Iron Chelates and Unwanted Biological Oxidations

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Introduction

- Versatility of oxidation state, reduction potential, coordination number, spin state, ligand type, ligand equilibria, ligand dynamics and structure give iron its special characteristics.

  Reed, C.A. (1985)
  in: The Biological Chemistry of Iron. (Dunford, H.B., Dolphin, D., Raymond, K.M., and Sieker, L., Eds.)
  D. Reidel, Boston, MA.

- Iron is an integral biological cofactor for many proteins. Iron is used in many different types of cofactors, *e.g.,* heme moieties, iron-sulfur clusters, di-iron centers, *etc*.

- Iron presents a significant paradox to the field of oxygen radicals in biology. It is required for many cellular functions yet it can also catalyze the deleterious oxidation of biomolecules.

 Aust, S.D. and Koppenol, W.H. (1991)
  in: Oxidative Damage and Repair: Chemical, Biological and Medical Aspects (Davies, K.J.A., Ed.), pp. 802-807, Pergamon Press.

Chelation of Iron

- The reactivity of iron is highly dependent upon its ligand environment. For example, chelators that contain oxygen ligands tend to stabilize Fe(III), while chelators that contain nitrogen or sulfur ligands tend to stabilize Fe(II).

- Ligation of iron by chelators that stabilize the ferrous form of iron, such as phenanthrolines, results in an increase in the reduction potential of the iron ($\approx$+1.1 V). Conversely, ligation of iron by chelators that stabilize the ferric form of iron, such as deferroxamine, results in a decrease in the reduction potential of the iron ($\approx$ -0.4 V).

Miller, D.M., Buettner, G.R., and Aust, S.D. (1990)
Free Radic. Biol. Med. **8**, 95-108.

The Effect of pH and Buffers on the Rate of Fe(II) Autoxidation
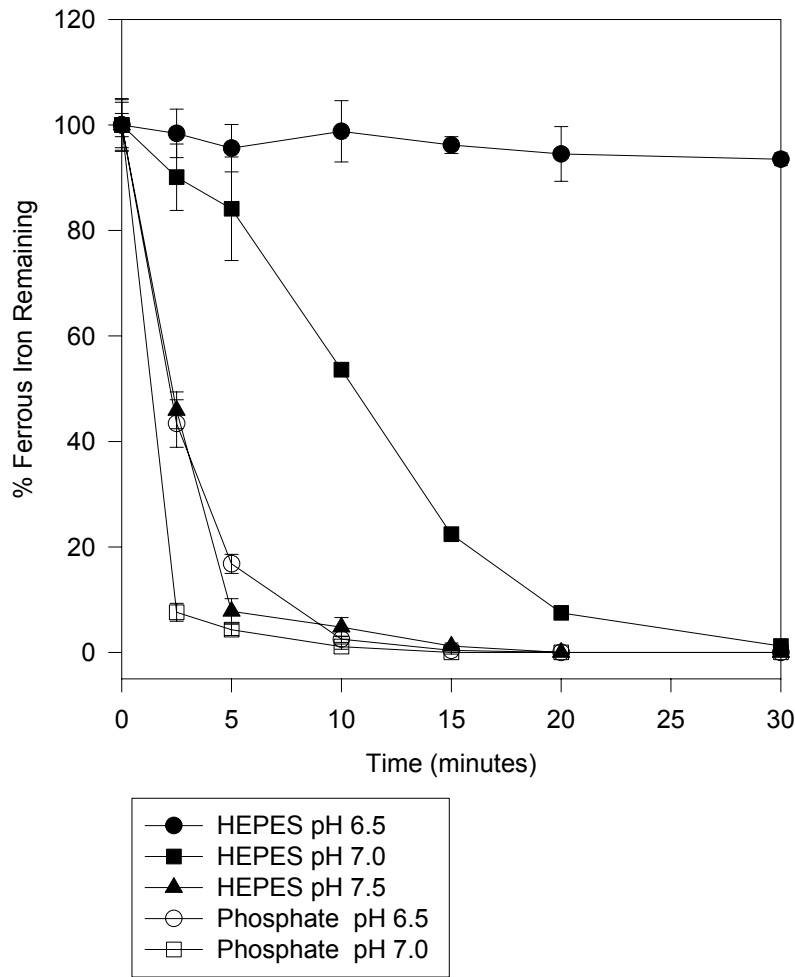
In general the rate of Fe(II) autoxidation in an aqueous solution (in the absence of any other chelators) is proportional to the square of $[OH^-]$.

$$rate = [Fe(II)][O_2][OH^-]^2$$

Harris, D.C. and Aisen, P. (1973) *Biochim. Biophys. Acta* **329**, 156-158.

However, relatively strong chelators such as phosphate, can somewhat override the effect of pH on the rate of Fe(II) autoxidation. For example, almost no Fe(II) autoxidizes in a HEPES buffered solution at pH 6.5, conversely, Fe(II) autoxidizes very rapidly in phosphate buffer, at pH 6.5.

Welch, K.D., Davis, T.Z., and Aust, S.D. (2002) *Arch. Biochem. Biophys.* **397**, 360-369.

The Effect of Chelation on the Rate and Stoichiometry of Fe(II) Autoxidation

The effect of chelation on the rate and
    stoichiometry of Fe(II) autoxidation

Chelator	Iron Oxidation[a] k (s^{-1})	Fe (II) / O_2[b]
None	1.2	3.4 ± 0.4
EDTA	> 11.5[d]	2.1 ± 0.1
NTA	> 11.5[d]	2.2 ± 0.1
Citric acid	7.2	2.5 ± 0.3
ADP	4.0	3.4 ± 0.1
Oxalic acid	2.3	3.4 ± 0.5
Histidine	2.0	3.8 ± 0.5
Histamine	1.0	3.3 ± 0.3
Glycine	1.5	3.3 ± 0.2
Deferoxamine	> 11.5[d]	2.1 ± 0.1
Hydroquinone	1.2	2.9 ± 0.2
Pyruvic acid	0.8	4.0 ± 0.2
Benzoquinone	10.7	none.[c]

Note. Reaction mixtures contained 110 µM Fe (II) and 550 µM chelator in 50 mM Tris buffer, pH 7.0.

[a] Iron oxidation rates are given as a 1st order rate constant for the initial rate (first 5 minutes).
[b] Fe (II) / O_2 refers to the number of Fe (II) oxidized per O_2 consumed.
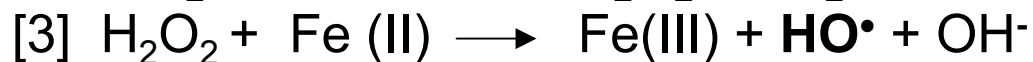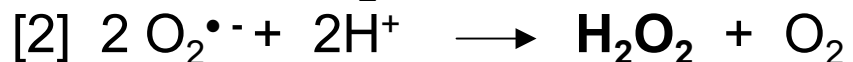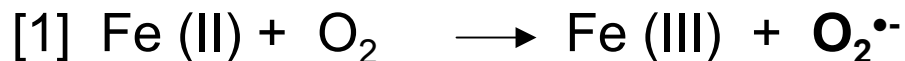[c] none = no oxygen consumed
[d] All the iron was oxidized by the first timepoint. Therefore, the rate constant for this reaction was greater than 11.5 s^{-1}.

It has been shown experimentally that chelators, in general, affect the stability of Fe(II) as expected, *i.e.,* chelators that ligate Fe(II) *via* oxygen ligands promote the autoxidation of Fe(II), whereas the autoxidation of Fe(II), ligated by chelators with nitrogen ligands, is slower. Interestingly, the stoichiometry of the autoxidation reaction appears to be inversely related to the rate, *i.e.,* the faster the rate of Fe(II) autoxidation the lower the stoichiometry.

Welch, K.D., Davis, T.Z., and Aust, S.D. (2002) *Arch. Biochem. Biophys.* **397**, 360-369.
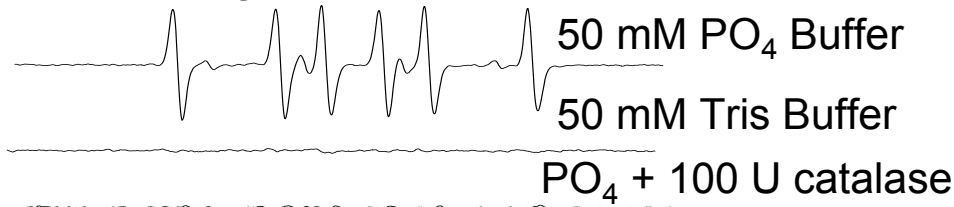
Mechanisms of Iron-Mediated Toxicity

- Iron can mediate the deleterious oxidation of biomolecules, which can have serious consequences for an organism, resulting in various diseases, *e.g.,* cancer, atherosclerosis, and diabetes.

  – Iron can oxidize numerous biomolecules indirectly *via* partially reduced oxygen species that can be produced in the presence of iron. One of the most commonly accepted mechanisms of iron-mediated oxygen radical production is described by the Haber-Weiss series of reactions shown below.

- Iron can also oxidize numerous biomolecules *via* direct transfer of an electron from the molecule to an iron complex, *e.g.,* ascorbate and dopamine.

$$[1] \ \ Fe \ (II) + O_2 \longrightarrow Fe \ (III) + \mathbf{O_2^{\bullet-}}$$
$$[2] \ \ 2 \ O_2^{\bullet-} + 2H^+ \longrightarrow \mathbf{H_2O_2} + O_2$$
$$[3] \ \ H_2O_2 + Fe \ (II) \longrightarrow Fe(III) + \mathbf{HO^{\bullet}} + OH^-$$
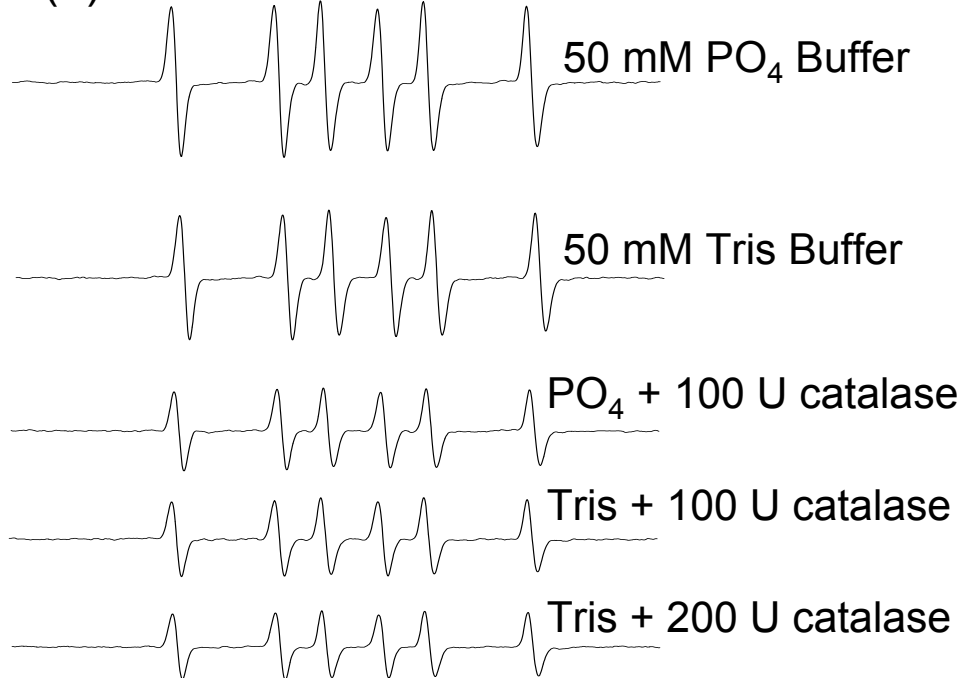
Reilly, C.A. and Aust, S.D. (2000) *in*: Toxicology of the Human Environment. The critical role of Free Radicals. C.J. Rhodes, Ed., Taylor & Francis Publ., London. pp 155-190.

Comparison of EtOH Oxidation by Fenton Reagent *vs* Fe:EDTA

Fenton Reagent

50 mM PO$_4$ Buffer

50 mM Tris Buffer

PO$_4$ + 100 U catalase

Fe(II):EDTA

50 mM PO$_4$ Buffer

50 mM Tris Buffer

PO$_4$ + 100 U catalase

Tris + 100 U catalase

Tris + 200 U catalase

The autoxidation of some iron chelates results in the production of a strong oxidant, which is not the hydroxyl radical. The DMPO adduct trapped during the oxidation of EtOH by HO$^\bullet$ is different than the adduct trapped during the oxidation of EtOH by Fe(II):EDTA. Additionally, the oxidation of EtOH by the HO$^\bullet$ is inhibited by both the presence of organic buffers such as Tris and the addition of catalase. Whereas the oxidation of EtOH by Fe(II):EDTA is not inhibited by the presence of organic based buffers. The oxidation of EtOH by Fe(II):EDTA is sensitive to, but not dependent upon, H$_2$O$_2$. These data suggest that a ferryl iron species may be formed during the oxidation of Fe(II):EDTA.

Welch, K.D., Davis, T.Z., and Aust, S.D. (2002) *Arch. Biochem. Biophys.* **397**, 360-369.

Direct Oxidation of Biomolecules

Many biomolecules are good Fe(III) reductants, however, they are also good iron chelators. Therefore, they will reduce Fe(III) to Fe(II) and subsequently chelate the Fe(II), which can promote the oxidation of the Fe(II) to Fe(III) with the concomitant reduction of O_2 to $O_2^{\bullet-}$. Thus, it appears as if the biomolecule autoxidized when in fact the oxidation of the biomolecule was mediated by a transition metal such as iron. The oxidation of ascorbate by iron is an excellent example.

Buettner, G.R. (1988) *J. Biochem. Biophys. Meth.* **16**, 27-40.

	Adrenochrome Formed (nmoles/min)	
Water Source	(-) Desferal	(+) Desferal
Tap water	1.20 ± 0.12	0
Deionized water	0.40 ± 0.08	0
Chelex treated water	0	0

The oxidation of epinephrine has been shown to be catalyzed by contaminating metals. Any treatment of the solutions to remove or chelate transition metals resulted in an inhibition of the oxidation of epinephrine.

Ryan, T.P., Miller, D.M., and Aust, S.D. (1993)
J. Biochem. Toxicol. **8**, 33-39.

# Schematic Representation of Lipid Peroxidation
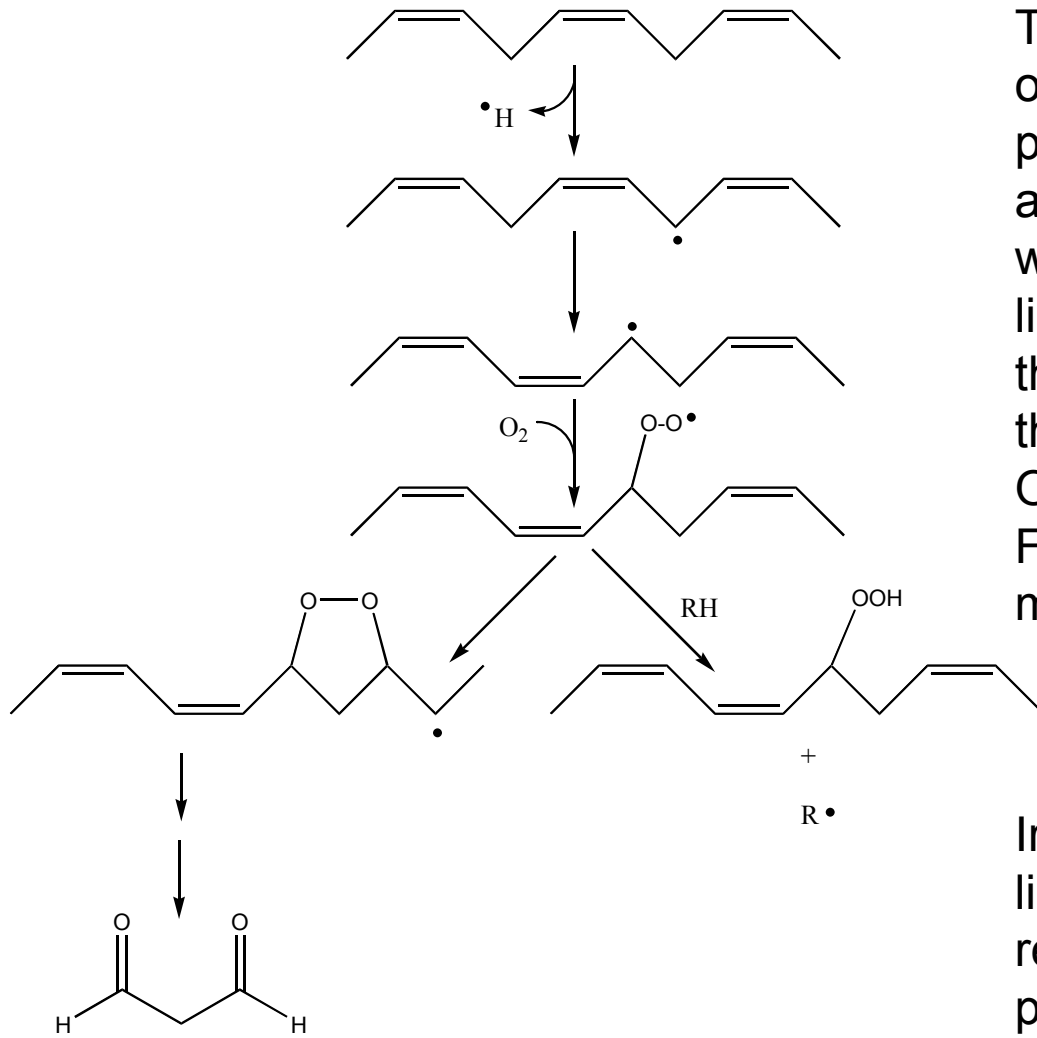
The process of lipid peroxidation occurs *via* a free radical mechanism promoted by iron. The first step is the abstraction of a hydrogen atom, which results in the formation of a lipid radical. There are different theories as to how iron is involved in the initiation of lipid peroxidation. One hypothesis is that a Fe(II)-O_2-Fe(III) species is required for iron-mediated lipid peroxidation.

Bucher, J.R., Tien, M. and Aust, S.D. (1983) *Biochem. Biophys. Res. Commun.* **111**, 777-784.

Iron can also breakdown existing lipid hydroperoxides, in a Fenton-like reaction, which will assist in the propagation steps of lipid peroxidation.

# The Dependence of Lipid Peroxidation on the Fe(II):Fe(III) Ratio
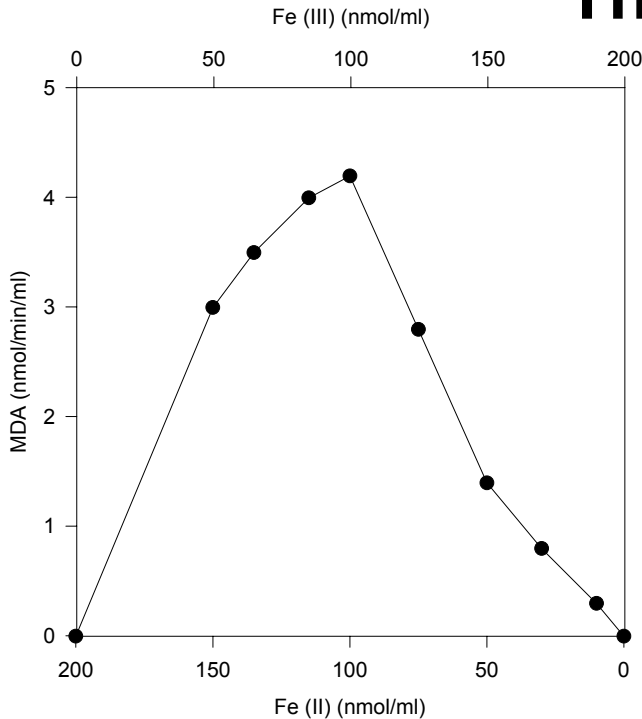
It has been shown that maximal rates of lipid peroxidation are observed when the the ratio of Fe(II):Fe(III) is 1:1.

Minotti, G. and Aust S.D. (1987)
J. Biol. Chem. 262, 1098-1104.

The addition of Fe(II), chelated by any one of a number of different iron chelators, in a liposomal system often results in a short lag period before lipid peroxidation starts. The addition of a second chelator results in an increase in the lag period or inhibition of lipid peroxidation. The degree of inhibition depends on the stability constant of the iron:chelator complex. The most pronounced effects were observed for the chelators with higher stability constants, which supports the hypothesis that a 1:1 ratio of Fe(II):Fe(III) is responsible for iron-mediated lipid peroxidation. Tang, L.X. *et al.* (1997) *J. Inorg. Biochem.* **68**, 265-272.

The Effect of Ascorbic Acid Concentration on ADP:Fe(III)-dependent Lipid Peroxidation
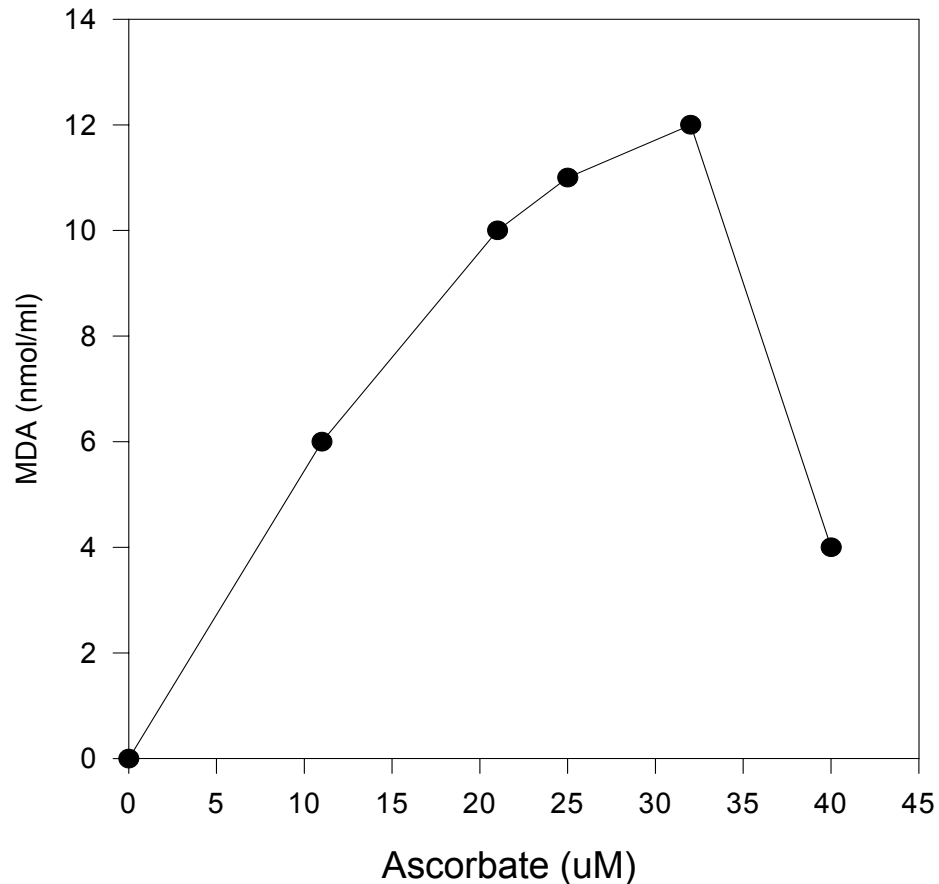
Ascorbic acid is able to promote the oxidation of lipids. The role of ascorbic acid in lipid peroxidation can be explained by the fact that ascrobate can reduce Fe(III) to give the 1:1 ratio of Fe(II):Fe(III). A high ratio of ascorbate to Fe(III) results in essentially all Fe(II), and less lipid peroxidation.

Miller, D.M. and Aust, S.D. (1988) *Arch. Biochem. Biophys.* **271**, 113-119.

Protein Oxidation

- Molecular oxygen, Fe(III), and an appropriate electron donor can catalyze the oxidative modification of proteins. Only a few amino acids are modified and relatively little peptide bond cleavage occurs when proteins are exposed to iron-mediated oxidation systems. The available data indicate that iron-mediated oxidation systems catalyze the reduction of Fe(III) to Fe(II) and of O_2 to H_2O_2 and that these products react at metal-binding sites on the protein to produce active strong oxidants (•OH, ferryl ion) which attack the side chains of amino acid residues at the metal-binding site.

<div align="right">

Stadtman, E.R. (1990)
Free Radic. Biol. Med. **9**, 315-325.

</div>

- Iron-binding sites on proteins serve as centers for repeated production of •OH that are generated via the Fenton reaction. Prevention of the site-specific free radical damage can be accomplished by using selective chelators for iron, by introducing high concentrations of HO• scavengers, and by adding enzymes that remove O_2•$^-$ to H_2O_2. Histidine, for example, is a compound that can intervene in free radical reactions in a variety of modes.

<div align="right">

Chevion, M. (1988)
Free Radic. Biol. Med. **5**, 27-37.

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DNA Oxidation

- It is thought that the genotoxicity of many chemicals is enhanced by their ability to decompartmentalize iron.

  Li, A.S. et al. (2001)
  Free Radic. Biol. Med. **30**, 943-946.

- One mechanism by which iron could be involved in the initiation or promotion of cancer is through the oxidation of DNA. The species responsible for oxidizing DNA is believed to be the $HO^\bullet$. Superoxide radicals have no effect on the oxidation of DNA in the absence of adventitious metals. This suggests that the role of $O_2^{\bullet-}$ in DNA oxidation is simply as a constituent of the Haber-Weiss reactions to produce the $HO^\bullet$.

- The addition of any chemical that will act as an alternate reactant for the $HO^\bullet$, such as organic-based buffers or $HO^\bullet$ scavengers, inhibits the oxidation of DNA. Conversely, the presence of chemicals which increase the iron-mediated production of $HO^\bullet$ will promote the oxidation of DNA.

  Djuric, Z. *et al.* (2001)
  J. Biochem. Mol. Toxicol. **15**, 114-119.

Conclusions

- The reactivity of iron is highly dependent upon its ligand environment. Any change in the ligand environment of iron, such as pH, buffer, or chelator, will effect its reactivity.

- Biomolecules do not autoxidize. Their oxidation is mediated by transition metals such as iron.

- Iron can mediate the oxidation of DNA and protein. DNA and most likely protein are oxidized by the hydroxyl radical.

- Iron appears to have a direct effect on the oxidation of lipids. Lipids can be oxidized by the hydroxyl radical but some type of $Fe(II)-O_2-Fe(III)$ complex also promotes lipid peroxidation.

- The iron-mediated oxidations of DNA, protein, lipid, and organic chemicals do not occur *via* the same mechanism.