This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

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Protein Hydroperoxide

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For 77:222, Spring 2005

26 Jan 2005

Abbreviations:

HO•— hydroxyl radical
H—hydrogen atom
PrH—protein molecule
$PrOO^-$ — protein peroxyl anion
ROS—reactive oxygen species
Pr•—protein radical
PrO•—protein alkoxyl radical
GSH — Glutathione
$O_2^{\bullet-}$—superoxide
$PrOO^\bullet$ —protein peroxyl radical
POOH—protein hydroperoxide
Ox — Oxidant
Protein-C•—protein carbon-centered radical

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Abstract

A major reaction product of HO• on proteins is the generation of protein hydroperoxide. Compared to most ROS, the peroxidized proteins have longer lifetimes and can initiate several kinds of damaging reactions. The protein hydroperoxides may constitute an intermediate stage in ROS-induced biological damage.

Introduction

It has been demonstrated that reactive oxygen species (ROS), the inevitable by-products of normal respiration [1], are involved in many diseases such as cancer, aging, inflammation and various forms of nervous system and skin damage. In order to design preventive and therapeutic methods to reduce or limit the deleterious effects of ROS, one needs to know the pathway of ROS production and the end points damage *in vivo*. Generation of supreoxide ($O_2^{\bullet-}$) by aerobes is a constant side effect of oxygen utilization. Being rather unreactive, the toxicity of $O_2^{\bullet-}$ was believed to depend on the production of hydroxyl radical ($HO^{\bullet}$). Since the half-life of $HO^{\bullet}$ is short, diffusion distance is limited,

which means that the most abundant cell constituent is the most likely target to be attacked. Research [2,3, 8] demonstrated that protein is the major initial cell target of hydroxyl free radicals. (Figure 1) A major reaction product of HO• on proteins is generation of hydroperoxside on protein. [7] Compared to most ROS, the peroxidized proteins have longer lifetimes, which enable them to diffuse a longer distance in cells or tissues. Protein hydroperoxides can initiate several kinds of damaging reactions, so it may constitute an intermediate stage in ROS-induced biological damage.

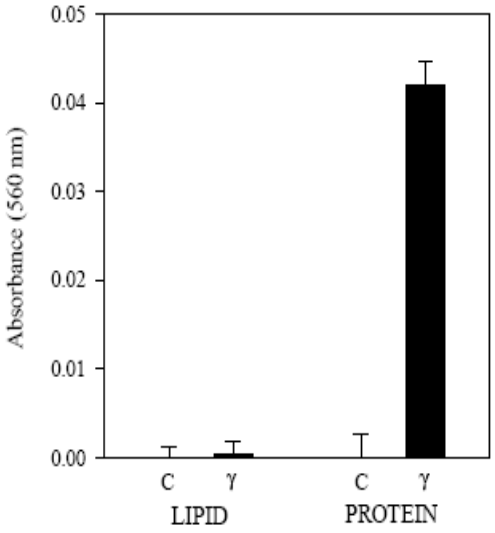

Figure. 1. Formation of protein and lipid peroxides in Sp2/0 cells. No lipid peroxidation or DNA damage was evident by the time of significant formation of protein peroxides [2].

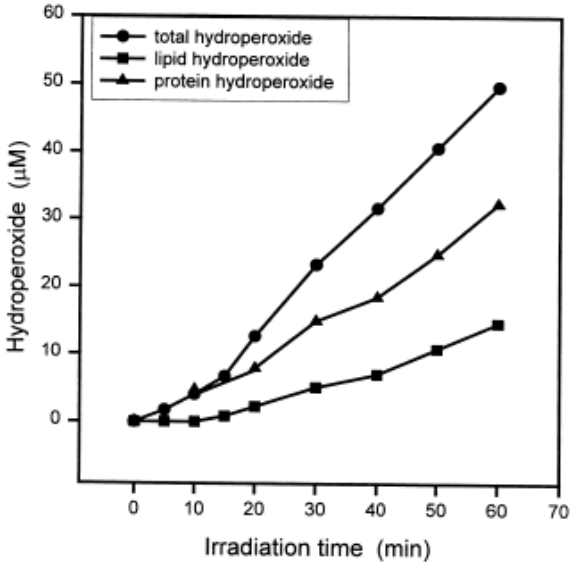

Figure 2. The kinetics of formation of protein and lipid hydroperoxides in serum. Human blood serum was exposed to γ rays under a stream of oxygen and the total, protein, and lipid hydroperoxides was measured in samples removed at intervals [8].

Formation of protein hydroperoxides

With an advantage of generating known quantities of defined free radicals, all initial studies of protein hydroperoxides employed X- or γ-irradiation of air- or O_2-saturated aqueous protein solutions [3]. Within the range of pH 5~9, the protein hydroperoxide yields are constant [3]. Protein hydroperoxide can be formed both on the backbone (at the α-carbon position) and side-chain [5~6]. (See figure 3 and 4).

$$PrH + Ox \longrightarrow Pr^{\bullet} + \text{reduced Ox} \quad (1)$$

$$Pr^{\bullet} + O_2 \longrightarrow PrOO^{\bullet} \quad (2)$$

$$Pr^{\bullet} + e^- \longrightarrow PrOO^- \quad (3)$$

$$PrOO^- + H^+ \longrightarrow PrOOH \quad (4)$$

$$PrOO^{\bullet} + PrH \longrightarrow PrOOH + Pr^{\bullet} \quad (5)$$

$$PrOO^{\bullet} + \text{radical} \longrightarrow POOH \quad (6)$$

When the oxidant (Ox) is $HO^{\bullet}$, its oxidizing power makes all hydrogen atoms (H) except those in aromatic rings, labile to abstraction (reaction 1). $Pr^{\bullet}$, a carbon-centered free radical, is formed. $Pr^{\bullet}$ has a longer lifetime that is long enough for reaction with O_2 to form protein peroxyl radicals ($PrOO^{\bullet}$) (reaction 2). Electrons can translocate freely in proteins and would be available to reduce the protein peroxyl radical in reaction 3. $PrOO^{\bullet}$ has a long lifetime, which allows it react with other protein molecules (PrH) quickly and initiate a chain reaction (reaction 5). Then reactions 1, 2 and 5 go on in endless cycle until terminated when $PrOO^{-}$ reacts with H^{+} or $PrOO^{\bullet}$ reacts with other radicals such as amino acid radical. (reaction 4,6) [3]

Stability of protein hydroperoxides

Protein hydroperoxides are long-lived in the absence of oxidizing or reducing agents, light, heat or transition metal ions, but are rapidly decomposed when treated with any of these agents.[9] Two-electron reduction of protein hydroperoxides (*e.g*. with borohydride) gives the corresponding unreactive alcohols [10].

Most common decomposition *in vivo* is hemolytic, and involves redox active agents such as transition metals. Exposure of the hydroperoxides to transition metal ions results in the formation of further reactive radicals, including alkoxyl (*e.g*. reaction 7), carbon-centered (from rearrangement or fragmentation of the initial alkoxyl species, reaction 8), and peroxyl (*e.g*. reaction 9) species [9].

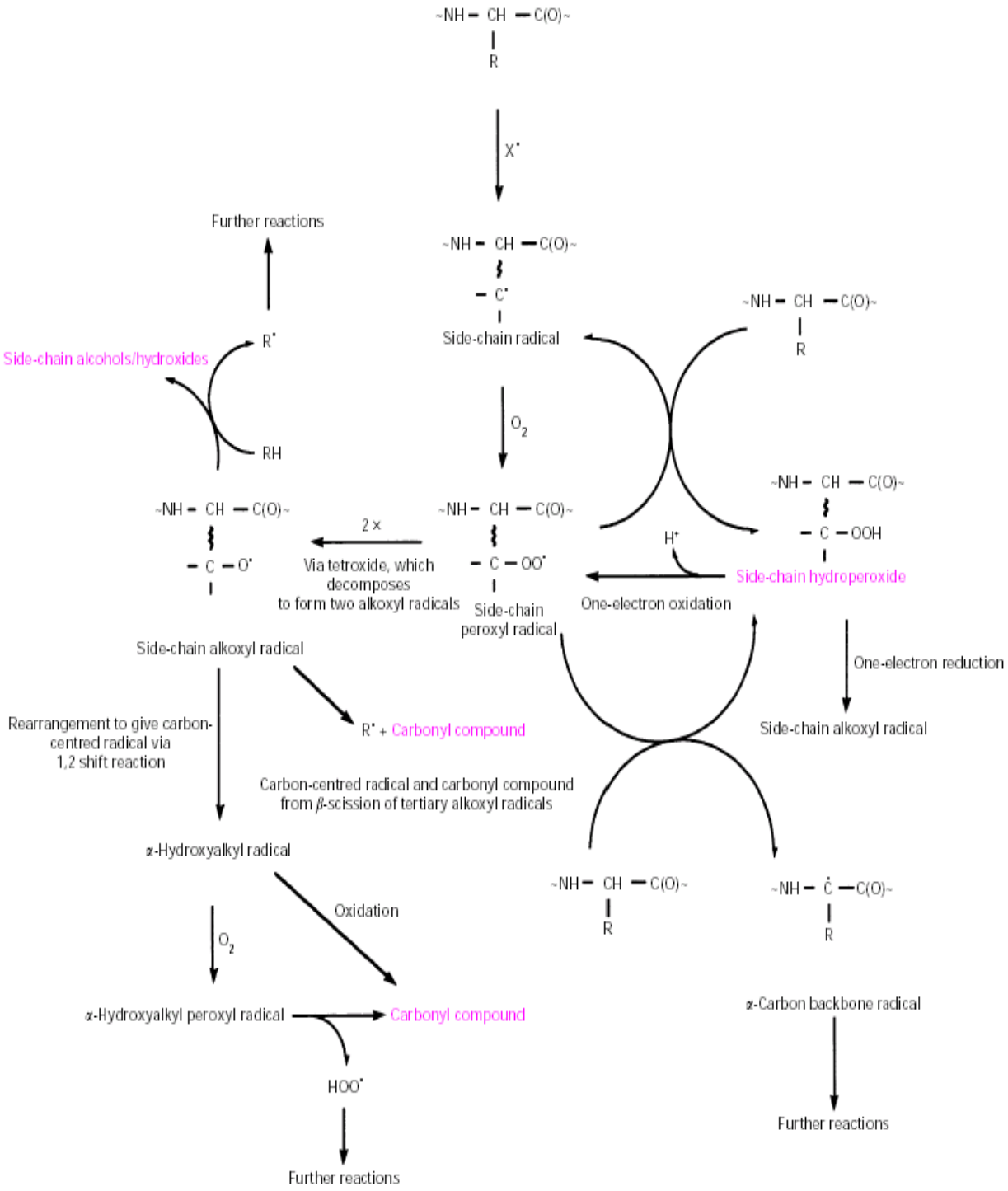

Figure 3. Major reactions of α side-chain radicals formed during protein oxidation in the presence of oxygen. (Species detected as products of side-chain oxidation are depicted in color).[5]

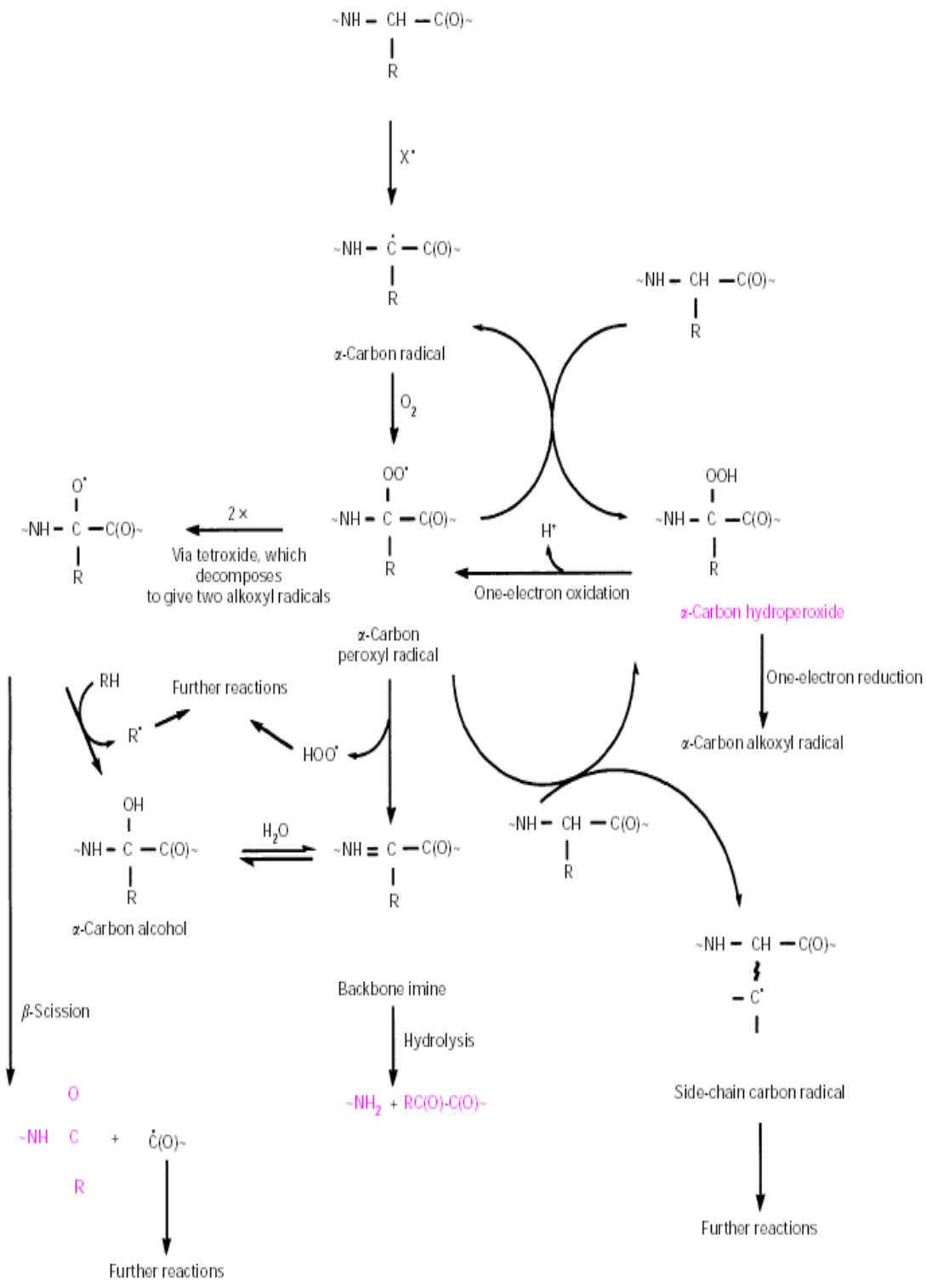

Figure 4. Major reactions of backbone radicals formed during protein oxidation in presence of oxygen. (Species detected as products of backbone oxidation are depicted in color. [5]

$$ROOH + Fe^{2+} \longrightarrow RO^{\bullet} + Fe^{3+} + HO^{-} \quad (7)$$

$$RO^{\bullet} + \longrightarrow R^{\bullet} \quad (8)$$

$$ROOH + Fe^{3+} \longrightarrow ROO^{\bullet} + Fe^{2+} + H^{+} \quad (9)$$

Reactivity of protein hydroperoxides with biomolecules

Protein-hydroperoxides can oxidize antioxidants and reducing agents such as ascorbate and GSH. They can also react with methionine residues, inactivate glutathione reductase, oxidize lipids, and crosslink with DNA [9].

The earliest report of the potential of protein hydroperoxides to cause biological damage is the oxidation of GSH and ascorbate [7, 10]. Inactivation of cellular caspases and inhibition of glyceraldehyde-3-phosphate dehydrogenase by protein hydroperoxides has also been reported [11].

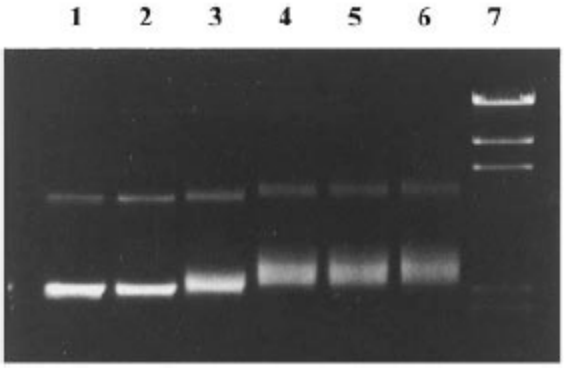

Figure 5 Gel shift of *pBR322* DNA incubated with increasing amounts of BSA-OOH at a constant BSA/DNA ratio
Samples of BSA (2 mg/mL in water) were irradiated for various times in a Co60 source at 30 Gy/min under oxygen and treated with catalase. Each sample contained different amounts of BSA-OOH. Lane 1, DNA alone. Lanes 2±6, DNA treated with 0, 41, 82, 119 and 161 μM protein peroxide. Lane 7, molecular-mass marker [4].

Table 1 The effect of radical scavengers and chelating agents on the migration of DNA incubated with peroxidized BSA [4].

DNA with . . .	Appearance of DNA bands
No addition	2, Narrow
BSA	2, Narrow
BSA-OOH	Retarded, diffuse
BSA-OOH + 26 mM formate	2, Narrow
BSA-OOH + 26 mM DMSO	Retarded, diffuse
BSA-OOH + 26 mM mannitol	Retarded, diffuse
BSA-OOH + 13 mM *t*-butanol	Retarded, diffuse
BSA + 1 mM trolox	Retarded, diffuse
BSA-OOH + 1 mM trolox	Retarded, diffuse
BSA-OOH + deferrioxamine	Less retarded and diffuse
BSA-OOH + neocuproine	Less retarded and diffuse
BSA-OOH + both chelators	Less retarded and diffuse

Exposure DNA to several peroxidized proteins resulted in formation of cross-links between the macromolecules (Figure 5) [4]. In order to get a view of the mechanism of cross linking, the authors also tested the effects of anti-oxidants, metal chelators, and free radical scavengers on the DNA-protein crosslinking (Table 1).

The action of formate was probably due to an increase in ionic strength. The effects of chelators suggested a role for metals in the cross-linking process [4]. The initial formation of alkoxyl radicals generated in metal-induced decomposition of protein peroxides (reaction 7) may shed some light on this process. Metal ions already bound to DNA assisted the crosslinking.

$DNA\text{-}M^{n+} + PrOOH \longrightarrow DNA\text{-}M^{(n+1)+} + PrO^{\bullet} + OH^{-}$ followed by

$PrO^{\bullet} + DNA \longrightarrow Pr\text{-}O\text{-}DNA^{\bullet}$ to give covalent crosslink [4].

Summary

Protein hydroperoxides can be produced by exposing proteins to ROS in the presence of O2. They are relatively long-lived. POOH can decompose to unreactive alcohols or reactive radicals. Protein-hydroperoxides have been shown to oxidize antioxidants and reducing agents, and inactivate glutathione reductase, oxidize lipids and crosslink with DNA.

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