Research Article

The Enzymatic Components of Antioxidative Defence System in Black Gram, Cluster Bean Seeds and Seedlings

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ABSTRACT

Biotic and abiotic factors in the environment cause stress to the seeds and seedlings of black gram (*Vigna mungo*) and cluster bean (*Cyamopsis tetragonoloba*) which in turn induces the generation of reactive free radicals through biological reactions. These radicals are unstable and highly reactive. Various antioxidant enzymes play an important role in scavenging these free radicals generated in plants. The most important free radicals are superoxide (O_2), hydrogeperoxide (H_2O_2), hydroxylradical ($OH^{\cdot}$), hydroperoxyl radical, nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{-}$). Lipids, nucleic acids, and proteins are the major targets of these free radicals for oxidation. The enzymatic components of the antioxidative defense system comprise antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), monodehydro ascorbate reductase (MDHAR), dehydro ascorbate reductase (DHAR), and glutathione reductase (GR) which operate in different subcellular compartments and respond when cells are exposed to oxidative stress. In the present investigation, molecular docking between antioxidants and free radicals was carried out and the energy values of interactions in terms of e negative values were calculated. The response of each antioxidant enzyme to free radicals was diverse in scavenging. The highest scavenging ability of catalase was with super oxide, guaiacol peroxidase with hydrogen peroxide, ascorbate peroxidase with peroxy nitrite, super oxide by both monodehydro ascorbate reductase and dehydroa scorbate reductase, hydrogen peroxide by glutathione reductase and super oxide by superoxide dismutase.

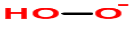
Keywords: stress, free radicals, antioxidant enzymes, molecular docking, *Vigna mungo, Cyamopsis tetragonoloba.*

INTRODUCTION

Reactive free radicals are constantly generated in plants through biological reactions. The presence of unpaired electron results in the formation of certain reactive oxygen species. These radicals are unstable and highly reactive. They accept electron from other molecules and behave as oxidants. The most important oxygen-containing free radicals are superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl radical and reactive nitrogen species (RNS) such as nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{-}$) (Fig.1). These are highly reactive species found in the membranes of cells. Lipids, nucleic acids, and proteins are the major targets of free radicals. Biotic and abiotic factors in the environment cause stress to biological systems. Stress in turn induces the production of reactive oxygen species.

Fig.1 Molecular structure of free radicals

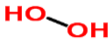
(i) Superoxide



(ii) Hydrogen peroxide

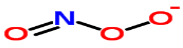
(iii) Hydroxy radicals

(iv) Hydroperoxy radical



(v) Nitric oxide

(vi) Peroxy nitrite



Black gram (*Vigna mungo*) seed is a rich source of protein which has been known to have interesting small molecules with antioxidant activity. However, their levels of enzymatic antioxidant properties against free radicals have not been explored. Catalase, a principal antioxidant enzyme activity from black gram seeds, has been studied. Day four sprouted black gram seeds were also found to have significant catalase content[1]. The molecular mechanism of seed deterioration is an important aspect in seed storage. Mounting research data pinpoints that the production of free radicals during storage is a major cause for disruption of cellular membranes and damage to protein and nucleic acid, which ultimately results in deterioration of cell organelles and seed ageing[2, 3]. It has been proved that proteins are the major targets of free radicals due to their abundance in biological systems[4]. The increasing NaCl concentration reduced the germination percentage, the growth parameters and the relative water content[5]. The germination percentage and the seedling growth parameters inhibited osmotic potentials in an increasing manner in *Phaseolus mungo* [6]. The decrease in seed germination of cluster bean can be attributed to the accelerated breakdown of stored food materials in seed by the application of zinc. Reduction in

seed germination can also be attributed to alterations of selection permeability properties of cell membrane. The decrease in seed germination of cluster bean due to zinc treatment is in conformity with the previous findings[7].

Environmental stresses are the most limiting factors to crop production. Seed germination and early seedling growth are considered as the most critical phases in the establishment of any species. Germination of seed is strongly influenced by variation in temperature, water stress and light requirement and these factors often show significant interaction in their effects on germination[8]. Water stress causes both reductions in the rate of protein synthesis as well as changes in the type of proteins produced. It is believed that these stress induced proteins allow plants to make biochemical and structural adjustments that enable plants to cope with the stress. All the plants have an inbuilt ability to adjust to environmental variables. Aboitic stress negatively influences survival, grain yield, biomass accumulation and production of most crops [9, 10].

The molecular mechanism of seed deterioration is an important aspect in seed storage. Mounting research data pinpoints that the production of free radicals during storage is a major cause for disruption of cellular membranes and damage to protein and nucleic acid, which ultimately results in deterioration of cell organelles and seed ageing[11, 12]. It has been proved that proteins are the major targets of free radicals due to their abundance in biological systems. Lowest catalase and peroxidase activities were recorded in *Cyamopsis tetragonoloba* seedlings[13]. Therefore, a mechanism to interrupt such an autocatalytic process is required.

The enzymatic components of the antioxidative defence system comprise of several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). These enzymes operate in different subcellular compartments and respond when cells are exposed to oxidative stress. Various antioxidant enzymes play important role in scavenging stress-induced ROS generated in plants.

MATERIALS AND METHODS

The Protein Data Bank (PDB) is a repository for the 3-D structural data of proteins from where antioxidant enzymes were retrieved[14]. Molecular structures of free radicals were retrieved from Chemspider[15]. The retrieved structures of enzymes were analyzed by using RasMol which is a molecular graphics program intended for the structural visualization of proteins[16]. 3-D structures of proteins are provided by the UniProtKB/Swiss-Prot database. Binding sites and active sites of proteins are associated with structural pockets and cavities. CastP provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins. It also measures the number of mouth openings, area of the openings and circumference of mouth and surfaces for each pocket. It provides measured parameters for pockets, cavities and mouth openings, as well as listing of wall atoms and mouth atoms for each pocket. TMHMM server is used to predict transmembrane helices in proteins[17]. Each selected amino acid sequence is subjected to transmembrane topology analysis using TMHMM prediction server. Hex is an interactive protein docking and molecular superposition program, written by Ritchie[18]. Hex understands protein structures in PDB format. Using Hex software, protein-ligand docking is possible. In this docking one molecule (always protein) acts as receptor and the other as ligand.

RESULTS

Catalase is one of the most active enzymes, found in plants like black gram and cluster bean. The major function of this enzyme is to decompose hydrogen peroxide (H_2O_2), to water and oxygen under normal and stressful conditions. The effectiveness of the catalase molecule was analyzed using TMHMM tool, which showed the enzyme outside the membrane in the cell showing exomembrane topology indicating its favorable position for effective function (Fig.3). Thus it could act as a most effective receptor enzyme in scavenging of free radicals.

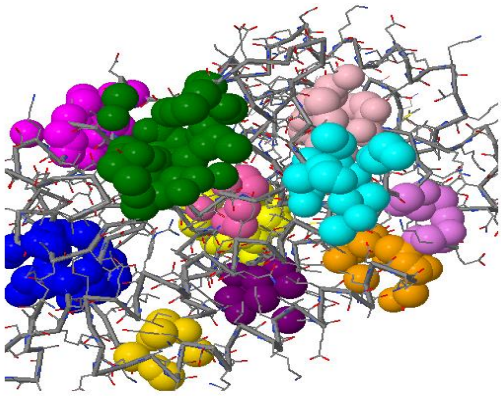


Figure 2: Ligand binding sites of Catalase

The receptor catalase showed many ligand binding sites with various sizes to facilitate entry of ligand into one of the binding sites for interaction, in which the inner wall of the cavity is lined with amino acid residues (Fig.2). During molecular docking, these amino acids play an important role by interacting with the ligand molecule. In the active site, atoms in the amino acids, lying within a specific distance of 5 Å or less than 5 Å , were involved in interaction and therefore, union of all such atoms formed the active force in the binding. In addition to this, electrostatic and vander walls forces were also involved in the binding. In order to find out the best effective interaction between ROS free radicals with antioxidant enzymes, Hex docking was carried out (Fig.4).

In this docking, four ROS radicals such as super oxide, hydrogen peroxide, hydroxy radical and hydroperoxy

radical and two reactive nitrogen species (RNS) such as nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{-}$) were used to act as ligand molecules which formed the targets for the antioxidant enzymes to inhibit their function.

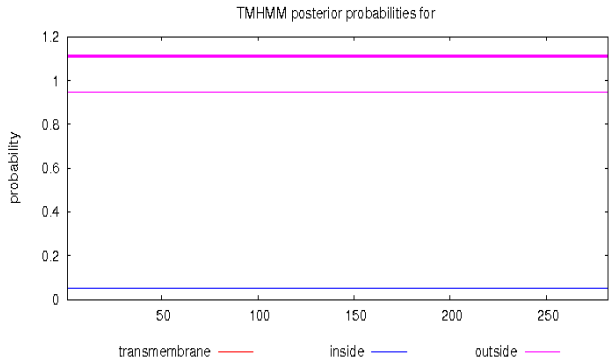


Figure 3: Topology prediction of Catalase

Figure 4: Molecular docking of Catalase with super oxide

The efficient nature of the ligand-receptor complex was identified via docking and the inhibition nature of the enzymes and their binding affinities were calculated using free energy simulations.

Docking results between antioxidant enzymes and free radicals of ROS and RNS were tabulated (Table.1). In this study with catalase, super oxide showed a maximum e-value (-401.30) followed by hydrogen peroxide (-320.00), hydroxy radical (-201.69), hydroperoxy radical (-201.08), peroxynitrite (104.60) and nitric oxide radical (-103.95) (Table.1). When reacting nitrogen species (RNS) such as nitric oxide radical (-103.95) and peroxynitrite radicalwere made to interact with catalase through molecular docking, the e values were less than that of ROS.The e negative values of peroxynitrite and nitric oxide ($NO^{\cdot}$) were only and -104.60 and -103.95 respectively indicating the ineffective nature of catalase against RNS. In this study, the order of the ability of free radical scavanging activity of catalase was super oxide > hydrogen peroxide > hydroxy radical > hydroperoxy radical > peroxynitrite > nitric oxide (Table 1).

In docking guaiacol peroxidase (GPX) receptor with free radicals, the order of the free radical scavanging activity was hydrogen peroxide (-303.19) > hydroxy radical (-203.13) > super oxide (-201.69) > hydroperoxy radical (-157.05) > peroxynitrite (– 200.00) > nitric oxide (-145.16) (Table-1).

Docking ascorbate peroxidase (APX) with free radicals, peroxynitrite ($ONOO^{-}$) showed a maximum e-value (-500.01) followed by super oxide (-329.00), hydrogen peroxide (-309.96), nitric oxide (307.03) hydroxy radical (-257.10) and hydroperoxy radical (-198.50) (Table.3). In this study, the order of the free radical scavanging activity of ascorbate peroxidase was peroxy nitrite > super oxide > hydrogen peroxide > nitric oxide > hydroxy radical > hydroperoxy radical (Table1).

Table 1: Showing e negative values of docking between free radicals and enzymic antioxidants receptor molecules

S.No	Free radicals	catalase (CAT)	guaiacol peroxidase (GPX),	ascorbate peroxidase (APX),	Mono dehydro ascorbate reductase (MDHAR),	dehydroascorbate reductase (DHAR)	glutathione reductase (GR)	superoxide dismutase
1	Super oxide	-401.30	-202.05	-329.00	-357.00	-395.00	-390.02	-580.00
2	Hydrogen peroxide	-320.00	-303.19	-309.96	-300.04	-259.13	-492.94	-385.04
3	Hydroxy radical	-201.69	-203.13	-257.10	-103.06	-216.05	-390.93	-360.03
4	Hydroperoxy radical	-201.08	-157.05	-198.50	-156.07	-150.08	-275.09	-295.05
5	Nitric oxide	-103.95	-145.16	-307.03	-249.00	-180.94	-100.81	-210.01
6	Peroxy nitrite	-104.60	-200.00	-500.01	-270.04	-106.05	-105.67	-115.07

Monodehydro ascorbate reductase (MDHAR) receptor, when docked with free radicals, super oxide showed a maximum e-value (-357.00) followed by hydrogen peroxide (-300.04), peroxynitrite (-270.04), nitric oxide (-249.00), hydroperoxy radical (-156.07) and hydroxy radical (-103.06) (Table.3). In this study, the order of the free radical scavanging activity of monodehydro ascorbate reductase was super oxide > hydrogen peroxide > peroxy nitrite > nitric oxide > hydroperoxy radical > hydroxy radical > (Table 1).

In docking dehydroascorbate reductase (DHAR) with free radicals super oxide showed a maximum e-value (-

395.00), followed by hydrogen peroxide (-259.13), hydroxy radical (-216.05) nitric oxide (-180.94) hydroperoxy radical (-150.08) and peroxy nitrite (-106.05)(Table.3). In this study, the order of the free radical scavanging activity of dehydroascorbate reductase was super oxide > hydrogen peroxide > hydroxy radicals > nitric oxide > hydroperoxy radical > peroxy nitrite (-106.05).

Glutathione reductase (GR) when docked with free radicals, hydrogen peroxide showed a maximum e-value (-492.94) followed by hydroxy radical (-390.93), super oxide (-390.02), hydroperoxy radical (-275.09), peroxy nitrite (-105.67) and nitric oxide (-100.81) (Table.3). In this study, the order of the free radical scavanging activity of glutathione reductase was hydrogen peroxide > hydroxy radical > super oxide > hydroperoxy radical > peroxy nitrite >nitric oxide (Table 1).

In docking superoxide dismutase (SOD) with free radicals, super oxide showed a maximum e-value (--580.00) followed by hydrogen peroxide (--385.04), hydroxy radical (--360.03) hydroperoxy radical (-295.05) , nitric oxide (-210.01) and peroxy nitrite (--115.07) (Table.3). In this study, the order of the free radical scavanging activity of superoxide dismutase was super oxide > hydrogen peroxide > hydroxy radical > hydroperoxy radical > nitric oxide > peroxy nitrite (Table 1).

Thus from the above, the highest scavenging ability of catalase was with super oxide, hydrogen peroxide by guaiacol peroxidise, peroxy nitrite by ascorbate peroxidase, super oxide by both monodehydro ascorbate reductase and dehydro ascorbate reductase, hydrogen peroxide by glutathione reductase and super oxide by superoxide dismutase.

DISCUSSION

The responses of plants to environmental stresses are complex and involve many kinds of physiological and biochemical reactions. Stress causes multiple adverse effects in plants. Production of a family of reactive free radicals is a common phenomenon. When plants are subjected to environmental stress, the balance between the production of reactive free radicals and the scavenging activity of antioxidants is disturbed resulting in an oxidative damage[19].

Recent developments in the free radicals and reactive oxygen species (ROS) in biology has made a remarkable revolution that promises a new age of disease management[20] It is well known that oxygen, an element essential for life, under certain situations, exhibits harmful effects on the human body. Most of the potentially harmful effects of oxygen are the formation of a number of free radicals, known as ROS. Reactive free radicals are constantly generated in living organisms through biological reactions. Reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), superoxide radical (O_2) and singlet oxygen ($O_2^{\cdot}$), and reactive nitrogen species (RNS) such as nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^{-}$) and nitrogen dioxide (NO_2) are the main sources of reactive free radicals[21]. These are highly reactive species found in the membranes of cells. Lipids, nucleic acids, and proteins are the major targets of free radicals. Biotic and abiotic factors in the environment cause stress to biological systems. Stress in turn induces the production of reactive oxygen species. Therefore, a mechanism to interrupt such an autocatalytic process is required. Under normal circumstances, concentrations of oxygen radicals remain low because of the activity of protective enzymes. The enzymatic components of the antioxidative defense system comprise several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), monodehydro ascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). These enzymes operate in different sub cellular compartments and respond when cells are exposed to oxidative stress. Various antioxidant enzymes play important role in scavenging stress-induced ROS generated in plants.

CONCLUSIONS

Even though large number of works has been reported on the scavenging activity of antioxidant enzymes against free radicals, the level of antioxidant activity and which enzyme is highly effective over other enzymes are not available. Therefore, in the present investigation molecular docking between antioxidants and free radicals was carried out and the energy values of interactions in terms of e negative values were calculated. The highest scavenging ability of catalase was with super oxide, guaiacol peroxidase with hhydrogen peroxide, ascorbate peroxidase with peroxy nitrite, super oxide by both monodehydro ascorbate reductase and dehydro ascorbate reductase, hydrogen peroxide by glutathione reductase and super oxide by superoxide dismutase.

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