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Role of reactive oxygen species in ultra-weak photon emission in biological systems

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ABSTRACT

Ultra-weak photon emission originates from the relaxation of electronically excited species formed in the biological systems such as microorganisms, plants and animals including humans. Electronically excited species are formed during the oxidative metabolic processes and the oxidative stress reactions that are associated with the production of reactive oxygen species (ROS). The review attempts to overview experimental evidence on the involvement of superoxide anion radical, hydrogen peroxide, hydroxyl radical and singlet oxygen in both the spontaneous and the stress-induced ultra-weak photon emission. The oxidation of biomolecules comprising either the hydrogen abstraction by superoxide anion and hydroxyl radicals or the cycloaddition of singlet oxygen initiate a cascade of oxidative reactions that lead to the formation of electronically excited species such as triplet excited carbonyl, excited pigments and singlet oxygen. The photon emission of these electronically excited species is in the following regions of the spectrum (1) triplet excited carbonyl in the near UVA and blue–green areas (350–550 nm), (2) singlet and triplet excited pigments in the green–red (550–750 nm) and red-near IR (750–1000 nm) areas, respectively and (3) singlet oxygen in the red (634 and 703 nm) and near IR (1270 nm) areas. The understanding of the role of ROS in photon emission allows us to use the spontaneous and stress-induced ultra-weak photon emission as a non-invasive tool for monitoring of the oxidative metabolic processes and the oxidative stress reactions in biological systems *in vivo*, respectively.

1. Introduction

Reactive oxygen species (ROS) are formed either during the metabolic processes linked to life-sustaining enzyme-catalyzing reactions or during the response to stress reactions when microorganisms, plants and animals including humans are exposed to biotic and abiotic stress factors. When ROS are effectively scavenged by the antioxidant defense system, the oxidative effect of ROS on biomolecules such as lipids, proteins and nucleic acids is fully prevented [1]. However, under circumstance, when the formation of ROS exceeds the capacity of antioxidant defense system, biomolecules are oxidized by ROS. The oxidation of lipids, proteins and nucleic acids leads to the formation of high-energy intermediates [2]. The decomposition of high-energy intermediates generates the electronically excited species which undergo the electronic transition from either the singlet or the triplet excited state to the singlet ground state [3]. The electronic transition of electronically excited species from the singlet or the triplet excited state to the ground state is accompanied by photon emission. As merely few photons are emitted per second per square centimeter, the photon emission is ultra-weak in nature. In order to envision the role of ultra-weak photon emission, the propagation of ultra-weak photons in biological system via through specialized primo vascular channels was hypothesized [4].

Based on the process during which ROS are formed comprising either metabolic processes or stress reactions, two types of ultra-weak photon emission are well described in the literature [5–7]. Spontaneous ultra-weak photon emission originates from the relaxation of electronically excited species formed in the biological systems during the oxidative metabolic process that are associated with the production of ROS during normal metabolic processes [5,6,8,9]. Stress-induced ultra-weak photon emission originates from relaxation of electronically excited species formed under abiotic (physical and chemical) and biotic (virus, bacteria, fungi) stress reactions.

Abbreviations: SOD, superoxide dismutase; NADPH, nicotinamide adenine dinucleotide phosphate; PMT, photomultiplier tube; UV, ultraviolet; ROS, reactive oxygen species.

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This review is focused on the involvement of ROS formed either during the oxidative metabolic processes or during the oxidative stress reactions in spontaneous and stress-induced ultra-weak photon emission, respectively. It is stated that ROS oxidize lipids, proteins and nucleic acids and thus initiate a cascade reactions that leads to the formation of electronically excited species responsible for the photon emission in near UVA, visible and near IR regions of the spectrum.

2. Reactive oxygen species production

Reactive oxygen species are formed either by the sequential one-electron reduction of molecular oxygen or by the triplet–singlet energy transfer from triplet excited pigments to molecular oxygen. The sequential one-electron reduction of molecular oxygen leads to the formation of superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$), each of which possess a differential redox potential and thus bears a varied degree of reactivity [10]. The triplet–singlet energy transfer from the triplet excited pigment to molecular oxygen results in the formation of singlet oxygen ($^1O_2$). Reactive oxygen species formed during the metabolic processes play an important role in the defense against infection, cell signaling, apoptosis and ageing [11]. On the other hand, ROS are able to oxidize biomolecules such as lipids, proteins and nucleic acids [1,10]. To prevent the dangerous effect of ROS on biomolecules, the non-enzymatic and the enzymatic antioxidant defense systems have been developed in the cells. The non-enzymatic antioxidant defense system comprises of the low molecular weight components such as carotenoids, coenzyme Q10, glutathione, lipoic acid, melanin, urocanic acid, porphyrin, bilirubin, flavins and pterins and vitamins (A, B, C, D and E) [1]. The enzymatic antioxidant defense is maintained by various types of antioxidant enzymes comprising superoxide dismutase (SOD), various types of peroxidases (glutathione peroxidase, ascorbate peroxidase, cytochrome c peroxidase), catalase and glutathione reductase [12–14]. Under the normal metabolic processes, the cells unceasingly produce ROS which exceed the threshold under the abiotic and the biotic stress as described in the next sections.

2.1. Metabolic production of reactive oxygen species

Metabolic processes (e.g. cellular respiration, photosynthesis) are the essential chemical reactions known to occur inside microbial, plant and animal cells. It is well known that metabolic processes are associated with the formation of radical ($O_2^{\cdot-}$, $HO^{\cdot}$) and non-radical (H_2O_2, $^1O_2$) ROS (Fig. 1). Superoxide anion radical is formed by the one-electron reduction of molecular oxygen or by the one-electron oxidation of H_2O_2. In animal cells, the one-electron reduction of molecular oxygen to $O_2^{\cdot-}$ occurs in the mitochondria during the cellular respiration. Electron donation to molecular oxygen is maintained by the complex I and the complex III located in the inner mitochondrial membrane [13,15]. Apart to mitochondria, the one-electron reduction of molecular oxygen is catalyzed by NADPH oxidase during the respiratory burst in the phagocytic cells, xanthine oxidase in the cytoplasm [16]. The one-electron oxidation of H_2O_2 catalyzed by flavin oxidases in peroxisomes is another source of $O_2^{\cdot-}$ in animal cells [17]. In plants, $O_2^{\cdot-}$ is formed by one-electron reduction of molecular oxygen by the stromal side of photosystem I [18] and photosystem II [19]. Hydrogen peroxide is formed either by the one-electron reduction of $O_2^{\cdot-}$ or by the two-electron reduction of molecular oxygen. The one-electron reduction of $O_2^{\cdot-}$ to H_2O_2 occurs either spontaneously or is catalyzed by SOD located in the mitochondria, chloroplast and cytoplasm [20]. The two-electron reduction of molecular oxygen occurs during the reaction in which specific substrates are oxidized by the various types of oxidases in the mitochondria and peroxisomes [10,21]. Hydroxyl radical formed by the one-electron reduction of H_2O_2 catalyzed by metal in the reaction known as Fenton reaction. Several types of metal ions such as iron, copper, manganese, zinc, chromium, cobalt, nickel and vanadium have been shown to reduce H_2O_2 to $HO^{\cdot}$ [22]. It is well known that metals are coordinated to the active enzyme site in metalloproteins or stored in a ubiquitous protein called ferritin, hemosiderin, transferrin and lactoferrin [12,23]. Singlet oxygen is formed by the triplet–singlet energy transfer from the triplet excited chlorophyll to molecular oxygen in chloroplast. The triplet chlorophyll is formed by the intersystem crossing from the singlet excited chlorophyll formed upon the light absorption in the light-harvesting complex in photosystem II [19].

2.2. Stress-induced production of reactive oxygen species

Various types of abiotic (physical and chemical stress factors) and biotic (virus, bacteria, fungi) stresses are known to significantly enhance ROS formation. Under such circumstances, the formation of ROS exceeds the capacity of antioxidant system and the hazardous ROS are inadequately eliminated. The inconsistency between the formation and the elimination of ROS causes the oxidation of lipids, proteins and nucleic acids. Below, the formation of ROS initiated by physical, chemical and biological stimuli is outlined.

2.2.1. Physical production of reactive oxygen species

It well known that both UV radiation and visible light results in the formation of radical ($O_2^{\cdot-}$, $HO^{\cdot}$) and non-radical ($^1O_2$) ROS by Type I and Type II reactions, respectively (Fig. 2) [24–28]. Endogenous pigments in plants, animals and humans such as porphyrins, bilirubins, melanins, pterins and urocanic acid are known to act as photosensitizers [27]. The absorption of UV radiation or visible light by photosensitizers leads to the formation of the singlet state of photosensitizer which forms the triplet excited state via intersystem crossing. The excited photosensitizer undergoes either the electron transport forming $O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$ (Type II reaction) or the energy transfer forming $^1O_2$ (Type II reaction) [27,29–31]. In Type I reaction, the electron transport leads to the formation of $O_2^{\cdot-}$ via the formation of photosensitizer anion radical and substrate cation radical or vice versa [30,32]. The spontaneous or enzymatically driven dismutation of $O_2^{\cdot-}$ leads to the formation of H_2O_2 which subsequently forms $HO^{\cdot}$ via Fenton reaction or other metals catalyzed reactions [14,30,32–34]. In Type II reaction, the triplet–singlet energy transfer from the excited photosensitizer to molecular oxygen forms $^1O_2$.

2.2.2. Chemical production of reactive oxygen species

Reactive oxygen species can be produced via different chemical systems either exogenously (independently to cell components) or endogenously (dependently to cell components). The exogenous source of ROS comprises of xanthine/xanthine oxidase system producing $O_2^{\cdot-}$, Fenton reagent (H_2O_2 and transition metal ions) known to generate $HO^{\cdot}$ and photosensitizer such as rose bengal, radachlorin and benzoporphyrin derivative known to produce $^1O_2$ upon photosensitization [10,35–37]. Besides the exogenous ROS production, ROS are also produced endogenously in the association with cell components. Paraquat anion radical formed by the reduction of paraquat by NAD(P)H reduces molecular oxygen to $O_2^{\cdot-}$ [38]. The addition of H_2O_2 to the cells can lead to the formation of $HO^{\cdot}$ upon reaction with endogenous metal ions coordinated to active enzyme site in metalloproteins or released from a ubiquitous protein called ferritin, hemosiderin, transferrin and lactoferrin [23]. Singlet oxygen is formed from lipid and DNA hydroperoxides in the presence of metal ions, cytochrome c, peroxynitrite, chloroperoxide and

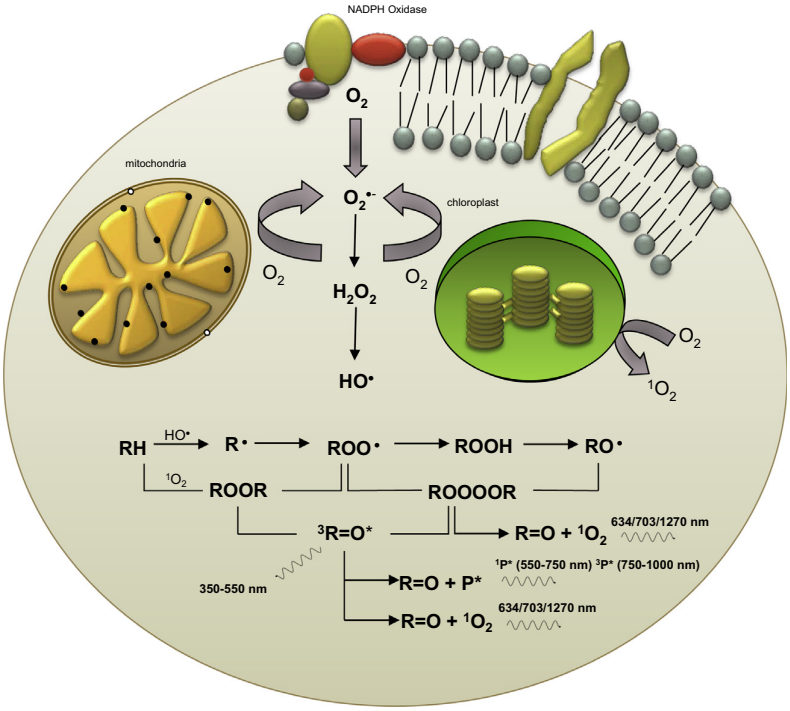

Fig. 1. A model showing the formation of reactive oxygen species (ROS) in different organelles of the cell. Superoxide anion radical ($O_2^{\cdot-}$) is produced via membrane-bound enzyme complex NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) which is found embedded within the plasma membranes and membranes of various organelles such as mitochondria, chloroplast and phagosomes. The dismutation of $O_2^{\cdot-}$ is accompanied by the formation of hydrogen peroxide (H_2O_2) and further hydroxyl radical (HO·) via Fenton reaction. The highly reactive HO· has the capability to oxidize all types of biomolecules such as lipids, proteins and nucleic acids. The oxidation of biomolecules is accompanied by the formation of high-energy intermediates such as dioxetane (ROOR) and tetroxide (ROOOOR) which further upon decomposition generates electronically excited species such as triplet excited carbonyl (^{3}R=O*), singlet (^{1}P*) and triplet (^{3}P*) excited pigments, and singlet oxygen ($^1O_2$).

hypochlorous acid formed as a by-product of myeloperoxidase/H_2O_2/halide system (chloride or bromide) [2,39,40]. It has been shown that hypochlorous acid formed by the myeloperoxidase system reacts with excess of H_2O_2 in the system to further yield $^1O_2$ [41].

2.2.3. Biological production of reactive oxygen species

Reactive oxygen species have been shown to be associated with the plant response to pathogen, herbivory and insects. The hypersensitive reaction is a defense response that causes cell death in the local region of infection and thereby restricting the growth of the pathogen in the organism. The first phase of hypersensitive reaction includes the change in the membrane potential and increasing pH in the extracellular matrix, whereas the second phase is known to be associated with the formation of ROS including $O_2^{\cdot-}$, H_2O_2 and HO· [42]. During the herbivore attack on plants, the defense response leads to the accumulation of secondary metabolites through the signaling pathways which are known to be governed by ROS and redox signaling. It has also been known that ROS burst occurs at the site of insect injury [43].

3. Ultra-weak photon emission

Ultra-weak photon emission is low-level chemiluminescence from biological systems generated in all living organisms during the oxidative metabolic and stress processes. The phenomenon is also referred to as biophoton emission, autoluminescence or low-level chemiluminescence [44–49]. Ultra-weak photon emission observed from various biological samples has been surveyed starting from the subcellular level, cellular level up to the individual organism including plants, animals and humans. Fig. 3 shows two-dimensional imaging of the spontaneous ultra-weak photon emission from *Saccharomyces* yeast (A), *Arabidopsis* plants (B) and upper part of human body (C). The detection of ultra-weak photon emission from the individual organism is possible due to the development of the low-noise and highly sensitive photon detection techniques. Several studies suggested the potential use of ultra-weak photon emission in a wide range of research area and bear the potential to prospect its way to the application in microbiology, plant biology and medicine.

The recent development of low-noise photomultiplier tube (PMT) and highly sensitive charge coupled device (CCD) camera has made possible to monitor spontaneous ultra-weak photon emission from microorganisms, plants and animals including humans. The choice of one-dimensional detection of ultra-weak photon emission using low-noise PMT or two-dimensional imaging of ultra-weak photon emission using highly sensitive CCD camera should be based on the objective of experiments. The one-dimensional detection of photon emission can serve as a better option for kinetic and spectral study of ultra-weak photon emission. On contrary, the two-dimensional imaging of photon emission is performed for the study of spatial and temporal properties of ultra-weak photon emission. Several lines of evidence have been demonstrated that the one-dimensional detection of photon emission from microorganisms [7], plants [50], animals [51] and humans [31,52,53] provides kinetic and spectral characterization of ultra-weak photon emission. The spatial and temporal properties of ultra-weak photon emission have been widely obtained by the two-dimensional imaging of photon emission from microorganisms [9], plants [54–57], animals [6] and humans [31,53,58].

Reactive oxygen species formed either during the metabolic processes or during the stress reactions have a capability to oxidize

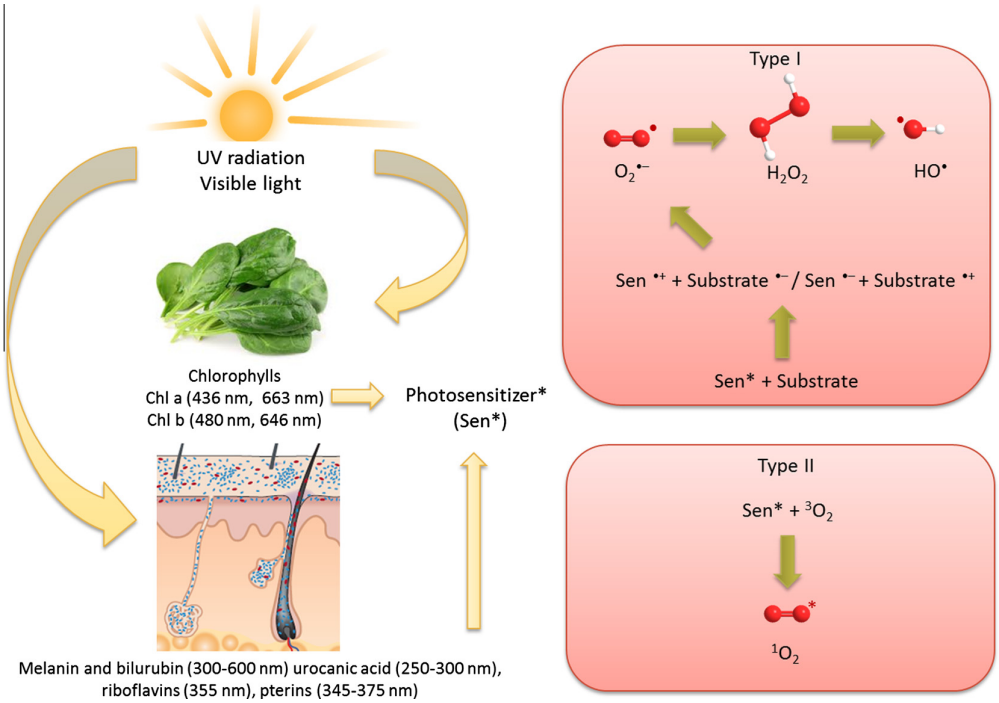

Fig. 2. Schematic illustration on the mechanism of photosensitization reaction in plants and animals (skin anatomy adopted from [112] with prior permission). The absorption of energy by the pigments such as chlorophylls in plants and bilirubins, melanins, pterins and urocanic acid in animals leads to the formation of excited state of photosensitizer (Sen^*) which can either undergoes Type I or Type II reactions. The type I reaction comprises of electron transport and is associated with the formation of superoxide anion radical ($O_2^{\bullet-}$) via formation of photosensitizer anion radical ($Sen^{\bullet-}$) and a substrate cation radical ($Substrate^{\bullet+}$) or vice versa. The $O_2^{\bullet-}$ further leads to the formation of hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$). Type II reaction proceeds via energy transfer from the excited photosensitizer molecule to molecular oxygen forming singlet oxygen ($^1O_2$).

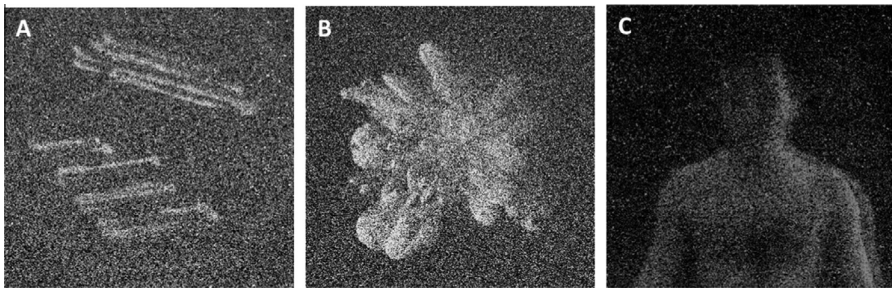

Fig. 3. Two-dimensional imaging of the spontaneous ultra-weak photon emission from different living system ranging from microorganisms, plants and animals. The two-dimensional images represent the spontaneous ultra-weak photon emission from (A) microorganisms (*Saccharomyces* sp.; adopted from [9]), (B) plants (*Arabidopsis* sp.) and (C) human body (adopted from [85]). Ultra-weak photon emission from *Arabidopsis* plant was measured with an integration time of 15 min. The images adopted (A and C) are reproduced with permission from John Wiley & Sons Ltd, Chichester, West Sussex, United Kingdom. PO19 8SQ.

lipids, proteins and nucleic acids. Based on the type of oxidative process initiated by ROS in the cells, spontaneous and stress-induced ultra-weak photon emissions are considered.

3.1. Spontaneous ultra-weak photon emission

When ROS are produced spontaneously during the metabolic processes, the ultra-weak photons are emitted spontaneously by the relaxation of electronically excited species formed during the oxidative metabolic processes. In the oxidative metabolic processes, the oxidation of biomolecules occurs by the hydrogen abstraction by $HO^{\cdot}$ or the cycloaddition of $^1O_2$. Hydrogen abstraction from lipids, proteins and nucleic acids by $HO^{\cdot}$ results in the formation of alkyl radical ($R^{\cdot}$) known to interact with molecular oxygen forming peroxyl radical ($ROO^{\cdot}$). The later abstracts an electron from another lipid, protein and nucleic acid while another $R^{\cdot}$ and hydroperoxide (ROOH) are formed. In the presence of metal, ROOH is reduced to alkoxyl radical ($RO^{\cdot}$). The cyclization of $ROO^{\cdot}$ and the recombination of two $ROO^{\cdot}$ form high-energy intermediates dioxetane (ROOR) and tetroxide (ROOOOR), respectively [59,60]. Apart to the cyclization of $ROO^{\cdot}$, dioxetane is formed by the cycloaddition of $^1O_2$ [61]. The decomposition of ROOR and ROOOOR leads to the formation of triplet excited carbonyls [$^3(R{=}O)^*$] [2,62–64]. The energy transfer from $^3(R{=}O)^*$ to pigments or molecular oxygen leads to the formation of excited pigment (P^*) or $^1O_2$. Apart to $^3(R{=}O)^*$, the decomposition of ROOOOR directly to $^1O_2$ occurs via Russell mechanisms [60,65]. The photon emission of $^3(R{=}O)^*$ is at near UVA and blue–green regions of the spectrum (350–550 nm) [64,66]. The photon emission of singlet and triplet excited pigments is in the green–red (550–750 nm) and red-near

IR (750–1000 nm) regions of the spectrum, respectively [66,67], whereas the dimol and the monomol photon emissions of $^1O_2$ are in the red (634 and 703 nm) and near IR (1270 nm) regions of the spectrum, respectively [68].

It has been observed that the spontaneous ultra-weak photon emission ranges from units to tens of photons $s^{-1}\,cm^{-2}$ [45]. The one-dimensional detection of spontaneous ultra-weak photon emission from isolated organelles, microorganisms, plants, animals and humans have been measured by several authors [31,69–71]. Besides this, the two-dimensional imaging of ultra-weak photon emission from microorganisms, plants, animals and humans has also been measured [5,6,31,47,70]. Spatial distributions of photon intensity have been observed together with the temporal variation measured in the time range of weeks to month [72–74].

3.2. Stress-induced ultra-weak photon emission

When ROS are produced during the stress reactions, the ultra-weak photons are emitted by the relaxation of electronically excited species formed during the oxidative stress processes. In the oxidative stress processes, the oxidation of biomolecules occurs either by hydrogen abstraction by HO· or by cycloaddition of $^1O_2$. As the formation of HO· and $^1O_2$ during stress processes is pronouncedly enhanced compared to ROS formation during the metabolic processes, the formation of ROOR by the cyclization of ROO· and the cycloaddition of $^1O_2$ is largely extended. Similarly, the concentration of ROO· formed by the interaction of R· with molecular oxygen is significantly enhanced. Under these circumstances, when the concentration of ROO· is high enough, the probability of recombination of two ROO· to high-energy intermediates ROOOOR is pronouncedly increased. The decomposition of ROOR and ROOOOR to $^3(R{=}O)^*$ and the decomposition ROOOOR to $^1O_2$ via Russell mechanisms are significantly enhanced compared to the spontaneous ultra-weak photon emission. Furthermore, the probability of collision of two $^1O_2$ responsible for the dimol photon emission of $^1O_2$ in the red region of the spectrum (634 and 703 nm) is pronouncedly enhanced compared to the spontaneous ultra-weak photon emission.

The stress-induced ultra-weak photon emission ranges from tens to thousands of photons $s^{-1}\,cm^{-2}$. The one-dimensional detection of stress-induced ultra-weak photon emission was presented from microorganisms, plants, animals and humans [7,31,51,70]. The measurement of kinetic profile of stress-induced ultra-weak photon emission from different biological systems have been found to possess a fast decay followed by photon emission close to spontaneous ultra-weak photon emission [75]. Based on different results obtained, it can be predicted that the duration of decay and the kinetic profile is most likely dependent on the nature and the duration of the stress. The two-dimensional imaging of ultra-weak photon emission from microorganisms, plants [54–57], animals [6] and humans [31,53,58] have also been measured. The two-dimensional images under stress condition have shown a marked difference as compared to the spontaneous ultra-weak photon emission as shown by different authors and thus, it can be pointed here that it can be a useful tool in stress detection.

4. Involvement of reactive oxygen species in ultra-weak photon emission

4.1. Involvement of superoxide anion radical in ultra-weak photon emission

4.1.1. Spontaneous ultra-weak photon emission

The addition of exogenous SOD to spinach chloroplasts suppressed pronouncedly ultra-weak photon emission [76]. Based on this observation, it has been proposed that $O_2^{\cdot-}$ is involved in the ultra-weak photon emission. As there is no $O_2^{\cdot-}$ production in the dark, the authors suggested that $O_2^{\cdot-}$ is formed by the one-electron reduction of molecular oxygen by NADPH during the chlororespiration in the chloroplasts. Indeed, the observation that the addition of a respiratory inhibitor salicylhydroxamic acid to the spinach chloroplast decreased the photon emission confirmed the involvement of $O_2^{\cdot-}$ formed during the chlororespiration. The authors proposed that plastoquinol formed by the reduction of plastoquinone by NADPH reduces molecular oxygen forming $O_2^{\cdot-}$. The two-dimensional imaging of ultra-weak photon emission from the tumor in nude mice transplanted with the carcinoma cell lines demonstrated that the photon emission from tumor is enhanced as compared to the photon emission observed before the transplantation [77,78]. The authors proposed that the enhanced $O_2^{\cdot-}$ production and the decreased level of antioxidant defense system such as SOD are responsible for enhanced photon emission in the tumor tissue. The involvement of $O_2^{\cdot-}$ in ultra-weak photon emission was supported by the observation that the topical application of non-enzymatic antioxidant of $O_2^{\cdot-}$ such as ascorbate, glutathione, α-tocopherol and coenzyme Q10 suppressed ultra-weak photon emission [53].

4.1.2. Stress-induced ultra-weak photon emission

4.1.2.1. Physically-induced ultra-weak photon emission. The observation that the addition of exogenous SOD to the cotyledons of etiolated seedlings of *Cicerarietinum* L. previously subjected to the sudden freezing and thawing suppressed ultra-weak photon emission indicates that $O_2^{\cdot-}$ is involved in ultra-weak photon emission [79,80]. An increase in ultra-weak photon emission was observed on the exposure of the dorsal and the palmar side of the hand to UVA radiation and visible light [31]. The absorption of UVA radiation or visible light by skin pigments such as urocanic acid (250–300 nm), porphyrin (protoporphyrins) (320–400 nm), flavins (345–375 nm), pterins (345–375 nm), melanin and bilirubin (300–600 nm) was proposed to form $O_2^{\cdot-}$ via Type I reaction. Type I reaction involves the transport of electrons leading to the formation of $O_2^{\cdot-}$ via formation of photosensitizer anion radical and substrate cation radical or vice versa.

4.1.2.2. Chemically-induced ultra-weak photon emission. Several reports have been presented on the involvement of $O_2^{\cdot-}$ in the ultra-weak photon emission based on the addition of chemically-generated $O_2^{\cdot-}$ using xanthine/xanthine oxidase system [79]. The addition of xanthine/xanthine oxidase to human leukocytes brings about a significant increase in ultra-weak photon emission [81]. The other group demonstrated that the addition of myristic acid to polymorphonuclear leukocytes caused pronounced enhancement in ultra-weak photon emission [82]. Based on the correlation between the enhancement in ultra-weak photon emission and oxygen uptake, the authors proposed that $O_2^{\cdot-}$ is involved in ultra-weak photon emission. Further evidence on the involvement of $O_2^{\cdot-}$ in ultra-weak photon emission has been provided by the application of non-enzymatic and enzymatic antioxidant. Kakinuma et al. [82] showed that the myristic acid-induced ultra-weak photon emission was partially suppressed by the exogenous application of SOD. The observation that the addition of electron acceptor paraquat to SOD-deficient mutant of yeast *Saccharomyces cerevisiae* enhanced the photon emission compared to wild type reveals that $O_2^{\cdot-}$ is involved in ultra-weak photon emission [7]. The photon emission from rice cells elicited by N-acetylchitooligosaccharide was shown to be twice compared to control rice cells [83]. N-acetylchitooligosaccharide is known to work as an elicitor at subnanomolar concentrations in suspension-cultured rice cells, inducing a variety of defense reactions such as the production of

$O_2^{\cdot-}$ [84]. More recently, the topical application of xanthine/xanthine oxidase on the human skin has been shown to cause enhancement in photon emission three times compared to the non-treated human skin [85]. The authors proposed that $O_2^{\cdot-}$ is formed by the one-electron reduction of molecular oxygen catalyzed by the complex I and the complex III during the respiration in the mitochondria, by NADPH oxidase during the respiratory burst in the phagocytic cells and xanthine oxidase in the cytoplasm of the cell.

4.1.2.3. Biologically-induced ultra-weak photon emission. Based on the observation that the topical application of chemically-generated $O_2^{\cdot-}$ by xanthine/xanthine oxidase in *Arabidopsis* plant brings no enhancement in the ultra-weak photon emission, Bennett et al. [54] proposed that the involvement of $O_2^{\cdot-}$ in ultra-weak photon emission might be ruled out. Furthermore, this proposal was supported by the observation that ultra-weak photon emission was unaffected by diphenyl iodonium, an inhibitor of NADPH oxidase. However, during the hypersensitive response elicited by cucumber mosaic virus in cowpea, the transient ultra-weak photon emission observed after several hours of inoculation was found to be suppressed by the exogenous addition of Tiron, a known $O_2^{\cdot-}$ scavenger [86]. The ultra-weak photon emission from maize leaf mechanically injured and chemically treated with oral secretion of caterpillar showed an enhancement in ultra-weak photon emission compared to the non-treated leaf. It can be hypothesized that the enhancement in photon emission is caused by NADPH activation which shows the involvement of $O_2^{\cdot-}$ in ultra-weak photon emission [71].

4.2. Involvement of hydrogen peroxide in ultra-weak photon emission

4.2.1. Spontaneous ultra-weak photon emission

Kobayashi et al. [6] demonstrated that the spontaneous ultra-weak photon emission from rat's brain correlates with the metabolic activity of brain cells as measured by electroencephalographic (EEG) activity. The photon emission from brain cells was enhanced by about 30% in the hyperoxia condition compared to the normal condition. The authors proposed that the enhancement in photon emission is due to the higher formation of H_2O_2 in hyperoxia condition.

4.2.2. Stress-induced ultra-weak photon emission

4.2.2.1. Physically-induced ultra-weak photon emission. The observation that the ultra-weak photon emission from the cotyledons of etiolated seedlings of *Cicerarietinum* L. subjected to the sudden freezing and thawing was found to be pronouncedly suppressed by the addition of exogenous catalase revealed that H_2O_2 is involved in ultra-weak photon emission [79]. Ultra-weak photon emission measured in water extract from soybean roasted in the presence of H_2O_2 was pronouncedly enhanced as compared to water extract from the boiled or autoclaved soybean [50]. The correlation between the photon emission and H_2O_2 formation measured using absorption spectroscopy with heating time indicates that H_2O_2 is involved in the ultra-weak photon emission and can be employed for the estimation of food quality.

4.2.2.2. Chemically-induced ultra-weak photon emission. The addition of exogenous catalase to the polymorphonuclear leukocytes treated with myristic acid partially suppressed ultra-weak photon emission [82]. The observation that the simultaneous addition of exogenous SOD and catalase almost completely diminished the myristic acid-induced ultra-weak photon emission reveals the involvement of H_2O_2 in ultra-weak photon emission. The ultra-weak photon emission from the rat perfused lung was found to increase by the addition of exogenous H_2O_2 and organic peroxide such t-butyl hydroperoxide [87]. The addition of H_2O_2 (several hundred mM) to the intact soybean seedlings caused an enhancement in the ultra-weak photon emission [88]. When H_2O_2 was added to the injured soybean seedlings, the photon emission was three time higher as compared to the intact soybean seedlings. It has been shown that the photon emission observed after the application of H_2O_2 to injured soybean seedling occurs in two phases, i.e. fast and slow phase. The authors proposed that photon emission in the fast phase is due to the direct effect of H_2O_2, whereas molecular oxygen produced during the decomposition of H_2O_2 is involved in the photon emission during the slow phase. It has been previously demonstrated that ultra-weak photon emission from rice cell culture is increased after the addition of H_2O_2 (several hundred mM) in a concentration-dependent manner [83]. Based on the correlation between H_2O_2-induced and elicitor-induced N-acetylchitooligosaccharide enhancement in ultra-weak photon emission, the authors proposed that ROS formed during the phospholipid signaling pathway are involved in the photon emission. The addition of H_2O_2 (several units mM) to Madin–Darby canine kidney (MDCK) cells results in the increase of ultra-weak photon emission [89]. The topical application of H_2O_2 and organic peroxide (benzoylperoxide) to the porcine ex vivo skin model caused pronounced increment in ultra-weak photon emission [90]. It has been reported that the ultra-weak photon emission in radish root cells increases with the application of exogenous H_2O_2 (several units mM) in concentration dependent manner [75]. With the application of non-enzymatic $HO^{\cdot}$ scavenger such as ascorbate, the enhancement in photon emission after the addition of exogenous H_2O_2 was prevented. Based on these observations, the authors proposed that $HO^{\cdot}$ formed by the reduction of exogenous H_2O_2 by endogenous metals is involved in the ultra-weak photon emission. The topical application of H_2O_2 (several tens to hundreds mM) on the dorsal and the palmar sides of the hand resulted in the increase in ultra-weak photon emission [52,85]. The authors demonstrated that the photon emission from the palmar side of the hand is four times higher compared to the photon emission from the dorsal side of the hand. The author proposed that the epidermis, which is 5–10 times thicker in the palmar side when compared to the dorsal side, participates in the higher photon emission from the palmar side of the hand. The authors suggested that H_2O_2 is formed by either one-electron reduction of $O_2^{\cdot-}$ catalyzed by SOD in mitochondria and cytoplasm or two-electron reduction of molecular oxygen in the reaction in which substrates such as monoamine, urate, polyamine, oxilate and fatty acyl-CoA are oxidized by various types of oxidases [85].

4.2.2.3. Biologically-induced ultra-weak photon emission. The infection of pathogen *Phytophthora infestans* to potato plant was shown to increase ultra-weak photon emission [56]. More detailed study showed that the enhancement in ultra-weak photon emission observed in the potato tuber infected with pathogen *P. infestans* correlates with the formation of H_2O_2 as confirmed by DAB staining [70].

Besides the role of ROS, reactive nitrogen species (RNS) have been proposed to participate in biologically-induced ultra-weak photon emission. With the infiltration of virulent pathogen to *Arabidopsis* plant, ultra-weak photon emission was found to be enhanced as compared to that infiltrated with avirulent pathogen [54]. Based on these observations, it was claimed that the ultra-weak photon emission is associated with hypersensitive reaction. The findings that the formation of H_2O_2 by exogenous glucose/glucose oxidase system caused no enhancement in the ultra-weak photon emission indicated that H_2O_2 unlikely participate in the ultra-weak photon emission. Also, it was observed that ultra-weak photon emission was completely suppressed by the exogenous

addition of nitric oxide inhibitors. Based on these observations, it was claimed that the hypersensitive reaction is dependent on RNS and intercellular calcium but not on ROS. Mansfield [55] extended the hypothesis of Bennett thereby making a correlation between the hypersensitive reaction leading to the generation of RNS and pointing that the ultra-weak photon emission is a phenomenon which occurs at the onset of the hypersensitive reaction.

4.3. Involvement of hydroxyl radical in ultra-weak photon emission

4.3.1. Spontaneous ultra-weak photon emission

As the lifetime of HO· is in the nanosecond time range, the experimental evidences on the involvement of HO· in spontaneous ultra-weak photon emission are limited. However, several authors hypothesized the participation of HO· in the spontaneous ultra-weak photon emission. Attempts have been made to study the involvement of HO· in the ultra-weak photon emission; however, no significant difference was observed when mannitol, a known HO· scavenger, was added to the unicellular green alga, *Chlamydomonas reinhardtii* [91]. It seems to be likely that the limiting factor is the low intensity of spontaneous ultra-weak photon emission from living cells.

4.3.2. Stress-induced ultra-weak photon emission

4.3.2.1. Physically-induced ultra-weak photon emission. The observation that the addition of mannitol and *t*-butyl alcohol suppressed significantly the ultra-weak photon emission from the cotyledons of etiolated seedlings of *Cicerarietinum* L. subjected previously to the sudden freezing and thawing reveals that HO· is involved in ultra-weak photon emission [80]. The addition of iron chelator, desferrioxamine, suppressed the ultra-weak photon emission, whereas the addition of exogenous ferric iron (Fe^{3+}) and iron-containing pigment (haematin) enhanced the ultra-weak photon emission. These observations indicate that HO· formed by one-electron reduction of H_2O_2 by transition metals is involved in the ultra-weak photon emission. The topical application of polyphenol antioxidant, oligomericproanthocyanidins, on the human skin exposed to UVA radiation was found to suppress the ultra-weak photon emission [92]. Considering that oligomeric proanthocyanidins has been shown to act as a scavenger of HO· [93], it is proposed here that HO· formed in the human skin under UVA exposure is involved in the ultra-weak photon emission. To monitor the effect of cell disruption on the ultra-weak photon emission in Chlamydomonas cells, the photon emission was measured in the cells injured mechanically with liquid nitrogen [91]. The ultra-weak photon emission was found to be enhanced subsequently upon warming at room temperature. These observations reveal that the cell disruption results in the enhancement in ultra-weak photon emission. The observation that mannitol suppressed ultra-weak photon emission in the disrupted cells reveals that HO· is involved in the ultra-weak photon emission under the mechanical injury.

4.3.2.2. Chemically-induced ultra-weak photon emission. When *Arabidopsis* plants were grown in the presence of copper, it was observed that the ultra-weak photon emission and MDA accumulation were observed to be higher as compared to the absence of copper [48]. Furthermore, the authors demonstrated that the ultra-weak photon emission observed in *vte1 Arabidopsis* plant lacking both tocopherol and plastochromanol was higher compared to WT. The likely mechanism responsible for the high ultra-weak photon emission observed in *Arabidopsis* plant is via HO· formation through Fenton reaction under copper toxicity. The topical application of Fenton reagent (H_2O_2 and Fe^{2+}) on the dorsal side of the human hand results in the enhancement in ultra-weak photon emission [85]. Based on the observation that the Fenton reagent provided the highest increase in photon emission compared to the topical application of xanthine/xanthine oxidase and H_2O_2 reveals that HO· is the strongest oxidant in the human body. Scavenging of HO· by mannitol considerably suppressed the ultra-weak photon emission measured after the addition of linoleic acid to Chlamydomonas cells [91]. The authors proposed that the oxidation of linoleic acid by HO· initiates the lipid peroxidation resulting in the formation of $^3(R{=}O)^*$ and $^1O_2$.

4.3.2.3. Biologically-induced ultra-weak photon emission. Evidence has been provided that in potato tuber infected by pathogen *P. infestans* for 3 weeks, the ultra-weak photon emission was found to be considerably enhanced compared to the non-infected potato tuber [70]. In parallel to the enhancement in the ultra-weak photon emission, the formation of HO· was pronouncedly enhanced as monitored using EPR spin-trapping spectroscopy. The correlation between the ultra-weak photon emission and HO· formation in the infected potato tuber confirmed the participation of HO· in the ultra-weak photon emission.

4.4. Involvement of singlet oxygen in ultra-weak photon emission

4.4.1. Spontaneous ultra-weak photon emission

The addition of lipid vesicles with different degree of fatty acid unsaturation to cytochrome c/hydroperoxide system increased the ultra-weak photon emission depending on the degree of fatty acid unsaturation [44]. The author proposed that the addition of $^1O_2$ formed by the decomposition of tetroxide via Russell mechanism to double bonds of unsaturated fatty acid leads to the formation of ROOR. The decomposition of ROOR results in the formation of $^3(R{=}O)^*$. The ultra-weak photon emission from two photosensitive mutants of *Arabidopsis* plant, the *vtc2* single mutant deficient in ascorbate and the *vtc2 npq1* double mutant deficient in both ascorbate and zeaxanthin was considerably higher as compared to wild type [48]. Similarly, the spontaneous ultra-weak photon emission from *vte1 Arabidopsis* plant lacking both tocopherol and plastochromanol was higher than from WT [94]. These observations indicate that $^1O_2$ is involved in the ultra-weak photon emission from *Arabidopsis* plant deficient in ascorbate and zeaxanthin [95,96].

4.4.2. Stress-induced ultra-weak photon emission

4.4.2.1. Physically-induced ultra-weak photon emission. Havaux et al. [48] demonstrated that the level of lipid peroxidation monitored by MDA level determined by HPLC correlates with the level of ultra-weak photon emission. These results indicate that the absence of zeaxanthin in the *vtc2 npq1* double mutant of *Arabidopsis* plant results in the pronounced formation of $^1O_2$ during the high light stress. Based on these considerations, it seems to be likely that $^1O_2$ formed during the high light stress initiates lipid peroxidation known to result in the formation of $^3(R{=}O)^*$ and $^1O_2$. Under high light stress, the ultra-weak photon emission from *vte1* mutant of *Arabidopsis* plant lacking both tocopherol and plastochromanol was higher as compared to WT [94]. Furthermore, the ultra-weak photon emission was found to correlate with MDA accumulation detected by HPLC. These observations lead to the conclusion that both tocopherol and plastochromanol act as efficient $^1O_2$ scavengers and $^1O_2$ play role in ultra-weak photon emission.

4.4.2.2. Chemically-induced ultra-weak photon emission. The chemical generation of $^1O_2$ using photosensitizers such as rose bengal, radachlorin and benzoporphyrin derivatives has been shown under illumination [97,98]. To avoid the contribution of monomol and dimol photon emission of $^1O_2$ formed by the photosensitization reaction to ultra-weak photon emission, the time period between the

photosensitization reaction and the detection of ultra-weak photon emission from biological sample is required. Because of the high reactivity of $^1O_2$, it can be involved in the oxidation of biomolecules in the close vicinity which subsequently leads to the formation of $^1O_2$. Experimental evidence on the direct involvement of $^1O_2$ in ultra-weak photon emission is limited and thus can be an area of extensive research.

4.4.2.3. Biologically-induced ultra-weak photon emission. During the pathogen infection, herbivore and insect attacks, the biological systems are known to respond by the formation of ROS [56,71]. Singlet oxygen has been demonstrated to be a major ROS formed during the pathogen infection which either can act as signaling molecule or can oxidize biomolecules. The oxidation of biomolecules leads to the formation of $^1O_2$. From the experimental point of view, it is difficult to discriminate between $^1O_2$ formation by the signaling or by the oxidation of biomolecules.

5. Role of biomolecule oxidation in ultra-weak photon emission

In order to understand the molecular mechanism of ultra-weak photon emission, it is crucial to determine the target for oxidative damage caused by ROS. The experiments held on the isolated molecules such as lipids, proteins and nucleic acids might provide the necessary information about the reactions ongoing within the biological systems. It has been demonstrated that ultra-weak photon emission originates from lipids, proteins and nucleic acids [90,95,99]. Two main mechanisms responsible for ultra-weak photon emission were proposed comprising either the formation of $^3(R{=}O)^*$ or the formation of $^1O_2$ through Russell mechanism. Based on the current knowledge, it is concluded here that both mechanisms proceed in lipids, proteins and nucleic acids (Fig. 4).

5.1. Role of lipid peroxidation in ultra-weak photon emission

It has been shown that the oxidation of linoleic acid catalyzed by soybean lipoxygenase led to the formation of $^1O_2$ as confirmed by $^1O_2$ monomol photon emission at 1270 nm [65]. The authors proposed that $^1O_2$ is formed by the recombination of ROO· via Russell mechanism. Several lines of evidence have been provided that the decomposition of lipid ROOH by metal ions, peroxynitrite, chloroperoxide and hypochlorous acid is a potential source of $^1O_2$ [2,39,87,100]. In this reaction, the lipid ROOH is proposed to be oxidized to lipid ROO·, the recombination of which results in the formation of highly unstable ROOOOR. The subsequent decomposition of ROOOOR leads to the formation of organic hydroxide, ground carbonyl, and $^1O_2$ (Fig. 4, reaction a). The experimental evidence indicates that $^1O_2$ is generated at a yield close to 10% by the Russell mechanism [101]. The oxidation of various lipids (monogalactosyldiacylglycerol, linoleic acid, β-carotene) either by $^1O_2$ formed by illumination in the presence of methylen blue or HO· formed by Fenton reagent was shown to result in the enhancement in the ultra-weak photon emission [95]. Based on the correlation between the ultra-weak photon emission from linoleic acid and *Arabidopsis* plant both induced by $^1O_2$, the authors proposed that the oxidized lipids are the main source of ultra-weak photon emission in *Arabidopsis* plant. The correlation between the photon emission from the lipid ROOH/horseradish peroxidase and EPR spin-trapping experiments revealed that ROOR is formed through the cyclization of ROO· rather than through the cycloaddition of $^1O_2$ [59].

5.2. Role of protein oxidation in ultra-weak photon emission

Evidence has been provided that the addition of H_2O_2 or t-butyl hydroperoxide to ferricytochrome c results in the ultra-weak photon emission [102,103]. The photon emission observed after the addition of H_2O_2 was ten times higher than after the addition of t-butyl hydroperoxide. The observation that the photon emission was suppressed by the addition of $^1O_2$ quencher (β-carotene) and enhanced by $^1O_2$ enhancer (1,4-diazabicyclo[2,2,2]-octane) revealed that $^1O_2$ participate in the photon emission. The authors proposed that the decomposition of ROOH by metalloproteins forms ROO·, the recombination of which results in the formation of unstable ROOOOR known to decompose to organic hydroxide, ground carbonyl, and $^1O_2$ (Fig. 4, reaction b). More support on the correlation between the protein oxidation and the ultra-weak photon emission was provided by Khabiri et al. [90]. The authors

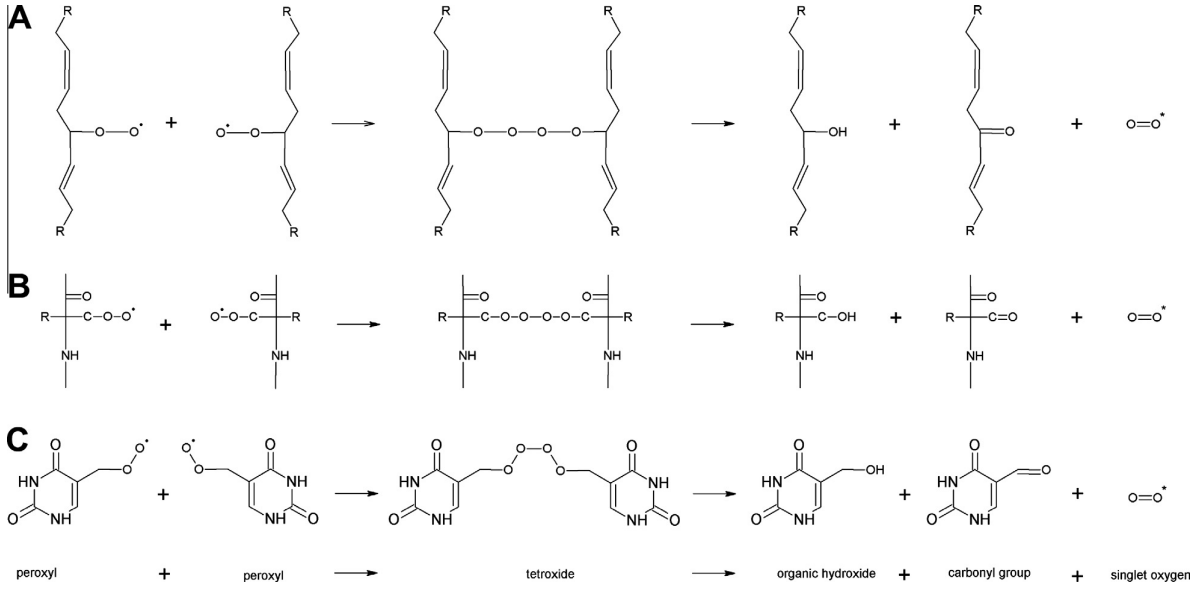

Fig. 4. Formation of singlet oxygen by recombination of two peroxyl radicals in lipids (A), proteins (B) and DNA (C). The recombination of two peroxyl radicals (ROO·) results in the formation of highly unstable tetroxide (ROOOOR). Subsequent decomposition of ROOOOR leads to the formation of organic hydroxide (ROH), ground carbonyl (R=O), and singlet oxygen ($^1O_2$).

reported that increasing concentration of H_2O_2 in an aqueous fetal bovine serum albumin solution induced both the ultra-weak photon emission and the formation of protein carbonyl compound in a concentration-dependent manner. The reaction between the Fenton reagent and the fetal bovine serum resulted in significantly higher photon emission and protein carbonylation compared to the oxidation by H_2O_2. The experimental data observed with the isolated amino acids showed that the addition of H_2O_2 to isolated amino acid induced the photon emission solely from Cys, whereas the addition of HO· formed by the Fenton reagent caused the pronounced photon emission from Phe, Trp, His and Cys. The authors proposed two phases in the ultra-weak photon emission. The first phase of ultra-weak photon emission origins from the reaction of unstable ROO· and RO·, whereas the second phase reflects the decomposition of more stable protein ROOH. The ultra-weak photon emission was observed after the protein oxidation mediated by $^1O_2$ produced after the irradiation of rose bengal [104]. The measurement of several peptides containing Trp, His or Tyr revealed that the photon emission was strongly dependent upon the peptide bonds of the compound. The dipeptide Trp-Ala showed a three magnitude higher photon emission than any other combination of amino acids. The fact that there was no difference in the extent of oxidation of all dipeptides suggests that the structural change of the dipeptide caused by the formation of peptide linkage at the amino group of the Trp moiety leads to the increase of ultra-weak photon emission.

5.3. Role of nucleic acid oxidation in ultra-weak photon emission

The addition of copper/ascorbate/H_2O_2 system to guanine caused a pronounced enhancement in ultra-weak photon emission, whereas other bases such as cytosine, adenine and thymine have no effect on ultra-weak photon. This experimental evidence showed that solely guanine is a source of ultra-weak photon emission [99]. Further, it has been demonstrated that the ultra-weak photon emission is enhanced in the following order: base (guanine) < nucleoside (guanine + ribose or deoxyribose) < nucleotide (guanine + ribose or deoxyribose + phosphate). Based on these observations, it is proposed here that in addition to guanine, the ultra-weak photon emission originates from the oxidation of ribose or deoxyribose. As the oxidation of phosphate unlikely leads to the ultra-weak photon emission, phosphate was suggested to cause a structural change that promotes the oxidation of ribose or deoxyribose. It has been shown that the HO· (sodium benzoate) and $O_2^{\cdot-}$ (SOD) antioxidants have minor effect on the ultra-weak photon emission, whereas the $^1O_2$ antioxidant (sodiumazide) suppressed the photon emission. Based on these observations, it is proposed that the oxidation of deoxyguanosine monophosphate by $^1O_2$ formed via Russell mechanism leads to the formation of ROOR known to decompose to ^{3}(R=O)*. The abstraction of hydrogen from the methyl group of thymine leads to the formation of thymine ROOH by a cascade of reactions. The addition of metal ions (Ce^{4+}, Fe^{2+}, Cu^{2+}) or hypochlorous acid to thymine ROOH resulted in the ultra-weak photon emission. The authors proposed that thymine ROOH undergoes through the oxidation to thymine ROO· in the presence of redox active compounds. The self-recombination of two thymine ROO· forms unstable ROOOOR known to decompose to $^1O_2$ by the Russell mechanism (Fig. 4, reaction c). The suppression of ultra-weak photon emission by $^1O_2$ antioxidant (sodiumazide) provides evidence on the involvement of $^1O_2$ in the photon emission [40]. The ultra-weak photon emission in the blue region of the spectrum has been shown from DNA treated with $^1O_2$ generated by $H_2O_2 + OCL^-$ system. The ultra-weak photon emission was shown to be suppressed by lycopene, β-carotene, vitamin C and vitamin E; however, no effect of mannitol, SOD and NaN_3 on the ultra-weak photon emission was observed [105].

6. Electronically excited species responsible for ultra-weak photon emission

The electronically excited species responsible for ultra-weak photon emission emits photons in the following regions of the spectrum 1) ^{3}(R=O)* in the near UVA and blue–green regions (350–550 nm), singlet and triplet excited pigments in the green–red (550–750 nm) and red-near IR (750–1000 nm) regions, respectively and 3) $^1O_2$ in the red (634 and 703 nm) and near IR (1270 nm) regions (Fig. 5) (Table 1).

6.1. Triplet excited carbonyl

Spectral analysis of the ultra-weak photon emission from the rat perfused lung induced by the addition of exogenous H_2O_2 showed that the photon emission is predominantly in the blue–green region of the spectrum [87]. These observations indicate that the photon emission originates predominantly from ^{3}(R=O)*. Spectral analysis of the t-butyl hydroperoxide-induced ultra-weak photon emission from rats brain and liver homogenate showed the small blue band between 410–440 nm [106]. The author suggested that the photon emission might be due to ^{3}(R=O)* formed by the decomposition of ROOR. The ultra-weak emission from the spinach mitochondria shows that the photon emission is in a wide spectral range from 450 nm to 750 nm [107]. The author proposed that ^{3}(R=O)* contributes to the photon emission from 450 nm to 540 nm. Spectral analysis of the ultra-weak photon emission from the cotyledons of etiolated seedlings of *Cicerarietinum* L. subjected previously to the sudden freezing and thawing shows the photon emission in the spectral range from 390 nm to 440 nm, which correspond to the photon emission due to de-excitation of ^{3}(R=O)* [80]. The ultra-weak photon emission measured after the addition of H_2O_2 and iron sulfate to the hemodialysis plasma cells was found to be predominantly in the blue region of the spectrum with a peak at 430 nm likely attributed to ^{3}(R=O)* [108]. The ultra-weak photon emission from the low-molecular mass fraction obtained by HPLC-gel chromatography showed that the photon emission origins from molecules such as vitamin B_{12} and uric acid. It is proposed here that HO· oxidized low-molecular-mass molecules to form ROOOOR which decomposes to ^{3}(R=O)* as a final emitter. The spectral analysis of the ultra-weak photon emission from

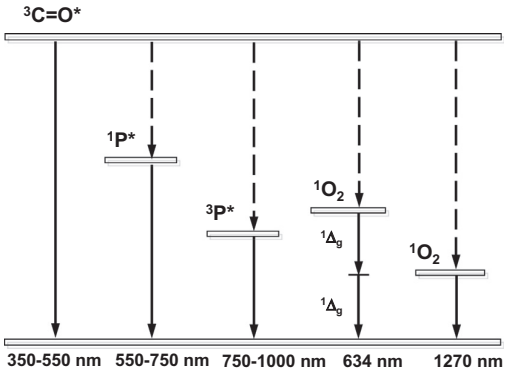

Fig. 5. The electronically excited species responsible for ultra-weak photon emission in the near UVA and blue–green regions (triplet excited carbonyl, ^{3}R=O*), in the green–red (singlet excited pigment, ^{1}P) and near IR (triplet excited pigment and 3) in the red and near IR (singlet oxygen, $^1O_2$) regions of the spectrum. The energy transfer from triplet excited carbonyl to pigments and molecular oxygen results in the formation of $^1P^*$,$^3P^*$ and $^1O_2$, respectively. The solid and dashed arrows represent electronic transition associated with photon emission and the energy transfer from triplet carbonyl to pigments and molecular oxygen, respectively.

Table 1
Spectral ranges of ultra-weak photon emission from various biological systems.

System	Treatment	Spectral range of photon emission [nm]	Refs.
Hemodialysis plasma cells	Fenton reagents	400–500	[108]
Rat brain/liver	Oxygenation	410–450, 475–485, 510–540, 560–580, 625–650	[106]
Cotyledon of etiolated seedlings of *Cicerarietinum*	Freezing and thawing	400–450, 634–703	[80]
Guanine	Phenathroline-Cu/ascorbat/hydrogen peroxide	440–480	[99]
Rice cells	N-acetylchitooligosaccharide	440–580	[83]
Spinach chloroplasts	None	450–750	[76]
DNA	Fenton reagents	455–475, 515–555	[90]
Human skin	UVA, H_2O_2, benzoylperoxide	455–555	[90]
Porcine ex-vivo skin	Hydrogen peroxide, benzoylperoxide	455–555	[90]
Trypthophan	Peroxynitrite	475–575	[113]
Esophageal carcinoma cell line (TE9)	None	500–700	[77]
Trypthophan	Fenton reagents	515–555	[90]
Rat perfused lung	H_2O_2	520–650	[111]
Rat perfused lung	t-Butyl hydroperoxide	650–720	[111]
Green algae *Chlamydomonas reinhardtii*	linoleic acid	680	[91]
Spinach mitochondria	Spontaneous emission	720–730	[107]
Linoleic acid	Soybean lipoxygenaseisozymes	1268	[65]
Ethyl hydroperoxide	Ferricytochrome c, chloroperoxide	1270	[114]
Linoleic acid hydroperoxide	Metal ions, HOCL, ONOO–	1270	[100,115]
Phosphatidylcholinehydroperoxide	HOCL	1270	[100]
Thymine hydroperoxide	Metal ions, HOCL	1270	[40]

DNA treated with copper/ascorbate/H_2O_2 system showed that the photon emission is from 390 to 430 nm [99]. These results showed that ^{3}(R=O)* formed through the ROOR mechanism is responsible for the photon emission. Using a rotating wheel with a set of short wavelength sharp cut-off glass filters, Takeda et al. [77] demonstrated that the ultra-weak photon emission from the esophageal carcinoma cell line (TE9) is attributed to the photon emission of ^{3}(R=O)* at around 450 nm. The spectral analysis of elicitor-induced photon emission showed that photons are emitted in the blue–green region of the spectrum (440–580 nm) [84]. The spectral analysis of ultra-weak photon emission measured after the topical application of H_2O_2 and organic peroxide (benzoyl peroxide) to the porcine ex-vivo skin model shows that the photon emission is predominantly in the blue region of the spectrum [90]. Similarly, the spectral analysis of ultra-weak photon emission measured after the addition of Fenton reagent (H_2O_2 and Fe^{2+}) to pure amino acid showed that Trp and Cys exhibit the comparable photon emission in the broad spectral ranges 420–550 nm, whereas His provides the pronounced photon emission in the spectral range 420–455 nm. When the ultra-weak photon emission was measured simultaneously from His to Trp, the photon emission in the spectral range 420–455 nm was suppressed. Based on this observation, the authors proposed that His transfers excitation energy to Trp.

6.2. Pigments

The ultra-weak photon emission from the singlet excited skin pigments such as melanin and billirubin is in the green region of the spectrum (550–600 nm), whereas the photon emission from the singlet excited chlorophylls is in the red region of the spectrum (670–740 nm) [109]. In spite of the fact that experimental evidence on the participation of triplet skin pigments and triplet chlorophylls in ultra-weak photon emission has not been provided yet, it cannot be rule out that triplet excited pigments contribute to photon emission in near IR region of the spectrum (750-1000 nm). It is proposed here that the short-wavelength photon emission originates predominantly from free chlorophylls, whereas long-wavelength photon emission is likely caused by the contribution of chlorophyll coordinated to the protein matrix or the reabsorption of ultra-weak photon emission by chlorophylls. The triplet–singlet energy transfer from ^{3}(R=O)* to chlorophylls has been proposed to proceed via the direct and the induced reaction pathways. In the direct reaction pathway, the triplet–singlet

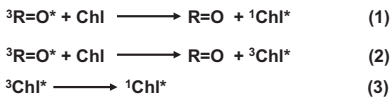

Fig. 6. The energy transfer from triplet excited carbonyl to chlorophyll. The triplet–singlet energy transfer from triplet excited carbonyl ^{3}(R=O)* to chlorophyll (Chl) results in the singlet excited chlorophyll (^{1}Chl*) (reaction 1). The triplet–triplet energy transfer from ^{3}(R=O)* to chlorophylls forms triplet excited chlorophyll (^{3}Chl*) (reaction 2) which further overcomes to ^{1}Chl* by the reverse inter-system crossing (reaction 3).

energy transfer from ^{3}(R=O)* to chlorophylls results in the formation of singlet excited chlorophyll (Fig. 6, reaction 1). Contrary, in the induced reaction pathway, the triplet–triplet energy transfer from ^{3}(R=O)* to chlorophylls brings about the formation of triplet excited chlorophyll (Fig. 6, reaction 2) followed by the overcome to its singlet excited state by the reverse inter-system crossing (Fig. 6, reaction 3). It has been demonstrated that ultra-weak photon emission from spinach leaf and isolated chloroplast predominates in the red region of the spectra with the maximum emission at around 720–730 nm [46]. The authors proposed that the photon emission originates from chlorophylls in the thylakoid membrane. The observation that the addition of sodium azide to the spinach chloroplasts has no effect on the ultra-weak photon emission reveals that the dimol photon emission of $^1O_2$ unlikely contributes to the ultra-weak photon emission in the red region of the spectrum [76]. It has been previously demonstrated that the addition of precursors of ^{3}(R=O)* to chloroplast bring about the photon emission in the red region of the spectrum [110]. Linoleic acid-induced ultra-weak photon emission in Chlamydomonas cells was shown to be predominantly at the red region of the spectrum with the emission maximum at 680 nm [91]. The authors proposed that ^{3}(R=O)* are the likely candidates for the primary excited species formed during the lipid peroxidation, whereas singlet excited chlorophylls formed by the triplet-singlet energy transfer from ^{3}(R=O)* to chlorophylls are the final emitters of photons.

6.3. Singlet oxygen

Ultra-weak photon emission induced by the addition of exogenous organic peroxides (cumenehydroperoxide, ethyl hydroperoxide, t-butyl hydroperoxide) to cytochrome c was observed to be

pronouncedly suppressed by $^1O_2$ scavengers such as b-carotene, histidine and trypthophan [103]. Contrary, the addition of $^1O_2$ dimol photon emission enhancer DABCO to cytochrome c treated with exogenous organic peroxides caused an enhancement in the ultra-weak photon emission. Based on these observations, the authors proposed that the dimol photon emission of $^1O_2$ is responsible for the ultra-weak photon emission. The spectral analysis of photon emission from cytochrome c/H_2O_2 and cytochrome c/t-butyl hydroperoxide systems showed that the maximum photon emission from cytochrome c/H_2O_2 system is at 600–612 nm, whereas the maximum photon emission from cytochrome c/t-butyl hydroperoxide system is shifted toward the longer wavelength range of 662–670 nm [103]. It seems likely that the shift in the photon emission is due to the different contribution of $^1O_2$ dimol photon emission for the 0,0 (634 nm) and 0,1 (703 nm) transitions. The authors proposed that in the cytochrome c/H_2O_2 system $^1O_2$ dimol photon emission at 634 nm is predominated, where in the cytochrome c/t-butyl hydroperoxide system $^1O_2$ dimol photon emission at 703 nm mainly contributes to the overall photon emission in the red region of the spectrum. These proposals have been confirmed by the spectral analysis of the ultra-weak photon emission from rat perfused lung induced by the addition of exogenous organic peroxides such as t-butyl hydroperoxide showing that the photon emission is predominantly in the red region of the spectrum [111]. These observations indicate that the photon emission is predominantly due to the $^1O_2$ dimol photon emission. The correlation between the succinate-induced ultra-weak photon emission in the spinach mitochondria and the formation of $^1O_2$ as detected by the bleaching of p-nitrosodimethylaniline at 440 nm revealed the involvement of $^1O_2$ in the ultra-weak photon emission [107]. Furthermore, the succinate-induced ultra-weak photon emission was suppressed by $^1O_2$ scavenger such as sodium azide and enhanced by the $^1O_2$ dimol photon emission enhancer DABCO. These proposals were confirmed by the observation that the succinate-induced ultra-weak photon emission in the spinach mitochondria appears predominantly in the red region of the spectrum. The observation that the addition of $^1O_2$ scavenger such as 2,5-dimethyl furan suppressed significantly the ultra-weak photon emission from the cotyledons of etiolated seedlings of *Cicerarietinum* L. subjected previously to the sudden freezing and thawing reveals that $^1O_2$ is a major source of the ultra-weak photon emission [80]. The spectral analysis of the ultra-weak photon emission showed that the photon emission band in the range of 620–710 nm corresponds to the spectral range of the dimol photon emission of $^1O_2$. Agatsuma et al. [108] demonstrated that the addition of H_2O_2 to the healthy and hemodialysis plasma cells results in the ultra-weak photon emission predominantly in the red region of the spectrum with a peak at 680 nm. The ultra-weak photon emission from high-molecular-mass fraction obtained by HPLC-gel chromatography showed that the photon emission origins from the proteins such as immunoglobin, albumin, cytochrome and microglobulin. It is proposed here that H_2O_2 oxidizes proteins to form ROOOOR know to decompose to $^1O_2$ as a final emitter. Similarly, the ultra-weak photon emission from the esophageal carcinoma cell line (TE9) at around 580 nm, 634 nm and 703 nm is attributed to $^1O_2$ [77]. The detection of $^1O_2$ monomol photon emission at 1270 nm from lipid or thymine ROOH in the presence of redox active compounds confirmed the role of $^1O_2$ as the emitter of ultra-weak photon emission [40,100].

7. Conclusion

The elucidation of the mechanistic aspects on the formation of electronically-excited species allows us to use ultra-weak photon emission as a diagnostic tool for study of the oxidative metabolic oxidative stress processes in the microbial, plant and animal cells. In this review, experimental evidence supporting the role of ROS in the ultra-weak photon emission is provided. It is proposed here that electronically excited species responsible for ultra-weak photon emission are formed by ROS-induced lipid peroxidation, protein and nucleic acid oxidation. The oxidation of biomolecules is proposed to form high-energy intermediates which are responsible for the formation of electronically excited species. The decomposition of either ROOR or ROOOOR results in the formation of $^3(R{=}O)^*$ known to emit photons in the near UVA and blue–green regions of the spectrum. The triplet-singlet and tiplet-triplet energy transfer from $^3(R{=}O)^*$ to pigments forms singlet and triplet excited pigments which emit photons in the green–red (550–750 nm) and near IR (750–1000 nm) regions of the spectrum, respectively. The triplet-singlet energy transfer from $^3R{=}O^*$ to molecular oxygen results in the formation of $^1O_2$ known to emit photons in the red and near IR regions of the spectrum.

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