

Monomeric Sarcosine Oxidase: 2. Kinetic Studies with Sarcosine, Alternate Substrates, and a Substrate Analogue[†]

Mary Ann Wagner and Marilyn Schuman Jorns*

Department of Biochemistry, MCP Hahnemann School of Medicine, Philadelphia, Pennsylvania 19129

Received February 15, 2000; Revised Manuscript Received April 21, 2000

ABSTRACT: Monomeric sarcosine oxidase (MSOX) is a flavoenzyme that catalyzes the oxidative demethylation of sarcosine (*N*-methylglycine) to yield glycine, formaldehyde, and hydrogen peroxide. MSOX can oxidize other secondary amino acids (*N*-methyl-L-alanine, *N*-ethylglycine, and L-proline), but *N,N*-dimethylglycine, a tertiary amine, is not a substrate. *N*-Methyl-L-alanine is a good alternate substrate, exhibiting a k_{cat} value (8700 min^{-1}) similar to sarcosine (7030 min^{-1}). Turnover with L-proline ($k_{\text{cat}} = 25 \text{ min}^{-1}$) at 25°C occurs at less than 1% of the rate observed with sarcosine. MSOX is converted to a two-electron reduced form upon anaerobic reduction with sarcosine or L-proline. No evidence for a spectrally detectable intermediate was obtained in reductive half-reaction studies with L-proline. The reductive half-reaction with L-proline at 4°C exhibited saturation kinetics ($k_{\text{lim}} = 6.0 \text{ min}^{-1}$, $K_d = 260 \text{ mM}$) and other features consistent with a mechanism in which a practically irreversible reduction step ($\text{E}_{\text{ox}}\cdot\text{S} \rightarrow \text{E}_{\text{red}}\cdot\text{P}$) with a rate constant, k_{lim} , is preceded by a rapidly attained equilibrium (K_d) between free E and the E·S complex. Steady-state kinetic studies with sarcosine and *N*-methyl-L-alanine in the absence or presence of a dead-end inhibitor (pyrrole-2-carboxylate) indicate that catalysis proceeds via a “modified” ping pong mechanism in which oxygen reacts with $\text{E}_{\text{red}}\cdot\text{P}$ prior to the dissociation of the imino acid product. In this mechanism, double reciprocal plots will appear nearly parallel (as observed) if the reduction step is nearly irreversible. A polar mechanism, involving formation of a covalent 4a-flavin–substrate adduct is one of several plausible mechanisms for sarcosine oxidation. Thiols are known to form similar 4a-flavin adducts. MSOX does not form a 4a-adduct with thioglycolate but does form a charge-transfer complex that undergoes an unanticipated one-electron-transfer reaction to yield the anionic flavin radical.

Monomeric sarcosine oxidase (MSOX)¹ converts sarcosine to glycine and formaldehyde. Sarcosine is a common soil metabolite and induces MSOX expression in various bacteria grown with sarcosine as sole source of carbon and energy (*1*). MSOX contains covalently bound flavin [8α -(*S*-cysteiny)FAD] and is a member of a family of enzymes that contain covalently bound flavin and catalyze similar oxidation reactions with amine substrates. Other family members

also binds various heterocyclic carboxylic acids, including pyrrole-2-carboxylate, the aromatic analogue of proline (*6*).

In this paper, we show that MSOX can oxidize other secondary amines, including L-proline, but tertiary amines are not substrates. The mechanism of MSOX catalysis has been investigated in steady-state and reductive half-reaction studies with sarcosine, other substrates, and a substrate analogue.



include *N*-methyltryptophan oxidase, pipecolate oxidase, and heterotetrameric sarcosine oxidase (*2–4*). The crystal structure of MSOX from *Bacillus sp. B-0618* has recently been determined (*5*). Studies with various sarcosine analogues strongly suggest that MSOX binds amino acids in their zwitterionic form and that the carboxylate group is essential for binding. The amino and methyl groups in sarcosine are not essential but do contribute to binding affinity. MSOX

EXPERIMENTAL PROCEDURES

Materials. Horseradish peroxidase, glucose oxidase (*Aspergillus niger*, type V-S) *o*-dianisidine, *N,N'*-dimethylglycine, methyl viologen, *N*-methyl-L-alanine, thioglycolic acid, pyrrole-2-carboxylic acid, and L-proline were purchased from Sigma. Sarcosine and *N*-ethylglycine were obtained from Aldrich.

Enzyme Purification and Assay. Purification of recombinant MSOX from *Bacillus sp. B-0618* and routine protein and activity assays were performed as described by Wagner et al. (*2*). Absorption spectra were recorded using a Perkin-Elmer Lambda 2S spectrometer. Enzyme concentration was determined using the previously determined extinction coefficient ($\epsilon_{454} = 12\,200 \text{ M}^{-1} \text{ cm}^{-1}$, pH 8.0) (*2*).

Anaerobic Experiments. Anaerobic experiments were conducted in a total volume of 500 μL , similar to that

[†] This work was supported in part by Grant GM 31704 (M.S.J.) from the National Institutes of Health.

* To whom requests for reprints should be addressed. Phone: (215) 991-8580; fax: (215) 843-8849; E-mail: marilyn.jorns@drexel.edu.

¹ Abbreviations: MSOX, monomeric sarcosine oxidase; FAD, flavin adenine dinucleotide.

described by Wagner and Jorns (6) except that reaction mixtures contained an oxygen-scavenging system consisting of glucose oxidase (8.4 U/mL), glucose (8 mM), and catalase (215 U/mL).

Steady-State Kinetics. Studies were conducted by monitoring hydrogen peroxide formation using a horseradish peroxidase-coupled assay. Reactions were performed at 25 °C in 100 mM potassium phosphate buffer, pH 8.0, containing amino acid, oxygen, and inhibitor, as indicated, 320 μ M *o*-dianisidine, and 1.8 U horseradish peroxidase in a total volume of 350 μ L. Assays were initiated by the addition of MSOX. Formation of oxidized *o*-dianisidine was monitored by the increase in absorbance at 460 nm ($\epsilon_{460} = 6765 \text{ M}^{-1} \text{ cm}^{-1}$). A standard semi-microcuvette was used for reactions in air-saturated buffer.

A special cuvette (Spectrocell) with a screw-cap equipped with a Teflon-silicone membrane was used in studies in which the oxygen concentration was varied. Gas mixtures containing 10.2%, 21%, 44%, or 100% oxygen (balance nitrogen) were passed through a gas washing bottle containing water at 25 °C and then split into four streams using a set of Hamilton valves that were connected, via luer-lock adaptors, to 28-gauge stainless steel needles. A screw-cap cuvette was filled with 340 μ L of reaction mixture containing all components except horseradish peroxidase and MSOX. The sealed cuvette was equilibrated to the desired oxygen concentration at 25 °C by bubbling for 15 min, using a second 28-gauge needle as an outlet vent. Horseradish peroxidase (5 μ L) was injected into the cuvette using a gastight Hamilton syringe. After the sample was mixed, the cuvette was placed into a thermostated cell holder of a Perkin-Elmer λ 3B spectrophotometer for a 2-min incubation at 25 °C. Reactions were initiated by injecting a 5- μ L aliquot of MSOX. Oxygen concentrations were calculated based on the dissolved oxygen concentration obtained after equilibration with 100% oxygen (1.3 mM) (7) and were corrected for the 10- μ L addition of enzymes in air-saturated buffer.

Data Analysis. Data were fit to eqs 1–4 using the curve fit function in Sigma Plot (Jandel Corporation). Equation 1 was used for (i) analysis of steady-state kinetic data in which amino acid substrate was varied at a single fixed oxygen concentration in air-saturated buffer. Y and A are the observed and apparent maximal turnover rates, X is the concentration of the varied substrate, and K is the apparent K_m , and (ii) analysis of the apparent first-order rate of enzyme reduction by L-proline (k_{obs}) as a function of the concentration of L-proline. Y and A are k_{obs} and k_{lim} , respectively. X is the L-proline concentration, and K is the dissociation constant of the E•S complex. Equation 2 was used to fit the first-order reduction kinetics with L-proline or thioglycolate ($k_{\text{obs}} = k$). Y is the observed absorbance at the selected wavelength and time = t , A is the maximal absorbance change, and B is the final absorbance at the selected wavelength. Equation 3 was used to fit the steady-state kinetic data obtained at various concentrations of sarcosine (or *N*-methyl-L-alanine) and oxygen. K_a and K_b are the K_m values for amino acid and oxygen, respectively. K_{ia} is the dissociation constant for MSOX•amino acid complex. Steady-state kinetic data obtained at a fixed sarcosine concentration and various concentrations of oxygen and pyrrole-2-carboxylate were fit to eq 4 using values determined for K_a , K_b , and K_{ia} in the absence of inhibitor. K_i is the dissociation constant for the

Table 1: Substrate Specificity Profile for MSOX^a

| compound | $k_{\text{cat}} (\text{app})$ (min^{-1}) | $K_m (\text{app})$ (mM) | k_{cat}/K_m $\text{mM}^{-1} \text{min}^{-1}$ |
|------------------------------|--|----------------------------|--|
| sarcosine | 2730 \pm 32 | 4.5 \pm 0.1 | 610 |
| <i>N</i> -methyl-L-alanine | 710 \pm 10 | 3.3 \pm 0.2 | 220 |
| <i>N</i> -ethyl-glycine | 2650 \pm 100 | 260 \pm 10 | 10 |
| <i>N,N</i> -dimethyl-glycine | no turnover | - | - |
| L-proline | 25 \pm 0.9 | 130 \pm 10 | 0.19 |

^a Apparent steady-state kinetic parameters were determined in air-saturated buffer (100 mM potassium phosphate, pH 8.0, at 25 °C). The values obtained with L-proline, however, represent true kinetic parameters since the oxygen concentration under these conditions is saturating for this substrate (G. Zhao and M. S. Jorns, unpublished results), as discussed in the text.

MSOX•pyrrole-2-carboxylate complex, and I is inhibitor concentration.

$$Y = \frac{AX}{X + K} \quad (1)$$

$$Y = Ae^{-kt} + B \quad (2)$$

$$v = \frac{V_{\text{max}}AB}{K_{ia}K_b + K_aB + K_bA + AB} \quad (3)$$

$$v = \frac{V_{\text{max}}AB}{(1 + I/K_i)(K_{ia}K_b + K_aB) + K_bA + AB} \quad (4)$$

RESULTS

Substrate Specificity. A substrate specificity profile for MSOX was constructed by determining apparent steady-state kinetic parameters with sarcosine and various analogues in air-saturated solution (Table 1). *N*-Methyl-L-alanine is a fairly good substrate for MSOX, exhibiting an apparent catalytic efficiency that is about one-third of that observed with sarcosine [$k_{\text{cat}} (\text{app})/K_m (\text{app}) = 220$ versus $610 \text{ mM}^{-1} \text{ min}^{-1}$]. The MSOX active site is therefore able to accommodate a methyl group in place of one of the α -hydrogens in sarcosine. Replacing the *N*-methyl group in sarcosine with an ethyl substituent is considerably less well-tolerated as judged by the 60-fold decrease in apparent catalytic efficiency, an effect entirely due to an increase in the apparent K_m . The lowest catalytic efficiency was observed with the cyclic analogue,

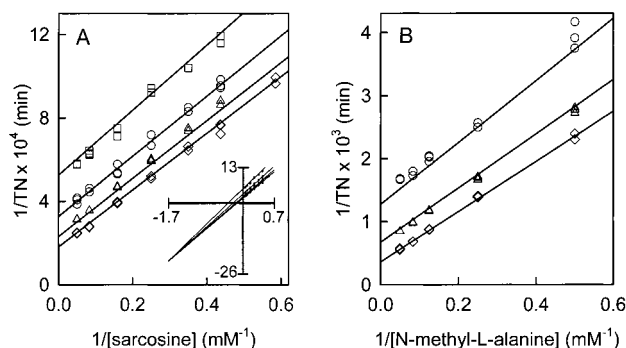
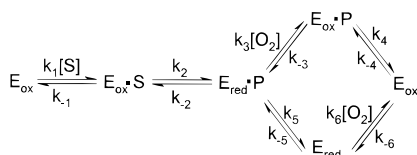


FIGURE 1: Steady-state kinetic data for MSOX with sarcosine (panel A) or *N*-methyl-L-alanine (panel B) as substrate. Measurements were made at 25 °C in 100 mM potassium phosphate buffer, pH 8.0, at various oxygen concentrations [1.27 (◇), 0.56 (△), 0.27 (○), and 0.13 mM (□)]. The lines represent fits of each data set to eq 3. The convergence of the fitted lines is illustrated in the inset of panel A where the sarcosine data have been replotted according to a different scale.

Scheme 1: “Modified” and Classic Ping Pong Mechanisms Are Illustrated by the Upper and Lower Loops, Respectively



L-proline [$k_{\text{cat}}(\text{app})/K_{\text{m}}(\text{app}) = 0.19 \text{ mM}^{-1} \text{ min}^{-1}$]. No effect on the rate of L-proline oxidation was observed when the oxygen concentration was varied from 0.14 to 1.3 mM at L-proline concentrations in the range of 31 to 180 mM (G. Zhao and M. S. Jorns, unpublished results). This means that the K_{m} value for oxygen is much less than the oxygen concentration in air-saturated buffer (0.27 mM) and that kinetic parameters for saturating oxygen are obtained with L-proline under these conditions. MSOX is unable to oxidize tertiary amines, as judged by the results obtained with *N,N*-dimethylglycine although the compound is bound at the active site (6).

Steady-State Kinetics with Sarcosine. Double-reciprocal plots of reaction rate versus sarcosine concentration at various oxygen concentrations are linear and almost parallel (Figure 1A). Linear regression analysis of the data at each oxygen concentration, however, showed a systematic variation in slope with oxygen concentration. This is not expected for a “classic” ping pong mechanism in which the first product dissociates prior to reaction of oxygen with the reduced enzyme. The results are consistent with a “modified” ping pong mechanism in which oxygen reacts with the reduced enzyme prior to the release of the first product (Scheme 1). The rate equation for a modified ping pong mechanism (eq 3) contains a $K_{\text{ia}}K_{\text{b}}$ term that is not present in case of a classic ping pong mechanism. The steady-state kinetic parameters obtained by fitting the data to eq 3 are similar to those estimated by linear regression analysis (Table 2).

Steady-State Kinetics with *N*-Methyl-L-alanine. Further evidence to distinguish between a classic versus a modified ping pong mechanism was sought in studies with a different amino acid substrate. In the case of a classic ping pong mechanism, the ratio $k_{\text{cat}}/K_{\text{m oxygen}}$ should be independent of the nature of the amino acid substrate, a feature not expected for a modified ping pong mechanism (8). The slopes of

Table 2: Steady-State Kinetic Parameters for MSOX with Different Amino Acid Substrates^a

| parameter | amino acid substrate | |
|--|------------------------|----------------------------|
| | sarcosine | <i>N</i> -methyl-L-alanine |
| k_{cat} (min^{-1}) | 7030 ± 180 (6650) | 8700 ± 1400 |
| $K_{\text{m amino acid}}$ (mM) | 9.4 ± 0.4 (8.9) | 33 ± 7 |
| $K_{\text{m oxygen}}$ (mM) | 0.35 ± 0.02 (0.31) | 2.7 ± 0.6 |
| $K_{\text{d amino acid}}$ (mM) | 0.6 ± 0.3 (0.6) | 1 ± 0.5 |
| $k_{\text{cat}}/K_{\text{m oxygen}}$ ($\text{mM}^{-1} \text{ min}^{-1}$) | 20 000 | 3200 |
| $k_{\text{cat}}/K_{\text{m amino acid}}$ ($\text{mM}^{-1} \text{ min}^{-1}$) | 750 | 260 |

^a Parameters were determined by fitting the data to an equation for a modified ping pong mechanism (eq 3), except for values shown in parentheses. The latter were determined by linear regression analysis of double reciprocal plots, as described in the text.

double-reciprocal plots of reaction rate versus *N*-methyl-L-alanine concentration varied depending on the oxygen concentration (Figure 1B). The data were fit to the equation for a modified ping pong mechanism. The k_{cat} value obtained with *N*-methyl-L-alanine ($8700 \pm 1400 \text{ min}^{-1}$) is similar to that observed with sarcosine ($7030 \pm 180 \text{ min}^{-1}$), but the K_{m} for oxygen is considerably larger ($2.7 \pm 0.6 \text{ mM}$ versus $0.35 \pm 0.02 \text{ mM}$). The ratio $k_{\text{cat}}/K_{\text{m oxygen}}$ with sarcosine is more than 6-fold larger than the value obtained with *N*-methyl-L-alanine (Table 2), consistent with a modified ping pong mechanism.

Inhibition Studies with a Dead-End Inhibitor. Additional evidence for a modified ping pong mechanism was sought in studies with pyrrole-2-carboxylate. Pyrrole-2-carboxylate forms a spectrally detectable complex with MSOX and acts as a dead-end competitive inhibitor with respect to sarcosine (6). In the case of a modified ping pong mechanism, this compound should act as a noncompetitive inhibitor with respect to oxygen whereas uncompetitive inhibition is expected for a classic ping pong mechanism (9). Double-reciprocal plots of velocity versus oxygen concentration at different concentrations of pyrrole-2-carboxylate are linear. The slopes and intercepts varied depending on the inhibitor concentration. The data were fit to an equation (eq 4) for a modified ping pong mechanism in the presence of a dead-end inhibitor that combines only with E (Figure 2). The value obtained for the inhibition constant ($K_{\text{i}} = 1.29 \pm 0.03 \text{ mM}$) is in excellent agreement with the K_{d} value obtained for MSOX-pyrrole-2-carboxylate complex by spectral titration ($K_{\text{d}} = 1.37 \text{ mM}$) (6).

Anaerobic Reduction with Sarcosine or L-Proline. MSOX is converted to a two-electron reduced form immediately upon mixing with excess sarcosine under anaerobic conditions, as judged by the characteristic bleaching of the oxidized flavin spectrum in a reaction that is fully reversible upon aeration of the sample. The spectrum observed for sarcosine-reduced MSOX is superimposable with a spectrum obtained after reduction with L-proline, a poor alternate substrate (Figure 3A, inset). Evidence for the formation of an intermediate during the reductive half-reaction was sought by monitoring the anaerobic reduction of the enzyme with a low concentration of L-proline. Under these conditions, the entire spectral course of the reaction could be monitored in a simple manual mixing experiment. No intermediate was detected upon reaction with 0.21 mM L-proline at 25 °C, as

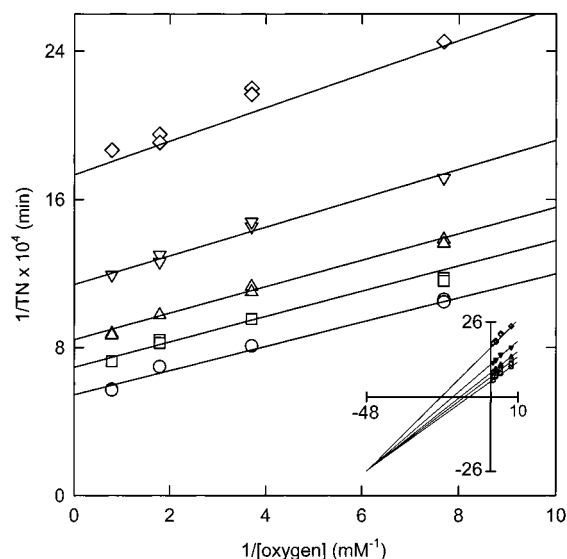


FIGURE 2: Pyrrole-2-carboxylate inhibition with respect to oxygen. Measurements were made at 4.0 mM sarcosine, the indicated oxygen concentrations, and the following inhibitor concentrations: 0 (○), 0.5 (□), 1.0 (△), 2.0 (▽), and 4.0 mM (◇). The lines represent a fit of the data set to eq 4. Data are replotted according to a different scale in the inset to illustrate the convergence of the fitted lines.

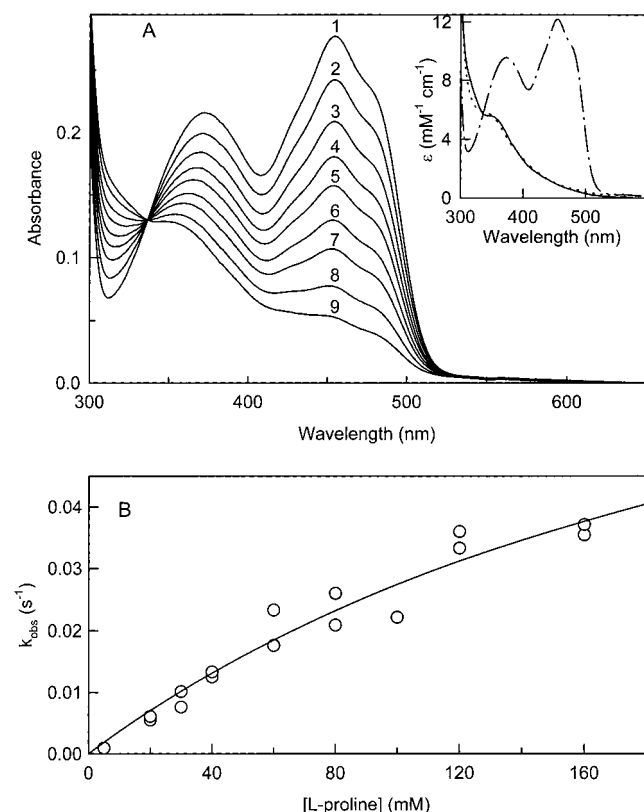


FIGURE 3: Reduction of MSOX with sarcosine or L-proline. Reactions were conducted under anaerobic conditions in 50 mM potassium phosphate buffer, pH 8.0, at 25 °C (panel A) or 4 °C (panel B). Panel A: Curve 1 is the initial spectrum of oxidized enzyme (22.7 μ M). Curves 2–9 were recorded 5, 15, 25, 35, 50, 66, 96, and 157 min after adding 0.21 mM L-proline. The inset shows spectra of oxidized enzyme (dot–dashed line) and enzyme reduced with 80 mM sarcosine (solid curve) or 80 mM L-proline (dotted line).

judged by the observed isosbestic conversion of the oxidized to the two-electron reduced enzyme (Figure 3A).

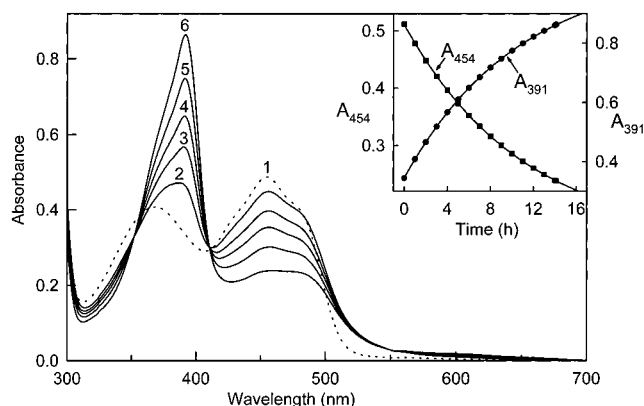


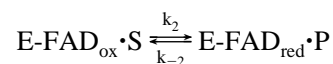
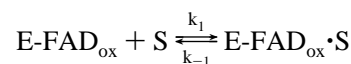
FIGURE 4: Anaerobic reduction of MSOX with thioglycolate. Curve 1 (dotted line) is the spectrum of the uncomplexed enzyme (40.0 μ M) in 50 mM potassium phosphate buffer at pH 9.0 and 25 °C. Curves 2–6 were recorded 2, 4, 6, 9, and 14.1 h after adding 150 mM thioglycolate. Inset: The solid lines show the fit of absorbance changes at 391 and 454 nm to a single-exponential expression.

Reductive Half-Reaction Kinetics with L-Proline. The reductive half-reaction at higher concentrations of L-proline was too fast to monitor in a manual mixing experiment at 25 °C but could be measured at 4 °C. The reduction of the enzyme by L-proline exhibited apparent first-order kinetics over a wide range of substrate concentrations (5–160 mM). A plot of k_{obs} versus L-proline concentration was hyperbolic (Figure 3B) ($k_{\text{lim}} = 6.0 \text{ min}^{-1}$, $K = 260 \text{ mM}$).

One-Electron Reduction of MSOX with Thioglycolate. MSOX binds thioglycolate ($K_d = 7.72 \text{ mM}$), forming an apparently stable charge-transfer complex under aerobic conditions (6). However, under anaerobic conditions, the charge-transfer complex is observed to undergo a slow, one-electron-transfer reaction (Figure 4), which results in the quantitative conversion of oxidized MSOX to the anionic flavin radical, as judged by the development of an intense new absorption band at 391 nm similar to that observed for the radical formation via 5-deazariboflavin-mediated photo-reduction (6). The one-electron reduction reaction with thioglycolate exhibits apparent first-order kinetics ($k_{\text{obs}} = 1.6 \times 10^{-3} \text{ min}^{-1}$, pH 9.0) (Figure 4, inset).

DISCUSSION

In addition to sarcosine, MSOX can oxidize other secondary amines, but *N,N*-dimethylglycine, a tertiary amine, is not a substrate. *N*-methyl-L-alanine is a good alternate substrate, exhibiting a k_{cat} value (8700 min^{-1}) similar to sarcosine (7030 min^{-1}). Turnover with L-proline ($k_{\text{cat}} = 25 \text{ min}^{-1}$) occurs at less than 1% of the rate observed with sarcosine. MSOX is converted to a two-electron reduced form upon anaerobic reduction with sarcosine or L-proline. The reductive half-



reaction with L-proline at 4 °C exhibited saturation kinetics ($k_{\text{lim}} = 6.0 \text{ min}^{-1}$, $K = 260 \text{ mM}$). A double reciprocal plot of the data was linear with a finite y intercept. According to Strickland et al. (10), the data are compatible with a reductive half-reaction where $k_{-2} \approx 0$ and $k_{-1} \gg k_2$. This means that

a practically irreversible reduction step is preceded by a rapidly attained equilibrium between free E and the E•S complex. Under these conditions, k_{lim} is the rate of the reductive step (k_2), and K is the dissociation constant for the E•S complex. The 4-fold lower rate observed for the reductive half-reaction at 4 °C, as compared with turnover at 25 °C (6 versus 25 min⁻¹) is likely due to the 21 °C temperature difference in the two sets of experiments. The results suggest that the reductive half-reaction with L-proline is rate-limiting. Steady-state kinetic studies conducted with sarcosine and N-methyl-L-alanine in the absence or presence of a dead-end inhibitor (pyrrole-2-carboxylate) indicate that catalysis proceeds via a modified ping pong mechanism in which oxygen reacts with E-FAD_{red}•P prior to the dissociation of the imino acid product. In this mechanism, double reciprocal plots will appear nearly parallel if the $K_{\text{ia}}K_{\text{b}}$ term in eq 3 is small, a condition that can arise when $k_{-2} \approx 0$.

Three plausible mechanisms for sarcosine oxidation have been suggested that are consistent with the crystal structure of MSOX•substrate analogue complexes: (i) a single electron transfer (SET) mechanism involving the intermediate formation of a flavin anion/amine cation radical pair (FAD^{•-}/CH₃N⁺CH₂CO₂⁻); (ii) a polar mechanism involving formation of a covalent flavin–substrate adduct intermediate via nucleophilic attack of substrate amine nitrogen at the 4a position of FAD; (iii) hydride transfer from the methyl group of sarcosine to flavin N(5) (5). No evidence for a spectrally detectable intermediate was obtained in reductive half-reaction studies with L-proline. The results are consistent with a hydride transfer mechanism but cannot rule out the polar or SET mechanism since the postulated intermediates may not accumulate in detectable amounts. Thiols are known to form 4a-flavin adducts (11) similar to the 4a-flavin–substrate adduct in the polar mechanism. We reasoned that an adduct formed with a monothiol, like thioglycolate, might be detectable since, unlike the flavin–substrate adduct, it could not undergo further reaction to yield the two-electron reduced flavin. MSOX does not form a 4a-adduct but does form a charge-transfer complex with thioglycolate. An unexpected, one-electron transfer reaction was observed under anaerobic conditions that resulted in quantitative conversion of oxidized MSOX to the anionic radical. This reaction would appear to be thermodynamically unfavorable as judged by comparing E° values for the RS[•]/RS⁻ or RS^{•+}H/RSH couple ($E^\circ = +0.78$ or $+1.35$ V, respectively) (12) with E° values observed for the EFl/EFl^{•-} or EFl/EFlH[•] couple with other

flavoenzymes ($E^\circ = -0.3$ to $+0.3$ V) (13, 14). A similarly unfavorable electron-transfer reaction, from substrate amino group to flavin, is postulated as the first step of the SET mechanism ($E^\circ = +0.7$ to 1 V for the RNH₂^{•+}/RNH₂ couple with aliphatic amines) (15). In the case of the thioglycolate reaction, the EFAD^{•-}•RS[•] complex is likely to exhibit only modest stability, as judged by the dissociation constant observed for the complex formed with oxidized enzyme and thioglycolate ($K_{\text{d}} = 7.7$ mM) (6). Dissociation of thiolate radical would prevent back electron transfer from EFAD^{•-}, a feature that might account for the observed quantitative electron transfer. The mechanism of the thioglycolate reaction is under investigation.

ACKNOWLEDGMENT

We thank Dr. John Schloss for helpful advice and useful discussions.

REFERENCES

1. Kvalnes-Krick, K., and Jorns, M. S. (1991) in *Chemistry and Biochemistry of Flavoenzymes* (Muller, F., Ed.) pp 425–435, CRC Press, Boca Raton, FL.
2. Wagner, M. A., Khanna, P., and Jorns, M. S. (1999) *Biochemistry* 38, 5588–5595.
3. Chlumsky, L. J., Zhang, L., and Jorns, M. S. (1995) *J. Biol. Chem.* 270, 18252–18259.
4. Reuber, B. E., Karl, C., Reimann, S. A., Mihalik, S. J., and Dodt, G. (1997) *J. Biol. Chem.* 272, 6766–6776.
5. Trickey, P., Wagner, M. A., Jorns, M. S., and Mathews, F. S. (1999) *Structure* 7, 331–345.
6. Wagner, M. A., Trickey, P., Zhi-wei, C., Mathews, F. S., and Jorns, M. S. (2000) *Biochemistry* 39, 8813–8824.
7. Ahmed, S. A., and Claiborne, A. (1992) *J. Biol. Chem.* 267, 25822–25829.
8. Rudolph, F. B., and Fromm, H. J. (1979) *Methods Enzymol.* 63, 138–159.
9. Fromm, H. J. (1979) *Methods Enzymol.* 63, 467–486.
10. Strickland, S., Palmer, G., and Massey, V. (1975) *J. Biol. Chem.* 250, 4048–4052.
11. Yokoe, I., and Bruice, T. C. (1975) *J. Am. Chem. Soc.* 97, 450–451.
12. Armstrong, D. A. (1999) in *S-Centered Radicals* (Alfassi, Z. B., Ed.) pp 27–61, John Wiley & Sons, New York.
13. Stankovich, M. T. (1991) in *Chemistry and Biochemistry of Flavoenzymes* (Muller, F., Ed.) pp 401–425, CRC Press, Boca Raton, FL.
14. Ramsay, R. R., Sablin, S. O., and Singer, T. P. (1995) *Prog. Brain Res.* 106, 33–39.
15. Chow, Y. L., Danen, W. C., Nelsen, S. F., and Rosenblatt, D. H. (1978) *Chem. Rev.* 78, 243–278.

BI000350Y