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Protein Hydroperoxides (POOH)

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Abbreviations:
Asc—ascorbate
BSA—bovine serum albumin
ESR—electron spin resonance
H—hydrogen atom
H_2O_2—hydrogen peroxide
O_2—Oxygen
$O_2^{\bullet -}$—superoxide
PH—protein molecule
$P^{\bullet}$—protein radical
Protein-$C^{\bullet}$—protein carbon-centered radical

PO•—protein alkoxyl radical
POO•—protein peroxyl radical
POO^-— protein peroxyl anion
POOH—protein hydroperoxid
ROS—reactive oxygen species
Oxidant—Ox

Outline ***page***

Abstract:

Protein hydroperoxides, a form of reactive oxygen species (ROS), are generated when proteins act on ROS and are damaged by free-radical-generating systems in the presence of oxygen. Several free radical species are implicated in protein peroxidiation and hydroxyl radical (•OH) is the most damaging species. Protein hydroperoxides are generated in high yields at both side-chain and backbone (α-carbon) sites. Research in recent years shows the protein hydroperoxides have ability to react with some biomolecules such as metals, antioxidants, GSH, enzymes and

DNA. These protein hydroperoxides can be determination by ferric-xylenol orange complex assay, iodometric assay, and EPR spectroscopy.

Introduction:

There is convincing evidence that protein is the critical and initial target when reactive oxygen species attack cells. Protein can produce oxidizing species after exposure to ROS in the presence of O_2 which leads to functional, structural and biological damage [1]. These oxidizing species, which have been identified as protein hydroperoxides, play an important role in physiology and pathology due to their ability to react with critical biological components [2]. The initial and most common proteins used in the studies of the formation of protein hydroperoxide group are bovine serum albumin (BSA) and lysozyme, but experiments show that most proteins have the ability to peroxidation by ROS. Protein Hydroperoxides can also generate protein-derived radicals (including a number of carbon-centred radicals, alkoxyl radicals and other species) which are predominantly present on amino acid side chains [3].

Formation of protein hydroperoxides

Mechanism of protein hydroperoxides:

Studies show that $HO^{\bullet}$ takes major responsibility for protein hydroperoxides formation. At the PH range of 5-9, protein hydroperoxides yields are constant [3]. $HO^{\bullet}$ can be generated by X or γ –irradiation, reduction of H_2O_2 with Fe^{2+} or Cu^{2+} and reaction of ozone with phenolics [4]. When $HO^{\bullet}$ or other ROS reacts with protein molecules, the hydrogen atom (H) can be abstracted and react with Oxidant Ox (reaction1). $P^{\bullet}$ is a carbon-centered free radical, which can react with

O_2 to form protein peroxyl radical ($POO^{\bullet}$) (reaction 2). $P^{\bullet}$ may also react with superoxide ($O_2^{\bullet -}$) and be oxidized to produce protein peroxyl anion (POO^{-}) (reaction 3). $POO^{\bullet}$ has a enough long lifetime which allows it react with other PH quickly and initiate a chain reaction (reaction 4). This reaction is a propagation process which would be terminated when $POO^{\bullet}$ reacts with H^{+} or $POO^{\bullet}$ reacts with other radicals (reaction5,6)[3].

$$PH + Ox \rightarrow P^{\bullet} + \text{reduced Ox} \quad (1)$$

$$P^{\bullet} + O_2 \rightarrow POO^{\bullet} \quad (2)$$

$$P^{\bullet} + O_2^{\bullet -} \rightarrow POO^{-} \quad (3)$$

$$POO^{\bullet} + PH \rightarrow POOH + P^{\bullet} \quad (4)$$

$$POO^{-} + H^{+} \rightarrow POOH \quad (5)$$

$$POO^{\bullet} + \text{radical} \rightarrow POOH \quad (6)$$

There are two major kinds of protein hydroperoxides can be formed in the chain reactions, α-carbon hydroperoxides and side-chain hydroperoxides [5]. α-carbon of peptide is the primary site attacked by ROS, and could form intermediate peroxyl radicals which would reduced to hydroperoxides (Figure 1). Some susceptible amino acids may also be peroxidized by ROS, such as Ile, Leu, Val, Glu, Pro and Lys with G values in excess of 0.7-OOH/100eV. The hydroperoxides fromed on α carbons of amino acids in polypeptide chain contribute to over 90% yields of protein hydroperoxides.[6] A little amount side-chain hydroperoxides also form in exposure of ROS and O_2 (Figure 2).

1. *α-Carbon hydroperoxides*

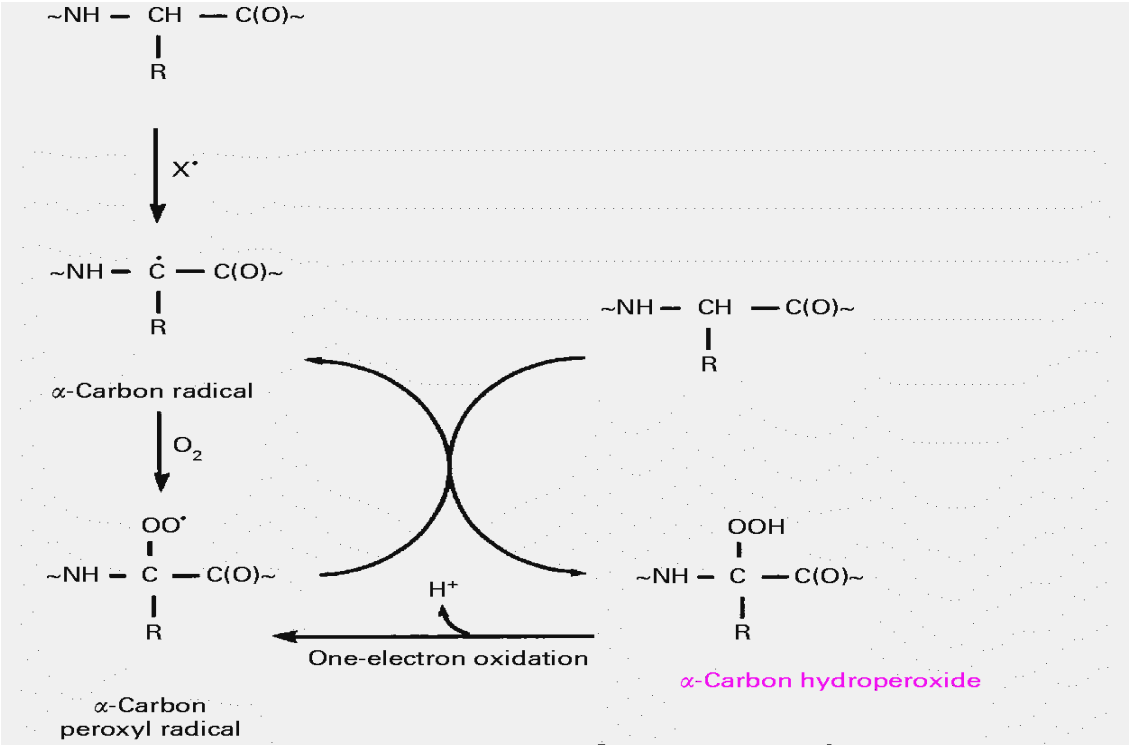

Figure1: Major reactions of aliphatic side-chain radicals formed during protein oxidation in the presence of oxygen [5]

2. *side-chain hydroperoxides*

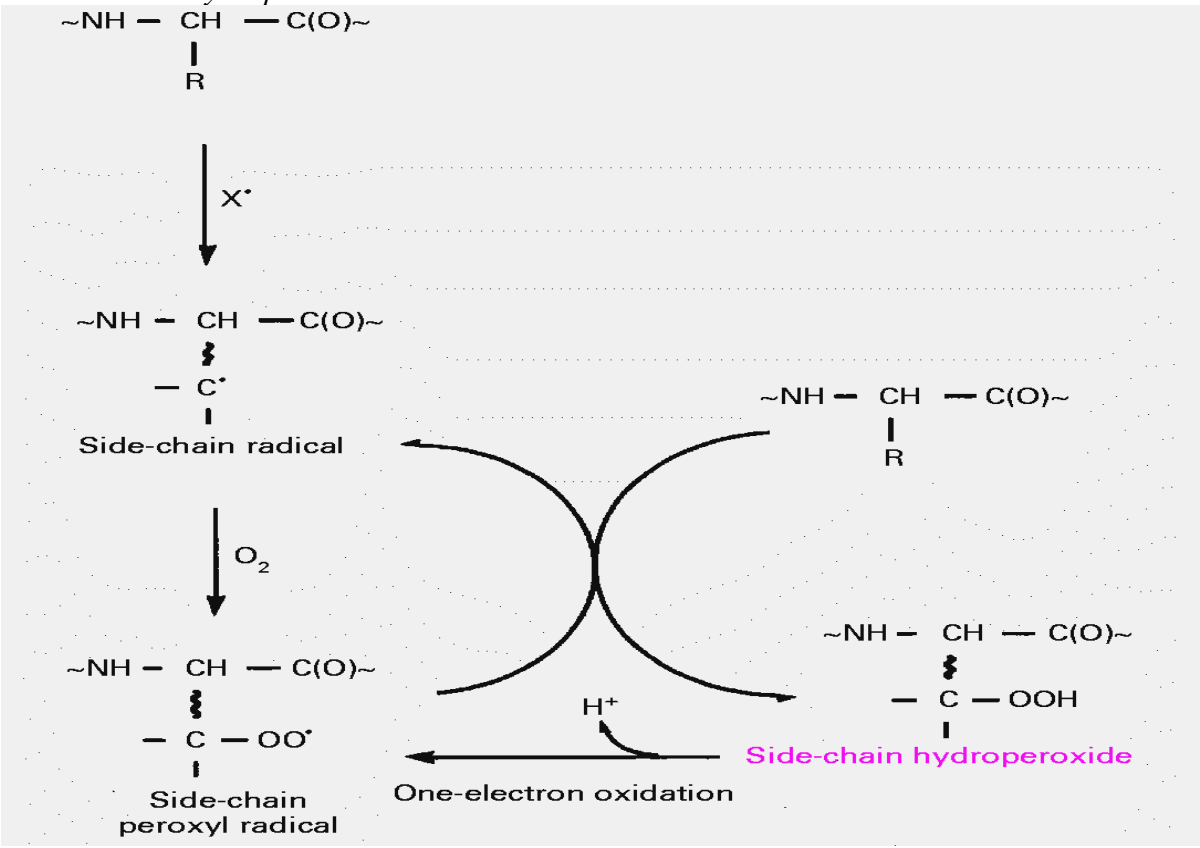

Figure 2: Major reactions of backbone radicals formed during protein oxidation in presence of oxygen [5]

Reaction of protein hydroperoxides

The stability of many kind protein hydroperoxides is measured in different condition which suggests that the decomposition of protein hydroperoxides occurs when the temperature is elevated or in presence of other molecules, such as oxidizing agent, reducin of these agents [7].

Reaction with metal:

Exposure of the hydroperoxides transition metal ions (Cu^{2+}, Cu^{+}, Fe^{2+} or Fe^{3+}) can lead to the formation of further reactive radicals, including alkoxyl radicals (reaction 7,8), and peroxyl radicals(reaction 9) [7].

$$POOH + Cu^{+} \rightarrow PO^{\bullet} + OH^{-} + Cu^{2+} \quad (7)$$

$$POOH + Fe^{2+} \rightarrow PO^{\bullet} + OH^{-} + Fe^{3+} \quad (8)$$

$$POOH + Fe^{3+} \rightarrow POO^{\bullet} + H^{+} + Fe^{2+} \quad (9)$$

Exposure in light:

Protein carbon-centered radical (Protein-C$^{\bullet}$) can be formed when protein alkoxyl radicals exposure in γ or α radiation (reaction 10) [7]

$$PO^{\bullet} \rightarrow Protein—C^{\bullet} \quad (10)$$

Reaction with DNA:

Previous studies have shown that protein hydroperoxides can give rise to further crosslink with DNA. The earliest observation is the formation of crosslink between DNA and BSA or lysozyme by radiation. Although the mechanisms of these processes are poorly understand, it might be expected that radicals including $HO^{\bullet}$, alkoxyl radicals and carbon-centred radicals formed from

protein hydroperoxides would give rise to mutagenic lesions on DNA (such as 8-oxodG), oxidization bases or base adducts and form protein–DNA cross links (reaction 11,12)[8] .

$$PO^{\bullet} + DNA \rightarrow PO— DNA^{\bullet} \quad (11)$$

$$POOH + DNA \rightarrow Protein —DNA \quad (12)$$

Detection and measurement of protein hydroperoxides

Several methods have been developed to measure and detection protein hydroperoxides.

Ferric-xylenol orange complex assay:

This method can apply to a variety of oxidized biological materials because its high sensitivity. We know that hydroperoxides can reaction with ferrous ions at low pH (reaction 8) in the presence of the dye xylenol orange (XO). The product of Fe-XO can be measures at the 560 nm (figure 3] [9]. Serum hydroperoxide concentrations can be calculated from the amounts of Fe^{3+} produced, providedthe values of Fe–XO and the number of Fe^{3+} ionsgenerated p –OOH group (reaction 13).

$$Fe^{3+} + XO \rightarrow Fe–XO \quad (13)$$

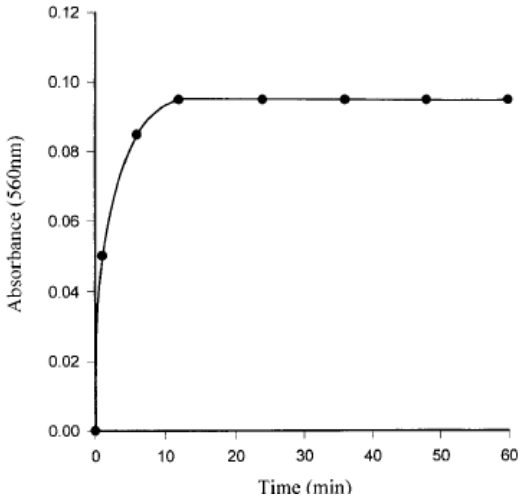

Figure. 3. Kinetics of the formation of the Fe–XO complex by peroxidized human blood serum. The serum was oxidized by irradiation ina 60Co source for 60 min under oxygen. After treatment with catalase, 200-ml serum samples were incubated with 100 mMFe21 and 250 mMSigma xylenol orange in 25 mM H2SO4. The absorbance was measuredFagainst a blank containing unoxidized serum, $Fe2^{+}$, and xylenolorange [9].

Iodometric assay:

In this assay, hydroperoxides oxidize iodide in acid solution. In presence of large excess of I_2, it converts to tri-iodide ion I_3^- which can be measured at 360nm. The advantages of the method are good specificity and exact 1:1 stoichiometry between the amount of peroxide reacting and iodine produced, allowing quantitation of the hydroperoxides. But the disadvantages are their low sensitivity in the normal form and also need to exclude oxygen from the reaction (reaction 14) [10].

$$POOH + I^- + 2H^+ \rightarrow I_2 + POH + H_2O \qquad \text{(reaction 14)}$$

EPR spectroscopy.

EPR is a type of absorption spectroscopy that can detect paramagnetic ions or molecules with at least one unpaired electron spin. In the reduction of protein hydroperoxides, it is shown that this process is accompanied by radical formation as detected by EPR spin trapping .The BSA radical concentration could be estimated by comparing the ESR signal intensity of samples containing BSA radicals with that of a known concentration (figure 4)[11].

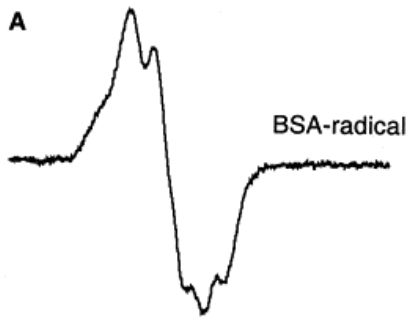

Figure 4. ESR spectra of BSA radicals[11].

Summary

Exposure of proteins to ROS in the presence of O_2 brings about multiple changes in the target molecules. These changes include oxidation of side chains, backbone chains, cross-linking changes, and formation of new reactive groups, including hydroperoxides. Protein hydroperoxides have the ability of initiating further radical chain reactions, protein alteration and side-chain fragmentation.

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