Journal of Photochemistry and Photobiology B: Biology 139 (2014) 24–33

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Singlet molecular oxygen generated by biological hydroperoxides

Sayuri Miyamoto [a,*], Glaucia R. Martinez [b], Marisa H.G. Medeiros [a], Paolo Di Mascio [a]

[a] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, CP26077, CEP 05513-970 São Paulo, SP, Brazil
[b] Departamento de Bioquímica e Biologia Molecular, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba-PR, Brazil

ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form 19 March 2014
Accepted 27 March 2014
Available online 2 June 2014

Keywords:
Ultra-weak photon emission
Singlet molecular oxygen
Lipid hydroperoxides
Peroxyl radicals

ABSTRACT

The chemistry behind the phenomenon of ultra-weak photon emission has been subject of considerable interest for decades. Great progress has been made on the understanding of the chemical generation of electronically excited states that are involved in these processes. Proposed mechanisms implicated the production of excited carbonyl species and singlet molecular oxygen in the mechanism of generation of chemiluminescence in biological system. In particular, attention has been focused on the potential generation of singlet molecular oxygen in the recombination reaction of peroxyl radicals by the Russell mechanism. In the last ten years, our group has demonstrated the generation of singlet molecular oxygen from reactions involving the decomposition of biologically relevant hydroperoxides, especially from lipid hydroperoxides in the presence of metal ions, peroxynitrite, HOCl and cytochrome c. In this review we will discuss details on the chemical aspects related to the mechanism of singlet molecular oxygen generation from different biological hydroperoxides.

1. Introduction

Light emission phenomena from biological organisms have intrigued people for centuries. Several reactions have been shown to produce electronically excited emitting species in the absence of light ("dark" chemiluminescent reactions), including electron transfer, radical–radical recombination, cyclic peroxide decomposition, and energy transfer from other electronically excited species [1–4]. These reactions can contribute for the phenomena of spontaneous or intrinsic ultra-weak photon emission (also known as low-level chemiluminescence or bioluminescence or biophoton emission) observed in isolated tissues/organs, microorganisms, plants and humans [5–14].

The long-lived $^1\Delta_g$ excited state of molecular oxygen and excited triplet carbonyl species are reported to be important sources of the ultra-weak photon emission observed in biological system. Substantial advancements have been made on the characterization of biochemical reactions involved in the generation of these excited species. Studies in the 1960–1970's [5–8,15–17] and 1980's [9,10,18,19] showed a correlation between tissue/organ low-level chemiluminescence and lipid peroxide content. Based on different evidences it has been proposed that reactions involving lipid derived peroxyl radicals would be responsible for the generation of excited species, in particular for the generation of singlet molecular oxygen [$O_2(^1\Delta_g)$] [17,20–23].

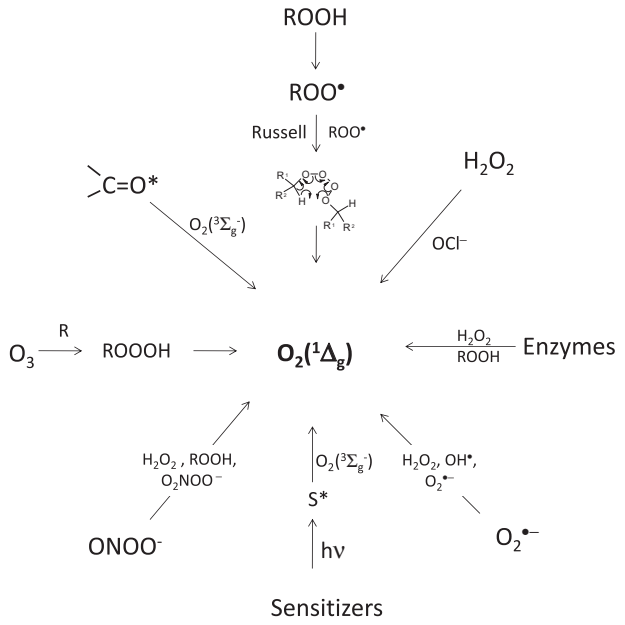
This review focuses on the production of $O_2(^1\Delta_g)$ from different types of biological hydroperoxides. Although excited carbonyl species can also be formed in several situations involving the production of hydroperoxides and peroxidases [4], this aspect is not discussed in details here. Studies demonstrating the generation of $O_2(^1\Delta_g)$ from different types of lipid hydroperoxides and chemical aspects related to the generation of $O_2(^1\Delta_g)$ from reactions involving peroxides will be discussed. Finally, mechanistic evidences obtained through 18-oxygen labeling studies showing the involvement of the Russell mechanism in the generation of $O_2(^1\Delta_g)$ will be highlighted.

2. Singlet molecular oxygen production in biological system

Singlet molecular oxygen [$O_2(^1\Delta_g)$] can be basically generated by light dependent and independent reactions [23,24]. Details on the several possible chemical and biochemical routes leading to $O_2(^1\Delta_g)$ generation and its detection in cellular systems have been reviewed elsewhere [23–25]. Scheme 1 summarizes some of the major routes that could generate $O_2(^1\Delta_g)$ in biological system. In photosensitized oxidation reactions, $O_2(^1\Delta_g)$ is generated by light induced excitation of endogenous sensitizers followed by energy transfer reactions to molecular oxygen [26]. Excitation reactions can also occur in the absence of light. This category of reactions include, $O_2(^1\Delta_g)$ production by hydrogen peroxide and

* Corresponding author. Tel.: +55 11 3091 9114; fax: +55 11 3815 5579.
E-mail addresses: miyamoto@iq.usp.br (S. Miyamoto), pdmascio@iq.usp.br (P. Di Mascio).

http://dx.doi.org/10.1016/j.jphotobiol.2014.03.028
1011-1344/



Scheme 1. Major biochemical reactions leading to the production of $O_2(^1\Delta_g)$.

hypochlorite during phagocytosis [27], superoxide anion [28–34], ozone reaction involving hydrotrioxide intermediates [35,36], peroxynitrite reactions [37–42], energy transfer reactions with excited carbonyl species [43], and reactions involving enzymes, such as, peroxidases and oxygenases [23,44–48].

3. Singlet molecular oxygen light emission

Singlet molecular oxygen in its first excited state, $O_2(^1\Delta_g)$, can decay to ground state by a forbidden transition, which has a characteristic band emission at 1270 nm in the near-infrared region of the electromagnetic spectrum (reaction (1)) [49]. This luminescence is extremely weak, with a quantum yield about 10^{-6}–10^{-3}, since most deactivating collisions of $O_2(^1\Delta_g)$ with solvent molecules generates heat via a nonradiative transition.

$$O_2\,^1\Delta_g\ (v=0) \rightarrow O_2\,^3\Sigma_g^-\ (v=0) + h\nu \quad (\lambda = 1270\ \text{nm}) \qquad (1)$$

The bimolecular transition of $O_2(^1\Delta_g)$ also occurs [50]. In this case, two molecules in the excited state simultaneously decay to the ground state emitting visible light with two characteristic bands (reactions (2) and (3)).

$$O_2\,^1\Delta_g\ (v=0) + O_2\,^1\Delta_g\ (v=0) \rightarrow 2\ O_2\,^3\Sigma_g^-\ (v=0) + h\nu \quad (\lambda = 634\ \text{nm}) \qquad (2)$$

$$O_2\,^1\Delta_g\ (v=0) + O_2\,^1\Delta_g\ (v=0) \rightarrow O_2\,^3\Sigma_g^-\ (v=0) + O_2\,^3\Sigma_g^-\ (v=1) + h\nu \quad (\lambda = 703\ \text{nm}) \qquad (3)$$

4. Russell mechanism

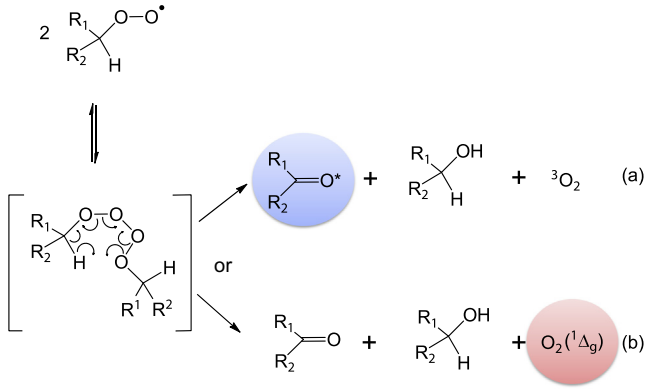
In 1957, Russell first proposed a mechanism in which two secondary or primary peroxyl radicals (ROO·) would react to generate a tetraoxide (ROOOOR) intermediate that rapidly decomposes through a mechanism involving α-hydrogen transfer to yield the corresponding ketone (R═O), alcohol (R—OH) and molecular oxygen [51] (Scheme 2). Later, Howard and Ingold (1968) confirmed the mechanism proposed by Russell and demonstrated that a fraction of molecular oxygen released from *sec*-butylperoxyl radical self-reaction is in the singlet excited state. In fact, based on the Wigner spin conservation rule, it would be expected that tetraoxide decomposition releases either oxygen in the singlet excited state or the carbonyl product in the triplet excited state. Quantitative analysis on the relative yield of these two excited species, revealed that $O_2\,^1\Delta_g$ is generated at a yield of 10% for most of the secondary and primary organic hydroperoxides, while triplet carbonyl species are generated at yields lower than 0.01% [52,53]. Based on these findings, it has been concluded that linear tetraoxide decomposition via a cyclic mechanism yields $O_2(^1\Delta_g)$ as the predominant excited species.

Bearing in mind that lipid peroxidation produces hydroperoxides as primary products and that peroxyl radicals are important intermediates in this process, researchers correlated the phenomena of luminescence to the production of excited species from lipid peroxidation process in biological tissues [8,9,16,18,54,55]. Indeed, a correlation between low-level chemiluminescence and content of endogenous peroxides in organs and tissues from normal animals and tumor-bearing animals has been observed in several studies [8,16,22,56]. Also the enhancement of light emission by the addition of external organic hydroperoxides [18] supported the hypothesis that lipid peroxidation should be an important source of the light observed in tissues undergoing spontaneous or metal/heme-induced oxidation.

5. Hydrogen peroxide as a source of singlet molecular oxygen

The observation of luminescence from subcellular fractions (mainly mitochondria and microsomes) and tissues stimulated other groups to look for emission in inflammatory cells. In 1972, Allen et al. observed the production of $O_2(^1\Delta_g)$ production from phagocytes by measuring the appearance of a chemiluminescence when human polymorphonuclear leukocytes were stimulated with opsonized latex or bacteria and attributed it to radioactive decay of $O_2(^1\Delta_g)$ [57]. Subsequent studies confirmed the generation of $O_2(^1\Delta_g)$ during phagocytosis [27,58–60]. Importantly, Steinbeck et al. [61,62] obtained additional evidences on the production of $O_2(^1\Delta_g)$ in neutrophyls using glass beads coated with 9,10-diphenylanthracene (DPA) as a chemical trap. Using this technique, the authors reported a relatively high conversion rate of 19% of the oxygen consumed during phagocytosis to $O_2(^1\Delta_g)$.

Although still controversial [63], the formation of $O_2\,^1\Delta_g$ during phagocytosis has been primarily associated to the reaction of H_2O_2 with HOCl produced by myeloperoxidase (MPO) in the presence of H_2O_2 and chloride ions (MPO/H_2O_2/Cl^- system, reaction (4)). Initial evidences for the production of $O_2(^1\Delta_g)$ by this system was obtained by Rosen and Klebanoff in an experiment using furan as chemical trap [27]. Later, Khan confirmed the formation of



Scheme 2. Mechanism proposed by Russell [51] for the termination reaction of peroxyl radicals. Reaction between two peroxyl radical yields a tetraoxide intermediate, which decomposes generating either excited carbonyl species (a) or $O_2(^1\Delta_g)$ (b) and the corresponding ketone and alcohol products.