Preface

In this book, the authors present topical research in the study of the biochemical characteristics, functions and potential applications of peroxidases. Topics discussed include the unique properties and biotechnological potential of ligninolytic peroxidases; the functions and regulation during pathogenesis of plant peroxidases; phospholipids and peroxidases in mitochondrial apoptosis; glutathione peroxidases in stem, cancer, and cancer stem cells; biochemical responses of aquatic macrophytes to two abiotic stresses; role of ascorbate peroxidase in antioxidant protection; and the route, dose and duration of exposure to cadmium-relevance to oxidative stress induction.

Chapter I – Ligninolytic fungi (white rot and litter-decomposing fungi) are a physiological rather than taxonomic group connecting fungal strains which are capable of extensively degrading lignin. The unique physiological and biochemical properties permit these fungi to transform and mineralize of wide range of organopollutants with structural similarities to lignin. They produce a complex of extracellular oxidative enzymes, including lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase, which are involved in degradative processes. This enzyme complex is produced by all ligninolytic fungi but its pattern is dependent on fungal species and cultivation conditions. Lignin peroxidase catalyzes oxidative cleavage of C-C bonds and ether (C-O-C) bonds in non-phenolic aromatic substrates with high redox potential. Manganese peroxidase oxidizes Mn^{2+} to Mn^{3+}, which facilitates the degradation of phenolic compounds or, in turn, oxidizes a second mediator for the breakdown of non-phenolic compounds. Versatile peroxidase is hybrid of lignin peroxidase and manganese peroxidase combining the catalytic possibilities of those two peroxidases. This review presents the most important molecular and catalytic properties of ligninolytic peroxidases including molecular structure, substrate specificity, optimal pH values and temperature, influence of effectors, catalytic activity towards lignin and xenobiotics. The features of catalytic mechanisms of these enzymes are comparing. The possible functions of ligninolytic peroxidases and supposed dependence the fungal degrading properties on pattern and properties to produce various peroxidases are discussed. Finally the potential of biotechnological use of these enzymes is under consideration.

Chapter II – Peroxidase (donor: H_2O_2oxidoreductase, EC 1.11.1.7) (PO) belongs to a class of widespread and vital enzymes. One of its specific features is the multiplicity of its molecular isoforms exhibiting various functions], and the highly variable genes encoding them are controlled by various *cis*-elements and *trans*-factors that are responsible for their differentiated expression activity depending on environmental conditions and stages of

ontogenesis. POs are localizing in cytoplasm, plasma membrane or secrete outside of cell. In cell wall POs presence in the ionic-bound or covalent bound fraction of proteins, and even freely circulated in apoplast. POs are key enzymes of lignification capable of binding with polysaccharides of plant and (or) pathogens. An important physiological sense of these reactions is the strengthening of the cell wall and construction on the pathogens' paths of physical barriers consisting of lignin- and suberin-containing polymers, which also possess high toxicity. This defense reaction is developing more or less automatically, thus these barriers are formed just in the zone of pathogen penetration and concurrently with the beginning of the active pathogen expansion into the tissues of the host plant. However, mechanisms of development of these events are still unclear. Expression of some genes encoding different isoPOs are regulated by pathogens (and their metabolites), elicitors, and stress-related hormones and hormone-like compounds, such as salicylic and jasmonic acids, ethylene and others.

Chapter III – Phospholipids constitute an essential part of cellular membranes, in particular, inner and outer membranes of mitochondria. They influence the function of mitochondrial proteins and cytosolic proteins associates with mitochondria. Phospholipids regulate the assembly and maintenance of the proteins embedded in the membranes, they modulate enzymes' ox-red properties, electron-transfer properties, kinase activity, and peroxidase activity. Phospholipids thus play a crucial role in all cellular processes such as protein biogenesis, protein import into mitochondria, energy production, and apoptosis. Over the last decade, a significant amount of research efforts has been devoted to mitochondrial apoptosis, in particular, early stages of apoptosis that precede mitochondrial permeability transition pore (PTP) opening and the release of proapoptotic factors into the cytoplasm, specifically cytochrome c (cyt c). Cyt c is a soluble protein located in the intermembrane space of mitochondria whose primary function is the electron shuttle between complexes III and IV of the respiratory chain. Cyt c is attached to the inner mitochondrial membrane (IMM) through a phospholipid molecule, cardiolipin (CL). It was demonstrated that cyt c, whose peroxidase function is negligible in a free state, exhibits a significant peroxidase activity upon binding to CL. This cyt c-CL complex catalyzes the peroxidation of CL. Peroxidized CL stimulates the formation of the permeability pore in the membranes and the release of cyt c into the cytoplasm through these pores. The increase of the peroxidase activity of cyt c in the CL-bound state is thus considered to be a prerequisite for apoptosis. Cells have also developed anti-apoptotic mechanisms. One of these anti-apoptotic functions is executed by a mitochondrial phospholipid hydroperoxide glutathione peroxidase or GPx4, a protein that defends cells from oxidative injury, attenuates cardiac disfunction, and participates in sperm development. GPx4 plays an essential role in preventing cyt c release from mitochondria. Specifically, it reduces oxidized phospholipids precluding cyt c detachment from CL and consequently suppressing apoptosis. The authors will describe the structure of cyt c and GPx4 as well as the catalytic cycles of the two peroxidases. The authors will highlight the recent advances and unresolved issues regarding biochemical mechanisms underlying cyt c-CL interactions. The authors will discuss the structural characteristics of cyt c-CL interactions responsible for the tight binding, unfolding of cyt c, and the enhancement of its peroxidase activity. The authors will also discuss how peroxidized CL triggers PTP opening and how the balance between the two peroxidases, CL-bound cyt c and GPx4, affects the early stages of mitochondrial apoptosis.

Chapter IV – Oxidative stress, as a disturbance in the equilibrium between free radicals (FR), reactive oxygen species (ROS), and endogenous antioxidant defense mechanisms is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Mounting evidence has implicated that oxidative stress is involved in various physiological and pathological processes, including DNA damage, proliferation, cell adhesion, and survival of cancer cells. Glutathione peroxidases (GPxs) (EC 1.11.1.9), which can reduce lipid hydroperoxides to their corresponding alcohols and free hydrogen to water, are enzyme family with peroxidase activity whose main biological roles are to protect the organism from oxidative damage. So far 8 sub-members of GPxs have been identified in humans, i.e. GPx 1–8. They all can react with H_2O_2 and soluble fatty acid hydroperoxides. A large number of publications have demonstrated that GPxs have significant roles in different stages of carcinogenesis. In this book chapter, the authors will review recent study progress of GPxs in stem, cancer and cancer stem cells. This knowledge will shed light on mechanisms that lead to better modes of cancer treatment.

Chapter V – Exposure to stress can lead to the disruption of cellular, molecular and physiological processes in plants. Plants generate ROS to cope with different forms of abiotic stresses. However, if a plant is exposed to stress conditions for a considerable period, the excess generation of ROS affects its radical scavenging ability in the long run. Thus the effects of environmental variability of a plant are reflected in its growth, reproduction and colonization. Aquatic plants are considered unchangeable biological filters and play an important role in the proper functioning of aquatic ecosystem. They often encounter abiotic stress of varying magnitudes and acquire with different adaptive characters. In this chapter, the authors will be focusing on the oxidative responses of aquatic macrophytes to various abiotic stresses with a special concern on flow velocity and redox states.

Chapter VI – The change in activities and numerous molecular forms of antioxidant enzymes is one of the parameters of plant biological tolerance against constantly changing environmental conditions. A group of genes involved in the synthesis of ascorbate is used in genetic engineering for the improvement of plant tolerance to biotic and abiotic stresses. The level of H_2O_2 in plant cells is controlled by two enzymes: catalase and ascorbate peroxidase (APX), though the key role in decomposition of H_2O_2 belongs to APX. Ascorbate peroxidase converts H_2O_2 into H_2O and O_2. The APX activity and electrophoresis spectra of its isoenzyme content have been investigated in plants subjected to abiotic (soil drought) and biotic (virus infections) stresses. The analysis of electrophoretic spectra of four isoforms in wheat leaves at the beginning of drought (flowering phase) revealed four isoforms. The amounts of APX isoforms increased to 7 during the wax ripeness phase both in irrigated plants and plants subjected to drought. The long-term drought led to the increase in the intensity of high-molecular isoforms and emergence of three additional isoforms of the enzyme. In this instance, the APX activity level remained higher in drought tolerant wheat genotypes compared with the sensitive ones. At the same time, in both tolerant and sensitive varieties, enhanced APX activity compared with the control variants was observed at the end of drought, which is apparently related to the synthesis of new molecules of the enzyme (i.e. it is stipulated by the changes of genome expression under stress). The immune enzymatic method revealed the presence of CMV virus in *C. sativus* L. plants and TMV in *L. esculentum* Mill. plants. The obtained results show significant differences in APX activities in all virus infected leaves compared with controls. The effect of the viral infection caused approximately

a 1.6-fold increase of the APX activity in *L. esculentum* Mill. and a 2.1-fold increase in *C. sativus* L. compared with corresponding controls. Pathogenesis was accompanied by an increase in the quantity of APX isoforms, which reached 5 in leaves of *L. esculentum* Mill. and by the formation of 2 new isoforms and intensification of the enzyme electrophoresis spectra in infected leaves of *C. sativus* L. compared with the control variants. Thus, an increase in ascorbate peroxidase activity and *de novo* synthesis of its numerous molecular forms under stress conditions testify its involvement in the antioxidant defense system.

Chapter VII – Cadmium (Cd) is a toxic metal currently ranked as seventh (out of 275) on ATSDR priority list of hazardous substances and it is considered as one of the most important occupational and environmental pollutants. This carcinogenic metal causes adverse effects in various tissues, particularly kidneys after prolonged exposure. However, the mechanisms of these toxic effects are still not completely understood. Literature data indicate different mechanisms of cadmium toxicity, oxidative stress being one of the most important. Experiments confirm that Cd, metal with no redox potential, can indirectly induce the production of reactive oxygen species (ROS) by affecting antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase), nonenzymatic antioxidants (glutathione, total sulfhydryl groups, antioxidative vitamines), and Fenton metals, iron and copper. This chapter summarizes our investigations on cadmium-induced oxidative stress conducted on three different animal species: rabbits, mice and rats. Application of a single oral dose of 50 mg Cd/kg b.w. in rabbits showed increased levels of lipid peroxidation in blood 4-6 hours after Cd intoxication indicating early development of oxidative stress in conditions of acute Cd poisoning. Experiments carried out on mice showed that single oral exposure to 20 mg Cd/kg b.w. resulted in intensive ROS production in liver while subacute oral dose of 10 mg Cd/kg b.w. induced more pronounced effect in kidneys, thus indicating different effect of Cd intoxication depending on duration of Cd exposure. Further experiments performed on rats provided strong evidence that Cd can induce oxidative stress in both blood and liver after oral (30 mg Cd/kg b.w.) and intraperitoneal (1.5 mg Cd/kg b.w.) acute intoxication, although intraperitoneal administration showed more pronounced negative effects. Results of these studies demonstrate that oxidative stress is an important mechanism of Cd toxicity and that Cd ability to induce oxidative stress in different tissues depends on the route, dose and duration of exposure to this toxic metal.