0014-4754/93/490523-07$1.50 + 0.20

Dealing with active oxygen intermediates: A halophilic perspective

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Abstract. Although oxygen tensions in the halophilic environment are diminished, they are nevertheless sufficient for the generation of active oxygen intermediates as byproducts of metabolism. Therefore, like all other aerobes, halophilic bacteria are compelled to possess the means to detoxify potentially lethal active oxygen intermediates. This review examines the superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen scavenging capacity of the halophiles, *Halobacterium* spp. Specifically, it looks at the potential of the bacteria to generate active oxygen intermediates and then examines the roles of superoxide dismutase, catalase, peroxidase and catalase-peroxidase. It also looks at some non-enzymatic means of neutralizing potentially lethal active oxygen intermediates.
Key words. Catalase; hydroperoxidase; oxygen; peroxidase; singlet oxygen; superoxide dismutase.

Introduction

Metabolic reactions in which oxygen is the terminal acceptor confer upon the organism an ability to oxidize a wide variety of substrates. This increased metabolic diversity is not, however, without potential danger. Although molecular oxygen is not toxic, it can produce metabolic byproducts whose reactivity might pose significant potential for cellular damage. Among the active oxygen intermediates are superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. Consequently, those organisms that exist in an oxygen environment require a system of defenses to counter the potentially lethal effects of oxygen intermediates.

Active oxygen intermediates

Oxygen is a triplet molecule in the ground state. It possesses two unpaired electrons with parallel spins. Reduction of the molecule may occur in one electron increments with the following possible products:

$$O_2 + 1e^- \longrightarrow O_2^- \quad (1)$$

$$O_2 + 2e^- + 2H^+ \longrightarrow H_2O_2 \quad (2)$$

$$O_2 + 3e^- + 3H^+ \longrightarrow OH^\cdot + H_2O \quad (3)$$

$$O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O \quad (4)$$

The simplest reaction is the univalent reduction of oxygen to produce the superoxide free radical ion (eq. 1). The protonated form of O_2^- is the perhydroxyl radical ($HO_2^\cdot$), a weak acid whose pK_a is 4.8. It can be produced by certain flavoprotein dehydrogenases or non-enzymatically through the autoxidation of ferredoxin, hydroquinones, reduced hemoproteins, and thiols[19,20,21,51,52]. With an $E^{0'}$ for the O_2/O_2^- reaction of -0.33 V[17], superoxide is a good reductant. However, superoxide can also serve as an oxidizing agent, abstracting an electron to form H_2O_2, in a reaction whose $E^{0'}$ is 0.87 V[17].

The most stable of the oxygen intermediates is hydrogen peroxide. It is a divalent reduction product of oxygen (eq. 2). Since the two outer orbitals of the molecule are completely filled, H_2O_2 does not exhibit radical properties. With a standard redox potential of 1.77 V, it is a strong nucleophilic oxidizing agent. However, hydrogen peroxide does not react efficiently with organic substrates, but does readily form transition metal complexes[11,15,17].

The trivalent reduction of oxygen produces the highly reactive hydroxyl radical $OH^\cdot$ (eq. 3). The $E^{0'}$ of this oxidant is in the range of 2.0 V and makes it one of the stronger oxidizing agents[10]. The half-life of the molecule in solution is short since the hydroxyl radical reacts at nearly diffusion-controlled rates ($K > 10^9\ M^{-1}s^{-1}$) with most organic molecules[14]. Due to the highly reactive nature of the hydroxyl radical, reactions will occur with the first substrate encountered. The potential for mutagenesis and destruction in biological systems is thus great; membranes, enzymes and nucleic acids are all at risk.

The tetravalent reduction of oxygen (eq. 4) results in the formation of water. This path is the most innocuous if done in a concerted fashion and is the preferred path for oxidations from a safety point of view since active oxygen intermediates would not be formed as byproducts. The terminal oxidase of mitochondria, cytochrome aa_3 oxidase, employs this means of an apparently harmless reduction of oxygen.

Although O_2^- can act as a destructive agent in membrane damage, lipid peroxidation and as a causative reactant in single stranded DNA breaks[22], most of its apparent toxicity is thought to be due to its interaction with H_2O_2 in a metal catalyzed reaction to produce the reactive hydroxyl radical[10].

$$O_2^- + M^{+3} \longrightarrow O_2 + M^{+2} \quad (5)$$

$$M^{+2} + H_2O_2 \longrightarrow M^{+3} + OH^\cdot + OH^- \quad (6)$$

The sum of the reactions is:

$$O_2^- + H_2O_2 \longrightarrow OH^{\cdot} + OH^- + O_2 \quad (7)$$

This reaction was first postulated by Haber and Weiss[27] and is believed to be the underlying mechanism for production of toxic oxygen intermediates[9]. That is, superoxide and hydrogen peroxide per se are not the predominant toxic species. Rather, through their interaction the highly reactive $OH^{\cdot}$ molecule is generated. It is the appearance of the latter molecule that must be minimized in aerobic organisms.

Excited states of oxygen can also be deleterious. When one of the outer shell electrons of oxygen is elevated to a higher orbital and the spin is inverted, the new electronically exicted species is termed the singlet state. There are two possible excited singlet states of oxygen. One is short-lived and is located 37.5 kcal above the triplet ground state and the other is long-lived and is located 22.5 kcal above the ground state. The former is designated $^1\Sigma g$ and the latter is designated as $^1\Delta g$[1,32]. As a result of its longer lifetime in aqueous solutions, the singlet state with the most significant potential for biological consequences is the $^1\Delta g$[32,33]. Relaxation from the excited state to the ground state can proceed by energy transfer to another molecule or by radiation.

Singlet oxygen can be formed in a number of chemical, photochemical and biochemical systems involving photooxidations, free radicals and lipid peroxides[46]. For example, singlet oxygen could be formed in a photochemical reaction if a pigment in the ground state absorbs a photon, thereby generating an excited singlet state of the pigment. The excited pigment could then undergo an intersystem cross to the triplet state. The excited triplet state could next transfer the exciton energy to dioxygen, producing singlet oxygen and regenerating the ground state pigment. The reactions can be written as follows:

$$P_0 + h\nu \longrightarrow P_1 \quad (8)$$

$$P_1 \longrightarrow T_1 \quad (9)$$

$$T_1 + {}^3\Sigma gO_2 \longrightarrow P_0 + {}^1\Delta gO_2 \quad (10)$$

where P_0 and P_1 are the ground and first excited singlet state of the pigment and T_1 represents the first excited triplet state of the pigment. The ground state of oxygen is written as the triplet, $^3\Sigma gO_2$.

Singlet oxygen is highly reactive and can participate in addition reactions to form hydroperoxides and endoperoxides[18,33]. In addition, peroxidation of lipids resulting in a weakened or altered membrane structure is a possible outcome of singlet oxygen interaction[33].

Active oxygen intermediate production by halophiles

All organisms that are exposed to an environment containing oxygen have the capability to generate active oxygen intermediates. The situation is exacerbated by those organisms that are exposed to light. Therefore, halophilic organisms that are floating near the surface of salt ponds carry the potential to generate O_2^-, H_2O_2, $OH^{\cdot}$ and $^1\Delta gO_2$ via metabolic oxidations or from photodynamic action.

Superoxide is produced from autoxidations of quinones, flavines, iron-sulfur centers, thiols, ferredoxin, hemoproteins, catecholamines and tetrahydropterins[21]. Enzymological generators of O_2^- include: xanthine oxidase, aldehyde oxidase and several flavoprotein dehydrogenases[19,20,21,51,52]. A disproportionation of O_2^- or a divalent oxidation would produce H_2O_2. This could be spontaneous or through enzymatic means. The combination of superoxide and hydrogen peroxide could produce $OH^{\cdot}$ (eq. 7). Finally, singlet states of oxygen can be formed through the action of light on pigment sensitizers. Therefore, halophilic organisms have a variety of means of generating active oxygen intermediates. Although the oxygen content in a salt environment is relatively low, the halophile would be exposed to increased oxygen tensions when it rises to the surface. Moreover, as it surfaces, the potential for photodynamic singlet oxygen generation is enhanced. Therefore, despite the apparent lowered oxygen environment, the halophilic organism is still at risk from active oxygen intermediates.

Biological defenses

In order to cope adequately with an oxygen environment, organisms would need either to prevent the formation of active oxygen intermediates or successfully scavenge and neutralize them once they are formed. Several different classes of proteins have evolved whose function is to sequester superoxide and hydrogen peroxide. In addition, there are low molecular weight compounds which serve as sequestering traps for singlet oxygen.

Superoxide dismutases

Superoxide dismutase (EC 1.15.1.1) is a class of metalloenzymes which effectively scavenges O_2^-[3,19,20]. The metal prosthetic group undergoes a reduction followed by an oxidation, both of which involve O_2^-.

$$O_2^- + M^{+2} \longrightarrow O_2 + M^{+1} \quad (11)$$

$$O_2^- + M^{+1} + 2H^+ \longrightarrow H_2O_2 + M^{+2} \quad (12)$$

The sum of these reactions is:

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (13)$$

The rate constants for superoxide dismutases are nearly at the level of being limited by substrate diffusion ($2 \times 10^9\ M^{-1}\ s^{-1}$). The superoxide dismutase group consists of three classes of metalloproteins; a copper-zinc containing enzyme, a manganese-containing enzyme and an iron-containing enzyme. The latter two

proteins are similar to each other and differ from the copper-zinc containing protein[3, 19, 20].

The copper-zinc containing superoxide dismutase is a dimer and consists of two subunits of about 16.5 kDa each. It is inhibited by cyanide and inactivated by H_2O_2. This enzyme is found predominantly in eukaryotes[3] although there have been several reports of the copper-zinc containing protein in prokaryotes. *Photobacterium leiognathi*[49], *Caulobacter crescentus*[58], several pseudomonads[59] and *Brucella abortus*[4], have been reported to possess bacteriocupreins, a bacterial form of the copper-zinc containing superoxide dismutase.

The manganese- and the iron-containing superoxide dismutases are both insensitive to cyanide inhibition. The manganese protein is 40–96 kDa, depending upon the organism, and consists of 2 or 4 equally sized subunits. Unlike the copper-zinc enzyme, the manganese enzyme is not inactivated by H_2O_2. The protein is found in the cytosol of prokaryotes whereas in eukaryotes, the manganese enzyme is found predominantly in the mitochondria[3] and glyoxysomes[12, 57].

Unlike the manganese enzyme, the iron-containing superoxide dismutase is inactived by H_2O_2. The protein ranges in size from 36–46 kDa and consists of two equally sized subunits. Analysis of X-ray crystallographic structure of the iron- and the manganese-containing proteins reveals similarities between the enzymes and a clear distinction from the copper-zinc proteins[3, 48]. Moreover, there are cases where bacteria can utilize either manganese or iron, depending upon the metal supplied[25, 26, 41, 42, 45]. Recently, a manganese-containing superoxide dismutase gene from the anerobic archaebacterium, *Methanobacterium thermoautotrophicum*, was cloned and expressed in *Escherichia coli* as an iron-containing superoxide dismutase with full activity[61].

Although there are exceptions, in general it is believed that the manganese-containing superoxide dismutase is associated with aerobic organisms whereas the iron-containing enzyme is associated with a facultative or relatively anaerobic environment[2, 3]. This might be a reflection of the higher redox potential of manganese compared to iron. Moreover, although the iron-containing superoxide dismutases were previously thought to be restricted to prokaryotes, there are several cases where the protein is found in eukaryotic photosynthetic organisms[35, 51, 52, 63]. The enzyme is associated predominantly with the chloroplast and is absent in mitochondria[53].

Superoxide dismutase in halophilic bacteria

Manganese-containing superoxide dismutases have been purified and characterized from *Halobacterium cutirubrum*[43, 44] as well as *Halobacterium halobium*[56, 60]. In no case was an iron or a copper-zinc form of superoxide dismutase reported. The M_r, subunit size, metal content and other physical characteristics of the enzymes did not set them apart from other manganese-containing superoxide dismutases from non-halophilic organisms. Interestingly, in *H. halobium*, the superoxide dismutase lies 3′ to a gene coding for the DNA repair enzyme, photolyase[60]. The photolyase and the superoxide dismutase can be cotranscribed or can be independently induced by various factors such as O_2 and UV light. This tandem arrangement of superoxide dismutase and a DNA photorepair enzyme appears to be unique to *H. halobium*, since other prokaryotes do not exhibit a similar phenomenon[60].

A characteristic of many enzymes isolated from halophilic organisms is their requirement for salt concentrations in excess of 1 M for stability and activity[36, 37, 38, 50]. An examination of the primary structure of the superoxide dismutases sequenced from halophilic bacteria shows remarkable similarity to non-halophilic manganese-containing superoxide dismutases[43, 55, 60]. There do not appear to be major alternations in sequence as a result of adaptation to a halophilic environment. A possible exception is at position 29 where there is a conserved lysine which is found in all non-halophilic manganese and iron-containing superoxide dismutases, as well as in other archeabacterial species. This position in the halophiles is occupied by threonine. Noteworthy in this regard is the fact that Benovic et al.[6] found that a lysine residue or residues might be responsible for reduced activity of *E. coli* manganese-containing superoxide dismutase at an increased ionic strength. Modification of the lysine residue or residues by acetylation reversed the negative effect of increasing ionic strength. Lysine 29, by virtue of its positive charge and location, has been implicated in directing O_2^- to the active site[48]. Substitution of this lysine by threonine in halophiles would replace the electrostatic steering of substrates with a weaker hydrogen bond steering.

Hydroperoxidases: catalases and peroxidases

Hydrogen peroxide, a relatively long-lived active oxygen intermediate, is neutralized in biological systems by a class of proteins termed hydroperoxidases. This class consists of catalases (EC 1.11.1.6) and peroxidases (EC 1.11.1.7). The heme-containing catalases and peroxidases efficiently scavenge H_2O_2 in similar type reactions.

Catalase:

$$H_2O_2 + H_2O_2 \longrightarrow 2H_2O + O_2 \qquad (14)$$

Peroxidase:

$$H_2O_2 + R(OH)_2 \longrightarrow 2H_2O + RO_2 \qquad (15)$$

The catalase reaction is one which is characterized by electron pair transitions in which H_2O_2 is decomposed to O_2 and H_2O[11]. The peroxidase reaction is characterized by single electron transfers resulting in the oxidation of various organic compounds by H_2O_2[15]. The first

transition product of both reactions is heme in an oxidation state of V (Compound I). Compound I is then reduced back to the original redox state in a concerted 2-electron step by H_2O_2 in the catalase reaction or by a series of 1-electron sequential steps by $R(OH)_2$ in the case of peroxidase.

Catalases from higher organisms resemble each other in that they have molecular weights in the range of 225,000–270,000, have 4 equally sized subunits each containing one ferric heme prosthetic group and show a broad pH range of 5–10.5 for activity[11]. Peroxidases are also heme-containing enzymes but are monomeric proteins which show a diversity in their molecular weights[15]. Additionally, the heme iron of catalase is not reducible whereas the heme iron of peroxidases can be reduced with dithionite[11,15]. Finally, catalases are specifically inhibited by 3-amino-1,2,4-triazole whereas peroxidases are not inhibited[40].

Recently, the hydroperoxidase class of proteins has been shown to contain enzymes which are unique in that they exhibit both catalase as well as peroxidase activity[47]. These proteins show characteristics that are typical of peroxidases in that their heme groups can be reduced readily by dithionite. Also, these bifunctional proteins show characteristics which are typical of catalases in that they have a tetrameric molecular weight in the range of 240,000 with equally sized subunits. However, these enzymes exhibit other properties which distinguish them from typical catalases. They possess a narrow pH range for maximal activity, a marked sensitivity to temperature, are inactivated by H_2O_2 and are resistant to inhibition by 3-amino-1,2,4-triazole[24,29]. Proteins with catalase-peroxidase activities have been found in *E. coli*[8], *Rhodopseudomonas capsulata*[31], *Klebsiella pneumoniae*[30], *Rhodobacter capsulatus*[29], *Chromatium vinosum*[47] and in *Bacillus* YN-200[64]. It would appear, therefore, that the novel group of hydroperoxidase with a dual function is found in a wide range of microorganisms.

Enzymatic removal of peroxides by halophiles

Halobacteria contain all three of the hydrogen peroxide scavenging enzymes described above. Catalase was partially purified from *H. cutirubrum*[37]. The halophilic catalase showed a remarkable similarity to beef liver catalase in molecular weight and kinetic properties. However, the halophilic catalase, as might be expected, showed a maximum of activity at about 1 M monovalent salt concentrations.

A peroxidase was purified from *H. halobium* L-33[23]. The protein is a monomer with a M_r of 110,000, its heme goup is capable of being reduced by dithionite and in addition to the peroxidase activity, it was reported to have a weak catalase activity. The protein is stable only in the presence of high salt concentrations, reaching a maximum activity at 3 M NaCl.

A catalase-peroxidase was recently purified from *H. halobium*[7]. The protein has a M_r of 240,000, and consists of 4 subunits of 60,000 each. Peroxidase activity is seen in the pH range of 6.5–8.0 and is maximal in salt concentrations above 1M NaCl. The catalase activity was observed over a narrow range, between 6.0 and 7.5, and requires 2M NaCl for optimal function. The halophilic protein demonstrates a thermotolerance not seen in typical catalase-peroxidases. The peroxidase activity decreases rapidly above a maximum of 40 °C whereas the catalase activity is greatest at 50 °C. Peroxidase activity is not completely inhibited until 80 °C while significant catalatic activity is still apparent after 5 minutes of incubation at 90 °C. An additional unique property of the halophilic protein is that the heme group is not capable of being reduced by dithionite.

Halophiles are not unique in having three enzymes to remove H_2O_2. Plants contain nearly a dozen different peroxidases with broad substrate specificities[15]. Is such an apparent redundancy necessary? Unique substrates for the *H. halobium* peroxidase and the catalase-peroxidase as well as unique microenvironments might necessitate the evolution of multiple forms of the same enzyme. Alternatively, it is possible that the catalase-peroxidase is an ancestral hydroperoxidase. Through evolution, separate and unique catalase and peroxidase proteins may have evolved from the hydroperoxidase. An insight into this possibility could be gained once amino acid sequence information on the various proteins is obtained. Finally, it is conceivable that some of the enzymes might be constitutive while others might be inducible by specific stress conditions. There is precedent for this in *E. coli* in which a catalase-peroxidase (HPI) increases in response to H_2O_2 levels while another form of the enzyme, an atypical catalase (HPII)[39] is induced in the stationary phase[29]. A similar instance was inferred in the case of a peroxidase and a catalase-peroxidase from *Rhodobacter capsulatus*[29]. It was suggested that the former enzyme might protect against H_2O_2 in exponentially growing cells whereas the latter enzyme might protect aging cultures against oxidative stress.

Non-enzymatic removal of active oxygen intermediates

Toxic oxygen species can also be removed by sequestration by endogeneous low molecular weight substances found within the cell. Glutathione and ascorbate can act as scavengers in enzymatic peroxidative reactions (gluthathione peroxidase, ascorbate peroxidase)[16,51]. However, other endogenous substrates play a direct, non-enzymatic role in scavenging toxic oxygen byproducts. By virtue of the fact that superoxide can act as either a reductant or an oxidant, it is capable of reacting with various oxidants or reductants present in the cell. Availability at the site of generation and cellular con-

centration would be controlling factors in these types of reactions. Examples of compounds that could react with O_2^- include: cytochromes and other hemes, metal complexes, hydroquinones, carotenoids, catecholamines, ascorbic acid, NAD(P)H and various sulphur containing compounds[16].

Due to its high reactivity, $OH^\cdot$ will react with virtually every substrate it encounters[10]. Addition reactions to aromatic molecules to form hydroxydienyl radicals and addition reactions to unsaturated aliphatics to form hydroxyalkyl radicals are possible consequences of hydroxyl radical interaction[14]. Moreover, hydrogen abstraction from unsaturated molecules to form water and carbon radicals is also possible. Oxidative deamination of aliphatic amino acids, hydrogen abstraction from thiol groups and addition reactions to nucleotides and nucleosides are other possible $OH^\cdot$ mediated reactions[14]. Encounter with hydroxyl radical by a scavenging substrate might serve to protect the organism, but if the reacting species is an integral membrane component or an essential protein or substrate, the consequences could prove toxic.

Singlet oxygen reacts readily with metal complexes, sulfides, carotenes, and unsaturated fatty acids[18]. However, in quenching singlet oxygen or in reacting with hydroxyl radical, the scavenging substrate might itself become radicalized, thereby propagating a chain reaction of radical mediated oxidations. Moreover, if depleted substrates are not replenished, repeated exposure to bursts of active oxygen intermediates could prove lethal due to an inadequate reservoir of protective substrates. If the substrates served the cell in some vital capacity, their oxidative depletion may harm the cell. It might be difficult then to differentiate between a truly protective effect of a substrate from that which appears to be beneficial, but is nevertheless deleterious in the long run.

Non-enzymatic removal of oxygen intermediates in halophiles

Halophilic organisms likely contain many of the non-enzymatic mechanisms for sequestering active oxygen intermediates as described. In addition to these substrates, halophiles are unique in that they possess molar concentrations of halide ion. It is conceivable that Cl^- might be oxidized by $OH^\cdot$ in a two step reaction to form Cl_2^-[14] as follows:

$$OH^\cdot + Cl^- \longrightarrow Cl^\cdot + OH^- \quad (16)$$

$$Cl^\cdot + Cl^- \longrightarrow Cl_2^- \quad (17)$$

Therefore, by virtue of the fact that there are large concentrations of halide, a large reservoir of endogenous hydroxyl radical scavenger is available.

In addition to the reactivity of halide ion with $OH^\cdot$, the major endogenous compatible solutes found in halophiles could easily react with hydroxyl radical. Betaines, glycerol, proline, glycine, glycine betaine and fatty acids could serve protective roles in a halophile. Carotenoid compounds are effective singlet oxygen scavengers[18,33]. In this regard, large amounts of C_{50} pigments, bacteriorubreins[34], as well as other carotenoids in the pigmented halophiles should serve as a potential reservoir of singlet scavenging agents. Bacteriorhodopsin likewise has the capacity to serve as a singlet trap.

Modulation of protective systems in halophilic bacteria

Superoxide dismutase levels in *H. halobium* are increased by an aerobic environment[56,60]. In *H. cutirubrum* as well as in *Haloferax* (*Halobacterium*) *volcanii* incubation with the superoxide producing substrate paraquat resulted in elevated levels of superoxide dismutase[44]. In addition, an exposure of *H. halobium* to 50 °C for 2.5 hours resulted in a more than two-fold enhancement in the amount of superoxide dismutase[5]. The superoxide dismutase can even be raised to five times the level found at 40 °C by subjecting *H. halobium* to 60 °C for up to 8 hours (Brown-Peterson and Salin, unpublished observations).

Although the increase in the amount of superoxide dismutase by exposure to higher temperature could be related to a heat-shock or stress-type effect, it is also possible that the increase could be explained by a more rapid metabolism at elevated temperatures. The rapid shunting of electrons through the respiratory chain at increased rates might elicit the signal to produce more of the oxygen radical scavenging protein as a precautionary defensive measure. We have been able to mimic this effect by subjecting *H. halobium* to micromolar amounts of respiratory inhibitors such as CN^-, N_3^-, antimycin A, and rotenone. In the presence of these compounds there is a correlation between increased oxygen consumption and the induction of superoxide dismutase (Brown-Petersen et al., submitted).

Preliminary experiments on the direct addition of H_2O_2 to cultures of *H. halobium* have shown that 20 mM H_2O_2 added to a growing culture resulted in increased amounts of superoxide dismutase, catalase and peroxidase after 24 hours (Brown-Peterson and Salin, unpublished results). No distinction was made as to whether the halophilic catalase, peroxidase or catalase-peroxidase was induced.

Future areas

Regulation of active oxygen protective systems in halophilic bacteria is an area in which there has been little study. There have not been in depth studies of how environmental factors such as light and dark, nutrient content, metal availability, growth stage and type of salt might affect the response to oxidative stress. A determination should be made as to whether oxygen tension,

metabolic rates, free radical production or redox state of the cell might be essential controls on induction of hydroperoxidases and superoxide dismutases in halophiles. Halobacteria offer a unique system in which to work, since not only is there an adaptation to a high salt environment but in addition, an insight will be gained into metabolic control in a member of the urkingdom, Archae.

Studies have not appeared on the presence of global regulators such as the ferric uptake regulator (Fur), aerobic respiratory control (Arc) or the fumarate nitrate reduction genes in the halophiles. These global regulators along with superoxide response (SoxR) have been shown to control iron-and manganese-containing superoxide dismutase levels in *E. coli*[13,28,62]. The control regions of the *H. halobium* superoxide dismutase gene contain no evidence of a Fur promoter sequence[54]. Although the complete gene sequence of the superoxide dismutases are known in *H. cutirubrum* and *H. halobium*[43,55,60] and the sequence of a photolyase is known along with associated promoter regions[60], the hydroperoxidase genes have not been sequenced.

A limiting factor in many studies on the halophilic proteins is that they can not be expressed adequately in the currently available expression vectors. As expression vectors specific for halophiles become available, studies can be initiated on effects of overproduction of oxygen detoxifying enzymes. In addition, studies need to be performed at the molecular level to determine how altering specific amino acids would affect the salt and thermal stability of the enzymes. Finally, the generation of various types of knock-out mutations in the hydroperoxidases and superoxide dismutase should lend insight into the necessity of these enzymes for halophilic bacteria survival.

Acknowledgments. This work was supported by funds made available from the Mississippi Agricultural and Forestry Experiment Station and has been approved as Journal Article No. J-8177.

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