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EFFECTS OF OXYGEN FREE RADICAL SCAVENGERS ON THE MEMBRANE MYOINOSITOL DEHYDROGENASE OF *BACILLUS PUMILUS* STRAIN 5

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Micromolar amounts of superoxide dismutase (SOD) or parabenzoquinone (PBQ) inhibit the membrane-bound myoinositol dehydrogenase of *Bacillus pumilus* strain 5 in the mode of this enzyme transferring electrons to 2,6-dichlorophenol indophenol (DCPIP). The inhibition trends are similar to those reported earlier by us for the inhibition by mannitol and benzoate. We postulate that the transfer of electrons from the enzyme to DCPIP involves in its rate-limiting step, a catalytic intermediate in the nature of superoxide (O_2^-) and/or hydroyl free radical (OH·). Scavenging of any one or both of these radicals, therefore, inhibits the electron transfer reaction. PBQ serves as an electron sink in the reaction preventing the reduction of DCPIP.

KEY WORDS: Oxygen free radicals, superoxide dismutase, parabenzoquinone, myoinositol dehydrogenase, *Bacillus Pumilus*.

INTRODUCTION

Superoxide, the anionic free radical (O_2^-) formed by one electron transfer to the oxygen molecule, is a common intermediate of oxygen reduction. This is because molecular oxygen in its ground state prefers univalent pathways of reduction.[1,2] A number of enzymes, including some that are membrane-bound, are known to involve O_2^- in their mechanism of action.[1,2,3] Thus, respiring biological systems produce O_2^-, and this could be dismutated spontaneously, or catalysed by superoxide dismutase (SOD), to hydrogen peroxide (H_2O_2). These two strong oxidants could form a yet more potent oxidant, the hydroxyl free radical (OH·) through a metal catalysed Haber-Weiss reaction[2,4] as follows:

$$O_2 + e^- \longrightarrow O_2^-$$

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

$$O_2^- + H_2O_2 \longrightarrow OH^- + OH\cdot + O_2$$

A number of enzymes protect the respiring cell from the toxicity of these partially reduced oxygen species.[2,4] Evidence is now accumulating to the effect that the production of some/all of these radicals by macrophages,[5] and granulocytes,[4] is part of the

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body defence mechanism of higher animals against pathogens.[2,4,5] An enzyme which forms O_2^-, H_2O_2 or $OH\cdot$ as obligatory intermediates in its rate-limiting electron transfer mechanism should, therefore, be expected to be inhibited in the presence of scavengers [of these oxygen centred species].

It has been reported that benzoate,[2,4] and mannitol[4] scavenge $OH\cdot$. In our earlier preliminary report,[6] we showed that each of these two compounds, in micromolar amounts, inhibits the membrane bound myoinositol dehydrogenase of *Bacillus pumilus* strain 5. We present herein, additional data to the effect that both superoxide dismutase and parabenzoquinone (PBQ) also inhibit this enzyme.

MATERIALS AND METHODS

Parabenzoquinone was supplied by British Drug Houses (BDH), Poole, England, while copper/zinc superoxide dismutase (SOD) type 1 was purchased from SIGMA Chemical Company, St. Louis, MO, USA. All other chemicals and reagents were of the highest purity available and were procured from standard sources previously reported.[6]

Bacillus pumilus strain 5 was grown and membrane extracts prepared essentially as previously reported.[6] The myoinositol dehydrogenase activity of the membrane fraction was assayed by the bleaching of 2,6-dichlorophenol indophenol (DCPIP) as detailed previously. SOD or PBQ was included as appropriate at myoinositol concentration of 0.2 mM in each case. The method of Lowry *et al.*[7] was employed for measuring the protein content of the membrane extract, using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Myoinositol dehydrogenase catalyses the first reaction in the metabolism of myoinositol.[8] Neither the pathway, nor the mechanism of re-oxidation of this enzyme in its reduced form in *B. pumilus* strain 5 membrane has so far been reported. The data on the inhibition, by SOD and PBQ, of the transfer of electrons from myoinositol to DCPIP in the presence of the membrane-bound enzyme is shown in Table I. It is

TABLE I

Effects of graded concentrations of superoxide dismutase and parabenzoquinone on membrane myoinositol dehydrogenase of *Bacillus pumilus* strain 5

Inhibitor	Concentration M	Inhibition %
Superoxide Dismutase	8.0×10^{-7}	35
	1.6×10^{-7}	14
Parabenzoquinone	28.6×10^{-5}	22
	14.3×10^{-5}	11
	7.3×10^{-5}	5

The reaction mixture was set up and decrease in absorbance at 600 nm with time recorded as previously reported.[6] When appropriate, superoxide dismutase or parabenzoquinone was included at the various concentrations indicated in the table. In each case, myoinositol concentration was maintained at 0.2 mM, and initial rates were calculated as detailed previously.[6]

obvious that the inhibition by these two agents displays the same trend as those exhibited by the inhibition by mannitol and benzoate reported earlier.[6] All these inhibitors are chemically unrelated in structure. They, however, share the common property of being scavengers of oxygen free radicals. Mannitol and benzoate scavenge OH· and SOD dismutes superoxide.[4]

If OH· is the cactalytically relevant species during the transfer of electrons from the reduced form of myoinositol dehydrogenase to DCPIP, this reaction would be directly inhibited by scavengers of OH·. If, on the other hand, the catalytically relevant free radical was O_2^-, scavenging of OH· would shift the equilibrium of the Haber-Weiss reaction towards continuous formation of OH· at the expense of O_2^-. The transfer of electrons from myoinositol dehydrogenase to DCPIP would be equally inhibited.

The data (see Table I) for the inhibitory effects of PBQ is consistent with these results. Since this quinone is capable of mono-electron reduction,[9] we postulate that two molecules of the semiquinone undergo a disproportionation reaction as reported in Fig. 1. Thus PBQ becomes, in this reaction, a sink for electrons, possibly preventing

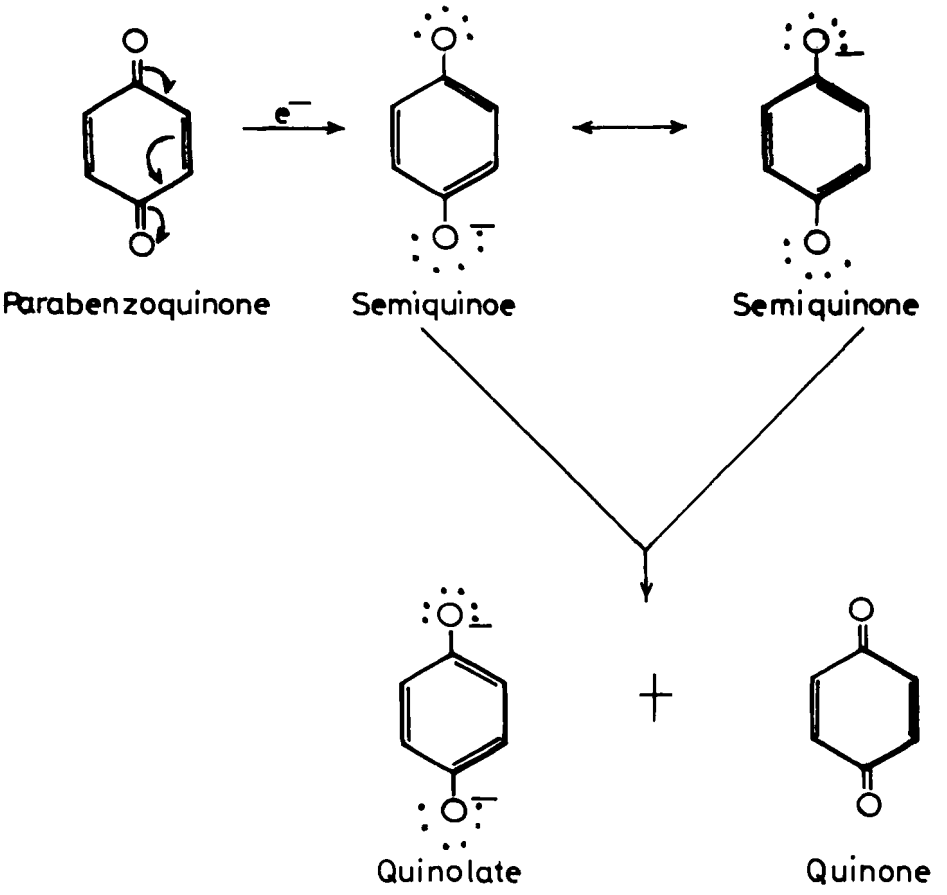

FIGURE 1 Mono-electron reduction of parabenzoquinone.

the formation of O_2^- and/or OH·. This mechanism may also generally account for reports that quinones are free radical scavengers.[10] Further investigations have been initiated to delineate the contributions of these findings to the understanding of the pathway and mechanism of electron transfer down the electron transport chain of *Bacillus pumilus* strain 5, from the dehydrogenation of myoinositol.

Loschen and Azzi[11] reported that the quinone, dibromothymoquinone (DBTQ), inhibits mitochondrial transport of electrons from either succinate or NADH, probably by competing with ubiquinone. On the basis of our "electron sink" postulate given above, this can be explained as follows: The inhibitory quinone (DBTQ) cannot anchor within the membrane lipid bilayer as (strategically as) ubiquinone would do *via* its long hydrophobic isoprenoid side chain.[10] Thus, electrons captured by the DBTQ (which now serves as an electron sink) are lost and, consequently, electron transport is inhibited.

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