GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

Review Article

LIPID OXIDATION IN BIOLOGICAL SYSTEMS: BIOCHEMICAL, BIOLOGICAL & BIOPHYSICAL ASPECTS

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ABSTRACT
Oxidation in a biological system is primarily concerned with the energy balance and homeostasis of oxidants and antioxidants reflecting the structural, functional efficacy of the biological system in totality. This ubiquitous process is related to molecular and biochemical interactions at various levels in biological system. The lipid oxidation appears to be an effectively applicable parameter for investigation of structural, biochemical, physiological aspects of cell biology, medicine and food technology. When lipids in either combined or free forms get derogated specifically in food and other edible products like meat, fish and fish-products *etc.*, a state of "off flavor" sets in rendering it unsuitable for use. Autoxidation, photo-oxidation and lipoxygenase activity, are the modes which accomplish the lipid oxidation although each occurs in varied conditions. Other factors which favor this process are the heavy metals and reactive species of oxygen and nitrogen produced due to either toxicants and/or xenobiotic *in vivo.* This process is among the main factors which results in oxidative stress *in vivo* and *vitro*. Oxidative stress has been observed during infection, Polymicrobial sepsis, neurodegenerative diseases, atherosclerosis *etc*, and indicator of hepatic health. Degree of oxidative stress reflects on the immunological, biological, structural aspects of cellular membrane. The biophysical implications of oxidative stress include change in conformation of lipids, derogative changes in bilipid layer, fluctuations in thermotropic phase, mobility of lipids *etc*. In this review the basic, biochemical, physiological, pathological and biophysical aspects of lipid oxidation has been appraised with respect to normal and pathogenic conditions in biological system.

KEY WORDS: Autoxidation, photo-oxidation, lipoxygenase activity, free radicals, eicosanoids, oxidative stress,

BASIC CONCEPT OF OXIDATION IN BIOLOGICAL SYSTEMS

Oxidation in a given biological system is basically involved in the production of energy at molecular level to be used for molecular, biochemical and physiological functions performed in a biosystem. Under normal conditions oxidation can involve either addition of oxygen, removal of hydrogen or removal of electron of these three, removal of electron is the most commonly takes place while removal of hydrogen is moderately involved and addition of oxygen is not so common. During the mode of removal of electron an acceptor of the released/removed electron is required because electrons cannot remain stable in free-state; thus, under the influence of oxidoreductase enzymes, removal of electron (oxidation) and acceptance of electron (reduction) take place simultaneously. Hence, these as one unit may be regarded as redox/oxidation-reduction reaction. The endogenous and exogenous molecules may be grouped in to two sets, one which includes molecules with low redox potential and the other in which molecules having higher redox potential are included; these molecules are responsible for the oxidation-reduction potential/reduction potential in a system under investigation. Oxidation reduction potential is maintained at an equilibrated state by combined and coordinated interactions between prooxidants, antioxidants, and inactivated free radicals. Suslow (2004) has suggested that redox potential or oxidation potential (ORP, ε, E_h) can be expressed as the quantitative ability of a chemical to accept electron and as a result of this the concerned chemical gets reduced. Volt (V) or millivolt (vM) is the unit to measure the reduction potential. Each chemical has its specific reduction potential; if reduction potential is more positive it reflects the higher affinity of the chemical for electron and the said chemical exhibits higher degree of reduction. This parameter is mostly exploited to check the oxidation-reduction potential (ORP) of water to gauge the quality of water. Hydrogen exhibits (-0.42 volt) as its redox potential and it is considered to be the lowest while oxygen exhibits (+0.82 volt) as its redox potential and it is considered to be the highest. Further, other chemicals/substances show respective oxidation-reduction potential between these two limits. Chemicals/substances having low redox potential release electron and a chemicals/substances having higher redox potential accept the released electrons. During this transfer of electron energy is liberated or yielded. The amount of energy released is proportional to the redox potential difference between the two chemicals acting as donor and acceptor, (Petrucci *et al*., 2002). In a biological system there is always competition between the action of prooxidants and the antioxidants; the equilibrium between these two rates is of great significance and must be coordinated meticulously to maintain normal vital functioning of cell and the molecules. In a given biological system this specific equilibrated state may be termed as

redox potential and it is specific for each cell, cell organelle and biological site. If this coordinated balance gets disturbed there is every chance of derogated consequences of the cell and the given biosystem, (Repetto *et al*., 2012).

LIPID OXIDATION IN BIOLOGICAL SYSTEM

Frünbeck *et al*. (2001) have suggested that in a biological system a continuous supply of energy is basically prime parameter for their survival in spite of the highly fluctuating energy supply in its ambient environment. Practically all the eukaryotes have ability to store excess energy in their respective adipocytes (lipocytes or fat cells) in the form of triglycerides to be released on demand as per the needs of biosystem. Thus, adipocytes are dynamic entities with respect to the process of storing and releasing energy, they also secrete molecules related to cytokine family like leptin (a hormone produced by adipocytes, it helps to regulate energy homeostasis by inhibiting hunger, tumor necrosis factor-α, interleukins-6 and others) their coordinated working accomplishes the peripheral storage of fuel, mobilization, combustion, and signaling pathway and homeostasis of energy. All these functionalities enable the biosystem to meet the varied metabolic challenges like starvation, stress, infection and fluctuations in energy requirements. Under normal day to day life an organism may face different types of stress and oxidative stress is of common occurrence. Lipid oxidation is a ubiquitous process in biological system basically related to molecular and biochemical interactions at various levels. There is an equilibrium state of lipid oxidation under normal physiological conditions. This process is kept under control *in vivo* by the antioxidants, pro-oxidants, inactivation of free radicals or use of endogenous plant antioxidants or by regulating oxidation substrate i e., lipid and oxygen. One of the prime functions of antioxidants is to protect biological membrane from oxidative damage thereby retaining the structural integrity of cell and its organelles.

A mixture of isomeric hydroperoxides is produced as result of abstraction of hydrogen from allylic methylene and rearrangement of electrons through either 3- carbon systems or 5-carbon system, oleate and linoleate and linoleate respectively during autoxidation, (Frankel, 1998c). Further, he suggested that more or higher complex mixture of hydroperoxides is produced when a polyunsaturated fatty acids undergoing autoxidation is having higher number of double bonds; the complexity of hydroperoxides formed is related to double bonds present in polyunsaturated fatty acids. Antioxidants can influence the termination step as these react with free radical chain reaction. Wasowicz *et al*. (2004) have suggested that in most of cases oxidative reactions are relatively slow in their initial stage; this period may be considered as induction period. During later period lipids get derogated at faster rate and results in 'off-flavor product'. Further, the removal/abstraction of hydrogen atom depends on its bondage, weakly bounded hydrogen atoms are readily abstracted than those bound strongly. The rate of hydroperoxidation is generally related to the number of double bonds present in the lipid undergoing this process. The hydrogen atoms present adjacent to carbon atoms could be the easy target; among monoeic acid (fatty acid), the hydrogen atoms in polyunsaturated fatty acids are easy target which belong to methylene groups having double bonds. Halliwel (2001) opined that radicals produced under these conditions are quite unstable and once hydrogen is removed the conjugated dienes are the result due to electronic rearrangement at the concerned site. Gordon (2001) has reported that hydroperoxides formed during earlier stages of autoxidation results in the formation of non-volatile, odorless and comparatively unstable compounds but their decomposition causes the formation of volatile aromatic compounds and these are considered to be 'off-flavors' rendering food products non-edible. Yin *et al*. (2004) reported that free radical initiated lipid autoxidation in case of low density lipoproteins, is related with atherosclerosis; a complex mixture of hydroperoxides, bicyclical endoperoxides, monocyclic peroxides and serial cyclical peroxides, is the result of oxidation of lipid components of low density lipoproteins. These oxidative compounds and/or their decomposed by-products are able to modify protein components. These fluctuations are the sources of different diseases. Hermans *et al*. (2005) have proposed that autoxidation is very good source of alcohol and ketones. Yin and Porter (2005) reported an interesting correlation between free radical initiated autoxidation of polyunsaturated fatty acids and human disease like atherosclerosis and cancer. Autoxidation of polyunsaturated fatty acids results in the formation of primary products such as hydroperoxides and if autoxidation proceed further then cyclic peroxides are formed as secondary oxidative products. Polyunsaturated fatty acids and sterols are the easy targets for free radical chain oxidation and this interaction is a part of lipid peroxidation. This process takes place under different conditions produced by oxidative stress. Peroxidation results in the formation of cyclic peroxides, peroxides, and hydroperoxides and epoxy alcohols. The mechanisms involved are reversible addition of oxygen to carbon radicals and rearrangement and cyclization of allyl and pentadienyl peroxyl radicals and hemolytic substitution of carbon radicals with peroxide bond, (Gordon, 2001). Repetto *et al*. (2010a) observed that poly unsaturated fatty acids can undergo lipid peroxidation enzymatic or nonenzymatic mode; the enzymatic mode is accomplished by lipoxygenase. These enzymes have ability to oxygenate free and form peroxy radicals by esterifying PUFA. The nonenzymatic process is brought about by molecular oxygen and Fe^{2+} ions. Chen and Niki (2011) are of the opinion that products of lipid peroxidation exhibit two aspects; these products have ability to change the membranes of the biological systems, cause alterations in proteins and nucleic acids. These alterations are the causes of many dysfunctions including acute and chronic stats within biological system. The products of lipid peroxidation can also interfere with the signaling transduction pathways including receptor and receptor dependant pathways. According to these researchers the second aspect of lipid oxidation is its ability to elevate the degree of defense capacity to face the future probable oxidative stress. Repetto *et al*. (2012) found that whenever superoxide anion O_2^- and H_2O_2 are formed then

biomolecules like amino acids, poly unsaturated fatty acids, nucleic acids, etc, are likely to be damaged. Thus, cell membrane and other organelles becomes the target of reactive species. The metal ions like arsenic, lead, mercury, cadmium and zinc have been detected and analyzed in rice bran oil up to µg/g; these ions are considered to be toxic metal contamination in the oil. The relative sizes of the metal ions are proportional to their common ionic radius, (Bakota *et al*., 2015).

BASIC CONCEPTUAL MEXHANISM OF LIPID OXIDATION

Wasowicz *et al.* (2004) proposed three basic mechanisms for lipid oxidation each yielding different products: these are autoxidation, photo-oxidation and oxidation due to lipoxygenase activity.

AUTOXIDATION/ FREERADICAL AUTOXIDATION

Frankel (1995), Hamilton *et al.* (1997) and Gordon (2001) have elaborated on the mechanism of autoxidation of lipid; it is also referred as 'free radical mechanism'. This mode of oxidation involves spontaneous interaction between oxygen and lipid resulting in distorted oxygenation and production in free radicals. Autoxidation is of common occurrence; it is accomplished in open air or in presence of oxygen and even ultra violet radiations can facilitate this oxidation. Autoxidation is carried out in three steps, namely initiation step, propagation step and termination step. During initiation step homolytic hydrogen atom *i.e*., symmetrical hydrogen atom, is removed from a methyl group and alkyl radical (R*) is formed in presence of an initiator. During the propagation step the alkyl radical (R*) reacts with oxygen and peroxy radical (ROO*) is formed. This in turn reacts with unsaturated fatty acids resulting in the formation of hydroperoxides (ROOH). This step can take place either at fast or slow rate; during fast mode R* (alkyl radical) reacts with oxygen forming ROO* (peroxy radical) and during slow mode the ROO* reacts with another RH alkyl group and forms ROOH and R* (alkyl radical).The termination step is concerned with conversion of free radical in to non-radical form; the alkyl radical may interact with other radical and forms R-R, peroxy radical may interact with alkyl radical and ROOR is formed and two peroxy radicals may interact and form ROOR and Oxygen. There are chances of formation of simple ether *i.e*., diethyl ether, (Hamilton *et al*., 1997, Frankel, 1998a, 1998b, Gordon, 2001). According to Porter (2013) free radicals have effective role in autoxidation of polyunsaturated fatty acids and sterol peroxidation; this interaction has its physical-organic chemical aspects. These aspects appear to be of future applications in lipid chemistry.

PHOTO-OXIDATION

Lipids when subjected to sensitizers, solar radiation and U V radiations are likely to undergo oxidation; hence it is referred as photo-oxidation. It is an alternative mode of oxidation resulting in the formation of hydroperoxides but not the free radicals as formed in autoxidation, (Gordon, 2001). The basic concept involves a singlet of oxygen ($^1O_2$) which is produced by light; this singlet is highly electrophilic in nature and it readily react with unsaturated lipids. This interaction is different than that involved in free radical autoxidation. Generally oxygen exists as triplet at ground level; an excited singlet of oxygen is available having energy 92kJ/mole above the ground level. Mostly a triplet of oxygen at ground level shows a tendency to interact as 'diradical' and it utilizes its semi occupied (easily available) orbitals to form a new bond having a preference for a radical from the substrate. The excited singlet of oxygen has a tendency to use completely occupied and/or empty orbitals for the purpose of formation of bond; this behavior indicates the nucleophilic and/or electrophilic nature of singlet of oxygen hence, exhibiting a tendency to interact with other molecular entities, (Frankel 1995; 1998d). This fact can be illustrated; when a singlet of oxygen interacts with oleic acid and it attacks the C- atom at 9 and 10 double bonds resulting in the formation of a mixture consisting of isomeric forms (equimolar compounds) of 9 and 10 hydroperoxides referred as R and S forms. There are two modes by which 'light' triggers lipid oxidation and both modes are mediated by sensitizers. First mode of photo-oxidation involves the photo sensitizers 'type -1 sensitizers': in this case light activated sensitizers directly react with the given substrate and results in the formation of radical and these radicals initiate the process of oxidation of lipids. The second mode of photo sensitizers involves 'type II'; in this mode the sensitizers activate the ground state/level oxygen to the first singlet state of oxygen and thereafter oxidation of lipids is brought about. The process of photo-oxidation involves both these modes and that too simultaneously. There are some parameters which help to study this process; these are availability of sensitizers, the structure of the sensitizers, concentration and structure of the substrate etc. Further, the hydroperoxides isomer formed as result of oxidation involving singlet of oxygen and triplet of oxygen are different; this helps to investigate both of the mechanisms by analyzing the end products. Thus, type II photo-sensitizers such as chlorophylls, pheophytins and riboflavin, when present in food, will exhibit type II oxidation of those lipids which contain unsaturated acyl group. The sensitizers such as chlorophyll, porphyrins, myoglobin, riboflavin, bilirubin, erythrosine, rose Bengal, methylene blue etc, are involved in photo-oxidation in addition to UV radiations or light. A singlet of oxygen reacts at the site of double bond; oxygen is added at the C-atom at both ends of double bond and this site of double bond attains *trans conformation*. Due to this reason there is a possibility of formation of 12- and 13- hydroperoxides when singlet of oxygen interacts with double bond present between C-12 and C-13 of any fatty acid. The life-time of a singlet of oxygen is greater in the hydrophobic cell membrane in comparison to that singlet of oxygen when in aqueous medium, (Frankel 1995; 1998d). Photo-oxidation is relatively faster process in comparison to autoxidation. When oleic acid and polyene are subjected to photo-oxidation and autoxidation at the same time the rate of photo-oxidation is around 30000 times and 1000-1500 times faster in comparison to autoxidation in the liposome and in intact cell membrane. Photo-oxidation is not a chain reaction instead it is accomplished in one interaction.

Carotenoids are among the primary inhibitory agents for photo-oxidation, thus, carotenoids act as protective agents against photo-oxidation in plants. The control mechanism or inhibitory mechanism of photo-oxidation appears to be an essential process in a given biological system for its normal functioning. Basically under this mechanism the production of singlet of oxygen from oxygen at ground level is regulated. Tocopherol acts as a quencher of singlet of oxygen formed in the system and a stable compound is formed. Frankel *et al*. (1979) showed that carotene/ carotenoids act as inhibitory agents to this type of interaction only when Tocopherol is present in vegetable oils. Carotenoids compete for the singlet of oxygen formed and deprive it from excess energy of double bond convert singlet of oxygen in to ground state of triplet of oxygen. In the process, carotenoids themselves become triplet but it thermally dissipates its excess energy and attains singlet state. This process is called quenching and in this case it is very fast ($k=3X10^{10}$ mole $^{-s^{-1}}$). This effectively proves that carotenoids act as protector of oils which are present in the biological system and may undergo II-type of photo-oxidation. Carotenoids present as auxiliary pigments play a role to nullify the pro-oxidant effect of chlorophyll present ubiquitously, Gordon (2001).

OXIDATION DUE TO LIPOXYGENASE ACTIVITY

Lipoxygenase is a group of enzymes which contain iron, these enzyme catalyze dioxygenation of those polyunsaturated fatty acids (PUFAs) among lipid which contain ciscis 1.4-pentadiene conformation; PUFAs + oxygen → hydroperoxide of fatty acids, (Wasowicz *et al*., 2004). These enzymes are present in plants, animals and fungi. The resultant products of lipoxidation play essential and varied roles in cellular function. Needham *et al*. (1986) have reported the involvement of lipoxygenase in the metabolism of eicosanoids (icosanoids); these eicosanoids are able to influence the signaling molecules which are produced as a result of oxidation of 20-carbon fatty acids and are derived from omega-3 and omega-6. Functionally these are concerned with inflammation, immunity caused due to toxins and pathogens, these icosnoids also act as messenger within central nervous system, further, these products influence cardiovascular diseases, triglycerides, blood pressure, arthritis etc. The lipoxygenase enzymes are present in plants and these play role in physiological aspects of growth, development, resistance to pest, senescence and response to wounds, (Vick and Zimmerman, 1987). Schewe and Kühn (1991) reported that lipoxygenase enzymes (LOX) have an ability to oxidize complex lipids; these enzymes also change the membranous structures present in the precursor cell destined to become effective structures in the respective cells such as red blood cells, lens epithelial cells, keratinocytes *etc*. Belitze and Grosch (1999) suggested that there is second type of enzyme interaction which involves lipase reaction on fatty acids thereafter substrate is esterified; these cause production of ketodiene fatty acids as an additional product. The parameters affecting lipid oxidation in dried microencapsulated oils on powdered food or gradients, these factors are types, concentration of matrix components and drying procedure ,other physic-chemical parameters like particles size, oil globalize, lipid distribution, water activity, pH, interactions between matrix components etc; Gong *et al* (2010) also reported on the factors affecting lipid oxidation and found that pH of the muscle, oxygen, temperature, pro-oxidants, antioxidants, amounts of inhibitors of lipid oxidation present in the aqueous fraction of muscles, amount of tocopherol in lipid phase, rate of depletion of tocopherol.

ROLE OF HEAVY METAL IONS IN OXIDATION OF LIPIDS AND OILS

Heavy metal ions are ubiquitous in fats, oils and food containing these components. Lahir (2013) mentioned that metals are likely to be present in lipids as impurities in a biological system as toxins and as contaminations along with raw materials from plants and animals, packaging of oils seeds, during processing and their total removal is not possible as they are found at least in traces. These metal ions have the ability to induce oxidation of lipids and catalyze the decomposition of hydroperoxides in to radicals which in turn initiate radical chain reaction leading to oxidation, (Petrucci, 2002). The metal ions react only when hydroperoxides group is present, according to Thanonkaew *et al* (2006) these metal ions catalyze the oxidation reaction because of decomposition of hydroperoxides resulting in initiation of new radical reaction. During initial state of reaction its rate is faster as compared to the later state of reaction; alkoxide radicals are essential to start the oxidative reaction as compared to peroxide. Ascorbic acid, a water soluble compound, acts as antioxidant; it is likely to act as pro-oxidant agent because these prooxidants have a tendency to interact with metal ions resulting in their ability to reduce the oxidative efficacy. This further helps in the extension of initial state of reaction. The pH of the medium is another parameter which affects the catalytic impact of redox reaction due to metal ions on peroxide decomposition; this step affects the initiation of radical chain reaction. Thanonkaew et al (2006) reported that Fe (II) enhanced lipid oxidation enormously while the concerned pro-oxidative effect appeared to be behaving in proportion to the relative concentration. The effect of copper I and II was found to be marginal on lipid oxidation but same metal ions declined sulfhydryl contents of proteins.

ROLE OF NITRIC OXIDE AND ITS INTERMEDIATES IN LIPID OXIDATION

In a classic research paper Moncada *et al*. (1991), it is reported that nitric oxide (NO) radicals act as endogenous stimulators for soluble guanylate cyclase enzyme and it exhibits the relaxing factor, this in turn is derived from endothelial cells; it acts *in vivo* as vasodilator. Further, NO is an endogenous product, its production is related to guanylate cyclase enzyme. Nitric oxide is formed in vascular endothelial cells because of nitric oxide synthase enzyme. It plays an effective role in many physiological functions such as regulation of vascular relaxing process, gene expression, post translational changes in protein and exhibiting cellular responses primarily inflammatory in nature, (Nair *et al*., 2007 and Valdez *et al*., 2011). Rubbo *et al*. (2009) have elaborated on the mechanism and consequences of nitration and peroxinitrite and suggest

that peroxinitrite may directly or indirectly mediate lipid oxidation. Repetto *et al*. (2012) observed that unsaturated fatty acids can readily be affected by nitration interaction. This process is facilitated further because the reactive species formed due to NO can easily diffuse through biological and/or cellular membrane and is likely to be retained around hydrophobic core of the membrane. During the lipid oxidation the lipoproteins accelerate the interaction between fatty acids and lipid peroxy radicals (ROO*) and free lipid are oxidized and converted into nitrated products; these products are arachidonic acid, arachidonate oleate, lenoleate, cholestryl linoleate (an esterified form). According to Repetto *et al*. (2012) the probable modes of nitration of lipids *in vivo* include (i) nitric oxide is converted into nitrite during auto-oxidation, nitrite acts as an oxidant and is capable of nitrating other molecules, (ii) Fatty acids get linked to related species which have been added electrophilically, (iii) the processes like oxidation, nitrosation (formation of nitroso derivatives, having R-NO functional group) and nitration (addition of nitro group) are selectively mediated by the free radicals species; these free radicals species are produced from peroxynitrite (ONOO*). In addition to this function these free radical species are known as mediators to facilitate inflammatory responses. Some of the major components of membranes are free fatty acids and esterified fatty acids namely arachidonic acid and linoleic acid, these undergo oxidation due to the oxidants but nitric oxide and its derived radicals interact with such fatty acids resulting in the formation products which are oxidized as well as nitrated namely nitroalkenes and nitroalcohols. When oxygen contents are low the quantity of peroxynitrite is relatively more and is very active, (Repetto *et al*., 2012). The process of nitro-alkylation takes place during redox process; cell signaling process involves the concept of reversible covalent bonding *i.e*. formation of reversible bond with one macromolecule (a protein or ligand) and the mass competes for the second molecule or ligand, wherein the competing second molecule /ligand has different mass than the first one. The sub-cellular distribution of protein, its structure and function depends on the changes occurred during post –translation process. Even when the cell is exposed to oxidants the peroxynitrite and its derivatives regulate pro-inflammatory responses of the affected cell. Repetto *et al*. (2011) reported that such inflammatory responses are exhibited by macrophages and neutrophils during injury and pathological conditions; during such conditions the involved cells get activated to produce nitric oxide and its derivatives to combat the situation. The super oxide dismutase (SOD) activity removes reactive species of oxygen and helps in extension of vaso-relaxing response due to nitric oxide (Rachmilewitze *et al*., 1993). The functional aspects of lipid oxidation and nitric oxide exhibit some specific pattern; these are related to some of the conditions. When there is a lipid environment and there is low polarity, high viscosity and packaging, the reaction of nitric oxide and reactive nitrogen species will be different as compared to aqueous phase. Further, these reactions may be categorized may be categorized according to the respective kinetics based on the diffusion and activation of lipid oxidation by nitric oxide. There can be some specific cases like (i) autoxidation of nitric oxide, (ii) hemolysis of peroxynitrite, (iii) inhibition of lipid oxidation by nitric oxide, (iv) nitration of protein and (v) products formed due to covalent lipoprotein, (Ignarro, 2010).

SPECIAL FEATURES OF HYDROPEROXIDES FORMED

Frankel (1995, 1998b, 1998c), Belitz and Grosch (1999) have reported some of the specific features of hydroperoxides formed under autoxidation. The hydroperoxides formed are unstable and are liable to decompose readily; during this decomposition monomolecular and/or bimolecular reactions are involved. Peroxy and alkoxyl radicals formed as a result of decomposition of hydroperoxides, are very reactive and initiate the autoxidation. The products of decomposition of hydroperoxides are non-volatile monomeric compound; these include di and tri oxygenated esters, these are the derivatives of corresponding keto-, hydroxy-, hydroperoxides- and epoxide esters. When unsaturated fatty acids produce mono-hydroxyperoxide under autoxidation these are able to form the precursors for volatile products of this decomposition e g, pentene, octane, octanal *etc*. When unsaturated aldehyde and ketones are subjected to autoxidation these produce volatile compounds like dimmers, oligomers, hydroperoxy epoxides, hydroperoxides diepoxides and dihydroperoxides. Even the secondary intermediate products are volatile compound in nature like those produced by monohydroxyperoxides.

LIPID OXIDATIOM AND ITS CONSEQUENCES IN BIOLOGICAL SYSTEMS

Oxidative stress of higher degree has been reported in erythrocytes during HIV infection in comparison to non-infectious condition, (Repetto *et al.* (1996). Halliwel (2001) proposed that among humans free radicals have tendency to induce pathogenesis, this is further affected by stress caused by environmental issues. Induced oxidative stress was observed in injured gastric mucosa, (Repetto *et al*., 2003). Fiszman *et al*. (2003) reported that oxidative stress is the cause of 'Familial amyloidotic polyneuropathy type-I. They observed enhanced superoxide dismutase type-I (SOD-I) activity. This activity indicates the interaction between the defense system and reactive oxygen species (ROS); the total reactive antioxidant potential (TRAP) values were found to be declined. This decline hints at the unavailability of antioxidants because these have been eliminated by either ROS or proactive ROS. Wu *et al*. (2007) studied the role of reactive nitrogen species in Polymicrobial sepsis induced renal peritubular capillary dysfunction and tubular injury and found that RNS generation has caused dysfunction in renal tubules and suggested that RNS may be considered as a potential parameter to investigate the renal tubular disorder. The non-equilibrium state between antioxidants and ROS along with pro-ROS reflected the destruction of the affected tissues. The mitochondria appear to be swollen and its matrix gets increased due to elevated rate of cellular oxidation, (Boveris *et al*., 2008). Dominguez *et al*. (2008) have indicated that lipid peroxidation is related to neurodegenerative pathological conditions. Navarro and

Boveris (2009) reported a possibility that lipid oxidation participates in the signaling pathway during pathogenesis; this process is a potential inflammatory agent ultimately resulting in death of the affected neural tissue specifically under those conditions that favor membrane lipid oxidation. The oxidation of cardiolipin of mitochondria (18% of the total phospholipids) caused the release of cytochrome-C from the inner membrane and this enhances the permeability of outer membrane of mitochondria. This condition along with other parameters initiates apoptosis in neural tissue. The release of cytochrome -C enhances the proteolytic cascade leading to cellular death due to apoptosis. Bashan *et al*. (2009) have shown that under normal conditions a balanced formation and use of reactive species of oxygen and nitrogen depends on the antioxidant system and it is needed for normal physiological functioning. They observed that ROS and RNS affect the pathway of insulin signaling. These two types of reactive species have a regulatory impact on degree of resistance of the individual. This parameter is an important risk factor along with others for type -2- diabetes. Dianzani *et al*. (2008) have expressed a view that lipid peroxidation takes place in animals and plants both and it involves the production of lipid radicals, up take of oxygen, rearrangement of double bonds present in unsaturated lipids; its most adverse effect is the destruction of lipids forming membrane involved in the formation of varied compounds such as alcohols, ketones, alkenes, aldehydes and ethers. They further reported that lipid peroxidation creates pathological conditions that favor the generation of reactive oxygen and nitrogen species at very high rate, specifically when α-tocopherol is either deficient or absent. Boveris et al (2008) have explained that the oxidative stress acts as an indicator of imbalance between the rate of activity of oxidants and antioxidants; when oxidants are active the oxidizing free radicals and other related products are formed at higher rate and are very active. Their activities are much higher in comparison to the activities of antioxidants at the site. They further suggested that due to the oxidative interaction affects the biological phospholipids present in membranous components of cell and cell organelles like mitochondria, microsomes, peroxisome, and plasma membrane. The impact of lipid peroxidation have been observed and explicated as neurotoxicity, hepatotoxicity and nephrotoxicity. Liver is a prime site of detoxification and toxicity due to hepatic lipid peroxidase is affected; hepatic cytochrome P-450 is involved in the conversion of aldehyde produced during lipid peroxidation to carboxylic acid (Boveris *et al*. 2008). This step becomes additional aspect of hepatic oxidation. Although cytochrome P-450 regulated metabolism works as a parallel coordinated metabolic transformations or interaction related to aldehyde elimination; thus when other aldehyde elimination pathways are affected adversely due to either pathological conditions or toxicity the hepatic cytochrome P-450 mechanism compensates the aldehyde elimination. Further they mentioned that 4-hydroxynonenal (HNE), unsaturated aldehydes like acrolene, trans- 2- hexenal, crotonaldehyde, food components, pollutants from environment get eliminated due to cytochrome P-450 and probably it also participates in signaling pathway. The endothelial cells demonstrate an endothelial derived relaxing factor which also acts as a vasodilator; this helps the vascular tissues to adjust with physicobiochemical fluctuations, (Moncada *et al*., 1991). Oxidized low density lipoprotein (LDL) is one of the prime factors to induce atherosclerosis. The vascular endothelial cells and macrophages are induced to produce nitric oxide, this in turn interacts with superoxide and peroxynitrite is formed. This peroxynitrite ($ONOO^-$) is effective in oxidizing LDL *in vitro*. Peroxynitrite interacts with free tyrosine and trinitrotyrosine is formed. It is a stable product and well established and very specific marker to track the oxidation of LDL; its presence in vivo indicates anti-atherosclerotic effects of nitric oxide, (Leeuwenburgh *et al*., 1997). Nitric oxide is capable of regulating non-enzymatic and enzymatic reactions of lipid oxidation. Further, nitric oxide is potent and non cGMP dependent mediator for signal transduction and free radical inflammatory responses. Nitric oxide along with prostaglandin endoperoxide synthase and lipoxygenase can regulate non-enzymatic lipid oxidation; it can also generate "eicosanoids"- the signaling molecules related to inflammation and vaso-activation, (Bloodworth *et al*., (2000). Nitric oxide is capable to accumulate macrophages at the specific site to oxidize LDL *in vivo,* thus, NO is considered as a prime determinant of pre and post oxidative status. Bloodworth *et al*. (2000) reported the presence of NO whenever higher amounts of oxidants are present, site of lipid oxidation and migration of monocytes to vasculature and NO produces antiatherogenic effects. They further mentioned that under those conditions under which oxidant defense is depleted and endogenous oxidants are formed in high amount then NO generates second oxidizing species, these factors can enhance membrane oxidation, lipoprotein oxidation and foam cell production. They suggested that NO exhibits and promotes pro-atherogenic effect. O'Donnell and Freeman (2001) have elaborated the role of nitric oxide in the signaling process and lipid oxidation and suggested that it is of prime importance in maintaining homeostasis in vascular tissues and also during vascular pathological conditions. During these two processes, the free radicals interact at faster rates; the rates are many folds higher and NO and oxidizing lipids result in the protecting the vascular injury and/or helping in exhibiting effective inflammatory responses. NO produced by vascular NO synthase performs two functions (i) it checks the progress of lipid radicals and (ii) it prevents the action of lipoxygenase, both of these aspects are protective in nature. Thus, O'Donnell and Freeman (2001) proposed that the enzymatic and non-enzymatic lipid oxidation are likely to influence the effect of NO by directly either removing or reducing the consumption of NO or can change the influence of catalytic enzyme nitric oxide synthase. Bashan et al (2009) observed that signaling pathway of insulin is positively and negatively is affected by reactive species of oxygen and nitrogen. Farooqui and Farooqui (2011) mentioned that lipid mediated oxidative stress can involve 4-hydroxynonenal, isoprostanes, isofurans, isoketones, neuroprostanes and neurofrans, They observed that activated microglial cells produce substances like cytokines like TNF-α and IL-1β, these are

proinflammatory in nature; even prostaglandins, platelets activator factors, all of these metabolites induce structural degeneration of neural tissue during Parkinson's disease. They also mentioned that dopamine also undergoes oxidation and the products of this oxidation are capable of changing functional and structural aspects of mitochondria, such as swelling and decline in electron transport chain interaction. When the products are derived from dopamine and variety of toxic quinines, these species cause increased disruption of mitochondrial membrane and induced oxidation of NADH but the amount of GSH remained unaffected. Lee and Yang (2012) observed that NADPH oxidase and reactive oxygen species mediated pulmonary and air ways pathogenesis and act as proinflammatory agents.

BIOPHYSICAL ASPECTS OF LIPID OXIDATION

There are numbers of reports on the physiological, clinical, pathological and toxicological aspects and the impact of lipid oxidation has been well illustrated; the biophysical aspect reveals the physicochemical parameters involved in these phenomena and it provided relatively better understanding of the principle and the mechanism of the structural and functional insight. This is expected to help in understanding the mechanism related to biological damage in the biological system. Oxidative stress reflects on the imbalance existing prior to the lipid oxidation. The related damages also reflect on the biophysical changes in the biological membrane and the physiological effectiveness of the antioxidant defenses of the affected biological system. Richter (1987) observed that the viscosity of the membrane was enhanced specifically at the 12 acyl-carbon depth, change in thermotropic phase behavior, electrical resistance of the membrane; the process of exchange of phospholipid between two monolayers was assisted and the membrane proteins were cross linked due to lipid oxidation. This resulted in the reduction of their respective rotational and lateral mobilities. Further, when membranes were studied with respect to microsomal cytochrome P-450, the protein aggregation was observed; this observation suggested that in given peroxide membrane the rise in lipid order was not the reason for protein mobilization. Jacob and Mason (2005) opined that lipid peroxidation contributes in modifying intermolecular packing, thermodynamics and phase parameters of the bilayer membrane. They observed a direct relationship between the cholesterol domain formation and the lipid hydroxide formation; this was found to be restricted or diminished when vitamin-E was used. Under the oxidative stress conditions the cholesterol domain were found to be restricted to C/P ratio of 1.0 or more. The oxidative stress also induced structural changes in membrane. All these changes lead to the functional disorder of membrane as observed in the aging process. Wong-Ekkabut *et al*. (2007) suggested that lipid peroxidation damaged the membrane; when oxidized functional group was introduced in the lipid tails it caused conformational changes in the lipids. The oxidized tails were found to be bent towards the aqueous phase while the oxygen atom from the hydrogen bond towards water and polar lipid head group. The resultant conformation of lipid layer exhibited specifically at the sites of higher concentration of oxidized lipid. They observed an enhanced average area per lipid, decline in thickness of the bilayer lipid, decrease in deuterium order parameter (this is a measure of motitional anisotropy of the particular C-D bond investigated and the yield its time –average orientation) for lipid tails, the water defects were found to be proportional to the concentration of oxidized lipids. These conformational changes exhibited increased water permeability of oxidized lipid tails, probably due to the bending of lipid tails toward water interface. Khandelia and Moritsen (2009) reported "extended lipid conformation' in phospholipid membrane. They also observed chain reversal in the affected membrane i e. profound change in structural, mechanical strength and dynamics of the biological membrane. Lipid oxidation and nitration are related to inflammatory aspect; peroxinitrite is the result of interaction between nitric oxide and superoxide anion. This causes mediation in the oxidative process that influences molecules physiologically specifically signaling for the enzymatic reaction for lipid metabolism. The oxidative stability fat globule membrane is important parameter in its functioning. Fat globules from different sources and their mode of formation show structural and functional variations, one can device these fat globules as their need. In this pursuit Zhu and Damodaran (2011) tried forming fat globules from milk using two different modes namely freeze drying and spray drying modes. They found the properties of the membranes of fat globules produced to be different. When fat globules were dried with freeze drying technique and spray drying technique the stability of these milk fat globule membrane changed; the samples obtained shoed morphological differences when viewed under light microscope. Thermotropic phase transition temperature ($T_{(m)}$) of lipids in freeze dried mode was 37.8˚C and in spry dried mode was 48˚C. This difference of 10.0˚C in ($T_{(m)}$) indicated change in the thermodynamic state of phospholipids in milk fat globules membranes. Milk fat globules membrane obtained from cheese showed higher amount of phosphotydilserine, sphingomyelin and bioactive protein CD36, butyrophilin, xanthine oxidase and mucin 1.In such cases oxidative stability in term of rate of lipid oxidation can be studied by measuring hexanal production. Patterson et al (2010 and Jurkiewicz *et al* (2012) have studied the oxidatively modified phosphobilipid using fluorescence technique; They observed structural and functional changes in oxidized lipid layer (phosphotidylcholine) such as (i) decline of lipid order, (ii) declined phase transition temperature,(iii) lateral extension of membrane and reduction in its thickness, (iv) hydration profile changed, (v) enhanced lipid mobility, (vi) starting of flip-flop pattern in bilayer, (vii) disturbed lateral phase organization (viii) more water defects, under extreme conditions this effects the bilayer and disintegration is the result. When omega-6-fatty acid was subjected to lipid peroxidation malondialdehyde and specifically 4-hydro-2-nonenal were the products formed. These products were found to affect physiological, protective aspects and gene expression were adversely affected these products even enhanced the cell death (Ayala *et al*., 2014). According to Makky and Tanaka (2015) aging and environmental stress plays an effective

role in oxidizing glycerophospholipids present in the membrane, this in turn brings about pathological and physiological dysfunctioning of the membrane, hence the cell. Whenever chemical oxidation of unsaturated hydrocarbon takes place many oxidized phospholipid end-products are formed. These products change physicochemical properties of cell membranes. The lipid oxidation causes change in the mechanics of membranes which are responsible for declined bending rigidity and increase the degree of permeability of membrane. When lipid bilayer is subjected to lipid peroxidation process the biomolecules like proteins and/or amino acids are likely to get covalently modified due to the product formed (Catala, 2015). He reported that lipid non-enzymatic changes of aminophospholipids are caused because of lipid peroxidation; the aldehydes and reducing sugars formed bring about deteriorating changes in bilipid layers that are related to aging. He proposed that during peroxidation the phospholipids and many of its oxidizing fatty acids are pushed to the surface affecting number of biological functions; for this condition he used the term “grow whisker”.

CONCLUSION

The primary object of lipid oxidation is to retain the structural and functional integrity of cell and its organelles. Oxidation reduction potential is maintained at an equilibrated state by combined and coordinated interactions between prooxidants, antioxidants, inactivated free radicals and oxidants. Autoxidation, photo-oxidation and lipoxygenase activity, are the modes which accomplish lipid oxidation although each occurs in varied conditions. Autoxidation involves spontaneous interaction between oxygen and lipids resulting in distorted oxygenation and production in free radicals. The control mechanism or inhibitory mechanism of photo-oxidation appears to be an essential process in a given biological system for its normal functioning. Icosanoids are the signaling molecules which are produced as a result of oxidation of 20-carbon fatty acids and are derived from omega-3 and omega-6. Functionally these are concerned with inflammation, immunity caused due to toxins and pathogens, these icosanoids also act as messenger within central nervous system, further, these products influence cardiovascular diseases, triglycerides, blood pressure, arthritis etc. The mitochondria appear to be swollen and its matrix gets increased due to elevated rate of cellular oxidation. The biophysical aspects reveal the physicochemical parameters involved in the phenomena and provide relatively better understanding of the principle and the mechanism of the structural and functional insight. When lipid bilayer is subjected to lipid peroxidation process the biomolecules like proteins and/or amino acids are likely to get covalently modified due to the product formed. In spite of the fact that lot of research has been conducted, there appears to be a greater need to pursue the role of lipid oxidation in more details to understand its mechanism in totality in normal and various pathogenic conditions in biosystem.

ACKNOWLEDGEMENT

I extend my gratitude to Prof (Dr) A V Chitre, Dept. Biophysics, Univ. of Mumbai, Santa Cruz (E), Mumbai, for critically going through this review and suggestions.

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