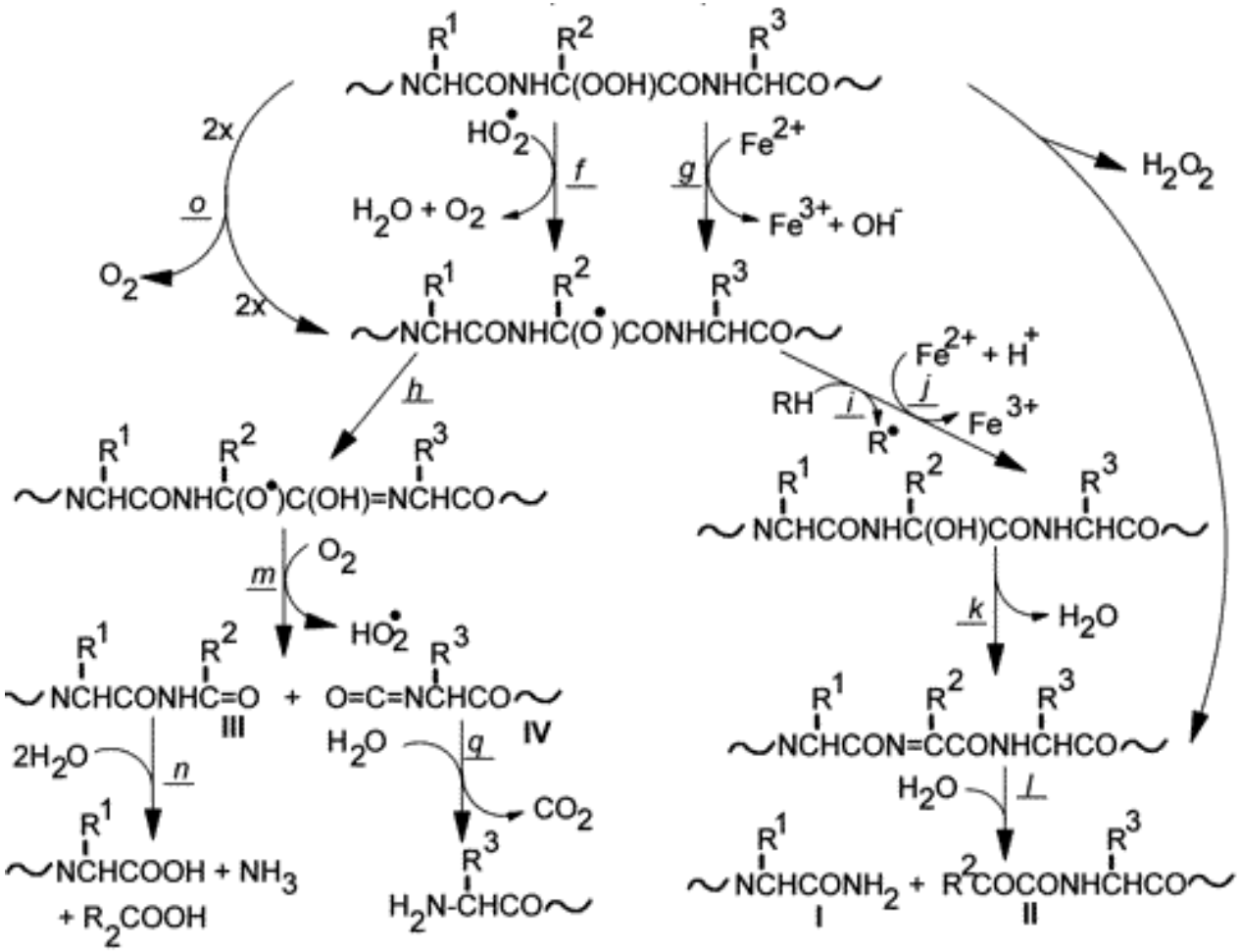
The alkyl radical thus formed may react with oxygen to form the alkylperoxy radical (reaction c) or with another alkyl radical to form inter- or intraprotein cross-linkages (reaction p).

Peptide Hydroperoxides

- **The protein peroxy radical can be converted to the alkyl peroxide by either reaction with:**
  - **free peroxy radical or SAD (reaction *d*),**
  - **reaction with Fe^{2+} (reaction *e*),**
  - **abstraction of a hydrogen from another source (not shown).**
- **Irrespective of how it is formed, the protein alkyl peroxide can be converted to the alkoxy protein derivative by either dismutation (reaction *o*),**
  - **reaction with free peroxy radical (reaction *f*), or**
  - **reaction with Fe^{2+} (reaction *g*).**



Further Evolution

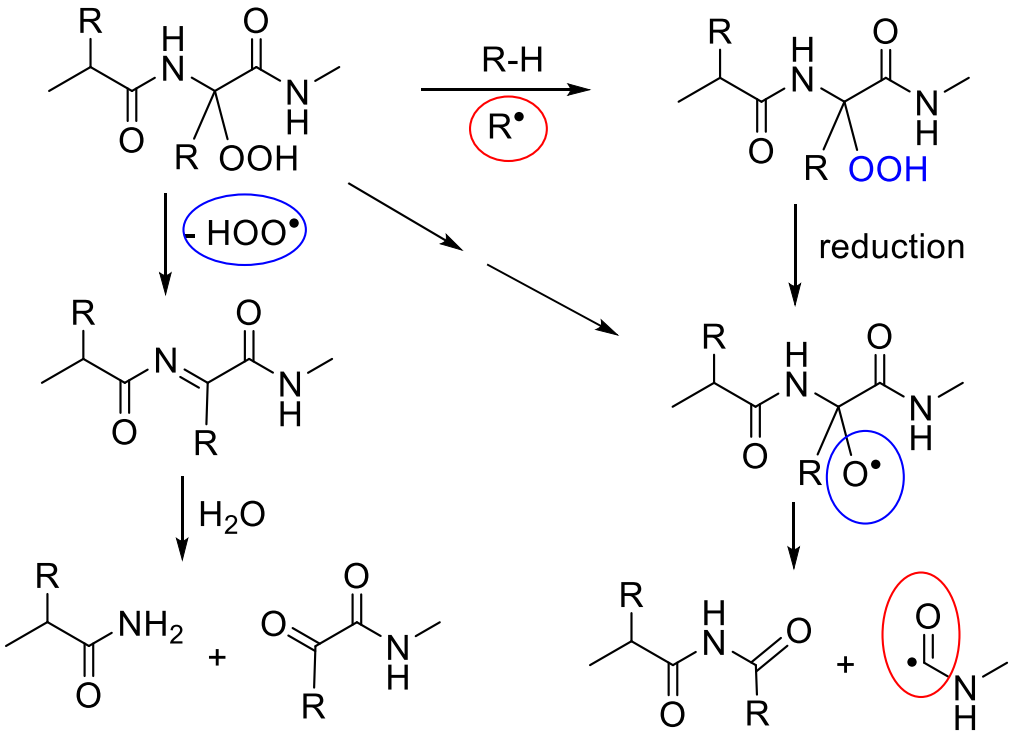


Finally, the alkoxy radical may undergo conversion to the hydroxy derivative (reactions i, j), which will undergo peptide bond scission by the so-called α-amidation pathway (reactions k, l). Alternatively, the alkoxy radical may undergo peptide bond cleavage by the so-called diamide pathway (reaction m).

Backbone Fragmentation Induced by Radicals

Common intermediate implicated in majority of mechanisms

- **Formation of α-carbon radical via direct, or indirect reaction - detection by EPR spin trapping (*e.g.* Chem. Res. Toxicol., 2000, 13, 1087-1095).**
- **Subsequent formation of peroxyl radical in presence of O_2.**
- **Little backbone fragmentation in absence of O_2.**

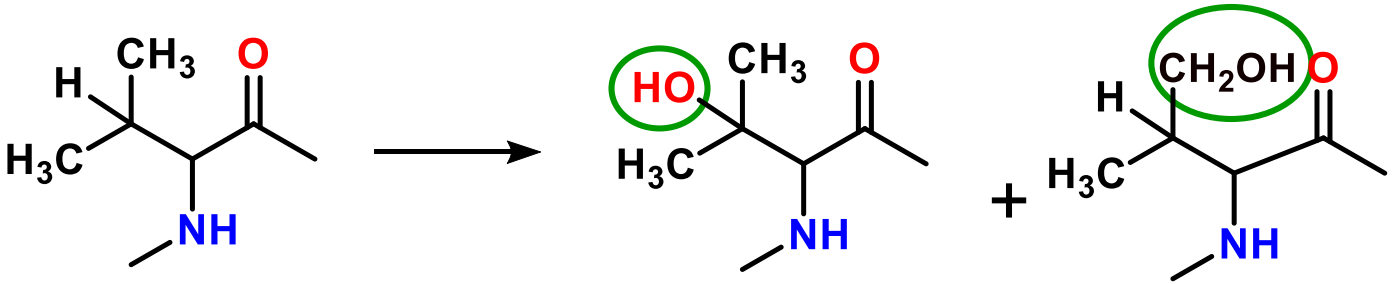


Mechanisms of Side-chain Oxidation by Radicals: Aliphatic Residues

Hydrogen atom abstraction gives common intermediates

but

ratio of intermediates formed at different C-H positions varies with attacking radical.



Valine side-chain

Via tertiary carbon-centered radical

Via primary carbon-centered radical

Direct, rapid-flow, EPR spectroscopy studies can give information on selectivity of initial radical attack for amino acids and peptides.

J. Chem. Soc., Perkin Trans. 2, 1998, 2617-2622; Biochim. Biophys. Acta, 2001, 1504, 196-219

Mechanisms of Side-chain Oxidation by Radicals: Aliphatic Residues

Initial carbon-centred radicals undergo rapid reaction with O_2 to give peroxyl radicals. Dimers formed in absence of O_2.

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \qquad \text{or} \qquad R^{\bullet} + R^{\bullet} \rightarrow R\text{-}R$$

Fate of peroxyl radicals:

- **Reaction with another peroxyl radical to give ROO-OOX (X = R, H).**
- **Hydrogen atom abstraction - gives hydroperoxide:**

$$\mathbf{ROO^{\bullet} + XH \rightarrow X^{\bullet} + ROOH \rightarrow \rightarrow ROH + carbonyls}$$

- **Elimination reactions - special case for Ser, Thr and few other residues**

$$\mathbf{-\underset{\underset{OH/NH}{|}}{C}\text{-}OO^{\bullet} \rightarrow -\underset{|}{C}{=}O + HOO^{\bullet}}$$

Major products from aliphatic side-chains: peroxides, alcohols and carbonyls.

Specific Aliphatic Side-chain Oxidation Products

Glutamic acid	**hydroperoxides**
Leucine	**hydroperoxides alcohols** **α-ketoisocaproic acid** **isovaleric acid** **isovaleraldehyde** **isovaleraldehyde oxime** **carbonyl compounds**
Glycine	**Aminomalonic acid**
Valine	**hydroperoxides alcohols** **carbonyl compounds**
Lysine	**hydroperoxides alcohols** **carbonyl compounds**
Proline	**hydroperoxides alcohols** **5-hydroxy-2-aminovaleric acid** **carbonyl compounds**
Arginine	**hydroperoxides** **5-hydroxy-2-aminovaleric acid**
Isoleucine	**hydroperoxides, alcohols,** **carbonyl compounds**
Methionine	**Methionine sulphoxide**
Cysteine	**Cystine,** **Oxy acids**

Free Radic. Biol. Med., 1999, 27, 1151-1163

Peroxides are Major Initial Products of $HO^{\bullet}$ Attack on Proteins in the Presence of O_2

Substrate	% yield of hydroperoxide groups formed from initial $HO^{\bullet}$
N-Ac-Lys-NH_2	26
Gly-Lys-Gly	34
Poly-lysine	64
Melittin	16
Protamine	33
Insulin	12
RNase A	53
BSA	36

Formation of Peroxides on Proteins

Does it matter what the attacking radical is ? - NO

High energy radiation (γ- or X-rays, UV, visible light with sensitizer)
Metal ions / ascorbate
Metal ions / peroxide systems ($HO^{\bullet}$, $RO^{\bullet}$)
Thermo-labile azo compounds + O_2 ($ROO^{\bullet}$)
Peroxynitrite
Activated white cells
Hemoprotein / peroxide systems

Does it matter what the amino acid / peptide / protein is ? - NO

Formed on most amino acids, and all peptides and proteins tested
Detected on both isolated proteins and proteins in cells

Yield and exact structure of peroxides formed is dependent on target, the attacking radical, O_2 concentration, presence of reductants / antioxidants / metal ions (*etc.* …)

Biochim Biophys Acta, 2001, 1504, 196-219

Fate of Protein Peroxides

$$\text{Protein-OOH} + 2e^- \rightarrow \text{Protein-OH}$$

$$\text{Protein-OOH} + M^{n+} \rightarrow \text{Protein-O}^\bullet + M^{(n+1)+} + HO^-$$

$$\text{Protein-OOH} + \text{UV / heat} \rightarrow \text{Protein-O}^\bullet + HO^\bullet$$

$$\text{Protein-O}^\bullet \rightarrow \text{Protein-C}^\bullet \text{ (1,2 hydrogen atom shifts)}$$

$$\text{Protein-O}^\bullet \rightarrow \text{Protein-carbonyl} + R^\bullet \text{ (}\beta\text{-scission reactions)}$$

$$\text{or} \quad \text{Protein-C}^\bullet + \text{released carbonyl}$$

$$\text{Protein-C}^\bullet + O_2 \rightarrow \text{Protein-OO}^\bullet$$

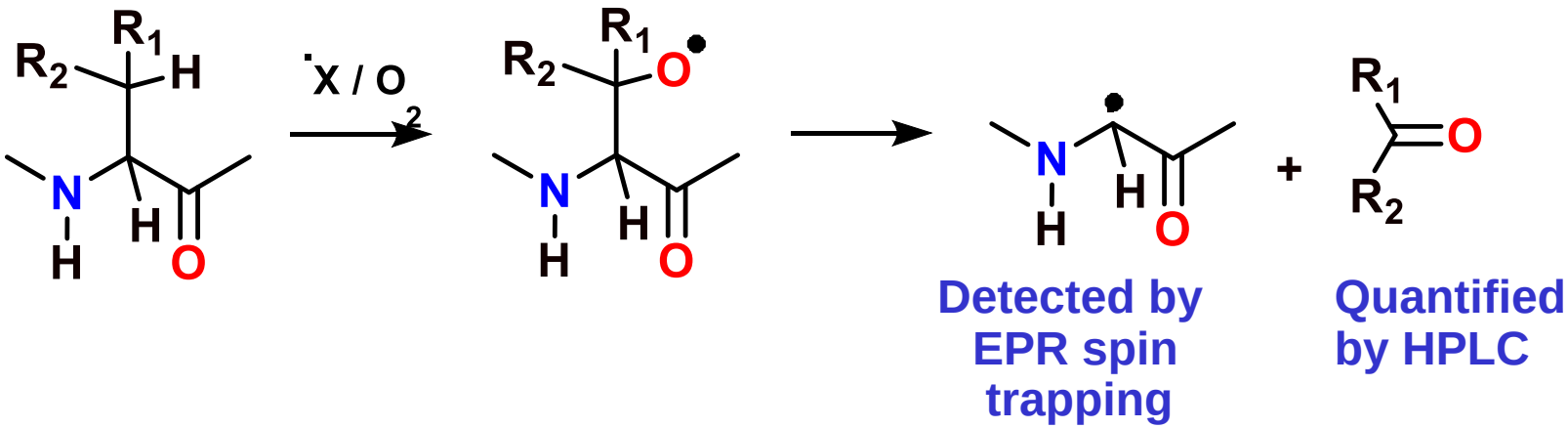
$$\text{Protein-OO}^\bullet + RH \rightarrow \text{Protein-OOH} + R^\bullet$$

Biochem. J., 1995, 305, 643-649; Arch. Biochem. Biophys., 1996, 336, 163-172; Chem. Res. Toxicol, 2000, 13, 1087-1095; Free Radic. Biol. Med., 2002, 32, 1171-1184.

Transfer of Damage within Proteins

Evidence for radical transfer from side-chains sites to backbone via mediation of alkoxy radicals

- **Favourable process due**
  - **to stability of α-carbon radical**
  - **relief of steric crowding**
  - **stability of carbonyl product**



Results in loss of side-chain group as reactive aldehyde / ketone and formation of backbone radical ⇨ backbone cleavage

Similar reactions with thiyl radical from cysteine ?

Chem Res Toxicol, 2000, 13, 1087; Free Radic Biol Med, 2002, 32, 1171, J Am Chem Soc, 2003, 125, 2042

Occurrence of Chain Reactions

Number of reactions of protein radicals that give rise to other radicals:

1) Decomposition of dimers formed between two peroxyl radicals

$$\mathbf{ROO\text{-}OOR \rightarrow 2RO^{\bullet} + O_2}$$

2) Hydrogen atom abstraction by an initial peroxyl radical

$$\mathbf{ROO^{\bullet} + XH \rightarrow ROOH + X^{\bullet}}$$

3) Fragmentation reactions of α-hydroxperoxy radicals on Ser and Thr
4) Decomposition of hydroperoxides to alkoxyl radicals

<u>All</u> of these reactions give rise to further radicals and hence either

- chain reactions on proteins, or
- damage to other biomolecules

Oxidation of Cys, Cystine and Met Residues

Important targets: rapid reaction with range of oxidants (both radical and non-radical) and easily oxidised.

- **Met converted to sulfoxide: -S- → -S(=O)- → -S(O_2)-**
- **Cys oxidised via two major pathways:**

  RSH → RSSR (cystine) via thiyl radical

  RSH → RS-X → → RSO_2H + RSO_3H (X=OH, Cl, etc.)

  ↓

  RSSR

- **Cystine can be oxidised to RSS(=O)R**
- **Both Met sulfoxide and cystine can be repaired**

- **Only major examples of repair of oxidised amino acid residues on proteins**

Free Radic Biol Med, 1995, 18, 93; PNAS, 2001, 98, 12920, Free Radic Biol Med, 1995, 31, 1432; Int J Radiat Biol, 1989, 55, 539; von Sonntag - The Chemical Basis of Radiation Biology

Consequences of Protein Thiol Oxidation

- **Oxidation of catalytic sites on proteins**
  - loss of function/abnormal function
  - BUT(!): sometimes it is gain in function!
- **Formation of mixed sulfide bonds**
  - Protein-protein linkages (RS-SR)
  - Protein-GSH linkages (RS-SG)
  - Alteration in 2o and 3o structure
- **Increased susceptibility to proteolysis**

Mechanisms of Side-chain Oxidation by Radicals: Aromatic Residues

1. **Major reaction is addition, though electron abstraction can also occur.**

2. **Electron abstraction reactions usually yield hydroxylated products.**

3. **Addition reactions tend to yield a greater diversity of products as this depends on the added species.**

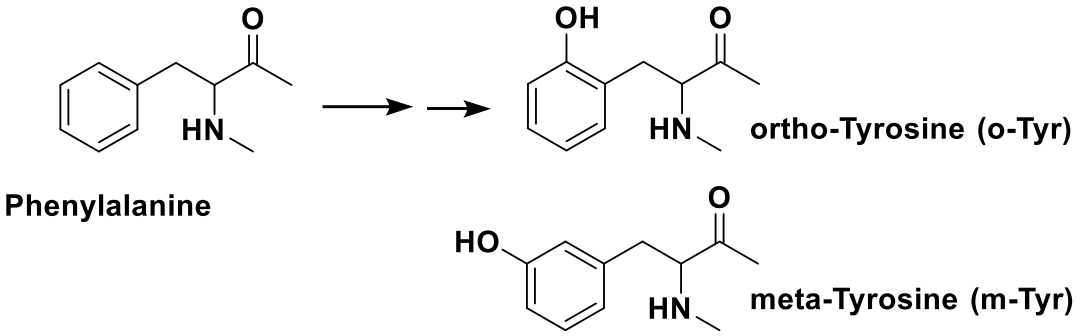
4. **Similar products tend to be formed in presence and absence of O_2.**

Specific Aromatic Side-chain Oxidation Products

Phenylalanine

o-, m-tyrosine

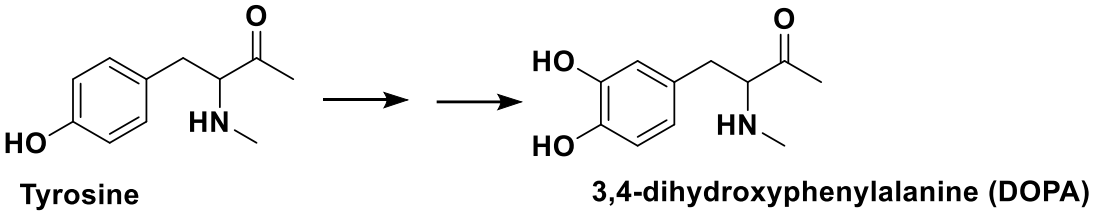
dimers



Tyrosine

DOPA

di-tyrosine



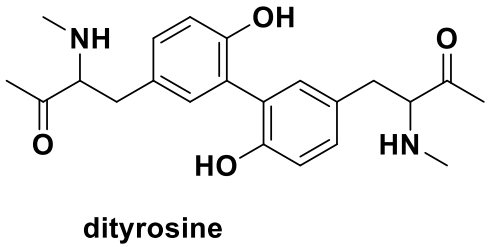
Tryptophan

N-formylkynurenine,

kynurenine,

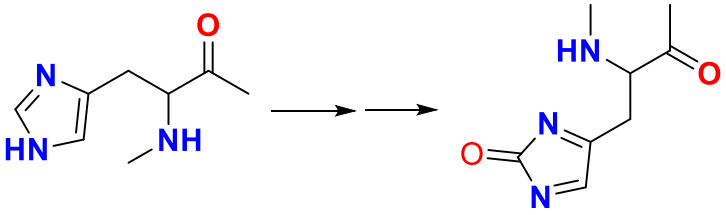
5-hydroxytryptophan,

7-hydroxytryptophan



Histidine

2-oxo-histidine



Free Radic. Biol. Med., 1999, 27, 1151-1163

Transfer of Damage within Proteins

Evidence for long range transfer of radical sites within proteins:

- transfer from initial site to a readily oxidised residue (Trp, Tyr, Met, Cys) to give more stable radical - can be equilibria.
- can occur over very large distances, but depends on protein structure
- occurs in competition with reaction of initial radical with O_2
- most common when initial radical is poorly, or unreactive, with O_2

Examples:

Trp ↔ Tyr, Trp ↔ Cys, Tyr ↔ Cys

Oxidised porphyrin ring from Trp, Tyr, Cys

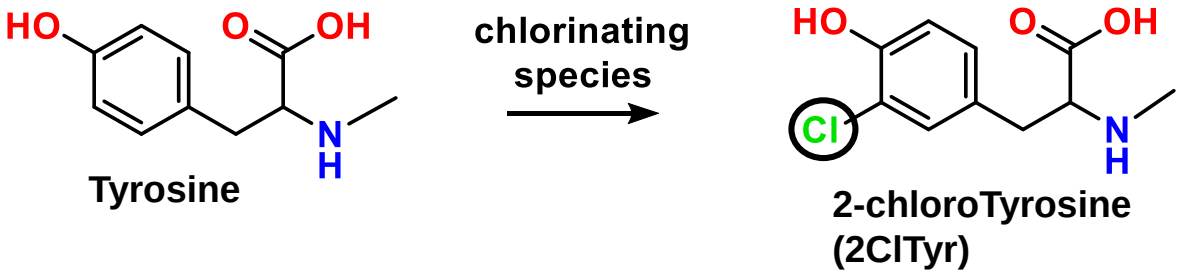
J. Am. Chem. Soc., 1989, 111, 5141-5145; J. Am. Chem. Soc., 1994, 116, 12010-12015; Int. J. Radiat. Biol., 1989, 55, 539-556; J. Biol. Chem., 1997, 272, 2359-2362

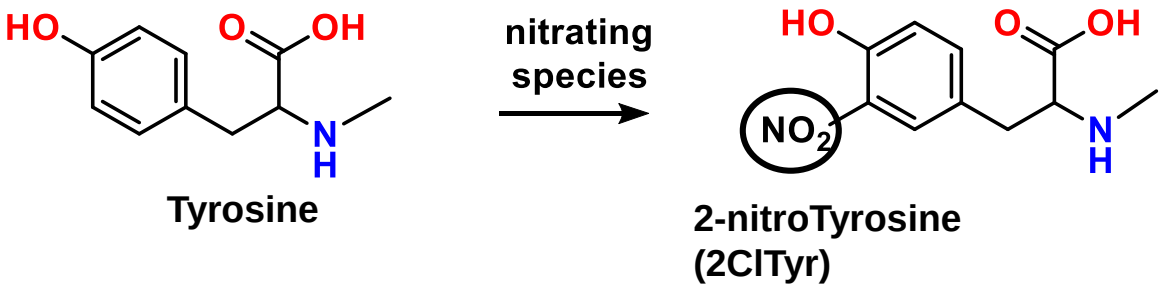
Mechanisms of Protein Oxidation by Non-Radical Oxidants

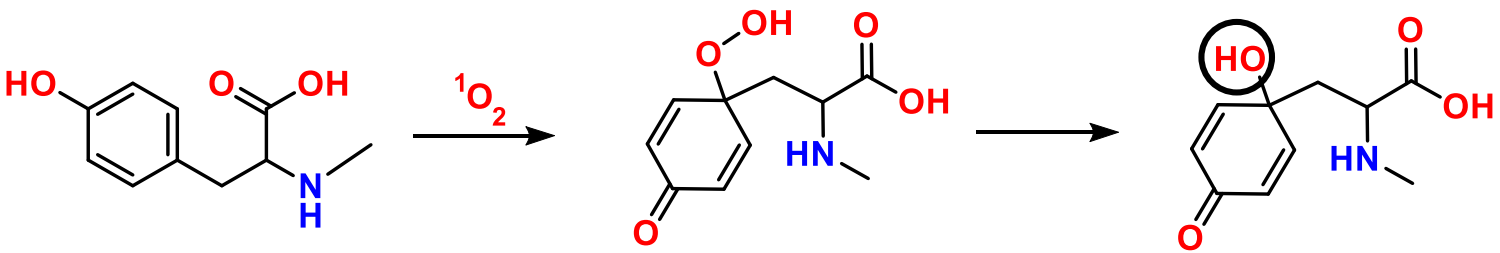
- Species such as $^1O_2$, HOCl, HOBr, peroxynitrite, O_3, UV light
- Reactions can be very selective and generally on side-chains

  Little fragmentation, but considerable aggregation
  - $^1O_2$ - damage to Cys, Met, Trp, Tyr and His.
  - HOCl / HOBr - primarily damage to Cys, Met, His, Lys, Trp, α-amino group.
  - Peroxynitrite - damage to Cys, Tyr, Trp.
  - UV light - damage to cystine, Trp, Tyr and His.
- Some products well-defined - *e.g.* 3-chloroTyr, 3-nitroTyr, methionine sulfoxide, disulphides.
- Some poorly defined ⇨ peroxides generated by $^1O_2$ and O_3.

Aromatic Side-chain Oxidation Products Generated by Non-Radical Oxidants







Free Radic. Biol. Med., 1999, 27, 1151-1163; Photochem. Photobiol, 2002, 76, 35-46

Oxidation of Other Residues by Non-Radical Reactions

- **Evidence for oxidation of cysteine residues by peroxides:**
  - **Protein thiols, peroxides and enzyme activity lost concurrently**
  - **Cysteine oxidised to disulphide and oxy acids**

- **Evidence for oxidation of methionine residues by peroxides:**
  - **Evidence for the generation of methionine sulfoxide**
  - **Inactivation of methionine-dependent enzymes**

Eur. J. Biochem., 2002, 269, 1916-1925; FEBS Lett., 2002, 527, 289-292; Redox Rep., 2003, 8, 81-86

Damage to other targets induced by reactive intermediates of protein oxidation

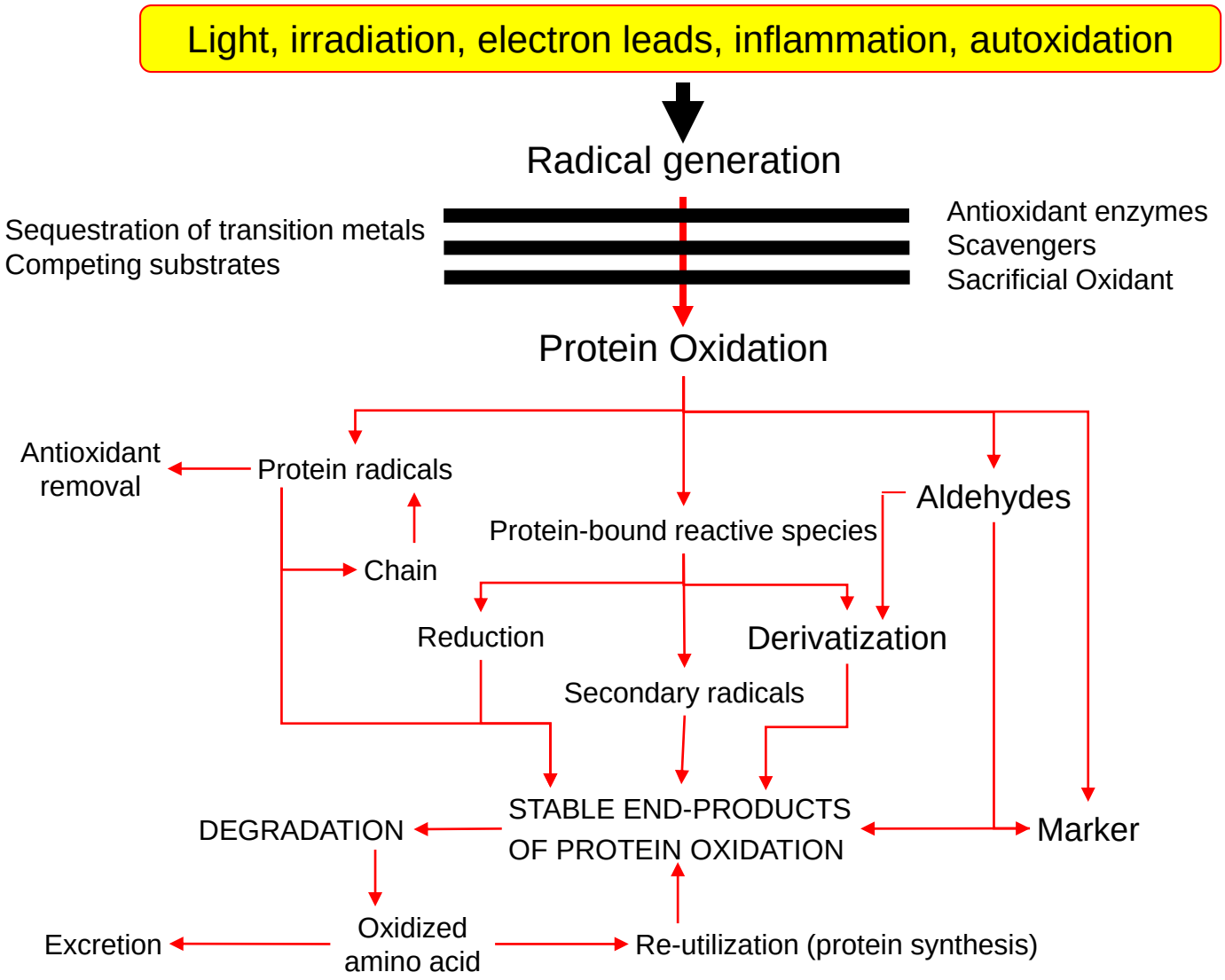
- **Protein peroxides can induce damage to other proteins by:**
  - Non-radical reactions (oxidation of susceptible thiols)
  - Radical-mediated reactions

- **Protein peroxides and DOPA can induce damage to DNA via radical-mediated reactions**
  - Oxidation of bases (*e.g.* 8-oxodG)
  - Induction of strand breaks
  - Formation of protein-DNA cross-links

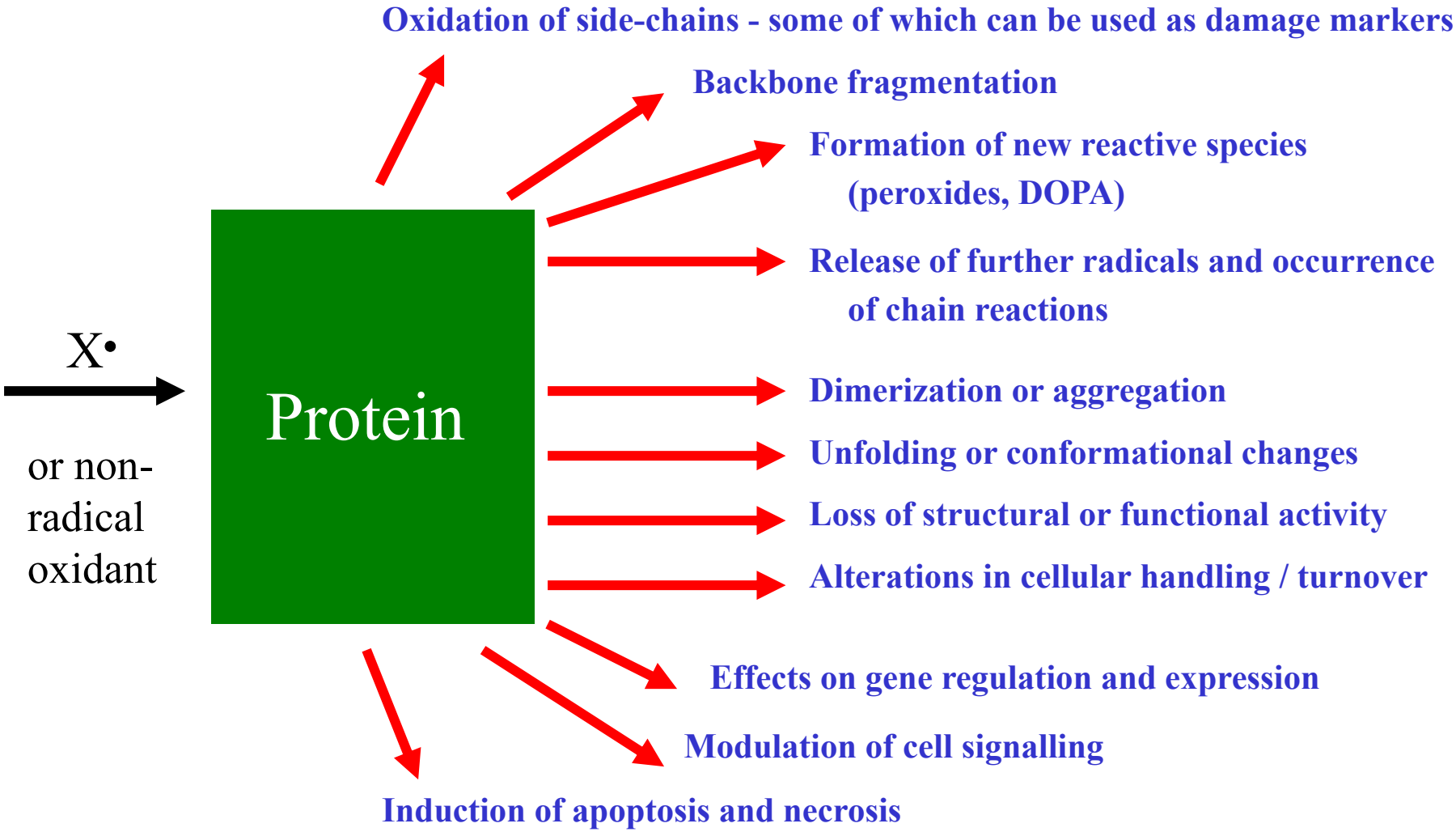
- **Protein peroxides can induce lipid oxidation via radical-mediated reactions**

Biochem. J., 1999, 338, 629-636; Biochem. J., 1999, 344, 125-134; Chem. Res. Toxicol., 2000, 13, 665-672; Biogerentology, 2002, 3, 95-102; Eur. J. Biochem., 2002, 269, 1916-1925; FEBS Lett., 2002, 527, 289-292.

Summarized Scheme of Protein Oxidation in vivo

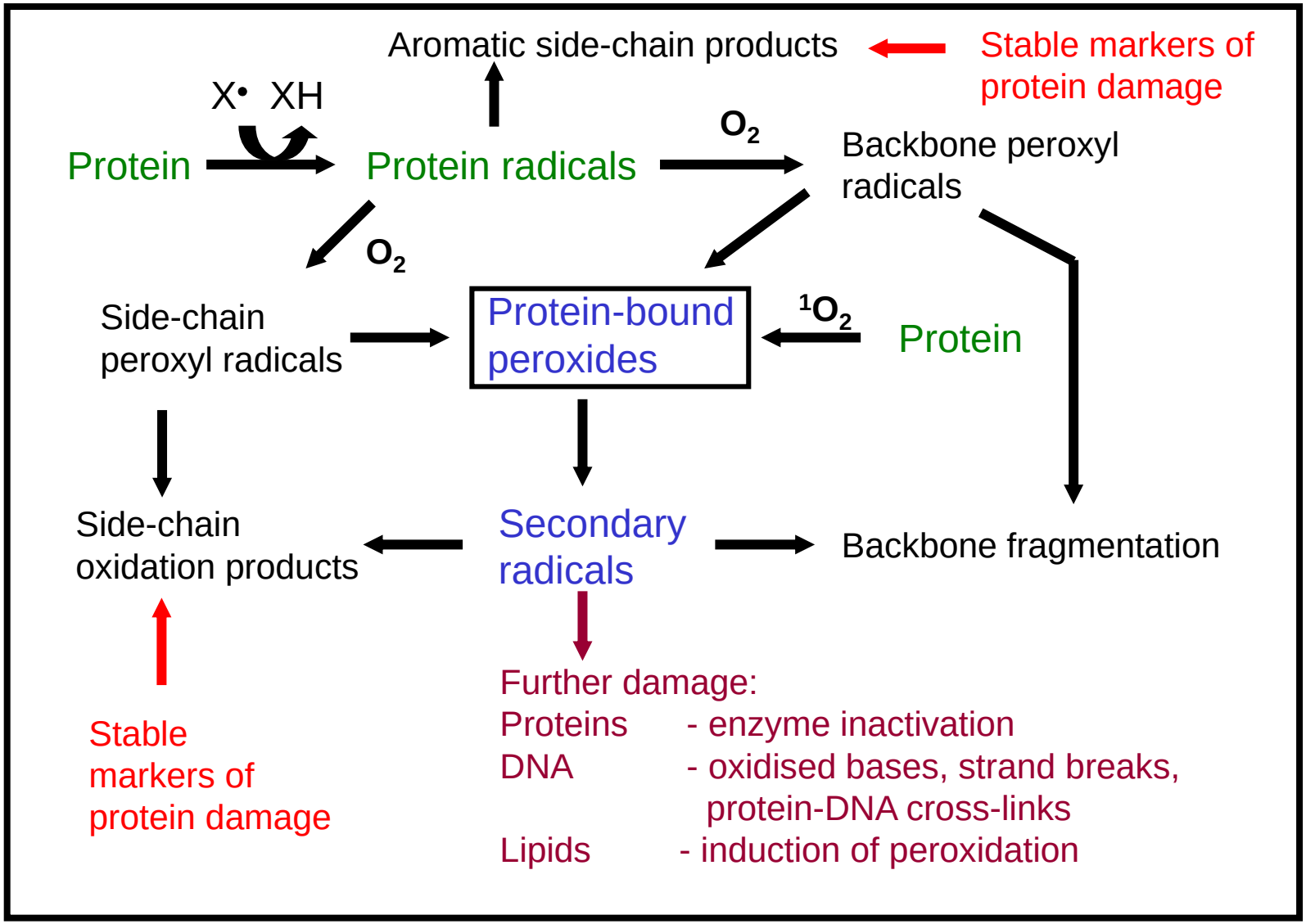


Consequences of Oxidation of Proteins

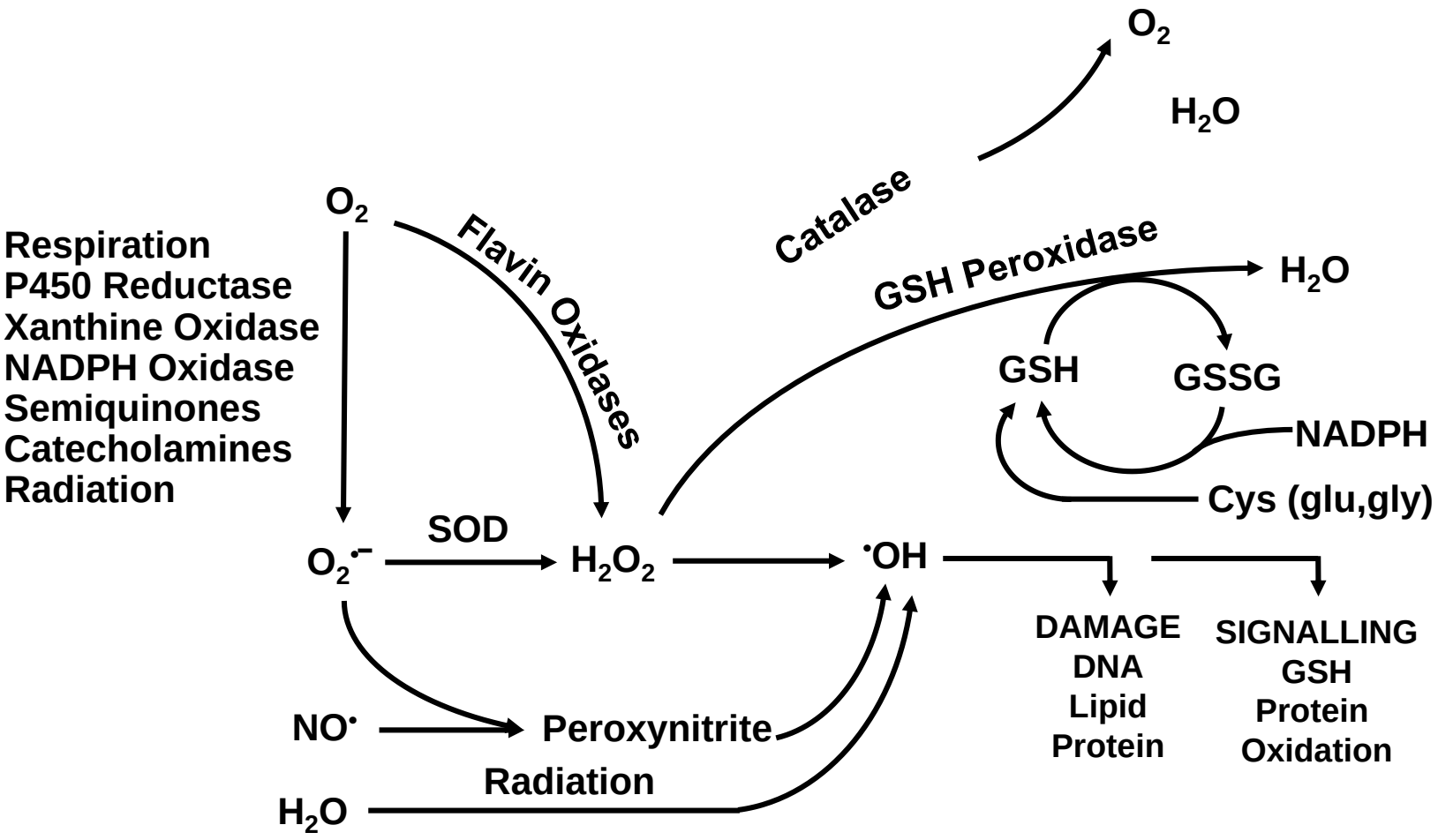


Biochim. Biophys. Acta, 2001, 1504, 196-219; J. Photochem. Photobiol. B, 2001, 63, 114-125.

Summary of Homolytic Reactions of Protein



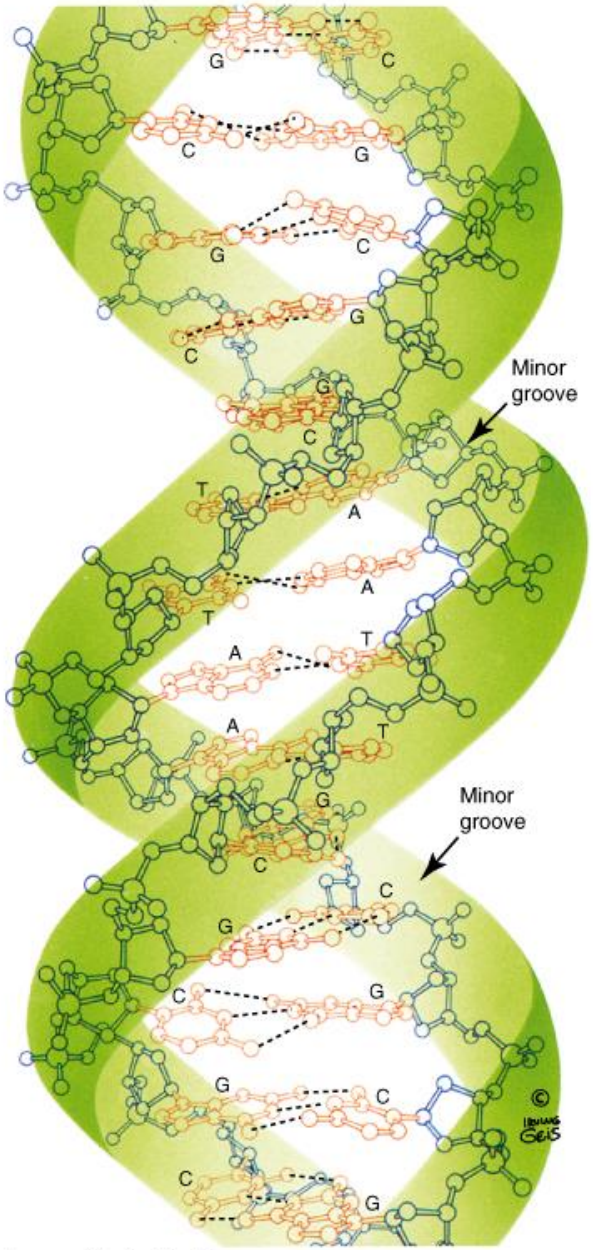
ROS Radicals Damage and Signaling



Cell Mol.Life Sci(2000) 57;1287-1305

Double Helical Structure of DNA

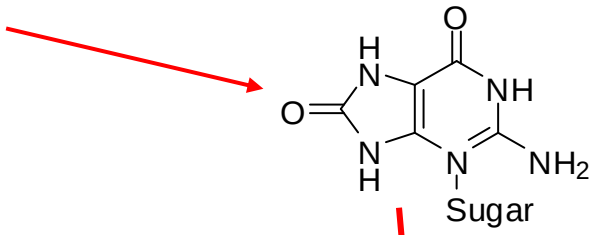
- **Forms a right-handed helix.**
- **The strands run antiparallel.**
- **There are about 10 base pairs per turn of the helix.**
- **One turn of the helix is 34 Å.**
- **The base pairs are 3.4 Å apart.**
- **Sugar phosphates on outside, base pairs on inside.**

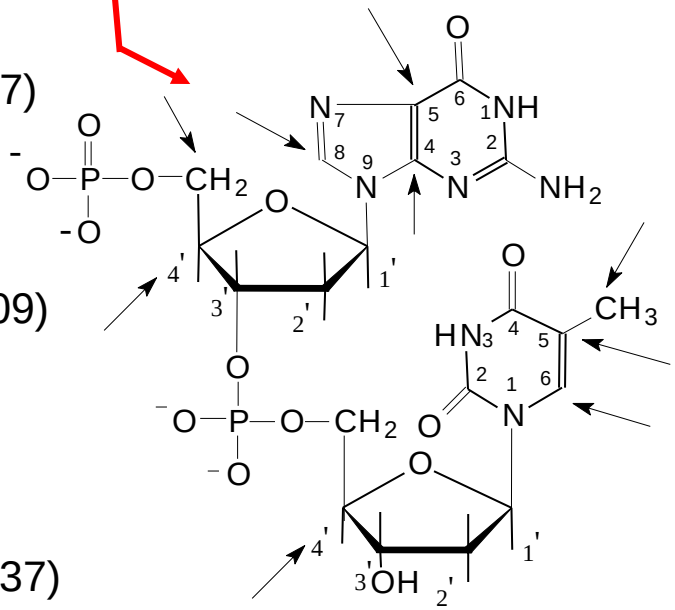


DNA Oxidation

Over 100 different types of oxidative damage to DNA:

- **8-Oxo-deoxyguanosine**
- **8-Oxo-deoxyadenosine**
- **5-Hydroxy-2-deoxycytidine**
- **Thymidine glycol**





- Single and double strand breaks
  Comet assay (Fairbairn *Mutat. Res.* 1995, 339, 37)
- Oxidative modification of DNA-bases
  (*e.g. 8-OH-guanine, 5-OH-uracil, etc*.)
  GS/MS based methods (Mutat. Res. 2003,531,109)
  Immunochemical methods (e.g. 8-oxodG)
- Nitrosative deamination of DNA bases
  Guanine - xanthine
  Cytosine - uracil (Burney Mutat. Res. 1999: 424, 37)
- Formation of DNA adducts with oxidized lipids/proteins (*e.g. dG-MDA, dT-Tyr*).

Oxidative Damage of DNA

Damage done to DNA by Reactive Oxygen species.

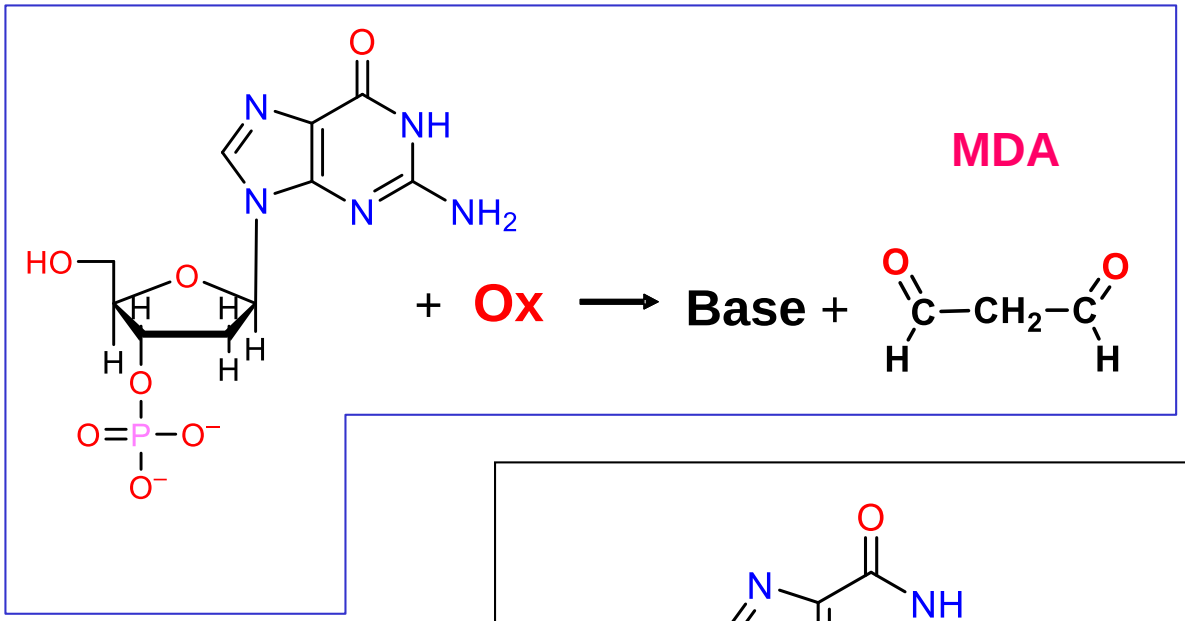
- **H_2O_2**. Not as reactive, but the most significant in terms of diffusion.
- **$^{\bullet}OH$**. It reacts with DNA directly.
- **$O_2^{\bullet -}$**.

Sources of radicals:

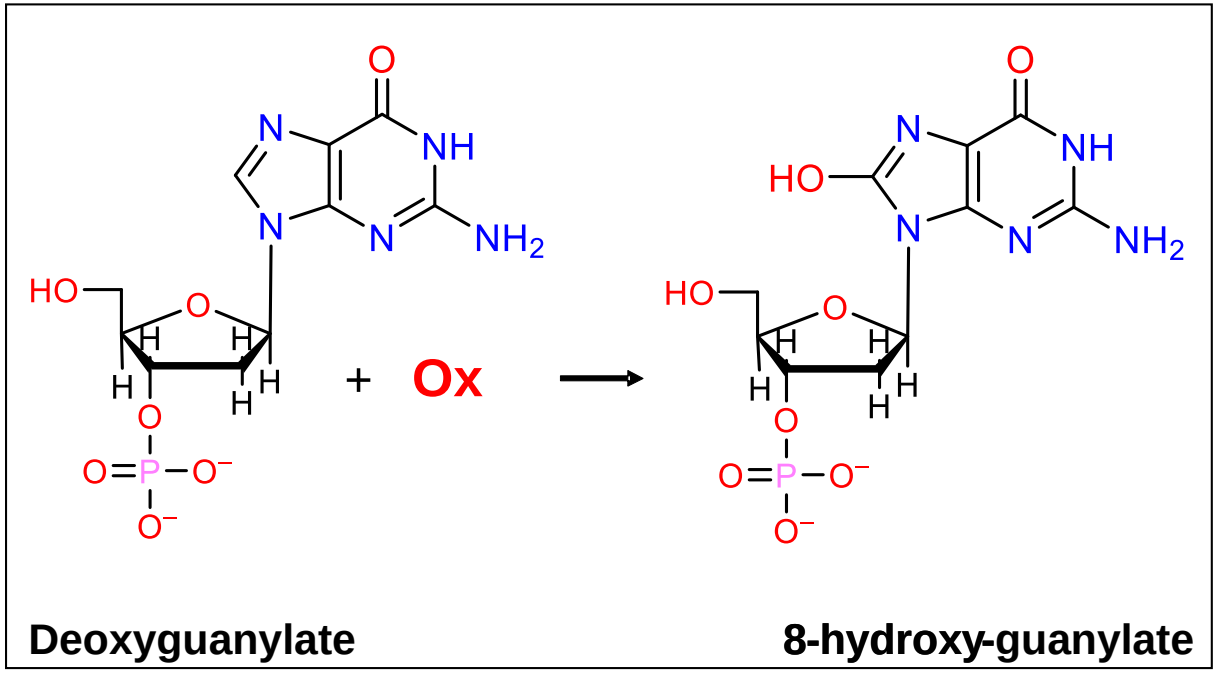
- Intracellular:
  - Respiration.
  - Peroxisomal metabolism.
- Extracellular:
  - Ionizing radiation.
  - UV.
  - Heat.
  - Some drugs.

The most damaging reaction: $RH_2 + {}^{\bullet}OH \rightarrow {}^{\bullet}RH + H_2O$

Oxidative Damage to DNA: Sugars and Bases



Sugar degradation

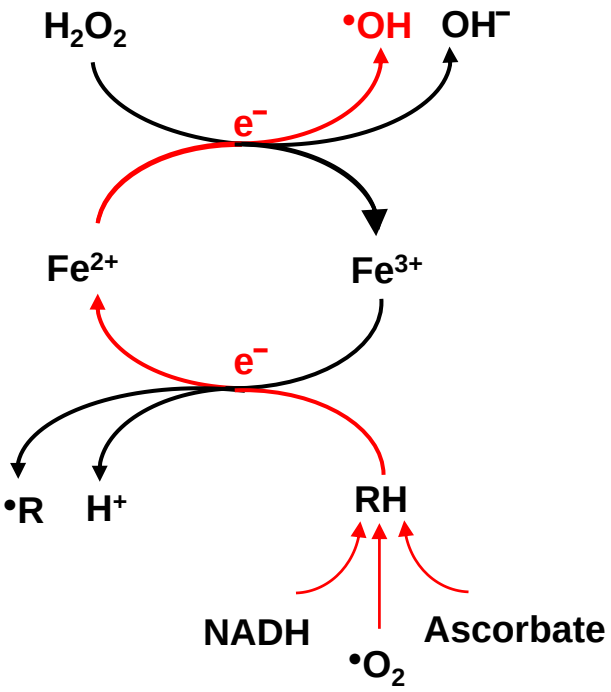


Aromatic Hydroxylation

Oxidative Damage of DNA

Fenton Reaction

Gives rise to highly reactive ·OH.
Caused by problematic transition metals functioning in a redox cycle

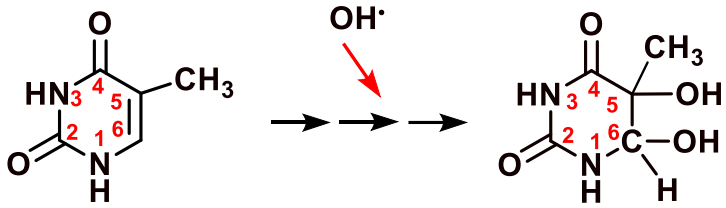


Defenses:

- Sequestration of transition metals: eg. Fe^{2+} as ferritin
- Catalase: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
- Superoxide dismutase (SOD): $2\ O_2^{\bullet -} + 2\ H^+ \rightarrow H_2O + O_2$
- Glutathione: $H_2O_2 + 2\ GSH \rightarrow 2\ H_2O + GS\text{-}GS$
- Other Radical Scavengers: Vit. C and Vit. E.

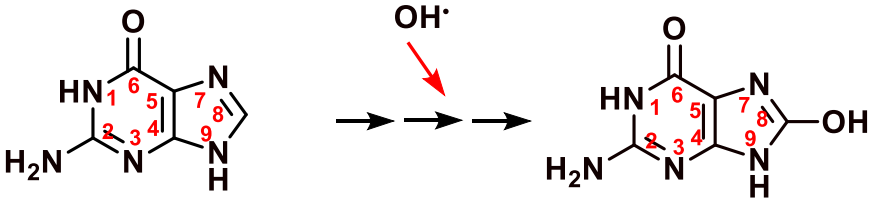
Oxidative Damage of DNA

- OH attack on the sugars leads to base loss and breaks.
- OH attack on the bases:
  - Glycol formation



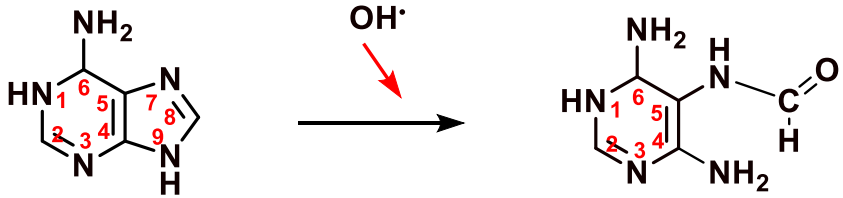
Thymine → (Thymine glycol) (DNA replication block)

  - Hydroxylation



Guanine → (8-hydroxy Guanine) (it leads to mispairing)

  - Ring opening

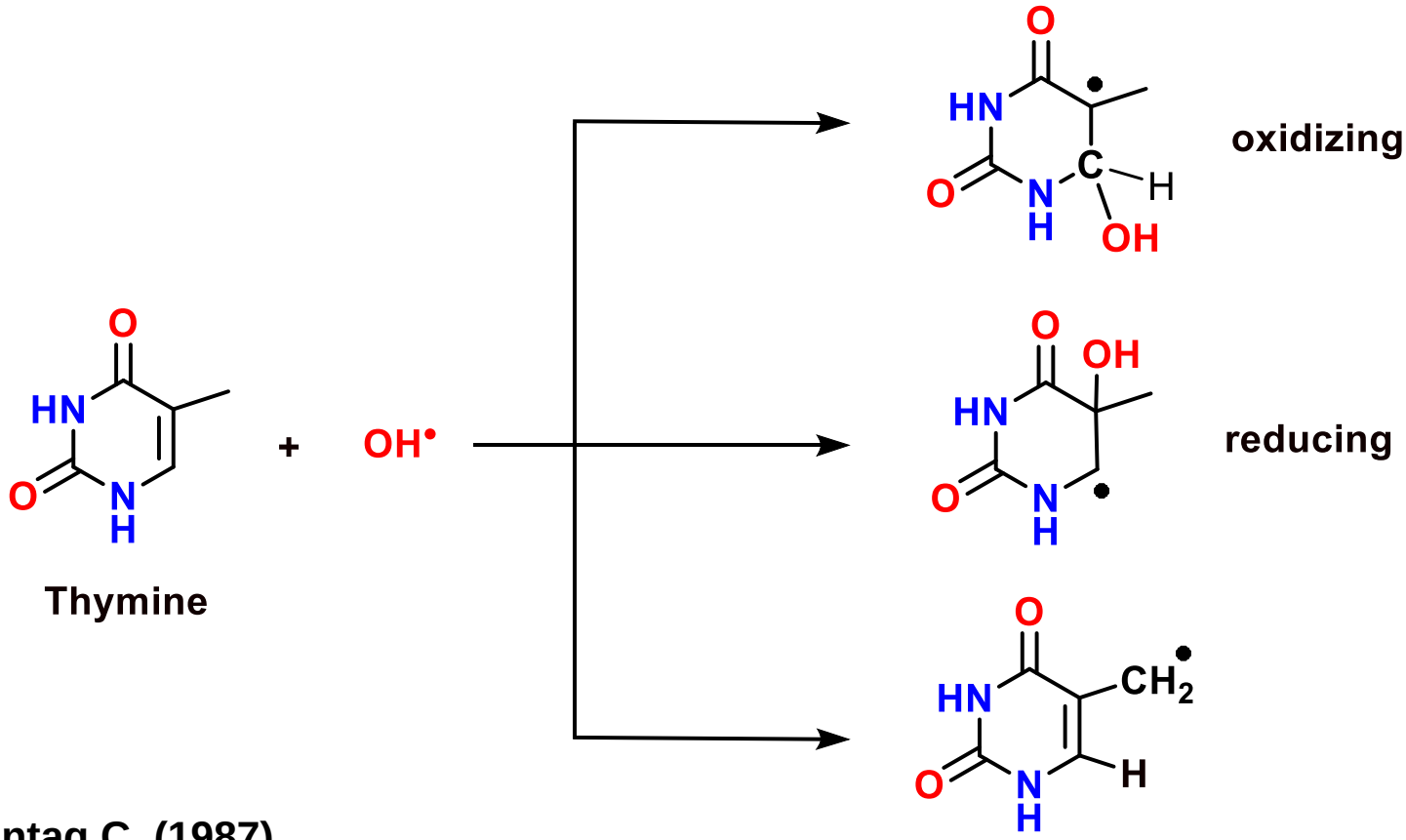


(2,6-diamino-4-hydroxy 5-formamido-pyrimidine)

Defenses: ***SoxRS*** and ***oxyR***, which turn on SOD, and Catalase, respectively.

Oxidative Damage to the Bases

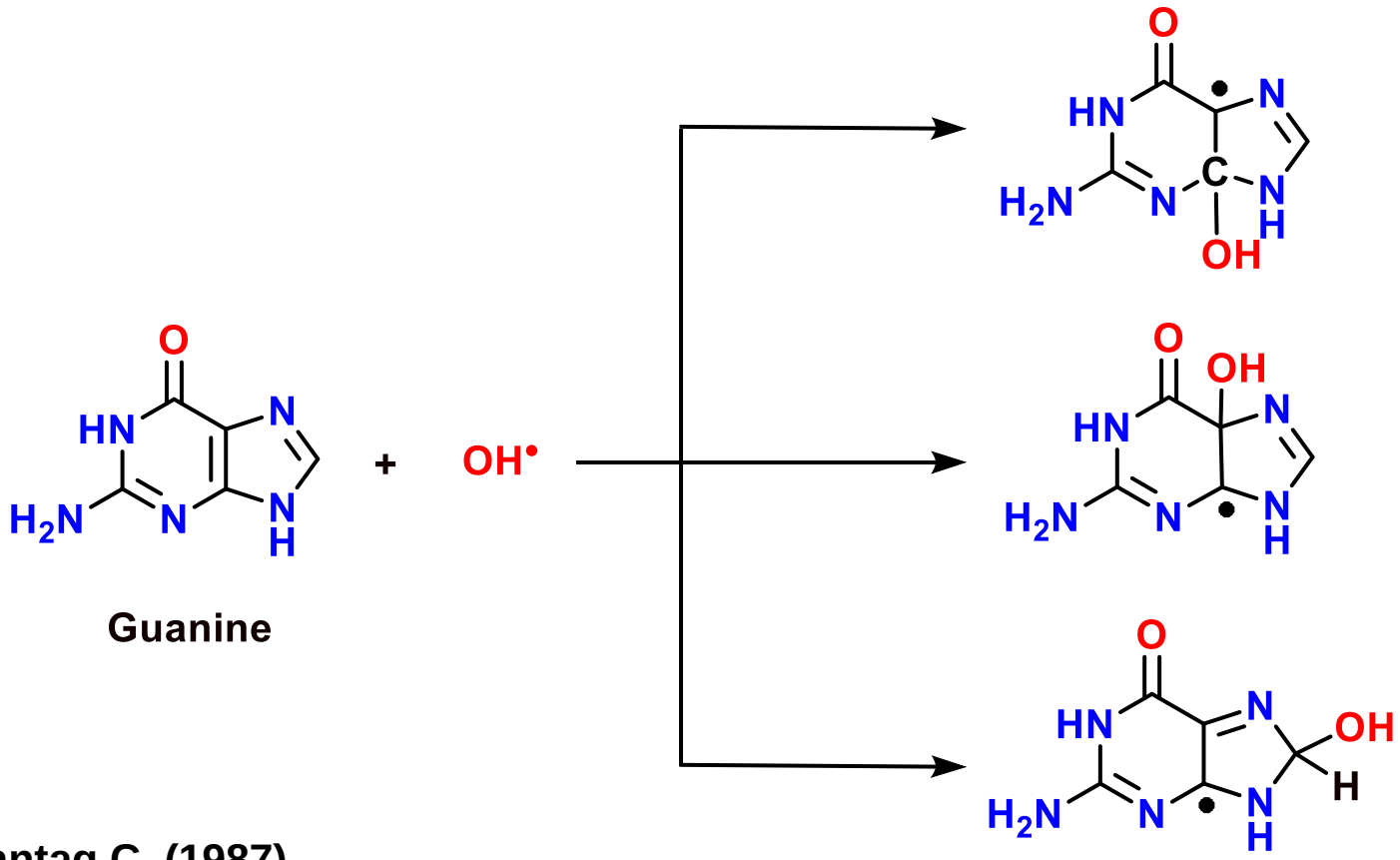
- **HO• attack on pyrimidines**



von Sonntag C. (1987)
The Chemical Basis of Radiation Biology.
Taylor & Francis London, NY.

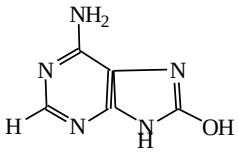
Oxidative Damage to the Bases

- **HO• attack on purines**

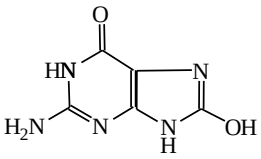


von Sonntag C. (1987)
The Chemical Basis of Radiation Biology.
Taylor & Francis London, NY.

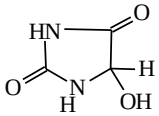
Examples of Oxidized DNA Bases



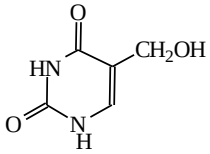
8-hydroxyadenine



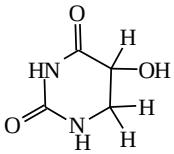
8-hydroxyguanine



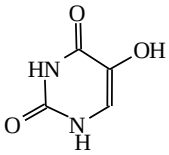
5-hydroxy hydantoin



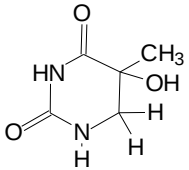
5-hydroxymethyluracil



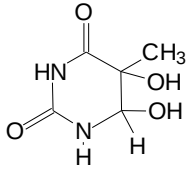
5-hydroxy-6-hydrouracil



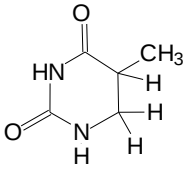
5-hydroxyuracil



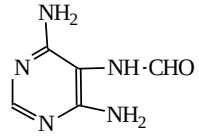
5-hydroxy-6-hydrothymine



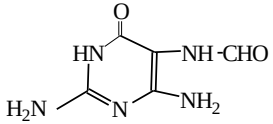
Thymine glycol (cis and trans)



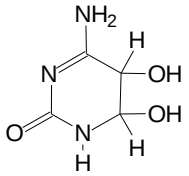
5,6-dihydrothymine



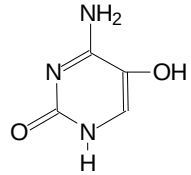
4,6-Diamino-5-formamidopyrimidine



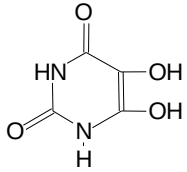
2,6-Diamino-4-hydroxy-formamidopyrimidine



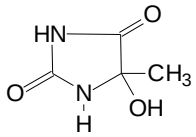
Cytosine glycol



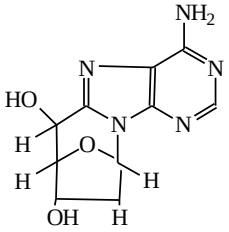
5-hydroxycytosine



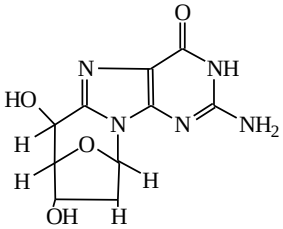
5,6-dihydroxy uracil



5-hydroxy-5-methylhydantoin



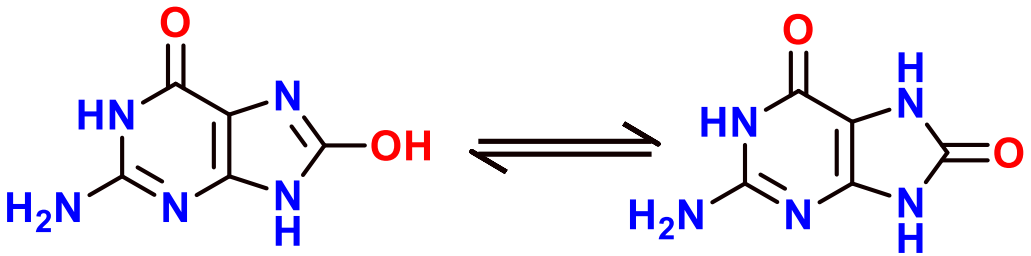
8,5'-cyclo-2'-deoxyadenosine



8,5'-cyclo-2'-deoxyguanosine

Adapted from: Dizdaroglu M. (1992)
***Free Radic Biol Med.* 10:225-242.**
Dizdaroglu M. (2002)
***Free Radic Biol Med.* 32:1102-1115.**

Oxidation of DNA can lead to Mutations



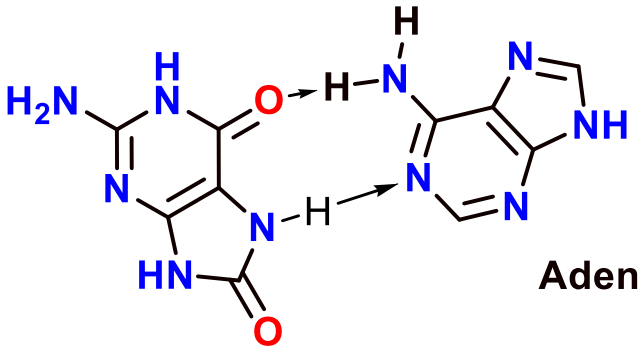
8-OH-Guanine (enol) **8-oxo-Gua** (keto)

Base pairs

A-T

G-C

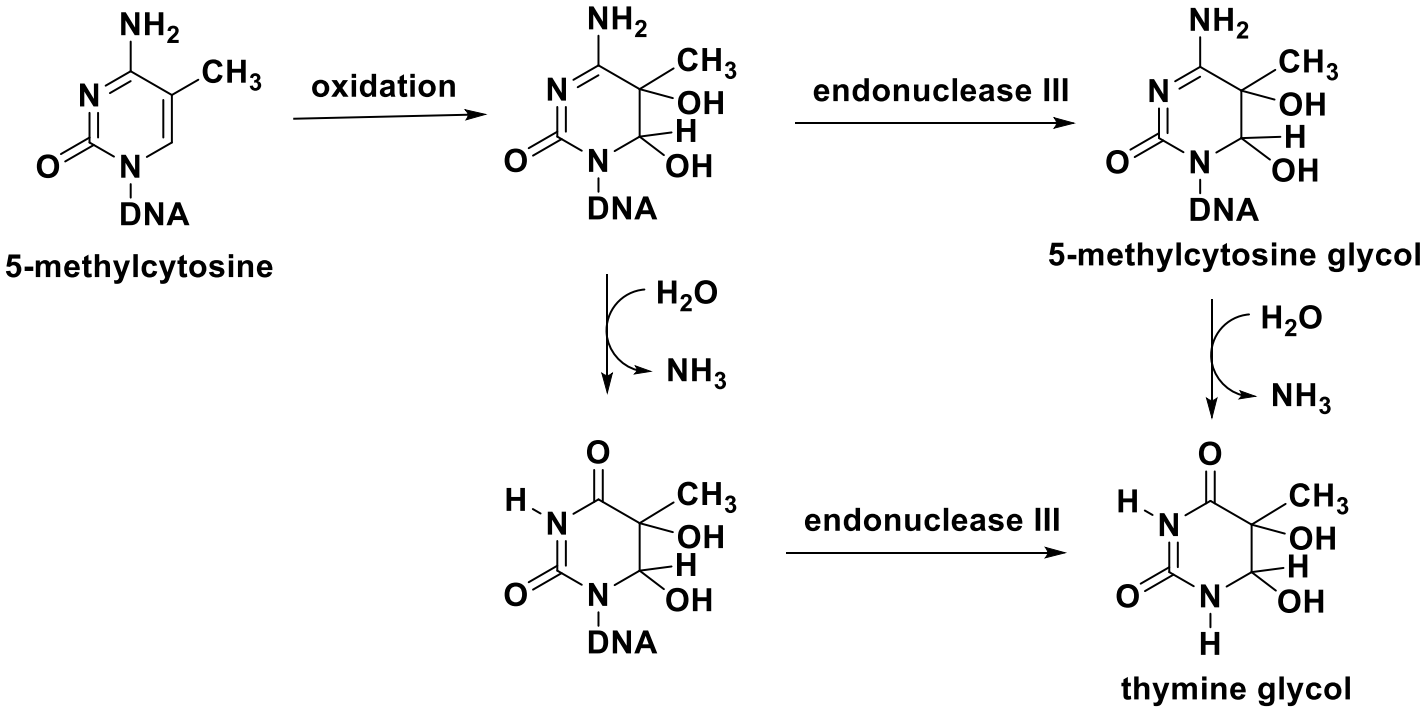
8 OH dG-A



8-OH-Gua **Adenine**

Misreading of 8-OHdGua can lead mutation (GC→AT transversion).

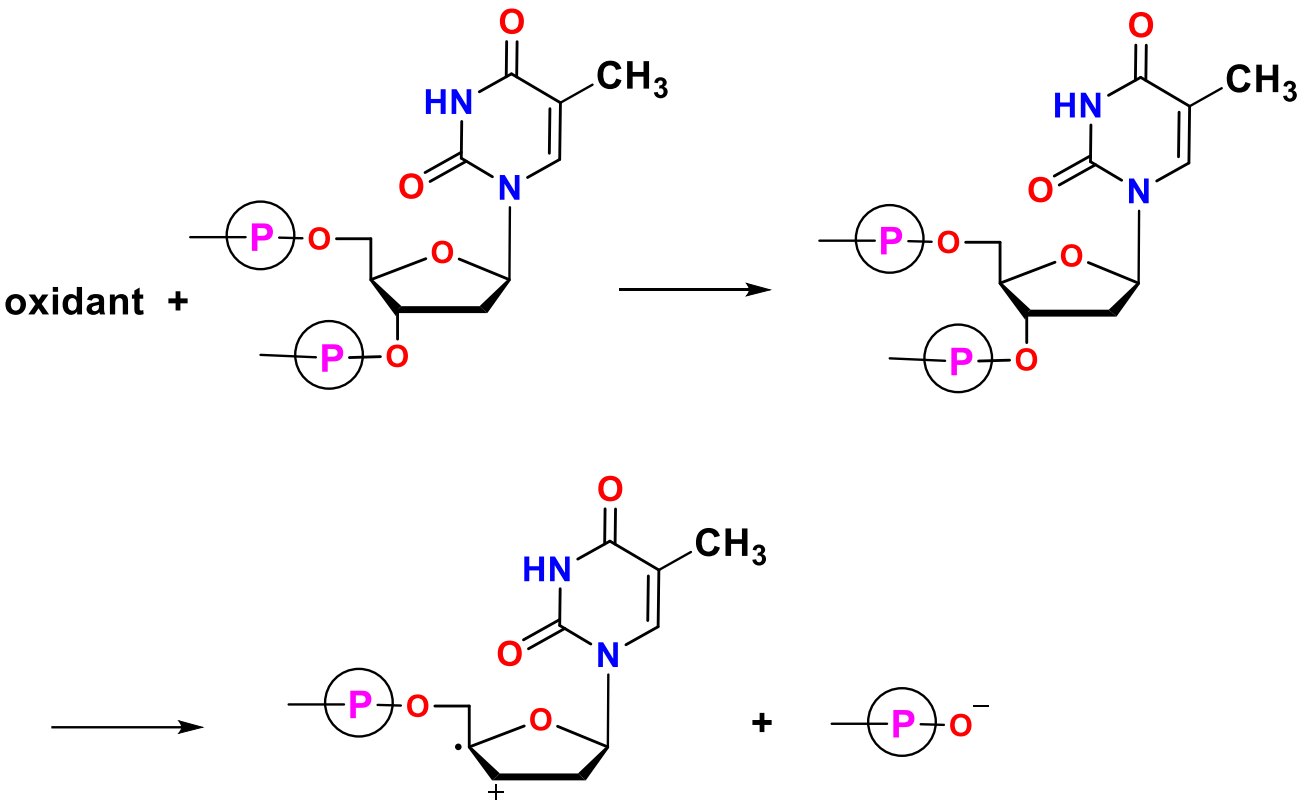
Oxidation of 5-methylcytosine leads to Rapid Deamination



Deamination can result in:
a) loss of methylation,
b) mutation (GC→AT transition).

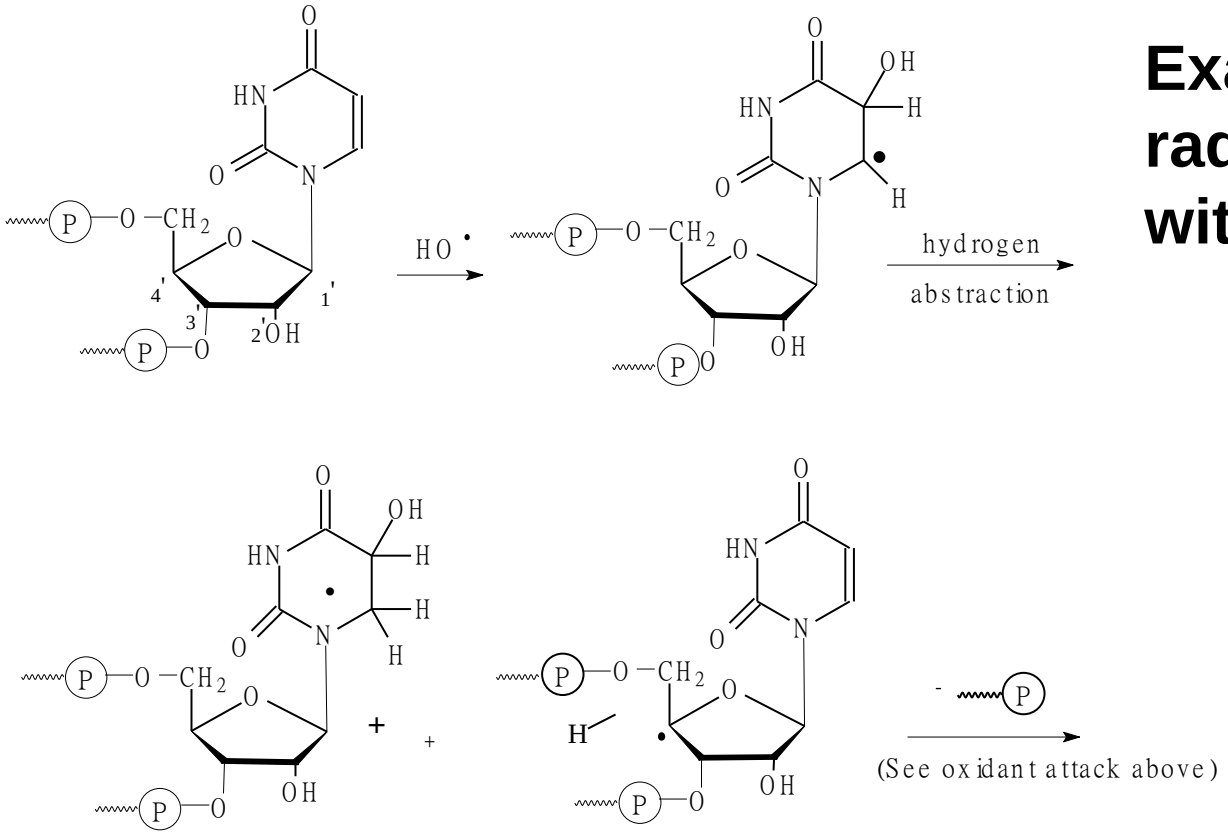
Adapted from: Zuo S, Boorstein RJ, Teebor GW. (1995) Oxidative damage to 5-methylcytosine in DNA. *Nucl Acids Res.* **23:**3239-3243.

Oxidant Attack on Sugar
(The 4'-hydrogen is the weak point)



Adapted from: C. von Sonntag (1987) *The Chemical Basis of Radiation Biology.* Taylor & Francis London, NY.

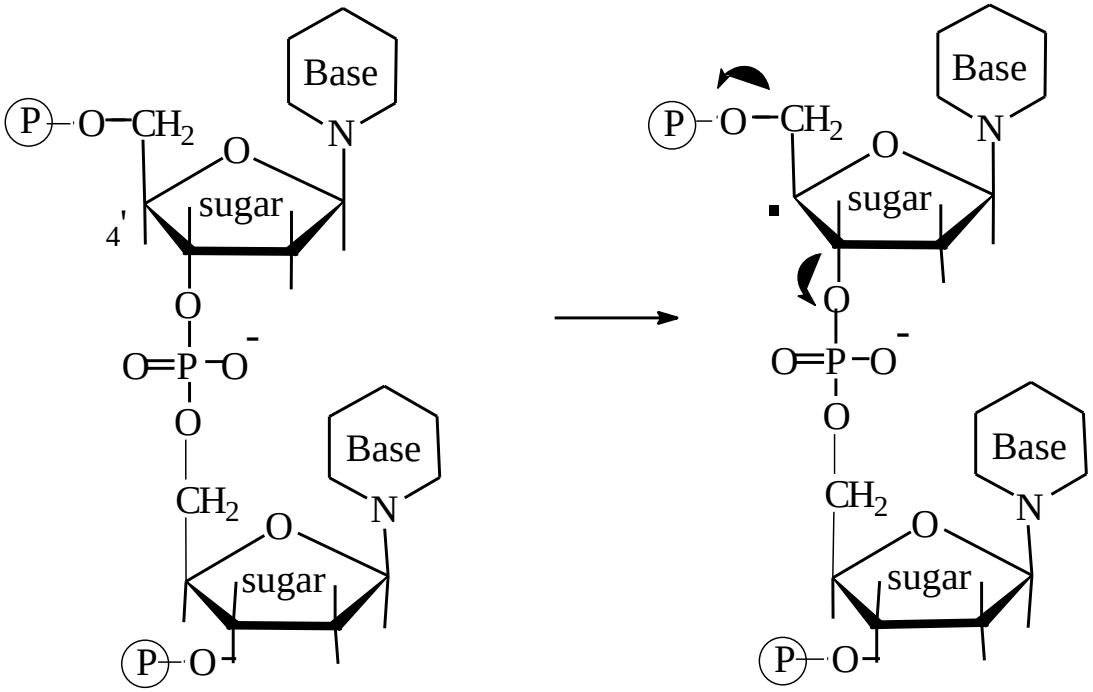
Transfer of Damage from a Base to a Sugar



Example: Uracil radical reacting with ribose

Adapted from: C. von Sonntag (1987) *The Chemical Basis of Radiation Biology.* Taylor & Francis London, NY.

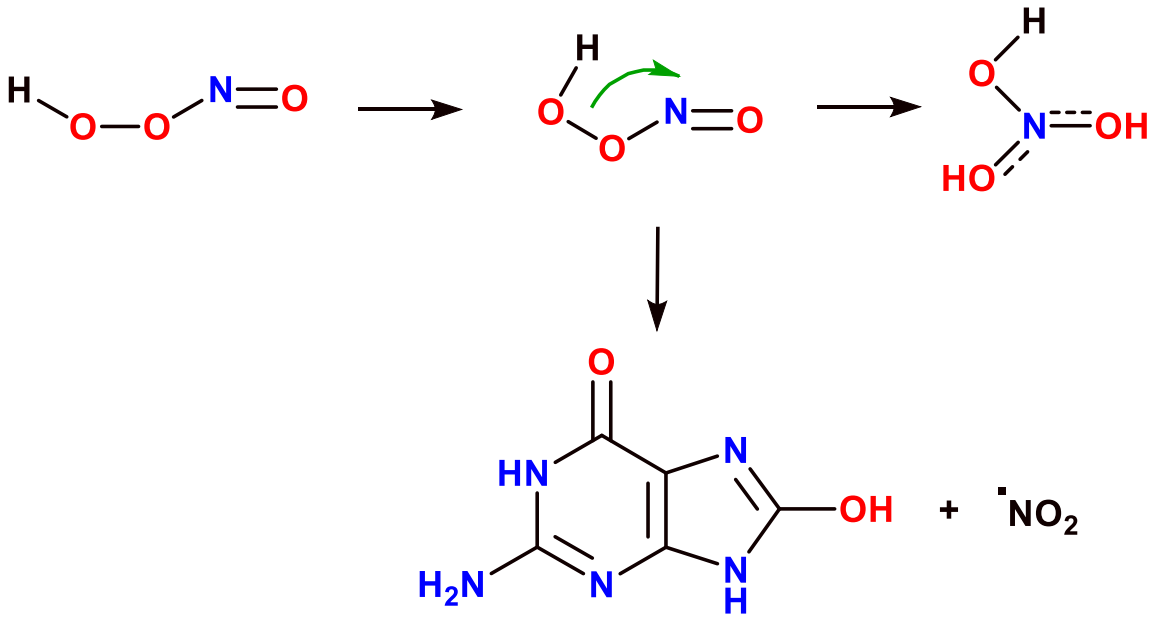
DNA Strand Breaks



DNA single strand breaks result because of the collapse of the sugar. They are the most common damage inflicted by ROS.

Damage by RNS

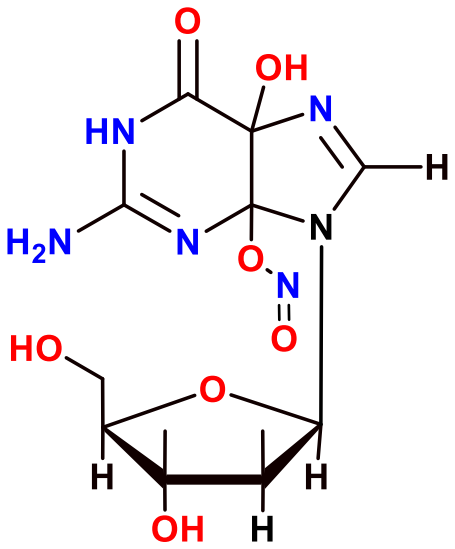
Peroxynitrite is a strong oxidant formed by reaction of nitric oxide with superoxide:



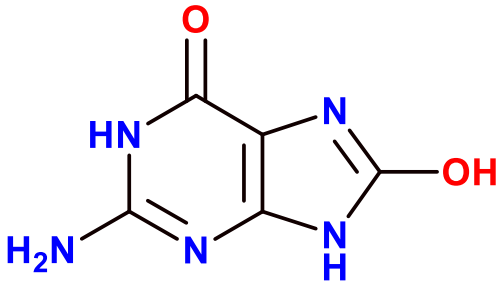
8-Hydroxy-2'-guanine

Spencer PE. *et al*. (1996) *Chem Res Toxicol.* **9:**1152-1158.

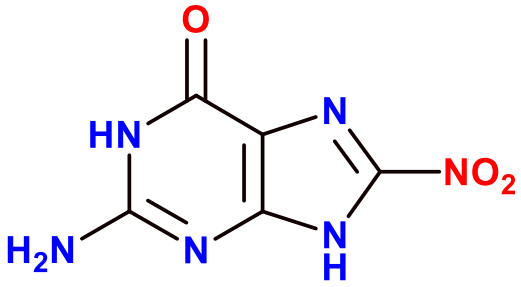
Reaction of Peroxynitrite with 2'-Deoxyguanosine Continued



4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2'-deoxyguanosine



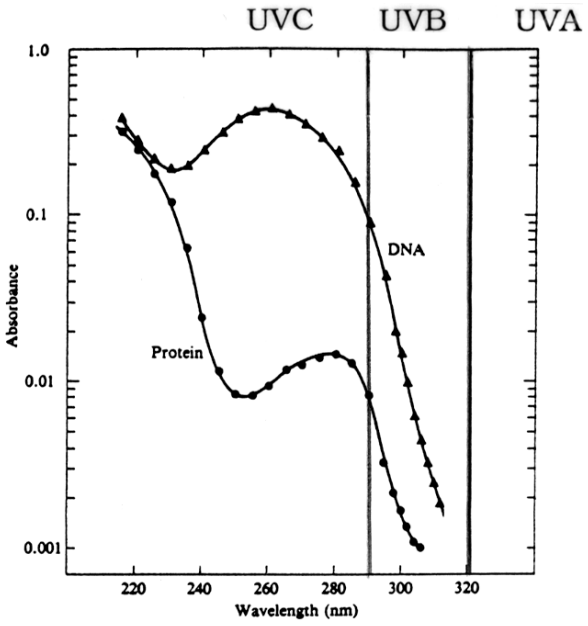
8-Hydroxyguanine



8-Nitroguanine

Adapted from Douki T. *et al.* (1996) *Chem Res Toxicol.* **9:**3-7.

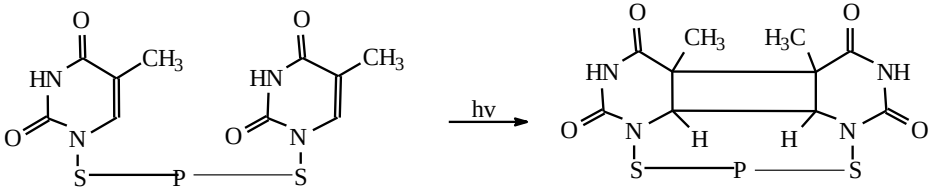
DNA Damage by UV light



Absorption spectra of DNA (calf thymus) and a protein (BSA) at equal conc. (≈ 20 μg/mL).

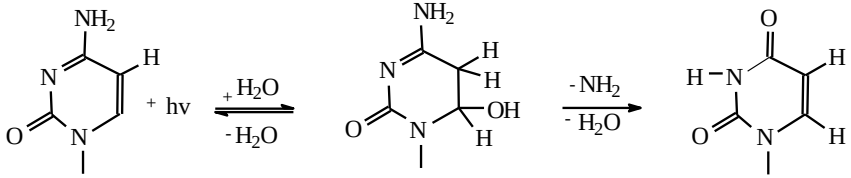
Adapted from: Harm W. (1980) *Biological Effects of Ultraviolet Radiation.* Cambridge University Press.

dimers:



pyrimidine dimers

hydrates:



cytosine

6-hydroxy-5,6-dihydrocytosine

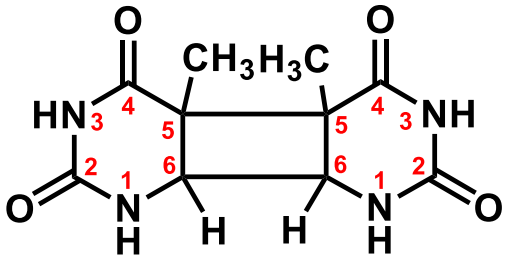
uracil

Adapted from: Halliwell B, Gutteridge JM. (1989) *Free Radicals in Biology and Medicine* Clarendon Press Oxford 2nd Ed.

Oxidative Damage of DNA by Ultraviolet Light

The main **photoproduct** formed by irradiation of DNA is **pyrimidine dimers**.

(Adjacent pyrimidines)



(Thymine dimer)

(Saturation of the C5=C6 double bond)

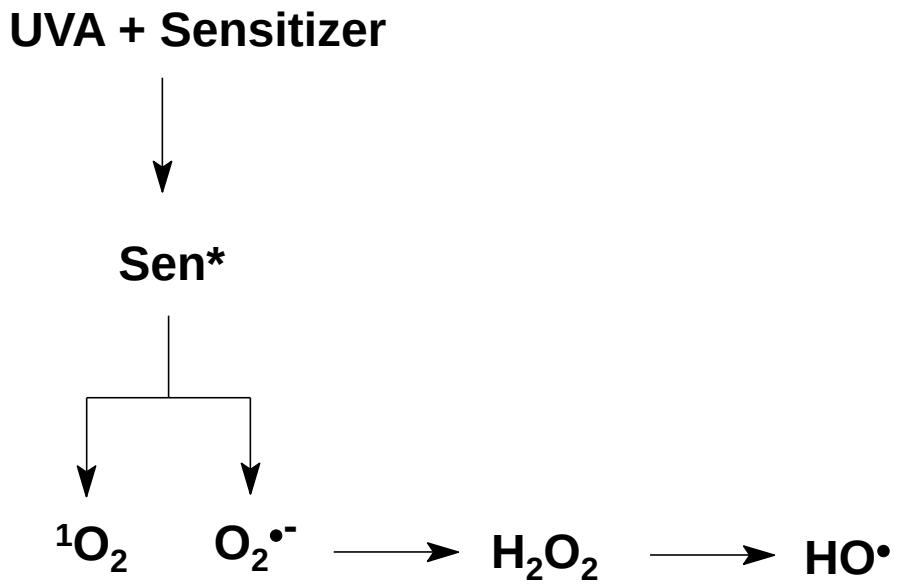
Adjacent pyrimidines in DNA become covalently linked by the formation of a 4-member ring. The consequences are **helix distortion**, but correct H-bonding can still occur.

- Reversible process: **Pyr + Pyr ⇄ Pyr-Pyr**

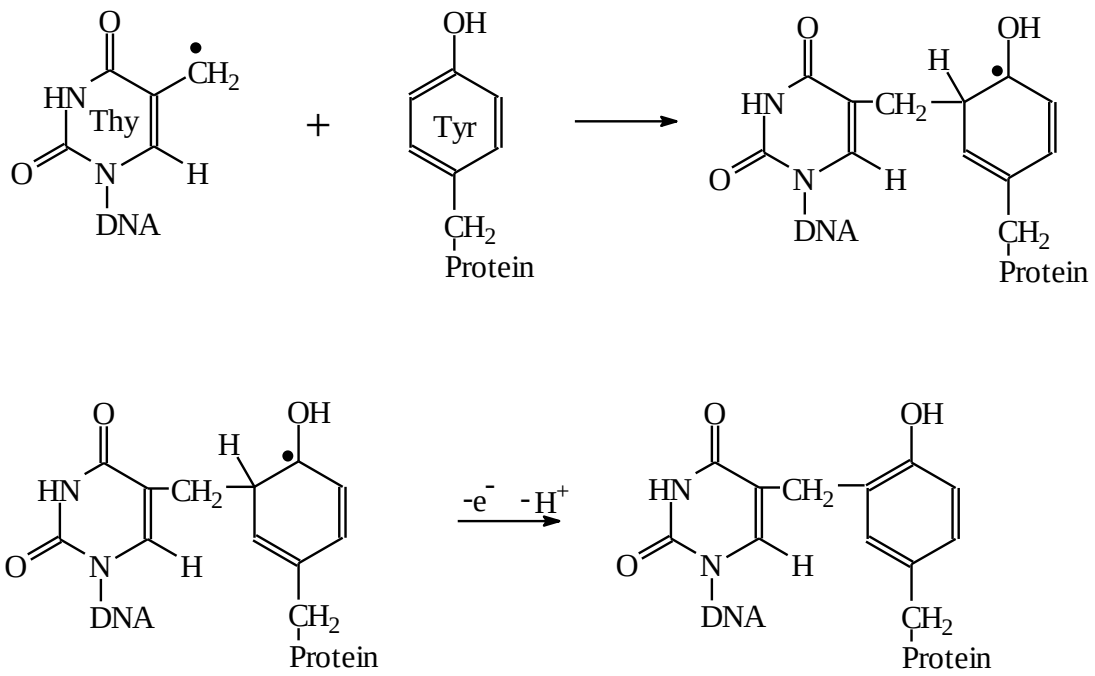
UV can also form:

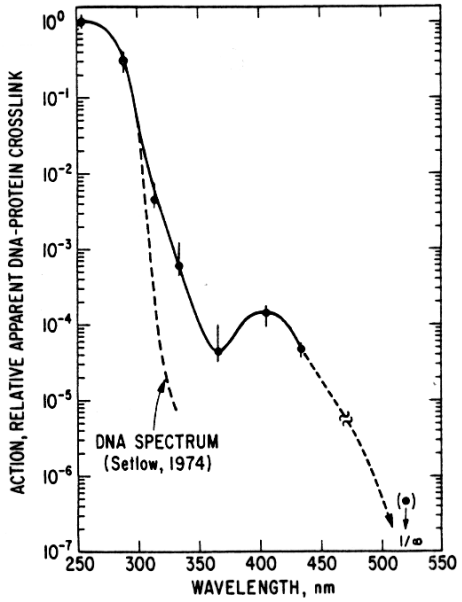
- Protein-DNA cross-links
- DNA-DNA cross-links.

Photosensitization can Produce Singlet Oxygen or Hydroxyl Radical



DNA-Protein-Crosslinking (DPC): Produced by UV Light or HO• Attack

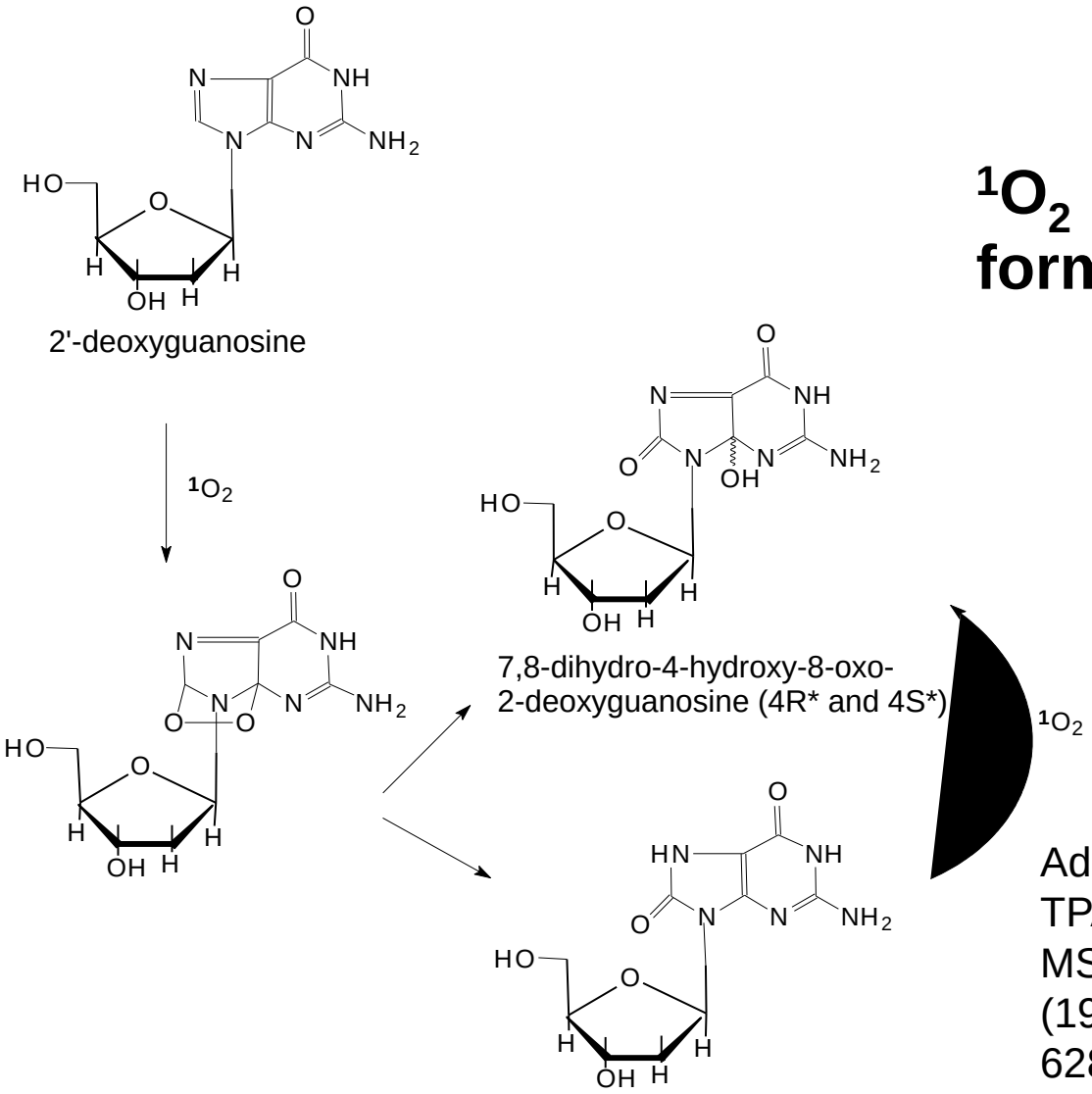




Action spectrum for the relative induction of DNA-protein crosslinks by UV and visible radiations

Peak GJ, Peak M J, Sikorski RS, Jones CA. (1985) *Photochem Photobiol.* **41:**295-302.

$^1O_2$ can React with DNA Bases



$^1O_2$ reacts with dG to form 8 OHdG

Adapted from: Devasagayam TPA, Steenken S, Obendorf MSW, Schulz WA, Sies H. (1991) *Biochemistry.* **30:**6283-6289.

Consequences of DNA Oxidation

- **DNA adducts/AP sites/Strand breaks**
  - Mutations
  - initiation of cancer
- **Stimulation of DNA repair**
  - can deplete energy reserves (PARP)
  - imbalanced induction of DNA repair enzymes
  - induction of error prone polymerases
  - activation of other signaling pathways

Summary

- **Oxidants can react with the DNA bases or sugars**
- **Guanine is the most sensitive base towards oxidative attack.**
- **More then 20 different oxidized base products are known; some can be mutagenic.**
- **Damage to the sugar can result in strand breaks.**
- **Electron rich moieties are the preferred sites of attack.**