The irreversible inactivation of two copper-dependent monooxygenases by sulfite: peptidylglycine α-amidating enzyme and dopamine β-monooxygenase

David J. Merkler[a,*], Raviraj Kulathila[a,**], Wilson A. Francisco[b], David E. Ash[c], Joseph Bell[c]

[a]*Analytical Protein and Organic Chemistry Group, Unigene Laboratories, Inc., 110 Little Falls Rd., Fairfield, NJ 07004, USA*
[b]*Department of Chemistry, University of California, Berkeley, CA 94720, USA*
[c]*Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140, USA*

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Abstract Peptidylglycine α-amidating enzyme (α-AE) and dopamine β-monooxygenase (DβM), two copper-dependent monooxygenases that have catalytic and structural similarities, are irreversibly inactivated by sodium sulfite in a time- and concentration-dependent manner. Studies with α-AE show that the sulfite-mediated inactivation is dependent on the presence of redox active transition metals free in solution, with Cu(II) being the most effective in supporting the inactivation reaction. Sulfite inactivation of α-AE is specific for the monooxygenase reaction of this bifunctional enzyme and amidated peptides provide protection against the inactivation. Consequently, the sulfite-mediated inactivation of α-AE and DβM most likely results from the transition metal-catalyzed oxidation of sulfite to the sulfite radical, $\dot{S}O_3^-$.

Key words: Sulfite-mediated inactivation; Peptide α-amidation; Dopamine hydroxylation; Copper-dependent monooxygenase

1. Introduction

Monoxygenases catalyze the reductive activation of O_2 with the concomitant insertion of an oxygen atom into a carbon–hydrogen bond. Peptidylglycine α-amidating enzyme (α-AE, EC 1.14.17.3) and dopamine β-monooxygenase (DβM, EC 1.14.17.1) are examples of a relatively rare subset of monooxygenases that require copper for catalysis. Each of these enzymes catalyzes a reaction of physiological importance in mammals. α-AE converts inactive, glycine-extended peptide precursors to the active, α-amidated peptide hormones in a two step reaction sequence (for recent α-AE reviews, see [1–3]) while DβM converts dopamine to the neurotransmitter, norepinephrine (for recent DβM reviews, see [4,5]).

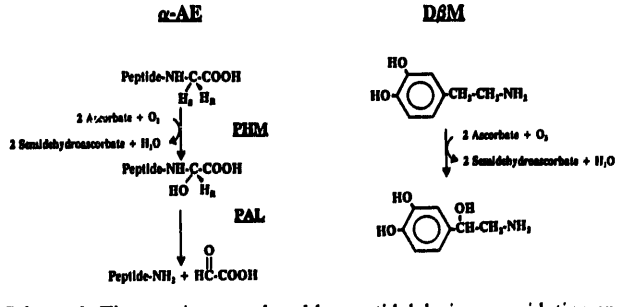
As shown in Scheme 1, both α-AE and DβM have obvious catalytic similarities, and alignment of their respective protein sequences reveals regions of extensive homology. In particular, two proposed His-X-His copper-binding motifs are conserved [6]. Mechanistic studies on DβM have shown that a substrate-based radical is formed during turnover [7,8]. Similarly, the formation of an α-centered glycyl radical is argued for α-AE based on the turnover-dependent inactivation of the enzyme by D-Phe-Phe-D-vinylglycine [9]. An α-centered glycyl radical has been discovered in the polypeptide backbone of pyruvate-formate lyase [10]; this radical reacts with hypophosphite leading to enzyme inactivation [11]. If a substrate-based glycyl radical were to form during α-AE turnover, it might be possible to trap the radical with hypophosphite or bisulfite, producing a substrate adduct that could be isolated and characterized.

In this communication, we report that the addition of micromolar concentrations of sulfite to α-AE or DβM results in the irreversible inactivation of the two enzymes and that the sulfite-mediated inactivation is turnover independent. More detailed studies with α-AE show that the sulfite-mediated inactivation requires exogenous transition metals and is the result of chemistry at the active site. The potent inactivation of α-AE and DβM by sulfite is important given that sulfite is a common food additive [12] and that millions of tons of sulfur (IV) compounds are dispensed into the atmosphere annually as a result of gaseous sulfur emissions from anthropogenic combustion sources and natural sources of gaseous sulfur compounds [13]. At physiological pH, sulfite and bisulfite will be the predominate forms of sulfur (IV) [14].

2. Materials and methods

2.1. Materials

Dansyl-Tyr-Val-Gly, D-Tyr-Val-Gly, substance P (RPKPQQFFGLM-NH_2), disodium fumarate; bovine catalase, $Cu(NO_3)_2$, $Mn(NO_3)_2$, $Mg(NO_3)_2$, and $Fe(NO_3)_3$ were from Sigma; (*R,S*)-α-hydroxyhippuric acid and Na_2SO_3 were from Aldrich; sodium ascorbate was from Spectrum; and tyramine-HCl was from Janssen Chimica. All other chemicals were of the highest quality available from commercial sources.



Scheme 1. The reactions catalyzed by peptidylglycine α-amidating enzyme (α-AE) and dopamine β-monooxygenase (DβM). α-AE is bifunctional with separate sites for peptide hydroxylation and α-hydroxyglycine-extended peptide dealkylation [2,3]. Peptide α-hydroxylating monooxygenase (PHM) is the monofunctional enzyme that catalyzes the hydroxylation half-reaction while peptidylamidoglycolate lyase (PAL) is the monofunctional enzyme that catalyzes the dealkylation half-reaction.

*Corresponding author. Fax: (1) (201) 882 8277.

**Current address:* Ciba-Geigy, Biophysical Chemistry Group Pharmaceutical Division, 556 Morris Ave., Summit, NJ 07901, USA.

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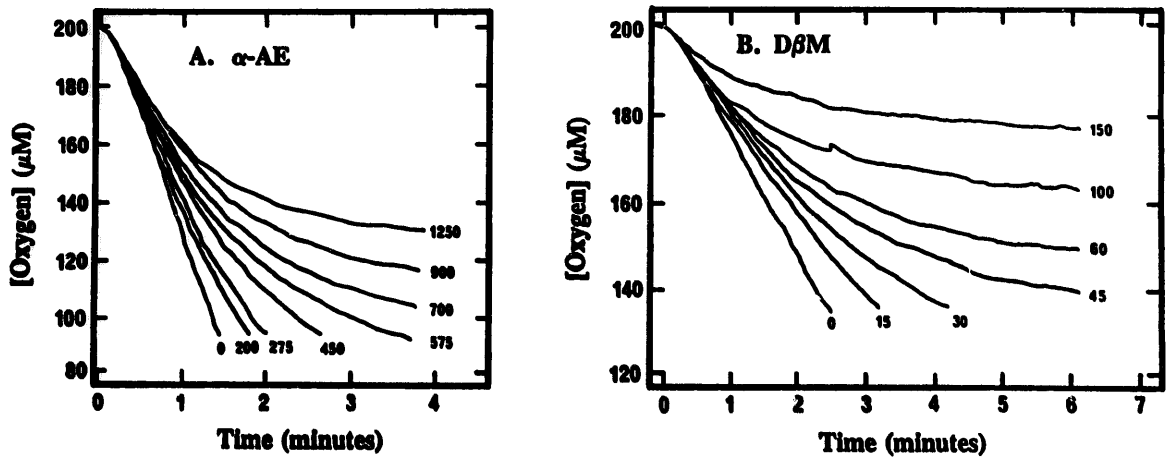


Fig. 1. Progressive inhibition of α-AE (A) and DβM (B) by Na_2SO_3. Reactions at 37°C were initiated at time = 0 by the addition of enzyme and the consumption of O_2 was measured as a function of time using an O_2 electrode. The assay conditions for the two enzymes are described in section 2. The numbers in panels A and B represent the concentration (μM) of Na_2SO_3 present in the assay solution.

2.2. Enzymes

2.2.1. α-AE. Chinese hamster ovary cells, which secrete recombinant type A rat medullary thyroid carcinoma α-AE into the culture media, were grown in a Wheaton stirred tank reactor [15]. The bifunctional 75-kDa enzyme was purified as described by Miller et al. [16] except that the final gel filtration step (Sephacryl S-300) was carried out using 50 mM HEPES/NaOH pH 7.8, 20 mM NaCl. The purified enzyme was ≥95% pure as judged by SDS-PAGE and unless otherwise noted, had a specific activity of ≥7.0 μmol of dansyl-Tyr-Val-Gly amidated/min/mg at 37°C.

2.2.2. DβM. Bovine adrenal dopamine β-monooxygenase was purified as described [17] and had a specific activity of 5.9 μmol of O_2 consumed/min/mg at 37°C.

2.3. Progressive inactivation of α-AE and DβM by Na_2SO_3

Reactions at 37 °C were initiated by the addition of enzyme, and O_2 consumption was measured using a Yellow Springs Instrument Model 35 monitor. The reaction conditions for α-AE were: 20 mM MES/NaOH pH 6.0, 100 mM NaCl, 1.0 μM $CuSO_4$, 10 μg/ml catalase, 500 μM sodium ascorbate, 270 μM D-Tyr-Val-Gly, 20 μg/ml α-AE, and 0–1250 μM Na_2SO_3. The reaction conditions for DβM were: 20 mM MES/NaOH pH 6.0, 100 mM NaCl, 1.0 μM $CuSO_4$, 10 μg/ml catalase, 10 mM sodium fumarate, 500 μM sodium ascorbate, 500 μM tyramine, 8.6 μg/ml DβM, and 0–150 μM Na_2SO_3.

2.4. Effect of Na_2SO_3 on the dealkylation activity of α-AE

Enzyme (63 μg/ml) was incubated at 37°C in 20 mM MES/KOH pH 6.0, 100 mM NaCl, 0.001% (v/v) Triton X-100, 1.0 μM $Cu(NO_3)_2$, and 100 μM Na_2SO_3. Controls lacking either Na_2SO_3 or Na_2SO_3 and α-AE were also included. To determine residual dealkylation activity, an aliquot (5 μl) was removed at the desired time and added to 495 μl of assay mixture such that the final solution contained 100 mM MES/KOH pH 6.0, 30 mM KCl, 0.001% (v/v) Triton X-100, 1% (v/v) ethanol, and 2 mM (*R,S*)-α-hydroxyhippuric acid. At regularly timed intervals, an aliquot (50 μl) was removed and added to 10 μl of 6% (v/v) trifluoroacetic acid to quench the reaction, and then applied to a C_{18} reverse-phase column (4.6 × 100 mm, Keystone Scientific Hypersil ODS) for analysis of the percent conversion of α-hydroxyhippuric acid to benzamide as described by Kulathila et al. [18].

2.5. Substance P protection against the Na_2SO_3-mediated inactivation of α-AE

Enzyme (2.5 μg/ml, specific activity = 4.4 μmol/min/mg) was incubated at 37°C in 20 mM MES/KOH pH 6.0, 100 mM NaCl, 0.001% (v/v) Triton X-100, 1.0 μM $CuSO_4$, 25 μM Na_2SO_3, and 0–500 μM substance P. A control containing no Na_2SO_3 and 500 μM substance P was also included. To determine residual amidation activity, an aliquot (5 μl) was removed at the desired time and added to 495 μl of assay mixture such that the solution contained 100 mM MES/KOH pH 6.0, 30 mM KCl, 1.0 μM $Cu(NO_3)_2$, 100 μg/ml catalase, 0.001% (v/v) Triton X-100, 1% (v/v) ethanol, 20 μM dansyl-Tyr-Val-Gly, and 10 mM sodium ascorbate. At regularly timed intervals, an aliquot (50 μl) was removed and added to 10 μl of 6% (v/v) trifluoroacetic acid, and then applied to a C_{18} reverse-phase HPLC column for analysis of the percent conversion of dansyl-Tyr-Val-Gly to dansyl-Tyr-Val-NH_2 as described by Jones et al. [19].

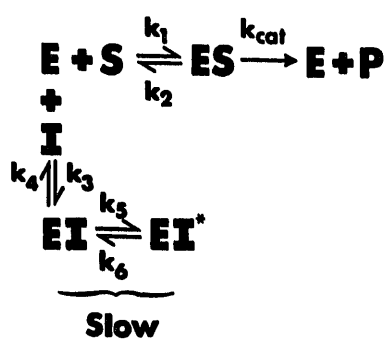
2.6. Metal ion dependence of the Na_2SO_3-mediated inactivation of α-AE

Enzyme (25 μg/ml, specific activity = 4.7 μmol/min/mg) was incubated at 37 °C in 20 mM MES/KOH pH 6.0, 100 mM NaCl, 0.001% (v/v) Triton X-100, 0–100 μM metal ion ($Cu(NO_3)_2$, $Mn(NO_3)_2$, $Mg(NO_3)_2$, or $Fe(NO_3)_3$) and 0 or 50 μM Na_2SO_3 for 20 min. Residual amidation activity was determined by the addition of an aliquot (5 μl) to 1.5 ml of 100 mM MES/KOH pH 6.0, 30 mM KCl, 1.0 μM $Cu(NO_3)_2$, 100 μg/ml catalase, 0.001% (v/v) Triton X-100, 1% (v/v) ethanol, 20 μM dansyl-Tyr-Val-Gly, and 10 mM sodium ascorbate. At regularly timed intervals, an aliquot (50 μl) was removed and added to 10 μl of 6% (v/v) trifluoroacetic acid. Conversion of dansyl-Tyr-Val-Gly to dansyl-Tyr-Val-NH_2 was determined by HPLC as described [19].

3. Results and discussion

3.1. Sulfite-mediated inactivation of α-AE and DβM

In the absence of Na_2SO_3, the steady-state rates for both α-AE and DβM are reached rapidly and are maintained for a



Scheme 2. Kinetic model for slow-binding inhibition.

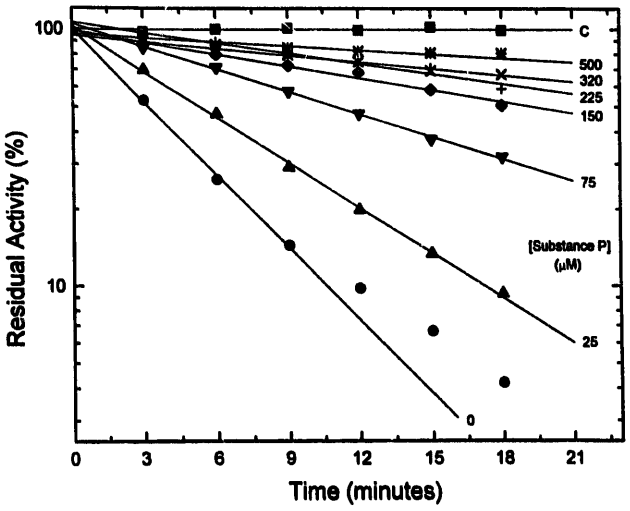


Fig. 2. Protection of α-AE against the sulfite-mediated inactivation by substance P. The inactivation rates (k_{obs}) as a function of substance P were obtained by incubating enzyme (2.5 μg/ml, specific activity = 4.4 μmol/min/mg) at 37°C in 20 mM MES/KOH pH 6.0, 100 mM NaCl, 0.001% (v/v) Triton X-100, 1.0 μM $CuSO_4$, 25 μM Na_2SO_3, and the indicated concentration of substance P. A control containing no Na_2SO_3 (line C) was included. At the indicated times, an aliquot was removed and assayed for residual dansyl-Tyr-Val-Gly amidation activity as described in section 2.

significant conversion of substrate to product. In the presence of Na_2SO_3, there is a time-dependent decrease in the steady state rate that is a function of the Na_2SO_3 concentration (Fig. 1). These progress curves are similar to those obtained for a slow-binding inhibitor [20,21] in which an inactive enzyme form accumulates slowly relative to catalysis (Scheme 2). Note that DβM is >10-fold more sensitive to Na_2SO_3 than α-AE under the conditions employed for these experiments (compare Fig. 1B to Fig. 1A).

The progressive inhibition observed in Fig. 1 might result from a reaction between sulfite and a substrate-based radical producing a reactive intermediate that modifies a key amino acid in the active site. This hypothesis requires that the sulfite-mediated inactivation be turnover-dependent. A reaction between the substrate-based radical and sulfite to form a substrate-sulfite adduct (without producing a reactive intermediate) would most likely not cause the inhibition of O_2 consumption shown in Fig. 1.

However, incubations of α-AE or DβM with sulfite lead to enzyme inactivation, demonstrating that the inactivation process is turnover-independent. Incubation of 3.4 μg/ml α-AE with 28 μM Na_2SO_3 and 1.0 μM $Cu(NO_3)_2$ for 40 min at 37°C resulted in a loss of ~90% of the original dansyl-Tyr-Val-Gly amidation activity. Amidation activity was not restored upon exhaustive dialysis against 20 mM MES/NaOH pH 6.0, 100 mM NaCl, 0.001% (v/v) Triton X-100. A control sample of α-AE incubated in buffer without Na_2SO_3 lost only 4% of its original activity during this procedure (including dialysis). In contrast to the results with Na_2SO_3, incubation of α-AE with 500 μM $NaNO_2$ or 500 μM NaH_2PO_2 for 20 min resulted in no loss of amidation activity. Incubation of DβM (8.9 μg/ml) with 100 μM Na_2SO_3 and 1.0 μM $CuSO_4$ for 30 min at 37°C resulted in a >90% loss of tyramine oxidation activity, which was not restored upon exhaustive dialysis against 20 mM MES/NaOH pH 6.0, 100 mM NaCl, 1.0 μM $Cu(NO_3)_2$, 10 mM sodium fumarate, and 500 μM tyramine. A control sample of DβM incubated in buffer without Na_2SO_3 lost 51% of its original activity during this procedure (including dialysis). These results show that the sulfite-mediated inactivation of both α-AE and DβM is turnover-independent and irreversible, and is thus not a consequence of chemistry between a sulfite and a substrate-based radical. In addition, the inactivation, at least for α-AE, is specific to sulfite. Also note that significantly lower concentrations of Na_2SO_3 inactivate α-AE under non-turnover conditions than the those concentrations shown in Fig. 1A. This result suggests that substrates (or competitive inhibitors) protect α-AE against the sulfite-mediated inactivation.

The rat 75-kDa α-AE is a bifunctional enzyme (see Scheme 1) with separate active sites for peptide hydroxylation (PHM site) and for α-hydroxyglycine-extended peptide dealkylation (PAL site) [2,3,22,23]. When incubated with 100 μM Na_2SO_3 and 1.0 μM $Cu(NO_3)_2$ for 15 min at 37°C, α-AE (63 μg/ml) retained 104% of the original α-hydroxyhippuric acid dealkylation activity. Since sulfite causes only the loss of amidation activity, the sulfite-mediated inactivation must result from chemistry at the PHM site. The inactivation mediated by ascorbate [24] and benzylhydrazine [25] was similarly localized to chemistry only at the PHM site.

3.2. Substance P protection against the sulfite-mediated inactivation of α-AE

One mechanism that could account for the sulfite-mediated inactivation of PHM and DβM would be the Cu(II)-dependent oxidation of sulfite to the sulfite radical, $\dot{S}O_3^-$, (Eqn. 1) [14,26] followed by a reaction between the radical and an active site amino acid. This is an attractive mechanism

$$Cu(II) + SO_3^{2-} \rightarrow Cu(I) + \dot{S}O_3^- \quad (1)$$

given that both enzymes are known to carry out redox chemistry at bound copper atoms [27,28] and that the sulfite-mediated inactivation of rhodanese [29] and papain [30] has been attributed to the oxidation of SO_3^{2-} to $\dot{S}O_3^-$. Inactivation medi-

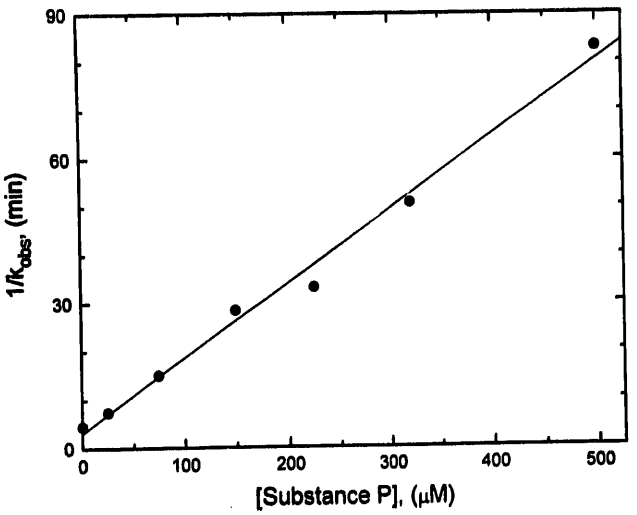


Fig. 3. Replot of $1/k_{obs}$ vs. [Substance P]. The observed inactivation rates (k_{obs}) as a function of the substance P concentration are from Fig. 2. The line represents a linear regression fit to the data.

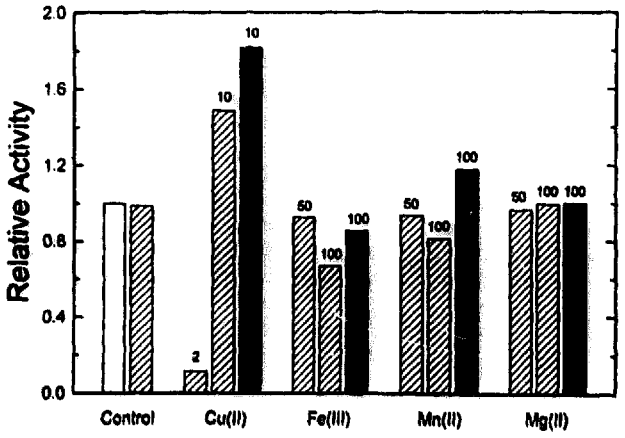


Fig. 4. Metal ion effects on the sulfite-mediated inactivation of α-AE. Enzyme (25 μg/ml, specific activity = 4.7 μmol/min/mg) was incubated in 20 mM MES/KOH pH 6.0, 100 mM NaCl, 0.001% (v/v) Triton X-100, 0–100 μM metal ion, and 0 or 50 μM Na_2SO_3. After 20 min at 37°C, an aliquot was removed and assayed for residual dansyl-Tyr-Val-Gly amidation activity as described in section 2. The slashed bars represent incubation solutions containing 50 μM Na_2SO_3, the filled bars represent incubation solutions containing the metal ion listed below and no Na_2SO_3, and the open bar represents the control containing no added metal ion and no Na_2SO_3. The numbers above the bars indicate the micromolar (μM) concentration of metal ion present in the incubation solutions. Each specific activity is the average of triplicate determinations with standard deviations of ≤9%. Preliminary experiments with a variety of metals evaluated at concentrations from 0–100 μM showed that these metals at the indicated concentrations provided the most pronounced effects.

ated by a radical might be viewed as being indiscriminate because such a reactive species could react with a number of amino acids causing a loss of activity by either altering the structure of the enzyme or by modifying a key catalytic residue. Protection by a competitive inhibitor would provide strong evidence that the sulfite-mediated inactivation is due to the modification of an active site amino acid. Substance P, an eleven amino acid amidated peptide involved in pain transmission [31], is a competitive inhibitor of α-AE with a K_{is} value of 33 ± 2 μM [32]. As shown in Fig. 2, substance P does protect against the sulfite- mediated inactivation. The concentration of substance P which halved the inactivation rate at 25 μM Na_2SO_3 was ~25 μM, a value approximately equal to the K_{is} value. The replot of $1/k_{obs}$ vs. [substance P] (Fig. 3) is linear indicating that the binding of 1 mol of substance P per mole of enzyme is sufficient to protect against the inactivation. The data presented in Figs. 2 and 3 indicate that sulfite must modify a group in the PHM site and confirms that the sulfite-mediated inactivation of α-AE is turnover-independent.

3.3. *Metal ion effects on the sulfite-mediated inactivation of α-AE*

In the absence of exogenously added Cu(II), 50 μM Na_2SO_3 had virtually no effect on α-AE activity (Fig. 4). This result is not surprising since α-AE binds copper relatively weakly [18] and the enzyme, as purified, is substantially underloaded with respect to Cu(II), containing only 0–0.2 mol of copper per mol of enzyme [2,18]. Addition of 1.0 or 2.0 μM Cu(II) results in a substantial loss of amidation activity in ≥15 min when Na_2SO_3 is ≥25 μM (Figs. 2 and 4). Concentrations of Cu(II) in excess of 4 μM protect α-AE against the sulfite-mediated inactivation. The residual amidation activity after a 15 min incubation in the presence of 50 μM Na_2SO_3 increased from 12% at 2 μM Cu(II) to 82% at 10 μM Cu(II) (Fig. 4), relative to untreated controls. These data are consistent with the hypothesis that the inactivation of α-AE and DβM results from the Cu(II)-catalyzed oxidation of sulfite to $\dot{S}O_3^-$ (Eqn. 1). The strongly reducing sulfite radical is likely to be consumed at [Cu(II)] >4 μM (Eqn. 2) [14,33], thereby explaining the protection seen at higher Cu(II) concentrations (Fig. 4).

$$Cu(II) + \dot{S}O_3^- \rightarrow Cu(I) + SO_3 \quad (2)$$

Transition metals other than cupric ions will catalyze the oxidation of SO_3^{2-} to $\dot{S}O_3^-$. Both Fe(III) [33] and Mn(II) [34] will also catalyze sulfite oxidation. As shown in Fig. 4, both Fe(III) and Mn(II) do support the sulfite-mediated inactivation of α-AE, but the extent of inactivation is not as dramatic as that observed with Cu(II). In contrast, Mg(II), which is not a redox active metal, does not support the sulfite-mediated inactivation of α-AE (Fig. 4). The data presented in Fig. 4 for Fe(III), Mn(II), and Mg(II) provide strong support for the hypothesis that a sulfite radical is responsible for the inactivation of α-AE and DβM shown in Fig. 1. Because of the relatively weak affinity of α-AE for Cu(II) [18] and the unknown affinity for Mn(II) or Fe(III), it is not possible at present to determine if enzyme-bound or free metal is responsible for the formation of the putative sulfite radical. Furthermore, the variable extent of sulfite inactivation in the presence of Cu(II), Mn(II), and Fe(III) (Fig. 4) may result from the effects of the diverse redox potentials of these metals on sulfite radical formation.

The increase in specific activity seen in Fig. 4 upon the incubation of α-AE with 10 μM $Cu(NO_3)_2$ is reproducible and represents a 1.8-fold increase from 4.7 μmol/min/mg to 8.5 μmol/min/mg. The original specific activity of this enzyme was 8.9 μmol/min/mg which had slowly decreased upon storage and repeated freeze/thaw cycles. The restoration of activity upon incubation with Cu(II) suggests that Cu(II) restores α-AE to a more active conformation which had been lost during storage.

In summary, we have found that two copper-dependent monooxygenases, α-AE and DβM, are irreversibly inactivated by low (micromolar) concentrations of sulfite. Additional studies with α-AE show that the sulfite-mediated inactivation is specific to the PHM active site and requires the presence of redox active transition metals. α-AE is unaffected by sulfite in the absence of added Cu(II), Mn(II), or Fe(III) and in the presence of Mg(II). These data suggest that the inactivation is the result of the modification of a key catalytic amino acid by $\dot{S}O_3^-$, which is formed by the transition metal catalyzed oxidation of SO_3^{2-}. More detailed studies to determine if the inactivated enzyme contains bound sulfur, to determine which amino acid(s) are modified, and to detect the sulfite radical by EPR are underway. The results presented here suggest that other enzymes that catalyze transition metal-dependent redox chemistry such as tyrosinase or cytochrome P-450 may also be inactivated by sulfite. The potent inactivation of α-AE, DβM, and perhaps other redox enzymes by sulfite is likely to be of physiological importance since sulfite is a common food additive [12]. Futhermore, sulfite is found in trace concentrations in the atmosphere [13], and its inhibition of redox enzymes may

provide an explanation for the hypersensitivity of asthmatics to sulfite [35].

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References

[1] Merkler, D.J. (1994) Enzyme Microb. Technol. 16, 450–456.
[2] Merkler, D.J., Kulathila, R., Young, S.D., Freeman, J. and Villafranca, J.J. (1993) in: Bioinorganic Chemistry of Copper (Karlin, K.D. and Tyeklár, Z., Eds.) pp. 196–209, Chapman and Hall, New York.
[3] Eipper, B.A., Milgram, S.L., Husten, E.J., Yun, H.-Y. and Mains, R.E. (1993) Prot. Sci. 2, 489–497.
[4] Stewart, L.C. and Klinman, J.P. (1988) Annu. Rev. Biochem. 57, 551–592.
[5] Klinman, J.P., Berry, J.A. and Tian, G. (1993) in: Bioinorganic Chemistry of Copper (Karlin, K.D. and Tyeklár, Z., Eds.) pp. 151–163, Chapman and Hall, New York.
[6] Southan, C. and Kruse, L.I. (1989) FEBS Lett. 255, 116–120.
[7] Fitzpatrick, P.F., Flory, D.R., Jr. and Villafranca, J.J. (1985) Biochemistry 24, 2108–2114.
[8] Miller, S.M. and Klinman, J.P. (1985) Biochemistry 24, 2114–2127.
[9] Zabriske, T.M., Cheng, H. and Vederas, J.C. (1992) J. Am. Chem. Soc. 114, 2270–2272.
[10] Wagner, A.F.V., Frey, M., Neugebauer, F.A., Schäfer, W. and Knappe, J. (1992) Proc. Natl. Acad. Sci. USA 89, 996–1000.
[11] Brush, E.J., Lippsett, K.A. and Kozarich, J.W. (1988) Biochemistry 27, 2217–2222.
[12] Taylor, S.L., Higley, N.A. and Brush, R.K. (1986) Adv. Food Res. 30, 1–76.
[13] Eatough, D.J., and Hansen, L.D. (1983) in: Advances in Environmental Science and Technology, Vol. 12 (Schwartz, S.E., Ed.) pp. 221–268, Wiley, New York.
[14] Neta, P. and Huie, R.E. (1985) Environ. Health Perspect. 64, 209–217.
[15] Matthews, D.E., Piparo, K.E., Burkett, V.H. and Pray, C.C. (1994) in: Animal Cell Technology: Products of Today, Prospects for Tomorrow (Spier, R.E., Griffiths, J.B. and Bethold, W., Eds.) pp. 315–319, Butterworth-Heinemann, Oxford, UK.
[16] Miller, D.A., Sayad, K.U., Kulathila, R., Beaudry, G.A., Merkler, D.J. and Bertelsen, A.H. (1992) Arch. Biochem. Biophys. 298, 380–388.
[17] Stewart, L.C. and Klinman, J.P. (1987) Biochemistry 26, 5302–5309.
[18] Kulathila, R., Consalvo, A.P., Fitzpatrick, P.F., Freeman, J.C., Snyder, L.M., Villafranca, J.J. and Merkler, D.J. (1994) Arch. Biochem. Biophys. 311, 191–195.
[19] Jones, B.N., Tamburini, P.P., Consalvo, A.P., Young, S.D., Lovato, S.J., Gilligan, J.P., Jeng, A.Y. and Wennogle, L.P. (1988) Anal. Biochem. 168, 272–279.
[20] Morrison, J.F. and Stone, S.R. (1985) Comments Mol. Cell. Biophys. 2, 347–368.
[21] Merkler, D.J., Brenowitz, M. and Schramm, V.L. (1990) Biochemistry 29, 8358–8364.
[22] Bertelsen, A.H., Beaudry, G.A., Galella, E.A., Jones, B.N., Ray, M.L. and Mehta, N.M. (1990) Arch. Biochem. Biophys. 279, 87–96.
[23] Merkler, D.J. and Young, S.D. (1991) Arch. Biochem. Biophys. 289, 192–196.
[24] Merkler, D.J., Kulathila, R., Tamburini, P.P. and Young, S.D. (1992) Arch. Biochem. Biophys. 294, 594–602.
[25] Merkler, D.J., Kulathila, R. and Ash, D.E. (1995) Arch. Biochem. Biophys. 317, 93–102.
[26] Conklin, M.H. and Hoffman, M.R. (1988) Environ. Sci. Technol. 22, 891–898.
[27] Brenner, M.C., Murray, C.J. and Klinman, J.P. (1989) Biochemistry 28, 4656–4664.
[28] Freeman, J.C., Villafranca, J.J. and Merkler, D.J. (1993) J. Am. Chem. Soc. 115, 4923–4924.
[29] Berni, R., Musci, G., Pallini, R. and Cannella, C. (1991) Free Rad. Res. Commun. 15, 203–209.
[30] Fujimoto, S., Nakagawa, T., Ishimitu, S. and Ohara, A. (1983) Chem. Pharm. Bull. 31, 992–1000.
[31] O'Donohue, T.L., Handelmann, G.E., Quirion, R., and Gaudreau, P. (1988) in: Peptide Hormones: Effects and Mechanisms of Action, vol. 2 (Negro-Vilar, A. and Conn, M.P., Eds.) pp. 79–150, CRC Press, Boca Raton.
[32] Tamburini, P.P., Jones, B.N., Consalvo, A.P., Young, S.D., Lovato, S.J., Gilligan, J.P., Wennogle, L.P., Erion, M. and Jeng, A.Y. (1988) Arch. Biochem. Biophys. 267, 623–631.
[33] Huie, R.E. and Peterson, N.C. (1983) in: Advances in Environmental Science and Technology, Vol. 12 (Schwartz, S.E., Ed.) pp. 117–146, Wiley, New York.
[34] Shi, X. and Dalal, N.S. (1990) Res. Chem. Intermed. 13, 103–115.
[35] Gunnison, A.F. and Jacobsen, D.W. (1986) CRC Crit. Rev. Toxicol. 17, 185–214.