D. C., Ladner, R. C., & Lipscomb, W. N. (1982) *J. Mol. Biol. 160*, 219-263.
Karplus, P. A., & Schulz, G. E. (1987) *J. Mol. Biol. 195*, 701-729.
Karplus, P. A., & Schulz, G. E (1989) *J. Mol. Biol. 210*, 163-180.
Laemmli, U. K. (1970) *Nature (London) 227*, 680-685.
Larimer, F. W., Lee, E. H., Mural, R. J., Soper, T. S., & Hartman, F. C. (1987) *J. Biol. Chem. 262*, 15327-15329.
Lowe, C. R., Hans, M., Spibey, N., & Drabble, W. T. (1980) *Anal. Biochem. 104*, 23-28.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Markert, C. L. (1963) *Science 140*, 1329-1330.
Matthews, R. (1990) in *Flavins and Flavoproteins 1990* (Curti, B., Ronchi, S., & Zanetti, G., Eds.) pp 593-597, Walter de Gruyter, Berlin and New York.
Monaco, H. L., Crawford, J. L., & Lipscomb, W. N. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5276-5280.
Pai, E. F., & Schulz, G. E. (1983) *J. Biol. Chem. 258*, 1751-1757.
Pai, E. F., Karplus, P. A., & Schulz, G. E. (1988) *Biochemistry 27*, 4465-4474.
Penhoet, E., Kochman, M., Valentine, R., & Rutter, W. J. (1967) *Biochemistry 6*, 2940-2949.
Robey, E. A., & Schachman, H. K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 361-365.
Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H., & Roe, B. A. (1980) *J. Mol. Biol. 143*, 167-178.
Scrutton, N. S., Berry, A., & Perham, R. N. (1987) *Biochem. J. 245*, 875-880.
Scrutton, N. S., Berry, A., Deonarain, M. P., & Perham, R. N. (1990) *Proc. R. Soc. London, B 242*, 217-224.
Soper, T. S., Larimer, F. W., Mural, R. J., Lee, E. H., & Hartman, F. C. (1989) *J. Protein Chem. 8*, 239-249.
Tabor, S., & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 1074-1078.
Taylor, J. W., Schmidt, W., Cosstick, R., Okruszek, A., & Eckstein, F. (1985) *Nucleic Acids Res. 13*, 8749-8764.
Wente, S. R., & Schachman, H. K. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 31-35.
Williams, C. H., Jr. (1976) *Enzymes (3rd Ed.) 13*, 89-173.

Apparent Oxygen-Dependent Inhibition by Superoxide Dismutase of the Quinoprotein Methanol Dehydrogenase†

Victor L. Davidson,* M. Arun Kumar, and Jinyun Wu
Department of Biochemistry, The University of Mississippi Medical Center, Jackson, Mississippi 39216-4505
Received June 13, 1991; Revised Manuscript Received October 29, 1991

ABSTRACT: Methanol dehydrogenase activity, when assayed with phenazine ethosulfate (PES) as an electron acceptor, was inhibited by superoxide dismutase (SOD) and by Mn^{2+} only under aerobic conditions. Catalase, formate, and other divalent cations did not inhibit the enzyme. The enzyme also exhibited significantly higher levels of activity when assayed with PES under anaerobic conditions relative to aerobic conditions. The oxygen- and superoxide-dependent effects on methanol dehydrogenase were not observed when either Wurster's Blue or cytochrome *c*-551i was used as an electron acceptor. Another quinoprotein, methylamine dehydrogenase, which possesses tryptophan tryptophylquinone (TTQ) rather than pyrroloquinoline quinone (PQQ) as a prosthetic group, was not inhibited by SOD or Mn^{2+} when assayed with PES as an electron acceptor. Spectroscopic analysis of methanol dehydrogenase provided no evidence for any oxygen- or superoxide-dependent changes in the redox state of the enzyme-bound PQQ cofactor of methanol dehydrogenase. To explain these data, a model is presented in which this cofactor reacts reversibly with oxygen and superoxide, and in which oxygen is able to compete with PES as an electron acceptor for the reduced species.

Inhibition of a process by superoxide dismutase (SOD)[1] is usually assumed to be due to blockage of a reaction involving superoxide as an intermediate (Fridovich, 1975). We report here that under certain conditions the activity of methanol dehydrogenase from *Paracoccus denitrificans* is inhibited by SOD in an unusual oxygen-dependent manner. Methanol dehydrogenase has long been considered to be an important enzyme due to its central role in bacterial methanol metabolism and the potential importance of methylotrophic bacteria to biotechnology (Anthony, 1982, 1986). Methanol dehydrogenase is also of great interest to enzymologists (Duine et al., 1987) who wish to elucidate its reaction mechanism and the role of its redox cofactor, pyrroloquinoline quinone (PQQ)[1] (Salisbury et al., 1979) (Figure 1), in catalysis and electron transfer. Although methanol dehydrogenase was first characterized in 1964 (Anthony & Zatman, 1964) and has subsequently been purified from several sources, the details of its reaction mechanism and its interaction with artificial and

† This work was supported by National Institutes of Health Grant GM-41574.
* Author to whom correspondence should be addressed at the Department of Biochemistry, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216-4505.

[1] Abbreviations: PQQ, pyrroloquinoline quinone; PES, phenazine ethosulfate; DCIP, 2,6-dichlorophenolindophenol; CuZnSOD, copper-zinc-containing superoxide dismutase; MnSOD, manganese-containing superoxide dismutase; O_2^-, superoxide; TTQ, tryptophan tryptophylquinone.

0006-2960/92/0431-1504$03.00/0

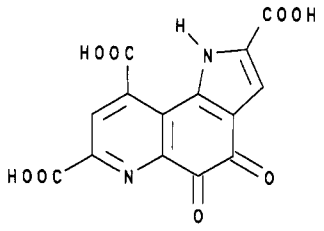

FIGURE 1: Structure of the PQQ prosthetic group of methanol dehydrogenase as deduced by Salisbury et al. (1979).

natural electron acceptors have remained controversial and poorly understood. Most methanol dehydrogenases are oligomeric proteins containing two noncovalently bound molecules of PQQ. The cofactors of these enzymes, as isolated, appear to be in the semiquinone form (Frank et al., 1989). Study of these enzymes has been complicated by the fact that incubation of methanol dehydrogenase with an electron acceptor in the presence of ammonia, which is an activator of the enzyme in vitro, leads to high rates of reaction in the absence of added substrate (Anthony & Zatman, 1964; Duine & Frank, 1980; Ghosh & Quail, 1981). Furthermore, if allowed to react in this manner, methanol dehydrogenase becomes inactivated. The most common explanation for this substrate-independent activity is that methanol dehydrogenase preparations are contaminated by an unidentified endogenous substrate. Although never fully characterized, this endogenous activity is common to all methanol dehydrogenases (Anthony, 1986). The endogenous activity is suppressed by CN^- (Duine & Frank, 1980), which also protects the enzyme against inactivation. Investigators have, therefore, routinely included CN^- in the assay mixture to allow the study of methanol-dependent activity.

We will present evidence that for methanol dehydrogenase, superoxide is produced reversibly by reaction of O_2 with reduced forms of the enzyme-bound cofactor and that SOD inhibits the enzyme by lowering the concentration of superoxide, thus allowing O_2 to compete for electrons with the electron acceptor which is routinely used in the assay of this enzyme.

Experimental Procedures

Methanol and methylamine dehydrogenases were purified from the periplasmic extract of *P. denitrificans* (ATCC 13543). Purification of methylamine dehydrogenase was as described previously (Davidson, 1990). The initial steps of the purification of methanol dehydrogenase were the same as those for methylamine dehydrogenase. Fractions containing methanol dehydrogenase eluted prior to methylamine dehydrogenase during chromatography over DEAE-Trisacryl. These fractions were pooled, concentrated, and subjected to gel filtration chromatography over AcA 34 for final purification. Cytochromes *c*-551i and *c*-550 were purified as described previously (Husain & Davidson, 1986). Wurster's Blue was synthesized from *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine according to the method of Michaelis and Granick (1943). All other enzymes and reagents were purchased from Sigma. The activities of the commercially obtained SODs were checked using the cytochrome *c* reduction assay in which xanthine and xanthine oxidase were used to generate superoxide (Flohe & Otting, 1984). Absorption spectra were recorded with a Milton Roy 3000 array spectrophotometer.

Methanol dehydrogenase was assayed with phenazine ethosulfate (PES) essentially as described by Anthony and Zatman (1964) with modifications which are detailed below. For the assay of endogenous activity, the reaction mixture contained 6 μg of methanol dehydrogenase, 3.6 mM PES, 0.17 mM DCIP, and 6.75 mM ammonium sulfate in 0.1 M potassium phosphate buffer, pH 9.0. The reaction was initiated by the addition of ammonium sulfate. For the assay of methanol-dependent activity in the absence of any endogenous reaction, the reaction mixture was identical to that described above but also contained 10 mM methanol and 6 mM KCN. Again, the reaction was initiated by the addition of ammonium sulfate. The rates of reaction were determined spectrophotometrically by monitoring the decrease in absorbance of DCIP at 600 nm (ϵ = 22 000 M^{-1} cm^{-1}). One unit is defined as the amount of enzyme activity required to reduce 1 μmol of DCIP per minute under the given experimental conditions. For the assay of methanol dehydrogenase with Wurster's Blue, the components of the assay mixture were as described above except that PES and DCIP were replaced with 0.18 mM Wurster's Blue. The rate of reaction was monitored by the decrease in absorbance of the dye at 560 nm. For the assay of methanol dehydrogenase activity with cytochromes *c*, the assay mixture contained 5 μg of methanol dehydrogenase, 6.75 mM ammonium sulfate, 1.1 μM cytochrome *c*-551i and 11 μM cytochrome *c*-550 in 0.1 M potassium phosphate buffer, pH 7.5. The reaction was initiated by the addition of the enzyme, and the reaction was monitored by the increase in absorbance of cytochrome *c*-550 at 420 nm (ϵ = 60 000 M^{-1} cm^{-1}). Methylamine dehydrogenase was assayed with PES and DCIP as described previously (Davidson, 1990).

Anaerobic experiments were performed using a Thunberg-type cuvette with an evacuation tube and ground-glass pouch having stopcock function. The cuvette was connected by a Firestone rapid purge valve (Ace Glass, Vineland, NJ) to a vacuum pump and a source of oxygen-free argon. Using the valve, it was possible to alternate cycles of evacuation and flushing of the cuvette with argon. Such cycling was repeated for approximately 25 min prior to each assay, which was performed under an argon atmosphere.

The figures in this paper contain raw data from representative experiments. To construct figures, data from spectrophotometric assays were collected and converted to ASCII files with software provided by the manufacturer (Milton Roy, Rochester, NY), edited with PC-Write (Quicksoft, Inc., Seattle, WA), and plotted with Tech Graph Pad (Binary Engineering Software, Inc., Waltham, MA).

Results

Effect of Oxygen on Methanol Dehydrogenase Activities. Before discussing the results of this study, it is necessary to comment on the assay of methanol dehydrogenase. As noted above, a complicating characteristic of methanol dehydrogenases is that they exhibit activity in the absence of added substrate. This endogenous activity is not lost on dialysis or gel filtration of the enzyme. The endogenous activity is often included when calculating rates of reaction in the presence of substrate. We have chosen to completely separate these activities. In the studies described below, two sets of experiments have routinely been performed. In one set, no substrate was included, and only the endogenous reaction studied. In the second set, a sufficiently high concentration of KCN was included in the assay mixture to completely inhibit the endogenous activity and to allow the assay of only methanol-dependent activity.

Methanol dehydrogenase has been assayed routinely under aerobic conditions as oxygen was not believed to participate in its reaction, and PES is the most commonly used electron acceptor for this enzyme. When methanol dehydrogenase was assayed in the present study with PES under aerobic and

Table I: Variation in Levels of Methanol Dehydrogenase Activities under Aerobic and Anaerobic Conditions

activity[a]	reaction rate (units/mg of protein) aerobic	anaerobic
endogenous	1.8 ± 0.1	2.5 ± 0.1
methanol-dependent	2.4 ± 0.4	4.8 ± 0.7

[a] Activities were assayed with PES and DCIP as described under Experimental Procedures. Values are means ± standard deviation for a minimum of three experiments.

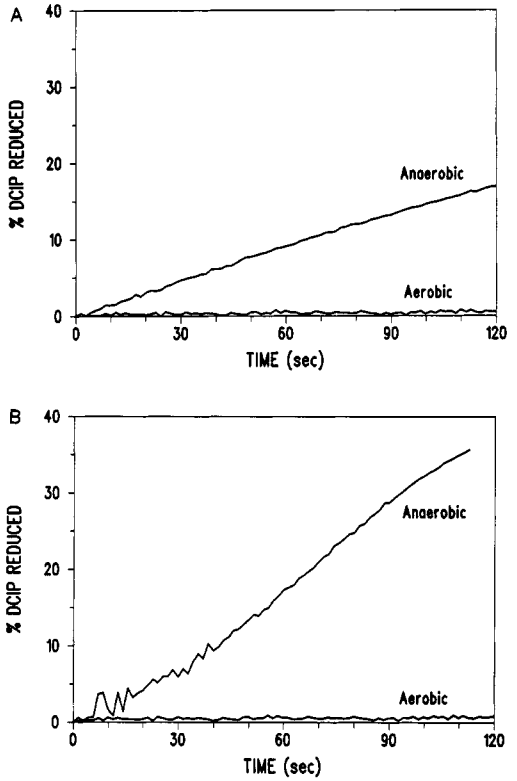

FIGURE 2: Oxygen-dependent inhibition by SOD of methanol dehydrogenase activities. (A) The endogenous activity of methanol dehydrogenase was assayed as described under Experimental Procedures, except that 200 units of CuZnSOD were included in the reaction mixture. (B) The methanol-dependent activity of methanol dehydrogenase was assayed as described under Experimental Procedures, except that 400 units of MnSOD were included in the reaction mixture. Assays were performed under aerobic or anaerobic conditions as indicated.

anaerobic conditions, clearly different reaction rates were observed. Activity was not diminished under anaerobic conditions, but surprisingly was enhanced relative to that measured under aerobic conditions. The endogenous activity increased by approximately 40%, and the methanol-dependent activity increased by approximately 100% (Table I).

Oxygen-Dependent Inhibition of Methanol Dehydrogenase by SOD. It should be noted that because the assay of methanol-dependent activity required the presence of KCN, it was not possible to use CuZnSOD, which is inhibited by cyanide. For that reason, MnSOD was used in studies in which KCN was present. When methanol dehydrogenase was assayed under aerobic conditions in the presence of SOD, significant inhibition was observed. This was true both for the endogenous and for the methanol-dependent activities. In contrast to the complete inhibition which was observed by SOD under aerobic conditions, essentially no inhibition of methanol dehydrogenase was observed under anaerobic conditions (Figure 2 and Table II). To ascertain whether the inhibition by SOD under aerobic conditions was due specifically to removal of superoxide

Table II: Effects of Various Agents on Methanol Dehydrogenase Activities

additions	% inhibition of activity[a] endogenous aerobic	endogenous anaerobic	methanol-dependent aerobic	methanol-dependent anaerobic
CuZnSOD (200 units)	97	8		
MnSOD (400 units)			98	0
catalase (1800 units)	5		0	
sodium formate (0.3 M)	0		0	
$MnCl_2$ (0.1 mM)	99	17	98	13
$MgCl_2$ (1 mM)	0		3	
$CaCl_2$ (1 mM)	0		3	
$ZnCl_2$ (1 mM)	0		0	
$CdCl_2$ (1 mM)	4		0	

[a] Activities were assayed with PES and DCIP as described under Experimental Procedures. The values corresponding to 100% activity in each of the four columns are those reported in Table I.

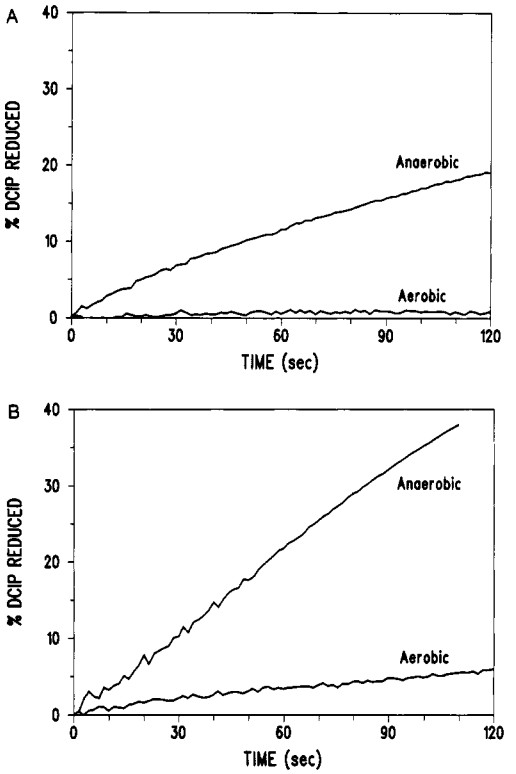

FIGURE 3: Oxygen-dependent inhibition by $MnCl_2$ of methanol dehydrogenase activities. The endogenous (A) and methanol-dependent (B) activities of methanol dehydrogenase were assayed as described under Experimental Procedures, except that 0.1 mM $MnCl_2$ was included in the reaction mixtures. Assays were performed under aerobic or anaerobic conditions as indicated.

from the reaction mixture, the effects of catalase and formate on methanol dehydrogenase activity were also examined (Table II). Addition of up to 1800 units of catalase or 0.3 M formate caused no significant inhibition of methanol dehydrogenase activity. This suggested that the observed inhibition by SOD was due specifically to removal of superoxide and not hydrogen peroxide or hydroxyl radical which would have been removed from the reaction mixture by catalase and formate, respectively.

Oxygen-Dependent Inhibition of Methanol Dehydrogenase by Mn. Like SOD, aqueous Mn^{2+} is also known to catalyze the dismutation of superoxide (Archibald & Fridovich, 1982). As such, the effects of added $MnCl_2$ on methanol dehydrogenase activity were examined. Results which were essentially identical to those obtained in the presence of SOD were observed in the presence of 0.1 mM $MnCl_2$ (Figure 3).

Both the endogenous and methanol-dependent activities of the enzyme were inhibited under aerobic, but not under anaerobic, conditions. The effects on methanol dehydrogenase activity of several other metals, which were of similar ionic radii but unable to catalyze superoxide dismutation, were also tested (Table II). Addition of these metals to the reaction mixture at concentrations up to 10-fold greater than Mn caused no significant inhibition of either the endogenous or the methanol-dependent activities under aerobic conditions. This inhibition, specifically by Mn, provides additional evidence that the inhibitory effects of SOD were due to superoxide dismutase activity and not some nonspecific effect due to interactions between SOD and methanol dehydrogenase, or any other component of the reaction mixture.

Insensitivity of the Quinoprotein Methylamine Dehydrogenase to SOD and Mn. To determine whether the above-mentioned effects of SOD and Mn were specific for methanol dehydrogenase, or a more general phenomenon, similar experiments were performed with another quinoprotein, the methylamine dehydrogenase from *P. denitrificans* (Husain et al., 1987). This enzyme, which catalyzes the oxidation of primary amines to their corresponding aldehydes, possesses tryptophan tryptophylquinone (TTQ), an *o*-quinone similar to PQQ, as its prosthetic group (McIntire et al., 1991; Chen et al., 1991). This enzyme is also routinely assayed in vitro using PES and DCIP as electron acceptors (Davidson, 1990). Incubation of methylamine dehydrogenase with SOD or $MnCl_2$ at concentrations which completely inhibited methanol dehydrogenase caused no inhibition of methylamine-dependent DCIP reduction by methylamine dehydrogenase and PES under aerobic conditions (data not shown). This suggests that the effects of SOD on methanol dehydrogenase are not a general feature of quinoproteins but a phenomenon peculiar to that enzyme. Furthermore, as the assay of methylamine dehydrogenase also employs PES and DCIP as electron acceptors, these data rule out the possibility that the observed effects on methanol dehydrogenase were due to factors affecting the transfer of electrons between PES and DCIP.

Assay of Methanol Dehydrogenase with Electron Acceptors Other than PES. Having established that the observed oxygen- and superoxide-dependent effects on methanol dehydrogenase activity were not an artifact related to the transfer of electrons from PES to DCIP, it was of interest to determine whether oxygen was interfering with the transfer of electrons from the reduced cofactor to PES. To examine this, the activity of methanol dehydrogenase was assayed with alternative electron acceptors to PES. In contrast to the results which were obtained with PES, the activity of the enzyme, when assayed with Wurster's Blue, was not inhibited by SOD or $MnCl_2$ and was essentially identical when assayed under aerobic and anaerobic conditions. The activity of methanol dehydrogenase was also assayed with its physiological electron acceptors cytochrome *c*-551i and cytochrome *c*-550. Neither SOD nor $MnCl_2$ affected the observed rates of methanol dehydrogenase-dependent cytochrome reduction. These data suggest that oxygen is interfering with the transfer of electrons from the enzyme-bound PQQ to PES and that this effect is enhanced on removal of superoxide by SOD or Mn.

Spectroscopic Studies. In an effort to determine whether the redox state of the PQQ cofactor of methanol dehydrogenase was being perturbed by the presence of oxygen or superoxide, a series of difference spectra were recorded. The visible absorption spectrum of *P. denitrificans* methanol dehydrogenase, as isolated (Figure 4), was very similar to that described by Frank et al. (1989) for the semiquinone form of

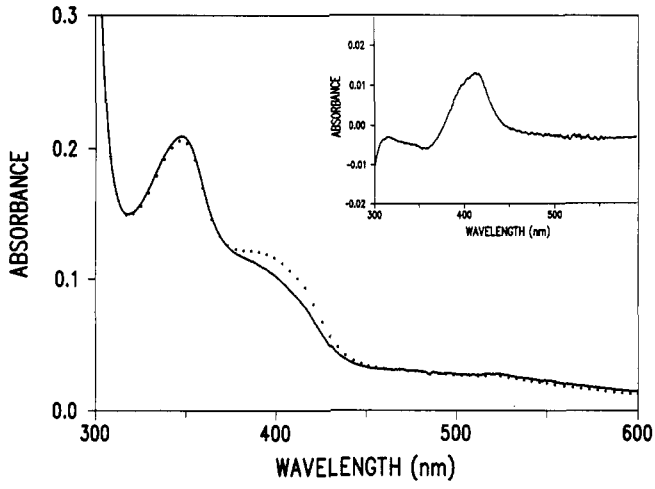

FIGURE 4: Absorption spectra of methanol dehydrogenase. The absorption spectrum of methanol dehydrogenase (0.8 mg/mL) was recorded in 0.1 M potassium phosphate, pH 9.0, containing 6.75 mM ammonium sulfate. Spectra were recorded in the absence (solid line) and presence (dotted line) of 6 mM KCN. A difference spectrum is shown in the inset.

the enzyme. No significant change in this spectrum was observed on addition of methanol. On addition of KCN, some increase in absorbance of the shoulder at 400 nm was observed (Figure 4). The visible absorption spectrum of methanol dehydrogenase was recorded under aerobic and anaerobic conditions, and in the presence and absence of SOD and of $MnCl_2$. These experiments were performed with enzyme incubated with and without KCN. No significant oxygen-, SOD-, or Mn-dependent changes in the spectrum of methanol dehydrogenase were observed. Under all conditions, spectra which were essentially identical to those shown in Figure 4 were obtained. Thus, no evidence was obtained to suggest that any oxygen- or superoxide-dependent changes in the redox state of PQQ had occurred.

Discussion

SOD catalyzes the dismutation of O_2^- to H_2O_2 and O_2 (Fridovich, 1975). Inhibition of a process by SOD is usually taken as strong evidence for a critical role for O_2^- in that process. That methanol dehydrogenase was not known to utilize oxygen as a substrate and is active under anaerobic conditions is in apparent contradiction to the observed inhibition by SOD under aerobic conditions. Oxygen-dependent inhibition by SOD has been observed in the reactions of certain semiquinones (Winterbourn et al., 1987; Winterbourn, 1981). To explain those phenomena, it was proposed that the active semiquinone and inactive quinone species were in reversible equilibrium with O_2 and O_2^- and that removal of O_2^- by SOD caused complete conversion to the quinone form. Those studies suggested that by such a process SOD was able to effectively, albeit indirectly, lower the concentrations of reactive intermediates other than O_2^-. The oxygen-dependent inhibition of methanol dehydrogenase by SOD which was observed when the enzyme was assayed using PES would appear to represent another example of such a process. This would be consistent with the observed lack of SOD inhibition under anaerobic conditions, as well as the observed higher levels of activity under anaerobic conditions relative to aerobic conditions. It would not, however, explain the observation that the enzyme is only inhibited when PES is used as an electron acceptor. This suggests instead that while O_2 and O_2^- are probably reacting reversibly with the enzyme-bound PQQ, O_2 may also be competing with PES as an electron acceptor for the enzyme.

The reduced and semiquinone forms of free PQQ are known to react with O_2 to form O_2^- in solution (Duine et al., 1987). No such reactions, however, have been reported with protein-bound PQQ in quinoprotein dehydrogenases. Our data suggest that this may be happening with the enzyme-bound PQQ in methanol dehydrogenase, as shown in eq 1–3. Data

$$E\text{–}PQQ + CH_3OH \rightarrow E\text{–}PQQH_2 + CH_2O \quad (1)$$

$$E\text{–}PQQH_2 + O_2 \rightleftharpoons E\text{–}PQQH^{\bullet} + H^+ + O_2^- \quad (2)$$

$$E\text{–}PQQH^{\bullet} + O_2 \rightleftharpoons E\text{–}PQQ + H^+ + O_2^- \quad (3)$$

suggest that the latter two reactions are normally at equilibrium and do not result in a net turnover of the redox state of the enzyme. These reactions with oxygen could also explain certain peculiar redox properties of the enzyme, such as its apparent isolation as a stable semiquinone species, and the lack of spectral change on addition of methanol. If the reaction of $PQQH_2$ with oxygen were to occur much faster than the reaction of $PQQH_2$ with PES, then inhibition could be observed in the presence of SOD, which would remove O_2^-, altering the normal equilibrium, and thus allowing O_2 to effectively compete with PES. As this is not observed with Wurster's Blue or cytochrome *c*-551i, the rates of electron transfer from $PQQH_2$ to these electron acceptors must be significantly faster than to O_2, and very much faster than to PES.

If PQQ is in direct equilibrium with oxygen and superoxide, it would be reasonable to expect to have observed some change in the visible absorption spectrum of the enzyme when that equilibrium was perturbed either by addition of SOD or $MnCl_2$ or by removal of air. This was not observed. It is possible that the apparent lack of spectral perturbation could be due to protein–PQQ interactions which mask such changes. It is also possible that protein–PQQ interactions can stabilize the semiquinone redox state of the enzyme which is formed after exposure to oxygen, so that subsequent removal of oxygen or superoxide has no apparent effect.

Quinoprotein oxidases such as plasma amine oxidase have been shown to possess quinones other than PQQ. For this enzyme, the prosthetic group is topaquinone (Janes et al., 1990). On the other hand, all of the enzymes which are known for certain to utilize PQQ are dehydrogenases. This study is the first to suggest that an enzyme-bound PQQ cofactor can be oxidized by O_2. This phenomenon was only observable in the present study because the rate of electron transfer from PQQ to O_2 in methanol dehydrogenase is significantly faster than to PES. The autoxidation of free quinones in solution is a well-known phenomenon, and the rates of autoxidation of quinones are strongly influenced by the nature of the substituents bound to the quinone (Buffinton et al., 1989). Another quinoprotein dehydrogenase, methylamine dehydrogenase, which possesses not PQQ but TTQ (McIntire et al., 1991; Chen et al., 1991), did not exhibit any oxygen- or superoxide-dependent effects on activity when assayed with PES. This suggests that the relative rates of electron transfer from the prosthetic group to PES and O_2 are different for TTQ and PQQ. It may also reflect differences in the active-site environment of the quinone prosthetic groups in each protein which influence the reactivity of the quinone with oxygen. It would be of interest to determine whether the phenomena which have been described for this methanol dehydrogenase are limited to methanol dehydrogenase or also observed with other PQQ-bearing quinoprotein dehydrogenases such as glucose dehydrogenase. The finding that PQQ in methanol dehydrogenase can react with oxygen must also be recognized as an important factor in evaluating possible reaction mechanisms for this enzyme.

ACKNOWLEDGMENTS

We thank Elizabeth Graichen, Brett Miller, Limei Hsu Jones, and Harold B. Brooks for technical assistance and helpful discussions. We also thank one of the reviewers for suggesting the possibility that oxygen could be competing with PES in these assays.

REFERENCES

Anthony, C. (1982) *The Biochemistry of Methylotrophs*, Academic Press, New York.
Anthony, C. (1986) *Adv. Microbiol. Physiol. 27*, 113–210.
Anthony, C., & Zatman, L. J. (1964) *Biochem. J. 92*, 614–621.
Archibald, F. S., & Fridovich, I. (1982) *Arch. Biochem. Biophys. 214*, 452–463.
Buffinton, G. D., Ollinger, K., Brunmark, A., & Cadenas, E. (1989) *Biochem. J. 257*, 561–571.
Chen, L., Mathews, F. S., Davidson, V. L., Huizinga, E., Vellieux, F. M. D., Duine, J. A., & Hol, W. G. J. (1991) *FEBS Lett. 287*, 163–166.
Davidson, V. L. (1990) *Methods Enzymol. 188*, 241–246.
Duine, J. A., & Frank, J. (1980) *Biochem. J. 187*, 213–219.
Duine, J. A., Frank, J., & Jongejan, J. K. (1987) *Adv. Enzymol. Relat. Areas Mol. Biol. 59*, 169–212.
Flohe, L., & Otting, F. (1984) *Methods Enzymol. 105*, 93–104.
Frank, J., Dijkstra, M., Balny, C., Verwiel, P. E. J., & Duine, J. A. (1989) in *PQQ and Quinoproteins* (Jongejan, J. A., & Duine, J. A., Eds.) pp 239–241, Kluwer Academic Publishers, Dordrecht, The Netherlands.
Fridovich, I. (1975) *Annu. Rev. Biochem. 44*, 147–159.
Ghosh, R., & Quail, J. R. (1981) *Biochem. J. 199*, 245–250.
Husain, M., & Davidson, V. L. (1986) *J. Biol. Chem. 261*, 8577–8580.
Husain, M., Davidson, V. L., Gray, K. A., & Knaff, D. B. (1987) *Biochemistry 26*, 4139–4143.
Janes, S. M., Mu, D., Wemmer, D., Smith, A. J., Kaur, S., Mautry, D., Burlingame, A. L., & Klinman, J. P. (1990) *Science 248*, 981–987.
McIntire, W. S., Wemmer, D. E., Christoserdov, A. Y., & Lidstrom, M. E. (1991) *Science 252*, 817–824.
Michaelis, L., & Granick, S. (1943) *J. Am. Chem. Soc. 65*, 1747–1755.
Salisbury, S. A., Forrest, H. A., Cruse, W. B. T., & Kennard, O. (1979) *Nature 280*, 843–844.
Winterbourn, C. C. (1981) *FEBS Lett. 136*, 89–94.
Winterbourn, C. C., French, J. K., & Claridge, R. F. C. (1978) *FEBS Lett. 94*, 269–272.