Photosynthesis

A New Approach to the Molecular, Cellular, and Organismal Levels

Edited by
Suleyman Allakhverdiev

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Abstract

This book is written by Russian and international authors in the field of photosynthesis research. It is dedicated to investigations of the problems of photosynthesis at different levels of organization: molecular, cellular and organismal. The book describes the multiple roles of various reactive oxygen species in photosynthetic organisms. Further, we have presented here a discussion of the structure and function of water oxidation complex (WOC) of PS II, and a possible role of Mn-bicarbonate complex in WOC. Other important topics in this book are: the structural and functional organization of the pigment-protein complexes, the structure and regulation of chloroplast ATP-synthase, the participation of molecular hydrogen in microalgae metabolism, the current concepts on the evolution and the development of photosynthetic carbon metabolism, and the adaptive changes of photosynthesis at increased CO_2 concentrations, as well as the photosynthetic machinery response to low temperature stress. The material available in this book is a unique report on the state of this trend in modern science. This book will be helpful not only for biophysicists, biochemists and experts in plant physiology, but also for a wider group of biologists; in addition, it is expected to be used in ongoing and future research work in the field. Lastly, and most importantly, it will serve to educate undergraduate, graduate and post-graduate students around the world.

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Irina R. Fomina, Karl Y. Biel

Preface

Existence of life on the Earth is supported by photosynthetic organisms which provide production of organic substances and oxygen evolution. In general, photosynthesis includes primary light reactions and secondary dark reactions. Light reactions begin with absorption of photons by light harvesting photosynthetic pigments, resulting in the formation of their singlet exited states. This process is followed by excitation energy transfer from one pigment molecule to the other. Then, charge separation occurs in the photosynthetic reaction centers. Excited electrons are transferred via the photosynthetic electron transport chain (ETC), providing production of the reducing power in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH). In anoxygenic phototrophs, external hydrogen compounds are a source of the electrons, and the light is absorbed in a single photosystem. The ETC of oxygenic photosynthetics contains two photochemical systems – PS II (water-plastoquinone oxido-reductase) and PS I (plastocyanin-ferredoxin oxido-reductase) – which transfer electrons from water to NADP, using one more complex, the cytochrome-b6f-complex. The source of electrons in this case is water molecules which are decomposed by water-oxidizing complex (WOC) of the PS II. Oxygen as a "waste" product of photosynthetic water cleavage led to the present-day aerobic atmosphere. From the very first moment the interaction with oxygen generated a new condition for the existing organisms starting an evolutionary adaptation process to this new oxydizing environment. Reactive oxygen species (ROS) became a powerful selector and generated a new hierarchy of life forms from the broad range of genetic mutations represented in the biosphere. During the electron transfer from water to NADP, protons are transferred from the stroma side (the positive (p) side) to the lumen side (the negative (n) side), and when this proton gradient is dissipated through the ATP-synthase, ATP is produced.

The next stage includes biochemical processes of fixation and reduction of CO_2 in photosynthetic carbon metabolism with using NADPH, and ATP. To date, the known metabolic pathways of carbon in photosynthesis can be classified into the 3-hydroxypropionat bicycle; the reductive citrate cycle, i.e., the Arnon-Buchanan cycle; C_3 or the reductive pentose phosphate cycle, i.e., the Benson-Bassham-Calvin cycle; C_4 or cooperative photosynthesis; Crassulacean acid metabolism (CAM); C_3/C_4 photosynthesis; and C_4-CAM photosynthesis. Some of them, for example the 3-hydroxypropionat bicycle and the Arnon-Buchanan cycle, are specific to anoxygenic phototrophs, others, such as C_4, CAM, and so on, have been in the

evolution of higher plants. The most important way of carbon in photosynthesis – the Benson-Bassham-Calvin cycle – is widespread in phototrophic organisms of different taxa. Eventually, fixation and reduction of CO_2 during photosynthesis leads to the formation of sugars and other organic compounds.

The present book has 8 chapters written mainly by the researchers of the Institute of Basic Biological Problems of the Russian Academy of Sciences (formerly the Institute of Photosynthesis). The each chapter describes photosynthesis at different levels of organization: molecular, cellular, and organismic. Among discussed problems in this book are: the structural and functional organization of the pigment-protein complexes; the evolutionary origin of the water-oxidizing complex of PS II; the hydrogen photoproduction coupled with photosynthesis; the structure and regulation of chloroplast ATP synthase; the formation, decay and signaling of reactive oxygen species in oxygen-evolving photosynthetic organisms during exposure to oxidative stress; the strategy of adaptation of photosynthetic carbon metabolism; the adaptive changes of photosynthesis under enhanced CO_2 concentration, and the photosynthetic machinery response to low temperature stress.

The material presented here reflects, mainly, the research interests and views of the authors. We do not claim to have produced all-inclusive views of the entire field. The book is intended for a broad range of researchers and students, and all who are interested in learning the most important global process on our planet – the process of photosynthesis.

We should like to believe that this book will stimulate future researchers of photosynthesis, leading to progress in our understanding of the mechanisms of photosynthesis and in its practical use in biotechnology and human life.

We express our sincere appreciation to the 17 authors for their outstanding contribution to this book. We are extremely grateful to Academician of the Russian Academy of Sciences (RAS) V.A. Shuvalov, Academician of the Azerbaijan National Academy of Sciences J.A. Aliyev, Corresponding Member of RAS A.B. Rubin, Corresponding Member of RAS Vl.V. Kuznetsov, and Professors D.A. Los, A.M. Nosov, V.Z. Paschenko, T.E. Krendeleva, A.N. Tikhonov, V.V. Klimov, A.A. Tsygankov, Dr. I.R. Fomina, and J. Karakeyan for their permanent help and fruitful advices.

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The Multiple Roles of Various Reactive Oxygen Species (ROS) in Photosynthetic Organisms[1]

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Abstract

This chapter provides an overview on recent developments and current knowledge about monitoring, generation and the functional role of reactive oxygen species (ROS) – H_2O_2, $HO_2^{\bullet}$, $HO^{\bullet}$, OH^-, $^1O_2$ and $O_2^{-\bullet}$ – in both oxidative degradation and

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signal transduction in photosynthetic organisms including a summary of important mechanisms of nonphotochemical quenching in plants. We further describe microscopic techniques for ROS detection and controlled generation. Reaction schemes elucidating formation, decay and signaling of ROS in cyanobacteria as well as from chloroplasts to the nuclear genome in eukaryotes during exposure of oxygen-evolving photosynthetic organisms to oxidative stress are discussed that target the rapidly growing field of regulatory effects of ROS on nuclear gene expression.

Keywords: photosynthesis, plant cells, reactive oxygen species, ROS, oxidative stress, signaling systems, chloroplast, cyanobacteria, nonphotochemical quenching, chromophore-activated laser inactivation, sensors

1.1   Introduction

About 3 billion years ago the atmosphere started to transform from a reducing to an oxidizing environment as evolution developed oxygenic photosynthesis as key mechanism to efficiently generate free energy from solar radiation (Buick, 1992; Des Marais, 2000; Xiong and Bauer, 2002; Renger, 2008; Rutherford *et al.*, 2012; Schmitt *et al.*, 2014a). Entropy generation due to the absorption of solar radiation on the surface of the Earth was retarded by the generation of photosynthesis, and eventually a huge amount of photosynthetic and other organisms with rising complexity developed at the interface of the transformation of low entropic solar radiation to heat. The subsequent release of oxygen as a "waste" product of photosynthetic water cleavage led to the present-day aerobic atmosphere (Kasting and Siefert, 2002; Lane, 2002; Bekker *et al.*, 2004), thus opening the road for a much more efficient exploitation of the Gibbs free energy through the aerobic respiration of heterotrophic organisms (for thermodynamic considerations, see (Nicholls and Ferguson, 2013; Renger, 1983).

From the very first moment this interaction with oxygen generated a new condition for the existing organisms starting an evolutionary adaptation process to this new oxydizing environment. Reactive oxygen species (ROS) became a powerful selector and generated a new hierarchy of life forms from the broad range of genetic mutations represented in the biosphere. We assume that this process accelerated the development of higher, mainly heterotrophic organisms in the sea and especially on the land mass remarkably.

The efficient generation of biomass and the highly selective impact of ROS lead to a broad range of options for complex organisms to be

developed in the oxydizing environment. The direct, mostly deleterious impact of ROS on the biosphere is thereby just a minor facet in the broad spectrum of consequences. Important and more complex side effects are for example given by the fact that the molecular oxygen led to generation of the stratospheric ozone layer, which is the indispensable protective shield against deleterious UV-B radiation (Worrest and Caldwell, 1986). ROS led to new complex constraints for evolution that drove the biosphere into new directions – by direct oxidative pressure and by long-range effects due to environmental changes caused by the atmosphere and the biosphere themselves as energy source for all heterotrophic organisms.

For organisms that had developed before the transformation of the atmosphere the pathway of redox chemistry between water and O_2 by oxygenic photosynthesis was harmful, due to the deleterious effects of ROS. O_2 destroys the sensitive constituents (proteins, lipids) of living matter. As a consequence, the vast majority of these species was driven into extinction, while only a minority could survive by finding anaerobic ecological niches. All organisms developed suitable defense strategies, in particular the cyanobacteria, which were the first photosynthetic cells evolving oxygen (Zamaraev and Parmon, 1980).

The ground state of the most molecules including biological materials (proteins, lipids, carbohydrates) has a closed electron shell with singlet spin configuration. These spin state properties are of paramount importance, because the transition state of the two electron oxidation of a molecule in the singlet state by $^3\Sigma_g^- O_2$ is "spin-forbidden" and, therefore, the reaction is very slow. This also accounts for the back reaction from the singlet to the triplet state.

In contrast to this majority of singlet ground state molecules the electronic configuration of the O_2 molecule in its ground state is characterized by a triplet spin multiplicity described by the term symbol $^3\Sigma_g^- O_2$. This situation drastically changes by two types of reactions which transform $^3\Sigma_g^- O_2$ into highly reactive oxygen species (ROS): i) Electronic excitation leads to population of two forms of singlet O_2 characterized by the term symbols $^1\Delta_g$ and $^1\Sigma_g^+$. The $^1\Sigma_g^+$ state with slightly higher energy rapidly relaxes into $^1\Delta_g O_2$ so that only the latter species is of physiological relevance (type I). ii) Chemical reduction of $^3\Sigma_g^- O_2$ (or $^1\Delta_g O_2$) by radicals with non-integer spin state (often doublet state) leads to formation of $O_2^{\cdot-}$, which quickly reacts to $HO_2^{\cdot}$ and is subsequently transferred to H_2O_2 and $HO^{\cdot}$ (*vide infra*) (type II). In plants, the electronic excitation of $^3\Sigma_g^- O_2$ occurs due to close contact to chlorophyll triplets that are produced during the photoexcitation cycle (Schmitt *et al.*, 2014a) (see Figure 1.1, Figure 1.2). Singlet oxygen is
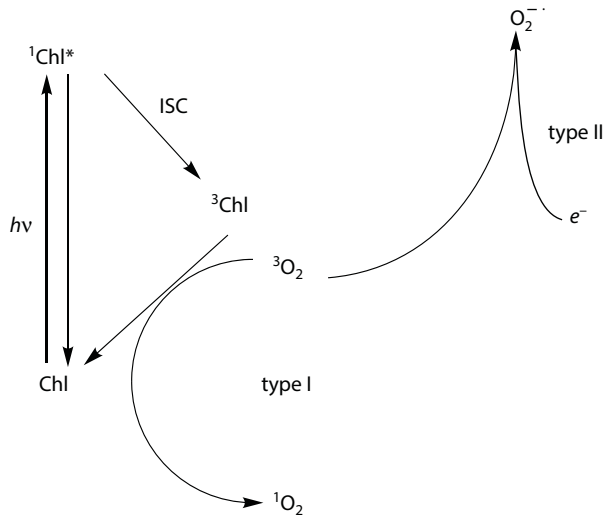
Figure 1.1 Production of ROS by interaction of oxygen with chlorophyll triplet states (type I) to $^1O_2$ or chemical reduction of oxygen to $O_2^{\cdot-}$ (type II)
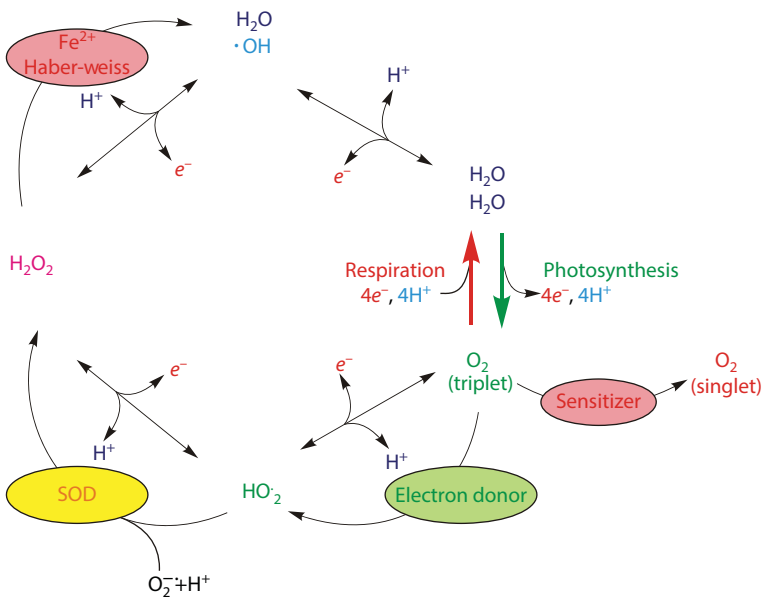
Figure 1.2 Scheme of ROS formation and water redox chemistry (water-water cycle, for details, see text)

predominantly formed via the reaction sensitized by interaction between a chlorophyll triplet (^{3}Chl) and ground state triplet $^3\Sigma_g^- O_2$:

$$^3Chl + {}^3\Sigma_g^- O_2 \rightarrow {}^1Chl + {}^1\Delta_g O_2 \tag{1}$$

^{3}Chl can be populated either via intersystem crossing (ISC) of antenna Chls or via radical pair recombination in the reaction centers (RCs) of photosystem II (PS II) (for reviews, see Renger, 2008; Vass and Aro, 2008; Rutherford *et al.*, 2012; Schmitt *et al.*, 2014a). Alternatively, $^1\Delta_g O_2$ can also be formed in a controlled fashion by chemical reactions, which play an essential role in programmed cell death upon pathogenic infections (e.g. by viruses).

Figure 1.2 schematically illustrates the pattern of one-electron redox steps of oxygen forming the ROS species HO$^\bullet$, H_2O_2 and $HO_2{}^\bullet/O_2{}^{\bullet-}$ in a four-step reaction sequence with water as the final product. The sequence comprises the water splitting, leading from water to $O_2 + 4H^+$ and the corresponding mechanism *vice versa* of the ROS reaction sequence. The production of $^1\Delta_g O_2$ is a mechanism next to that.

In biological organisms, the four-step reaction sequence of ROS is tamed and energetically tuned at transition metal centers, which are encapsulated in specifically functionalized protein matrices. This mode of catalysis of the "hot water redox chemistry" avoids the formation of ROS. In photosynthesis, the highly endergonic oxidative water splitting ($\Delta G^\circ = + 237.13$ kJ/mol, see Atkins, 2014) is catalyzed by a unique Mn_4O_5Ca cluster of the water-oxidizing complex (WOC) of photosystem II and energetically driven by the strongly oxidizing cation radical P680$^{+\bullet}$ (Klimov *et al.*, 1978; Rappaport *et al.*, 2002) formed via light-induced charge separation (for review, see Renger, 2012).

Correspondingly, the highly exergonic process in the reverse direction is catalyzed by a binuclear heme iron-copper center of the cytochrome oxidase (COX), and the free energy is transformed into a transmembrane electrochemical potential difference for protons (for a review, see Renger, 2011), which provides the driving force for ATP synthesis (for a review, see Junge, 2008). In spite of the highly controlled reaction sequences in photo-synthetic WOC and respiratory COX, the formation of ROS in living cells cannot be completely avoided. The excess of ROS under unfavorable stress conditions causes a shift in the balance of oxidants/antioxidants towards oxidants, which leads to the intracellular oxidative stress (Kreslavski *et al.*, 2012b). Formation of ROS (the production rate) as well as decay of ROS (the decay rate) with the latter one determining the lifetime, both bring about the concentration distribution of the ROS pool (Kreslavski *et al.*, 2013a). The activity of antioxidant enzymes, superoxide dismutase (SOD), catalase, peroxidases, and several others, as well as the content of low molecular weight antioxidants, such as ascorbic acid, glutathione,

tocopherols, carotenoids, anthocyanins, play a key role in regulation of the level of ROS and products of lipid peroxidation (LP) in cells (Apel and Hirt, 2004; Pradedova *et al.*, 2011; Kreslavski *et al.*, 2012b).

The exact mechanisms of neutralization and the distribution of ROS have not been clarified so far. Especially the involvement of organelles, cells and up to the whole organism, summarizing the complicated network of ROS signaling (see chap. 6 and 7) are still far from being completely understood (Swanson and Gilroy, 2010; Kreslavski *et al.*, 2012b, 2013a).

Photosynthetic organisms growing under variable environmental conditions are often exposed to different types of stress like harmful irradiation (UV-B or high-intensity visible light), heat, cold, high salt concentration and also infection of the organisms with pathogens (viruses, bacteria) (Gruissem *et al.*, 2012). Under these circumstances, the balance between oxidants and antioxidants within the cells is disturbed. This imbalance leads to enhanced population of ROS including singlet oxygen ($^1\Delta_g O_2$), superoxide radicals ($O_2^{-\bullet}$ or $HO_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$). Other highly reactive oxygen species like atomic oxygen or ozone are either not formed or play a role only under very special physiological conditions and will not be considered here. In this sense, the term ROS is used in a restricted manner. In addition to ROS, also reactive nitrogen- and sulfur-based species play an essential role in oxidative stress (OS) developed within the cells (Fryer *et al.*, 2002; Benson, 2002). However, this interesting subject is beyond the scope of this chapter.

It is obvious that ROS exert deleterious effects. Oxidative destruction by ROS is known and has been studied for decades. However, ROS also act as important signaling molecules with regulatory functions, which have been unraveled only recently. ROS were found to play a key role in the transduction of intracellular signals and in control of gene expression and activity of antioxidant systems (Desikan *et al.*, 2001; Desikan *et al.*, 2003; Apel and Hirt, 2004; Mori and Schroeder, 2004; Galvez-Valdivieso and Mullineaux, 2010; Foyer and Shigeoka, 2011). Being implicated in reactions against pathogens, (e.g. by respiratory bursts) and by the active participation in signaling, ROS have a protective role in plants (Bolwell *et al.*, 2002; Dimitriev, 2003).

ROS contribute to acclimation and protection of plants, regulate processes of polar growth, stromatal activity, light-induced chloroplast movements, and plant responses to biotic and abiotic environmental factors (Mullineaux *et al.*, 2006; Pitzschke and Hirt, 2006; Miller *et al.*, 2007; Swanson and Gilroy, 2010; Vellosillo, 2010). In animals, recent studies have established that physiological H_2O_2 signaling is essential for stem cell

proliferation, as illustrated in neural stem cell models, where it can also influence subsequent neurogenesis (Dickinson and Chang, 2011). This chapter will describe generation and decay of ROS and their monitoring in cells including novel microscopic techniques. Additionally the rapidly growing field of regulatory effects and pathways of ROS will be described although a complete description of the multitude of roles of ROS from nonphotochemical quenching (NPQ) to genetic signaling is impossible. However, this chapter provides an overview about the existing knowledge aiming to include the most important original literature and reviews. The book chapter is based on the review of (Schmitt *et al.*, 2014a); however, it is significantly broadened to cover the fields that were not mentioned in (Schmitt *et al.*, 2014a).

1.2   Generation, Decay and Deleterious Action of ROS

The interaction between chlorophyll triplets (^{3}Chl) and triplet ground state of molecular oxygen ($^3\Sigma_g^- O_2$): ^{3}Chl $+ ^3\Sigma_g^- O_2 \rightarrow ^1$Chl $+ ^1D_g O_2$ is the predominant reaction forming singlet oxygen ($^1\Delta_g O_2$) in photosynthetic organisms (see Figure 1.1). ^{3}Chl is populated either via intersystem crossing (ISC) of antenna Chls or via radical pair recombination in the reaction centers of photosystem II (PS II) (for reviews, see Renger, 2008; Rutherford *et al.*, 2012). Alternatively, ROS can also be formed by direct reduction of oxygen, most probably at PS I and by controlled chemical reactions, which play an essential role in programmed cell death upon pathogenic (e.g. viral) infections. The general water-water cycle which is mostly responsible for the subsequent formation of $O_2^{-\bullet}$ or $HO_2^\bullet$, H_2O_2 and $HO^\bullet$ is shown in Figure 1.2.

Under optimal conditions, only small amounts of ROS are generated in different cell compartments. However, exposure to stress can lead to a drastic increase of ROS production and sometimes to inhibition of cell defense systems (Desikan *et al.*, 2001; Nishimura and Dangl, 2010). As a consequence of unfavorable conditions, oxidative stress is developed due to the generation of ROS via both the sensitized $^1\Delta_g O_2$ formation and the reductive pathways leading to production of $O_2^{-\bullet}$, H_2O_2 and $HO^\bullet$ radicals (see Figure 1.2).

Rapid transient ROS generation can be observed and is called "oxidative burst" (Bolwell *et al.*, 2002). In this case, a high ROS content is attained within time periods from several minutes up to hours. Oxidative bursts occur during many plant cell processes, especially photosynthesis, dark respiration and photorespiration.

Studies using advanced imaging techniques, e.g. a luciferase reporter gene expressed under the control of a rapid ROS response promoter in plants (Miller *et al.*, 2009), or a new H_2O_2/redox state-GFP sensor in zebrafish (Niethammer *et al.*, 2009; see chapter 4, *"Monitoring of ROS"*), revealed that the initial ROS burst triggers a cascade of cell-to-cell communication events that result in formation of a ROS wave. This wave is able to propagate throughout different tissues, thereby carrying the signal over long distances (Mittler *et al.*, 2011). Recently, the auto-propagating nature of the ROS wave was experimentally demonstrated. Miller *et al.* (2009) showed by local application of catalase or an NADPH oxidase inhibitor that a ROS wave triggered by different stimuli can be blocked at distances of up to 5-8 cm from the site of signal origin. The signal requires the presence of the NADPH oxidase (the product of the *RbohD* gene) and spreads throughout the plant in both the upper and lower directions.

The lifetime of $^1\Delta_g O_2$ in aqueous solution is about 3.5 ms (Egorov *et al.*, 1989). On the other hand, the lifetime is significantly shortened in cells due to the high reactivity of $^1\Delta_g O_2$, which rapidly attacks all relevant biomolecules (pigments, proteins, lipids, DNA), thus leading to serious deleterious effects. Values in the order of 200 ns were reported for $^1\Delta_g O_2$ in cells (Gorman and Rogers, 1992) so that the species can diffuse up to 10 nm under physiological conditions (Sies and Menck, 1992), thus permitting penetration through membranes (Schmitt *et al.*, 2014a). Distances up to 25 nm have been reported (Moan, 1990) suggesting that $^1\Delta_g O_2$ can permeate through the cell wall of *E. coli*. The singlet oxygen chemistry significantly depends on the environment, solvent conditions and the temperature (Ogilby and Foote, 1983). Higher values of up to 14 µs lifetime and 400 nm diffusion distance in lipid membranes suggest that $^1\Delta_g O_2$ can indeed diffuse across membranes of cell organelles and cell walls (Baier *et al.*, 2005). But as most proteins are prominent targets (Davies, 2003) with reaction rate constants in the range of 10^8-10^9 $M^{-1}s^{-1}$ the potential of $^1\Delta_g O_2$ to work directly as a messanger is rather limited (Wilkinson *et al.*, 1995). Among the canonical amino acids, only five (Tyr, His, Trp, Met and Cys) are primarily attacked by a chemical reaction with $^1\Delta_g O_2$, from which Trp is unique by additionally exhibiting a significant physical deactivation channel that leads to the ground state $^3\Sigma_g^- O_2$ in a similar way as by quenching with carotenoids. The reaction of $^1\Delta_g O_2$ with Trp primarily leads to the formation of peroxides, which are subsequently degraded into different stable products. One of these species is N-formylkynurenine (Gracanin *et al.*, 2009). This compound exhibits optical and Raman spectroscopic characteristics that might be useful for the identification of ROS generation sites (Kasson and Barry, 2012). The reactivity of Trp in proteins was shown

to markedly depend on the local environment of the target (Jensen *et al.*, 2012). Detailed mass spectrometric studies revealed that a large number of oxidative modifications of amino acids are caused by ROS and reactive nitrogen species (Galetskiy *et al.*, 2011).

The wealth of studies on damage of the photosynthetic apparatus (PA) by $^1\Delta_g O_2$ under light stress and repair mechanisms is described in several reviews and book chapters on photoinhibition (Adir *et al.*, 2003; Allakhverdiev and Murata, 2004; Nishiyama *et al.*, 2006; Murata *et al.*, 2007; Vass and Aro, 2008; Li *et al.*, 2009, 2012; Goh *et al.*, 2012, Allahverdiyeva and Aro, 2012). Such high reactivity leads to an extensive oxidation of fundamental structures of PS II where oxygen is formed in the water-oxidizing complex. $^1\Delta_g O_2$ is directly involved in the direct damage of PS II (Mishra *et al.*, 1994; Hideg *et al.*, 2007; Triantaphylidès *et al.*, 2008, 2009; Vass and Cser, 2009), destroying predominantly the D1 protein, which plays a central role in the primary processes of charge separation and stabilization in PS II. The resulting photoinhibition of PS II (Nixon *et al.*, 2010) leads to dysfunction of D1 and high turnover rates during the so-called D1-repair cycle. D1 by far exhibits the highest turnover rate of all thylakoid proteins and underlies complex regulatory mechanisms (Loll *et al.*, 2008).

Carotenoids play a pivotal role in ^{3}Chl suppression and quenching (Frank *et al.*, 1993; Pogson *et al.*, 2005). In addition, NPQ developed under light stress also reduces the population of ^{3}Chl in antenna systems as well as PS II of plants (Ruban *et al.*, 1994; Härtel *et al.*, 1996; Carbonera *et al.*, 2012) (see chapter 3). The interaction between $^1\Delta_g O_2$ and singlet ground state carotenoids does not only lead to photophysical quenching, but also to oxidation of carotenoids by formation of species that can act as signal molecules for stress response (Ramel *et al.*, 2012). Likewise, lipid (hydro)peroxides generated upon oxidation of polyunsaturated fatty acids by $^1\Delta_g O_2$ can act as triggers to initiate signal pathways, and propagation of cellular damage (Galvez-Valdivieso and Mullineaux, 2010; Triantaphylides and Havaux, 2009). Detailed studies of the damage of the PA by $^1\Delta_g O_2$ are additionally found in (Allakhverdiev and Murata, 2004; Nishiyama *et al.*, 2006; Wakao *et al.*, 2009; Allahverdiyeva and Aro, 2012; Goh *et al.*, 2012; Li *et al.*, 2012).

Among all ROS, the $O_2^{-\bullet}/H_2O_2$ system is one of the key elements in cell signaling and other plant functions (see Figure 1.1). $O_2^{-\bullet}$ and H_2O_2 are assumed to initiate reaction cascades for the generation of "secondary" ROS as necessary for long-distance signaling from the chloroplasts to or between other cell organelles (Baier and Dietz, 2005; Sharma *et al.*, 2012; Bhattacharjee, 2012).

The initial step in formation of redox intermediates of the H_2O_2/O_2 system in all cells is the one-electron reduction of O_2 to $O_2^{-\bullet}$ (see Figure 1.2). $O_2^{-\bullet}$

and H_2O_2 are mainly formed in chloroplasts, peroxisomes, mitochondria and cell walls (Bhattacharjee, 2012). Enzymatic sources of $O_2^{\bullet-}$ / H_2O_2 generation have been identified such as cell wall-bound peroxidases, aminooxidases, flavin-containing oxidases, oxalate and plasma membrane NADPH oxidases (Bolwell et al., 2002; Mori and Schroeder, 2004; Svedruzic et al., 2005). In particular, sources of ROS in the apoplast are oxidases bound to the cell wall, peroxidases, and polyamino oxidases (Minibayeva et al., 1998, 2009b).

The major source of $O_2^{\bullet-}/H_2O_2$ production in chloroplasts is the acceptor side of photosystem I (PS I) (Asada, 1999, 2006). The exact mechanism of O_2 reduction is still a matter of discussion. It was assumed that O_2 mainly is reduced by transfer of electrons from reduced ferredoxin (Fd) to O_2 via ferredoxin-thioredoxin reductase (Gechev et al., 2006) although this assumption was challenged since a long time (Asada et al., 1974; Goldbeck and Radmer, 1984). New findings showed that reduced Fd was only capable of low rates of O_2 reduction in the presence of $NADP^+$ with contribution to the total O_2 reduction not exceeding 10% (Kozuleva and Ivanov, 2010; Kozuleva et al., 2014). NADPH oxidase (NOX) is considered to be involved into ROS production both in animal and plant cells (Sagi and Fluhr, 2001, 2006) according to the reaction $NADPH + 2\ O_2 \rightarrow NADPH^+ + 2\ O_2^{\bullet-} + H^+$.

Under conditions of limited NADPH consumption due to impaired CO_2 fixation rates via the Calvin-Benson cycle in photosynthetic organisms, some components of the electron transport chain (ETC) will stay reduced and can perform $^3\Sigma_g O_2$ reduction to $O_2^{\bullet-}$. It is suggested that H_2O_2 formation takes place in the plastoquinone (PQ) pool, but with a low rate (Ivanov et al., 2007), studies on mutants of Synechocystis sp. PCC 6803 lacking phylloquinone (menB mutant) show the involvement of phylloquinone in O_2 reduction (Kozuleva et al., 2014).

Recent literature suggests very short lifetimes for $O_2^{\bullet-}$ radicals in the μs regime (1 μs half-life is published in (Sharma et al., 2012), while 2-4 ms are found in (Gechev et al., 2006) - which is about one order of magnitude longer than that of $^1\Delta_g O_2$ (vide supra). $O_2^{\bullet-}$ radicals are rapidly transformed into H_2O_2 via the one-electron steps of the dismutation reaction catalyzed by the membrane-bound Cu/Zn-superoxide dismutase (SOD) (see Figure 1.2) (Asada, 1999, 2006).

Three forms of SODs exist in plants containing different metal centers, such as manganese (Mn-SOD), iron (Fe-SOD), and copper-zinc (Cu/Zn-SOD) (Bowler et al., 1992; Alscher et al., 2002), from which Cu/Zn-SOD is the dominant form. The non-enzymatic $O_2^{\bullet-}$ dismutation reaction is very slow (Foyer and Noctor, 2009; Foyer and Shigeoka, 2011). Earlier literature