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EFFECT OF SOME FREE RADICALS ON SUPEROXIDE DISMUTASE AND CARBOXYPEPTIDASE- A REVIEW

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ABSTRACT
Free radicals are species with unpaired electron in their outermost shell. Most free radicals come from oxygen or nitrogen atoms. Radical species such as superoxide radical, hydroxyl radicals and hydrated electron are called the primary radicals of water radiolysis and can be produced by irradiating water molecule. Inorganic radicals on the other hand are formed by irradiating aqueous solution of enzyme containing sodium or potassium salt of the radical to be produced. Superoxide dismutase, an important antioxidant enzyme can be inactivated by hydrogen peroxide at His-118 of the active site of the enzyme, while hydrated electron was found to be completely ineffective as inactivating specie. Hydrated electron was also found to be completely ineffective in the inactivation of carboxypeptidase, while there appears to be some relative efficiency with hydrogen atom.
Keywords: hydroxyl radical ($^{\cdot}OH$); hydrogen atom ($H^{\cdot}$); hydrated electron (e^{-}_{aq}); Superoxide dismutase SOD; Superoxide radical ($O_2^{\cdot-}$)

INTRODUCTION
A free radical is any specie with at least one unpaired electron in the outermost shell (Valko *et al*., 2006). Free radicals are highly reactive and once formed can start a chain reaction. Their chief danger comes from the damage they do when they react with important cellular components such as enzymes, DNA or even the cell membranes. Those radicals derived from oxygen represent the most class of such species generated in living systems (Karlsson, 1997).
Free radicals can be generated via:

- Irradiation by UV light, X-rays and gamma rays;
- products of metal-catalysed reaction;
- pollutants in the atmosphere;
- produced by neutrophils and macrophages during inflammation;
- by- products of mitochondria- catalysed electron transport reaction and other mechanisms (Cadenas, 1989).

Primary radicals of water radiolysis are produced when water is irradiated; the water molecules are electronically excited and ionized (Sule, 1995). The radicals species produced include: the hydroxyl radical ($^{\cdot}OH$), hydrogen atom ($H^{\cdot}$), and the hydrated electron (e^{-}_{aq}) with a yield of 2.75, 0.55 and 2.7 respectively per 100eV of gamma radiation (Von Sonntag, 1987; Buxton, 1968).

Superoxide Dismutase
Superoxide dismutase (SOD, EC 1.15.1.1) is one of the key antioxidant enzymes which provide an essential defence against oxygen toxicity to the cell (Mc Cord and Fridovich, 1969). They function by catalytically converting superoxide radical ($O_2^{\cdot-}$) to oxygen (O_2) and hydrogen peroxide (H_2O_2) (Fridovich, 1995). There are several isoforms of superoxide dismutase, differing in the nature of the active metal center and amino acid constituents, as well as in their number of subunits and cofactors. In humans, SOD exists in three (3) forms, namely:

- Mitochondrial Mn- SOD;
- Cystolic Cu, Zn- SOD; and
- Extracellular EC- SOD (Landis, 2005).

Superoxide dismutase destroys superoxide radical with remarkably high reaction rates, by successive oxidation and reduction of transition metal ion at the active site in a 'Ping Pong' type mechanism (Mates *et al*., 1999). Cystolic Cu,Zn-SOD; a homodimeric metalloenzyme of 32KDa with each subunit containing as the active site, each monomer binds one copper and one zinc ion. The reaction mechanism involves the sequential reduction and reoxidation of Cu^{2+} , for which both reaction are relatively pH- independent and proceed with rate constants of 2×10^9 $M^{-1}s^{-1}$ (Alwarez *et al*., 2004).

$$O_2^{\cdot-} + \text{Cu (II)Zn SOD} \longrightarrow O_2 + \text{Cu(I)Zn SOD} \quad ....1$$
$$O_2^{\cdot-} + \text{Cu(I)Zn SOD} \longrightarrow H_2O_2 + \text{Cu (II)Zn SOD} \quad .....2$$

Mn SOD, on the other hand is a homotetramer of 96KDa containing one manganese atom per subunit (Mates *et al*., 1999)). This enzyme cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide.

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad .....3$$

Free radical inactivation of SOD has been studied. In their study, Uchida and Kawakishi (1994) reported that treatment of Cu,Zn-SOD with H_2O_2 resulted in an increase in 2-oxohistidine and a concomitant decrease in histidine content, suggesting loss of activity as histidine was found to be the crucial amino acid residue responsible for the activity of the enzyme (Roberts *et al*., 1974). In similar study, Yim *et al* (1990) suggested that H_2O_2 reacts with enzyme-bound transition metal ions, producing hydroxyl radicals, which presumably then mediates the inactivation of SOD leading to the loss of peroxidase activity of Cu,Zn-SOD, leading to deleterious effect on living cells. They showed that H_2O_2 selectively damages the active site and it is selective at His- 118 of the active site of SOD.

At the active site of SOD, four histidine residues are bound to the Cu (II) ion and also bounded is the zinc (II), and additional two more histidines and one aspartate. Enzyme inactivation could be caused through the reaction of H_2O_2, or its conjugate base (HO_2^-) in the process reducing Cu (II) to Cu (I), followed by the reaction of Cu (I) with another H_2O_2 forming an active site oxidant, described as copper- bound hydroxyl radical (Hodgson and Fridovich, 1975) leading to the formation of 2-oxohistidine (Uchida and Kawakishi, 1994; Gunther , 2002).

$$\text{SOD- Cu (II)} + HO_2^- \longleftrightarrow \text{SOD- Cu (I)} + H^+ + O_2^- \quad .....4$$
$$\text{SOD- Cu (II)} + O_2^- \longleftrightarrow \text{Cu (I)} + O_2 \quad .....5$$
$$\text{SOD- Cu (I)} + HO_2^- + H^+ \longleftrightarrow \text{SOD- Cu (II) OH} + HO^- \quad ...6$$
$$\text{SOD- Cu (II) OH} + CO_2 \longleftrightarrow \text{SOD-Cu(II)} + CO_3^{\cdot-} + H^+ \quad .7$$

SOD - Cu (II) OH is the bound oxidant and can also be written as SOD- Cu (I)O or SOD- Cu(III); its responsible for attacking a histidine residue in the ligand field of copper and thus inactivating the enzyme (Liochev and Fridovich, 2010).

Roberts *et al* (1974) reported that in the inactivation of SOD, the solvated electron is almost completely inactive as an inactivating species. However, the probabilities of hydroxyl radical and hydrogen atom inactivating a native enzyme molecule at pH 7.2 are about 0.04 and 0.01 respectively. Hydrogen atom is more effective when the enzyme copper is in the cuprous state, suggesting a conformational difference between the two oxidation states of the enzyme. Inactivation by inorganic radical $Br_2^{\cdot-}$ is negligible at pH 7 but increases sharply above pH 10. $Br_2^{\cdot-}$ was shown to inactivate the holoenzyme only when reaction at some histidine residues occurred. The suggestions that; in the native enzyme below pH 10, the copper protects histidines from damaging reactions with $Br_2^{\cdot-}$ and that strong copper-histidine interactions are necessary for activity have been supported by studies on the KCN- inhibited and copper- free enzymes. Also, free radicals from ethanol oxidation (1-hydroxyl radical) have been detected (Knetch *et al*., 1990; Reinke *et al*., 1991).

$$e^-_{aq} + N_2O \xrightarrow{H_2O} {}^{\cdot}OH + OH^- + N_2 \quad .....8$$
$$CH_3\text{-}CH_2OH + {}^{\cdot}OH \longrightarrow CH_3\text{-}{}^{\cdot}CH\text{-}OH + H_2O \quad ...9$$

1- hydroxyl radicals were generated by gamma radiolysis of a N_2O- saturated ethanolic solution (10^{-2}M) in phosphate buffer (10^{-3}, pH 7.4) (Santiard *et al*., 1995). 1- hydroxyl radicals can inactivate superoxide dismutase with a $K_{inactivation}$ of 1.13×10^4 $M^{-1}s^{-1}$.

Carboxypeptidase

Carboxypeptidase is an enzyme synthesized in the pancreas and secreted into the small intestine. It hydrolyses the first peptide or amide bond at the carboxyl or C- terminal end of proteins and peptides. The first carboxypeptidases to be discovered are carboxypeptidase A and carboxypeptidase B. These two enzymes differ in the proteins they will cleave each preferring different amino acids at the C-terminal group of the peptide. The final products of the enzymes are free amino acids, and small peptides of two or three amino acids. These products are absorbed by the epithelial cells of the small intestines. Another carboxypeptidase of great significance in mammalian physiology is carboxypeptidase E, also known as carboxypeptidase H, which activates peptide hormones, such as insulin and neurotransmitters. It does this by cleaving off C-terminal amino acids that are basic. Other carboxypeptidases have functions that range from wound healing to blood clotting (George, 2011). In 1953, Thompson preliminarily determined the N-terminal amino acid sequence, establishing that carboxypeptidase A was a single polypeptide chain, it has a molecular weight of 35.3 (Bradshaw *et al*., 1969). Through x-ray diffraction studies, Rees *et al.* (1983) determined the structure of Carboxypeptidase A as composed of 307 amino acid residues. The mechanism of its activation was used as a model to investigate the role of zymogens in biological systems (Neurath, 1964). The enzyme contains zinc (II) ions as a metal ion cofactor (Ophardt, 2003), with tyrosine as the crucial amino acid in its activity (Roberts, 1973). Due to the non-specific nature of primary radical attack, it has been observed that there are low efficiencies in their inactivation of carboxypeptidase A. The hydrated electron was found to be completely ineffective while there appears to be some relative efficiency with H atom (Roberts, 1973). Rapid localization of e^-_{aq} to the disulphide bond has been shown earlier, and it can be concluded that either the disulphide bond is not vital to activity, for which there is some chemical evidence (Walsh *et al*., 1962).

Table 1: Inactivating efficiency of some radiation- produced radicals at pH 7.8

Inactivating radicals	Inactivating efficiency
$H^{\cdot}$	0.20
$OH^{\cdot}$	0.06
e_{aq}^{-}	0
$O_2^{\cdot -}$	0.03

However, in N_2O- equilibrated solutions at neutral and alkaline pH, about 75% of the enzyme inactivation arises from $OH^{\cdot}$ attack, the remainder being supplied by H atoms. This is due to low yield of H atoms above pH 4 (Roberts, 1973).

Inactivation of carboxypeptidase A by secondary radicals was also studied and was found to be pH dependent (Roberts, 1973). At pH 7.8, the inactivating efficiency for $(CNS)_2^{\cdot -}$ and $Br_2^{\cdot -}$ was found to be 0.06 and 0.16 respectively. $Br_2^{\cdot -}$ is more efficient because it is less specific than $(CNS)_2^{\cdot -}$ and hence reacts at tryptophan, tyrosine and histidine residues in carboxypeptidase (Roberts, 1973). Inactivation by the secondary radicals centred about pH9.4 is linked with the increase in the reaction rate.

Studies of carboxypeptidase inactivation by $(CNS)_2^{\cdot -}$ and $Br_2^{\cdot -}$ in the presence of a strong competitive inhibitor of 3- phenyl propionate, in which the enzyme is at least 95% in the form of the enzyme-inhibitor complex (Elkins-Kaufman and Neurath, 1948) was found to reduce by a total factor of more than two. A smaller effect on the rate compared with inactivation is to be expected, since the inhibitor shields only a few of the total reactive residues in carboxypeptidase A (Roberts, 1973).

CONCLUSION

Free radicals are species with unpaired electron in their outermost shell. They are highly reactive and dangerous when they react with bio-molecules and they can lead to the inactivation of many enzymes. Their degree of inactivation can be studied using pulse radiolysis, and due to the specificity of the reactions of the free radicals, they can be used to determine the crucial amino acids responsible for the catalytic activity of an enzyme. Superoxide dismutase destroys $O_2^{\cdot -}$ in a 'Ping Pong' type mechanism. Studies have shown that H_2O_2 selectively damages superoxide dismutase and it is selective at His- 118 of the active site of SOD. The hydrated electron is almost completely inactive in the inactivation of superoxide dismutase. While the probabilities of hydroxyl radical and hydrogen atom in the inactivation of the native enzyme at pH 7.2 is about 0.04 and 0.01 respectively. Carboxypeptidase is an enzyme that is synthesized by the pancreas and secreted into the small intestine; it hydrolyses the first peptide or amide bond at the C-terminal end of proteins. It has been observed that there are low efficiencies in the inactivation of carboxypeptidase A by the primary radicals of water radiolysis. However, at pH 7.8 the inactivating efficiency for $(CNS)_2^{\cdot -}$ and $Br_2^{\cdot -}$ was found to be 0.06 and 0.16 respectively.

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