SINGLET MOLECULAR OXYGEN AND PEROXIDES IN CHEMICAL AND BIOLOGICAL SYSTEMS

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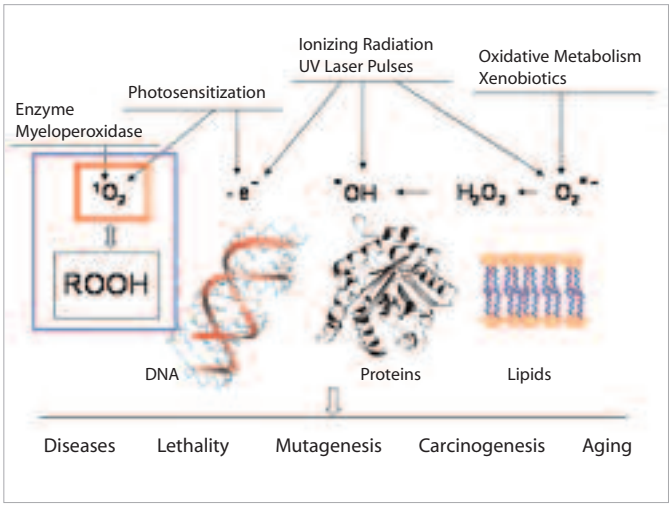

Singlet molecular oxygen ($^1O_2$) has been shown to be generated in biological systems and have been implicated in cell defense mechanisms against viruses and bacteria. Our studies focus on providing the mechanism by which $^1O_2$ and other reactive oxygen species play their physiological and pathological roles. We have been devoted to develop suitable $^1O_2$ generators based on the thermolysis of endoperoxides. These compounds are chemically inert and have been employed as versatile sources of $^1O_2$. This approach has been used in our studies for the detection of $^1O_2$-induced damage in cells (DNA, lipids and proteins) and for screening of biologically occurring compounds for quenching $^1O_2$.

Evidence has been accumulated during the last three decades on the strong implication of reactive oxygen species and one-electron oxidants in the generation of hydroperoxides from several nucleobases, amino acids and unsaturated lipid components. Singlet oxygen is also a major source of peroxidation of several key cellular components. The breakdown products of the rather unstable hydroperoxide (ROOH) precursors thus produced from exposure to endogenous or exogenous oxidizing agents may be implicated in deleterious biological effects such as cellular lethality, aging, mutagenesis and carcinogenesis. It may be added that oxidative processes to biomolecules are also involved in the etiology of other diseases including arteriosclerosis, arthritis, cataract and diabetes.

The purpose of the present project is to extend our understanding of the reactions between reactive oxygen species, specifically $^1O_2$ and ROOH with biomolecules *in vitro* and *in vivo* emphasizing the following aspects. Description of the main peroxidation reactions initiated by $^1O_2$ and ROOH within key cellular targets including pyrimidine and purine nucleobases, several lipid components and amino acids. Studies on the molecular effects of the initial formation of the above hydroperoxides within cellular components. Search of stable degradation products of biomolecules (ex. nucleobase) hydroperoxides that may be considered as the chemical signature of the formation of the latter unstable compounds that can be measured within cellular structure (ex. DNA, lipids, proteins). Indicators of lipid peroxidation that may involve cholesterol hydroperoxides and several degradation products including aldehydes. Measurement of adducts between amino substituted nucleobases and reactive compounds as aldehydes arise from the breakdown of initially generated unstable peroxides. Measurement of several side-chain altered amino residues that often arise from the fate of initially generated peroxides is used as bio-indicators of protein oxidation within cells.

Major efforts have been devoted to the elucidation of the mechanisms of peroxidation of major cellular biomolecules including nucleic acids, lipids and proteins. Relevant peroxidation pathways are now available at least for the main components of the key cellular biomolecules although there is still a need of further studies, particularly for isolating and characterizing putative hydroperoxides. Attempts should also be made to validate in the whole biomolecules the mechanisms of formation of hydroperoxides that were inferred from model studies. Another relevant major topic deals with the search of molecular signature of the peroxide/$^1O_2$ formation in targeted biomolecules within cells upon exposure to oxidative conditions. It may be anticipated that gentle and sensitive mass spectrometric methods such as tandem mass spectrometry (MS/MS) in association with HPLC and the use of ^{18}O-labeled peroxide/$^1O_2$ should constitute powerful tools for this purpose.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our studies have focused on identifying the mechanism by which singlet oxygen ($^1O_2$) and other reactive oxygen/nitrogen species play their physiological and pathological roles. We have devoted efforts to develop suitable $^1O_2$ generators based on the thermolysis of endoperoxides. Few years ago, we synthesized the first water-soluble naphthalene endoperoxide isotopically labeled as a source of $^{18}[^1O_2]$. Synthesis and the use of the ^{18}O-labeled hydro peroxides derivatives (ROOH: lipids, proteins and nucleic acids) were also performed. The application of sensitive and specific methods, using mass spectrometry with electro spray ionization/MALDI-ToF developed in our laboratory, allows for the study of $^1O_2$/ROOH reaction in biological media, aiming to respond to the strong interest in the role of nutrition in the prevention and pathogenesis of cancer.

In our recent study, two cis and trans tryptophan hydro peroxide (WOOH) isomers were completely characterized by HPLC/mass spectrometry and NMR analyses as the major W-oxidation photoproducts. Using ^{18}O-labeled hydro peroxides ($W^{18}O^{18}OH$), it was possible to confirm the formation of a two-oxygen-labeled FMK molecule derived from $W^{18}O^{18}OH$ decomposition. In addition, these reactions are chemiluminescent (CL), indicating a dioxetane cleavage pathway. In summary, photo oxidation of W gives rise to a mixture of trans and cis hydro peroxides. Mechanistic aspects of WOOH decomposition were identified by different techniques: ^{18}O-isotopic labeling studies coupled to mass spectrometry analyses and light emission measurements.

Another interesting result, is the generation of 3β-hydroxy-5β-hydroxy-B-norcholestane-6β-carboxaldehyde (ChAld) by reaction of cholesterol with $^1O_2$ produced by photosensitization or by thermo decomposition of 1,4-dimethylnaphtalene endoperoxide, a defined pure chemical source of $^1O_2$. On the basis of chemiluminescence's measurements and derivatization studies, we proposed that the mechanism of ChAld formation involves initial formation of a 1,2-dioxetane by $^1O_2$ attack at the 5¢ position. Our results add a new pathway that might explain the presence of significant amount of ChAld and 3β-hydroxy-5-oxo-5,6-secocholestan-6-al in neurodegenerative and cardiovascular diseases.

MAIN PUBLICATIONS

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