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Inactivation of Monoamine Oxidase A by the Monoamine Oxidase B Inactivators 1-Phenylcyclopropylamine, 1-Benzylcyclopropylamine, and *N*-Cyclopropyl-α-methylbenzylamine†

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ABSTRACT: Three known mechanism-based inactivators of beef liver mitochondrial monoamine oxidase (MAO) B are tested as inactivators of human placental mitochondrial MAO A. 1-Phenylcyclopropylamine (1-PCPA), 1-benzylcyclopropylamine (1-BCPA), and *N*-cyclopropyl-α-methylbenzylamine (*N*-CαMBA) are time-dependent irreversible inactivators of MAO A. The K_I values for 1-PCPA and *N*-CαMBA, analogues of the MAO B substrate benzylamine, are much higher with MAO A than with MAO B. Evidence is presented to show that 1-PCPA inactivates MAO A by attachment to the flavin cofactor, unlike the reaction with MAO B in which 1-PCPA can attach to both a cysteine residue and the flavin [Silverman, R. B., & Zieske, P. A. (1985) *Biochemistry 24*, 2128-2138]. The reaction of 1-BCPA with MAO A was too slow to study in detail. *N*-CαMBA exhibits the same properties toward inactivation of MAO A that it does for inactivation of MAO B. Attachment in both cases is shown to be to one cysteine residue per enzyme molecule. The results with 1-PCPA indicate that the active site topographies of MAO A and MAO B are different. The ability of *N*-CαMBA to undergo attachment to a cysteine residue in both MAO A and MAO B may lead the way toward peptide mapping of the two isozymes in order to determine differences in their primary structures.

Monoamine oxidase (MAO)[1] is one of the enzymes responsible for the catabolism of biogenic amines. Inhibitors of MAO have been used clinically for the treatment of depression for almost 30 years (Zeller, 1959). Tranylcypromine (*trans*-2-phenylcyclopropylamine), a member of the cyclopropylamine class of MAO inactivators that is currently in use as an antidepressant drug (Baldessarini, 1985), was shown to be a mechanism-based inactivator (Silverman, 1988) of MAO (Paech et al., 1980; Silverman, 1983). Other cyclopropylamine analogues, e.g., *N*-cyclopropylbenzylamine

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[1] Abbreviations: MAO, monoamine oxidase (EC 1.4.3.4); 1-PCPA, 1-phenylcyclopropylamine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); 1-BCPA, 1-benzylcyclopropylamine; *N*-CαMBA, *N*-cyclopropyl-α-methylbenzylamine.

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(Silverman & Hoffman, 1980; Silverman et al., 1980; Vazquez & Silverman, 1985; Yamasaki & Silverman, 1985), *N*-(1-methylcyclopropyl)benzylamine (Silverman & Hoffman, 1981; Silverman & Yamasaki, 1984), *N*-cyclopropyl-α-methylbenzylamine (Silverman, 1984; Vazquez & Silverman, 1985), 1-phenylcyclopropylamine (Silverman & Zieske, 1985a, 1986), and 1-benzylcyclopropylamine (Silverman & Zieske, 1985b), also were determined to be MAO inactivators. All of these inactivation studies suggest that radical intermediates are involved in MAO catalysis and inactivation, and recently a radical intermediate was spin-trapped and observed by ESR spectroscopy (Yelekci & Silverman, unpublished results).

In 1968 Johnston demonstrated that there are two isozymic forms of MAO, now termed MAO A and MAO B. The reaction catalyzed by the two forms is the same; substrate specificities, however, are different. For example, 5-hydroxytryptamine and norepinephrine are substrates principally for MAO A, and benzylamine is metabolized by MAO B. Furthermore, selective inhibitors and inactivators for the two isozyme forms have been discovered (Fowler & Ross, 1984). Several lines of evidence indicate that MAO A and MAO B are distinct enzymes, not the same enzyme whose activity is modulated by its phospholipid environment. MAO A from placental trophoblast tissue and MAO B from platelets of a male newborn (Cawthon et al., 1981) or MAO A and MAO B from rat liver (Smith et al., 1985) were radiolabeled with [^{3}H]pargyline. NaDodSO$_4$–PAGE gave two distinct radioactive bands; limited proteolysis of the two labeled bands gave different patterns of ^{3}H-peptides. NaDodSO$_4$ and phospholipase A$_2$ were used by White and Stine (1982, 1984) to show that MAO A and MAO B are two distinct enzymes embedded in a phospholipid environment in which MAO A activity is critically dependent on associated phospholipids, but MAO B is not. MAO A and MAO B from human brain were separated by ion-exchange chromatography after solubilization with octyl glucoside (Pearce & Roth, 1984). Human liver MAO A and MAO B were separated by immunoaffinity chromatography with a MAO B specific monoclonal antibody (Denney et al., 1982). Furthermore, the brain regions containing MAO A and B could be localized with the use of [^{11}C]clorgyline and [^{11}C]-L-deprenyl, which are selective MAO A and B inactivators, respectively, and positron emission tomography (Fowler et al., 1987) or with monoclonal antibodies for MAO A and B (Westlund et al., 1985).[2]

Although MAO A and B are, apparently, distinct enzymes, it has been shown (Yu, 1980; Nagy & Salach, 1981) that the amino acid sequence at the covalently bound flavin site is identical, namely, Ser-Gly-Gly-Cys(flavin)-Tyr, for both MAO A and MAO B from several species.

In order to determine the similarity of the active site in MAO A and B, we investigated the reactions of three known mechanism-based MAO B inactivators, i.e., 1-phenylcyclopropylamine, 1-benzylcyclopropylamine, and *N*-cyclopropyl-α-methylbenzylamine, with purified MAO A. All three compounds inactivate MAO A, but various differences in these reactions relative to those with MAO B were observed, suggesting different active site topographies for the two enzymes.

Materials and Methods

Analytical Methods. Optical spectra were recorded on a Beckman DU-40 UV/vis scanning spectrophotometer. MAO assays were carried out on a Perkin-Elmer Lambda 1 UV/vis or a Beckman DU-40 UV/vis scanning spectrophotometer. An Orion Research Model 601 pH meter with a general combination electrode was used to measure pH values.

Reagents. The syntheses of 1-PCPA (Silverman & Zieske, 1985a), 1-BCPA (Silverman & Zieske, 1985b), and *N*-CαMBA (Silverman, 1984) were reported. 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent) was purchased from Aldrich. Urea and sodium hydrosulfite were bought from Sigma Chemical Co. and BCA protein assay solutions were purchased from Pierce Chemical Co. *N*-[1-^{3}H]CαMBA was prepared previously (Silverman, 1984) and had a specific activity of 4.0×10^6 dpm/μmol.

Enzymes and Assays. Human placental MAO A, isolated and stored according to the procedures of Weyler and Salach (1985), was kindly donated by Dr. Thomas P. Singer (UCSF). The enzyme was homogeneous by NaDodSO$_4$–PAGE. The enzyme assay was modified as follows: enzyme activity was determined at 25 °C in 50 mM sodium phosphate buffer, pH 7.2, containing 0.2% Triton X-100 plus 0.5 mM kynuramine, and the increase in absorbance at 314 nm was measured. While working with 1-PCPA, we found that MAO A retains activity longer in the presence of glycerol. Consequently, the studies with *N*-CαMBA were carried out in 100 mM sodium phosphate buffer, pH 7.2, containing 20% glycerol and /.2% Triton X-100. Most of the experiments with 1-PCPA, however, did not contain glycerol. All protein concentrations were determined by using the BCA protein assay method (Pierce Chemical Co.).

Inactivation of MAO A by 1-PCPA, 1-BCPA, and N-CαMBA. A 5-μL aliquot of 16.2 μM MAO A was added to 95 μL of varying concentrations of 1-PCPA (0, 1.67, 2.0, 2.5, 3.33, 5.0, and 10.0 mM), 1-BCPA (0, 0.1, 0.125, 0.167, 0.20, 0.25, 0.33, 0.5, and 1.00 mM), or *N*-CαMBA (0, 0.33, 0.40, 0.50, 0.67, 1.0, and 2.0 mM) in 100 mM sodium phosphate buffer, pH 7.2, containing 0.2% Triton X-100 and was incubated at 25 °C. Aliquots (8 μL) were removed at timed intervals and assayed for residual enzyme activity as described above.

Determination of the Partition Ratio for 1-PCPA Inactivation of MAO A. A 25-μL aliquot of 16.2 μM MAO A was added to 25 μL of varying concentrations of 1-PCPA (0, 20, 40, 60, 100, 140, 180, 220, 260, 300, 400, and 1000 μM) in 50 mM sodium phosphate, pH 7.2, buffer containing 0.2% Triton X-100. The solutions were incubated in the dark at 25 °C over an 11-day period. Aliquots (3 μL) were removed periodically and assayed for residual activity. Activity was expressed relative to the control solution without inactivator.

Change in the Flavin Spectrum during Inactivation of MAO A by 1-PCPA. An 84-μL aliquot of the 16.2 μM MAO A was added to a self-masked microcuvette containing 230 μL of 1-PCPA (0 or 10 mM) in 50 mM sodium phosphate, pH 7.2, with 0.2% Triton X-100. Spectra were recorded over a 4-h period. Aliquots (5 μL) were removed at 3 h and at 4 h and assayed to determine enzyme activity. A 350-μL aliquot of saturated urea was added to the control and the inactivated MAO A solutions to denature the enzyme, and a spectrum was recorded. A 15-μL aliquot of 40 mM sodium hydrosulfite was added to the inactivated MAO A solution to test for further reduction of the flavin, and then another 15-μL aliquot was added to the control solution to reduce the flavin. Spectra were recorded during the experiment to determine the oxidation states of the flavin in the control and inactivated MAO A solutions.

Sulfhydryl Titration of 1-PCPA- and N-CαMBA-Inactivated MAO A. Aliquots (150 μL) of 16.2 μM MAO A were

[2] Added in proof: Recently, Bach et al. (1988) isolated cDNA clones that encode human liver MAO A and MAO B and showed that these proteins are different.

added to 150-μL aliquots of a solution of 1-PCPA (0 or 20 mM) or *N*-CαMBA (0 or 10 mM) in 100 mM sodium phosphate, pH 7.2, buffer containing 20% glycerol and 0.2% Triton X-100 and incubated at 25 °C. When the inactivated enzyme activity was less than 10% of the control activity, each enzyme solution was dialyzed for 6 h against three changes (200 mL each) of 100 mM sodium phosphate, pH 7.2, buffer solution containing 20% glycerol and 0.2% Triton X-100. The contents of the dialysis bags were transferred to micro test tubes and vortexed, and aliquots were removed for enzyme activity assays (3 μL) and for protein concentration analyses (2 × 10 μL). Thiol titrations with DTNB were performed according to a modification of the literature procedures (Fernandez Diez et al., 1964). A 200-μL aliquot of either the control or the inactivated MAO A was added to a solution comprised of 380 μL of deionized H_2O, 200 μL of 100 mM sodium phosphate, pH 8.0, and 100 μL of 20% $NaDodSO_4$ with 1 mg/mL EDTA. The solution was mixed, and the absorbance at 412 nm was recorded as an enzyme absorbance background reading (spectrophotometer prezeroed by using a solution comprised of 380 μL of deionized H_2O, 200 μL of 100 mM sodium phosphate, pH 8.0, 100 μL of 20% $NaDodSO_4$ with 1 mg/mL EDTA, and 200 μL of 20% glycerol in 50 mM sodium phosphate containing 0.2% Triton X-100). A 20-μL aliquot of 4 mg/mL DTNB in 100 mM sodium phosphate, pH 8.0, was added to the enzyme solution, and the absorbance at 412 nm was recorded over a 45-min period. A 20-μL aliquot of the DTNB solution was added to the solution used to zero the spectrophotometer, and the absorbance at 412 nm was used as a DTNB absorbance background. The total amount of free 5-mercapto-2-nitrobenzoate produced was calculated from the absorbance at 412 nm of the DTNB-treated MAO A solution minus the two background readings.

Determination of the Partition Ratio and Tritium Incorporation for N-[1-^{3}H]CαMBA Inactivation of MAO A. A 100-μL aliquot of 15.2 μM MAO A was added to 100 μL of varying concentrations of *N*-[1-^{3}H]CαMBA (0, 9, 18, 27, 36, 45, 54, 63, 72, 81, and 90 μM) in 100 mM sodium phosphate, pH 7.2, buffer containing 20% glycerol and 0.2% Triton X-100. The solutions were incubated in the dark at 25 °C for 5 days, and then a 5-μL aliquot was removed from each sample and assayed for residual enzyme activity. The activity was expressed as a percent of the control solution (no inactivator) and plotted versus the molar ratio of *N*-CαMBA to MAO A. The enzyme solutions were dialyzed individually for 6 h at 4 °C against three changes (500 mL each) of 100 mM sodium phosphate, pH 7.2, buffer containing 20% glycerol and 0.2% Triton X-100. The enzyme solutions were transferred back into capped tubes, and aliquots were removed for protein assays (40 μL) and scintillation counting (100 μL). The amount of tritium incorporation was expressed as micromoles per milligram of enzyme and plotted versus the concentration of *N*-CαMBA.

Treatment of Tritium-Labeled MAO A with Urea. A 300-μL aliquot of MAO A was added to 300 μL of 10 mM *N*-[1-^{3}H]CαMBA in 100 mM sodium phosphate containing 20% glycerol and 0.2% Triton X-100 and incubated for 60 min at 25 °C. The inactivation mixture was microdialyzed for 6 h against three changes (6 mL each) of 100 mM sodium phosphate, pH 7.2, containing 20% glycerol and 0.2% Triton X-100 and then exhaustively dialyzed for 4 h against two changes (500 mL each) of the same buffer. A 300-μL aliquot of the enzyme solution was transferred to a new dialysis bag and dialyzed for 3 h against 8 M urea (500 mL) in 100 mM sodium phosphate containing 20% glycerol and 0.2% Triton X-100 and then against 100 mM sodium phosphate buffer, pH 7.2, containing 20% glycerol and 0.2% Triton X-100 (500 mL) for 3 h. The contents of both the control-inactivated MAO A solution and the urea-treated MAO A solution were transferred to capped tubes. Aliquots were removed for protein assay (40 μL) and scintillation counting (100 μL). Tritium incorporation was expressed in micromoles of tritium incorporated per milligram of MAO A.

Table I: Comparison of Kinetic Properties for 1-PCPA, 1-BCPA, and *N*-CαMBA with MAO A and MAO B

	MAO A			MAO B		
inactivator	K_I (mM)	k_{inact} (min^{-1})	PR[a]	K_I (mM)	k_{inact} (min^{-1})	PR
1-PCPA	10	0.28	11	0.20[b]	2.0[b]	7
1-BCPA	0.16	0.004	3	0.48[c]	0.005[c]	1
N-CαMBA	2.5	0.35	2	0.044[d]	0.25[d]	2

[a] Partition ratio. [b] Taken from Silverman and Zieske (1985a). [c] Taken from Silverman and Zieske (1985b). [d] Taken from Silverman (1984).

Changes in the Flavin Spectrum upon Inactivation of MAO A by N-CαMBA. Two 150-μL aliquots of 15.2 μM MAO A were added to two self-masked microcuvettes containing 150 μL of 100 mM sodium phosphate, pH 7.2, buffer with 20% glycerol and 0.2% Triton X-100 and with and without 2.10 mM *N*-CαMBA. Difference spectra were recorded periodically over a 4.5-h period during which time aliquots (3 μL) were removed from the cuvettes and assayed. An aliquot (350 μL) of saturated urea was added to each of the microcuvettes, and difference spectra were recorded over a 60-min period.

Reactivation by Various Amines of MAO A Inactivated with N-CαMBA. A 3.5 μM solution of MAO A was incubated with 9 mM *N*-CαMBA in 100 mM sodium phosphate buffer, pH 7.2, containing 20% glycerol and 0.2% Triton X-100 until no more activity remained (~5 h). After dialysis against three changes (350 mL each) of the same buffer, 50-μL aliquots were removed and diluted with an equal volume of solutions containing 10 mM β-mercaptoethanol and 20 mM of the following amines: 5-hydroxytryptamine, norepinephrine, tyramine, dopamine, kynuramine, and benzylamine. Periodically, aliquots (7.5 μL) were removed and assayed as usual in a total volume of 700 μL.

RESULTS

Inactivation of MAO A by 1-PCPA. 1-PCPA is a time- and concentration-dependent inactivator of MAO A; benzylamine protects the enzyme from inactivation. The K_I and k_{inact} values were determined (Kitz & Wilson, 1962) by plotting the $t_{1/2}$ values for each inactivator concentration versus the reciprocal of the inactivator concentration. The K_I and k_{inact} values are given in Table I. Neither dialysis nor gel filtration of inactivated enzyme regenerated any enzyme activity.

Partition Ratio for 1-PCPA Inactivation of MAO A. A titration of MAO A with varying concentrations of 1-PCPA showed that it requires approximately 12 equiv of 1-PCPA to inactivate the enzyme (Figure 1); i.e., the partition ratio is about 11.

Effect on the Flavin Spectrum during MAO A Inactivation by 1-PCPA. Treatment of MAO A with 1-PCPA resulted in the bleaching of the flavin spectrum as a function of time (Figure 2). Addition of sodium hydrosulfite gave an additional 10% reduction in the flavin. Denaturation of inactive enzyme with urea resulted in no reoxidation of the flavin.

Inactivation of MAO A by N-CαMBA. Inactivation of MAO A by *N*-CαMBA is pseudo first order. The kinetic constants for inactivation were obtained as described above

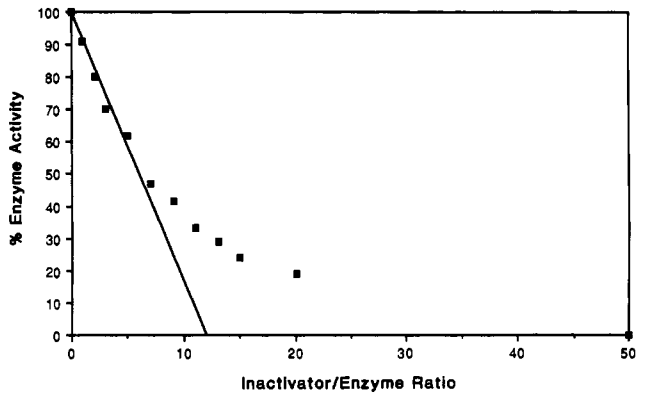

FIGURE 1: Determination of the partition ratio for 1-PCPA. See Materials and Methods.

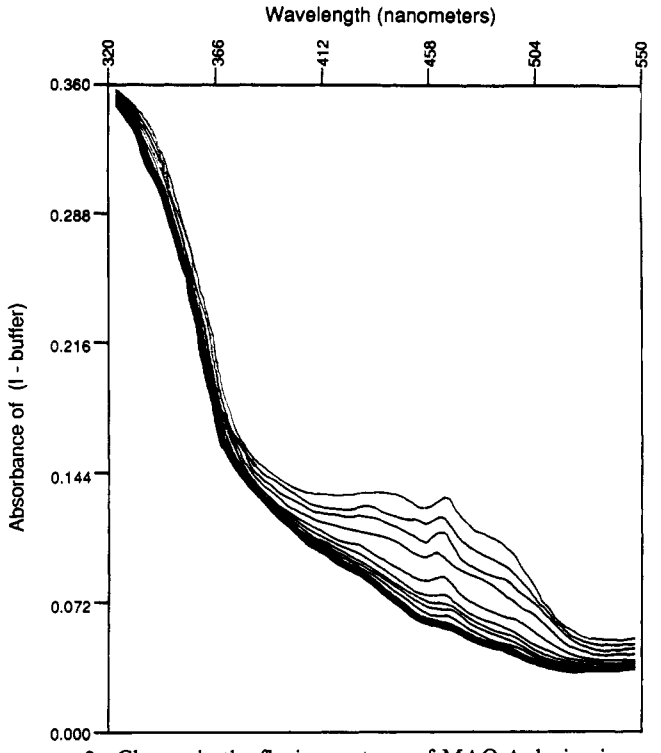

FIGURE 2: Change in the flavin spectrum of MAO A during inactivation by 1-PCPA. The scans from top to bottom shown by thin lines were recorded after 0, 4, 8, 15, 30, 45, 60, 90, and 120 min. The thick trace is the 180- and 240-min scan. See Materials and Methods.

for 1-PCPA inactivation of MAO A and are given in Table I.

Effect of N-CαMBA Inactivation of MAO A on the Flavin Spectrum. Inactivation of MAO A by *N*-[1-^{3}H]CαMBA resulted in the bleaching of the flavin spectrum (Figure 3). However, urea denaturation returned the spectrum to that of oxidized flavin.

Partition Ratio and Stoichiometry for N-CαMBA Inactivation of MAO A. *N*-[1-^{3}H]CαMBA was used to titrate MAO A so that the stoichiometry of labeling could be determined with the partition ratio. A plot of percent enzyme activity remaining versus the ratio of inactivator/enzyme (Figure 4) gave a partition ratio of about 2 (turnover number of about 3). Incorporation of tritium into MAO A is proportional to the loss of enzyme activity and plateaus at approximately 1.2 equiv of inactivator per enzyme molecule (Figure 4). After urea denaturation 1.1–1.2 equiv of tritium remained bound to MAO A.

Sulfhydryl Titration of 1-PCPA- and N-CαMBA-Inactivated MAO A and MAO B. The cysteine contents of MAO A and MAO B before and after inactivation by 1-PCPA and *N*-CαMBA are listed in Table II.

Reactivation of N-CαMBA-Inactivated MAO A by Various Amines. Incubation of *N*-CαMBA-inactivated MAO A with 5-hydroxytryptamine resulted in a time-dependent unimolec-

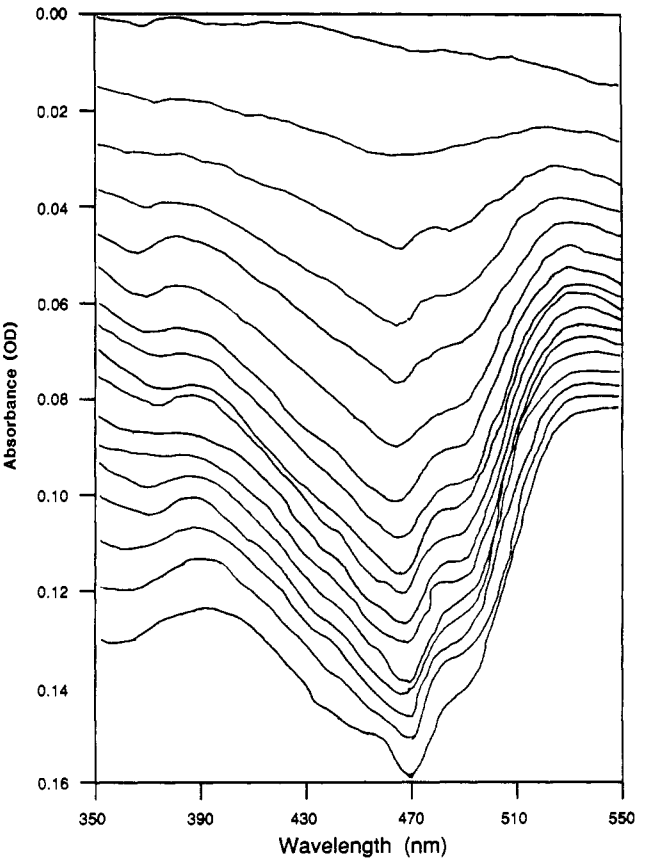

FIGURE 3: Change in the flavin difference spectrum during inactivation of MAO A by *N*-CαMBA. The spectra shown are difference spectra obtained by subtraction of the native enzyme spectrum from that of the enzyme in the presence of *N*-CαMBA. The traces from top to bottom were recorded after 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 150, and 180 min after the addition of *N*-CαMBA. See Materials and Methods.

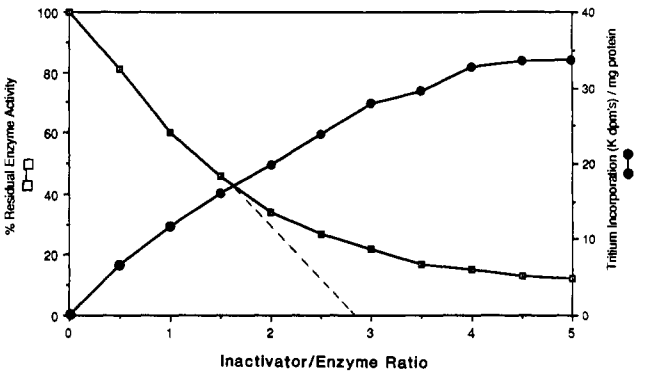

FIGURE 4: Concentration-dependent loss of MAO A activity and concomitant incorporation of tritium into the enzyme by *N*-[1-^{3}H]-CαMBA. See Materials and Methods.

Table II: Cysteine Content[a] of MAO A and MAO B before and after Inactivation by 1-PCPA and *N*-CαMBA

	MAO A	MAO B
native	8.2	6.1
1-PCPA-inactivated	8.2	5.2
N-CαMBA-inactivated	6.9	5.0

[a] Number of cysteine residues per enzyme molecule (average of three experiments). See Materials and Methods for the procedure.

ular return of enzyme activity with a half-life of about 70 min. The half-lives for tyramine, dopamine, kynuramine, and benzylamine were 30, 90, 120, and 50 min, respectively. Norepinephrine reactivated only 10% of the enzyme activity in 8 h.

Release of ^3H from N-[1-^3H]CαMBA-Inactivated MAO A by Benzylamine. When the reactivation experiment described above with benzylamine was repeated with *N*-[1-^{3}H]CαMBA, 57% of the bound tritium was released when 60% of the enzyme activity had been regenerated.

Discussion

A comparison of the kinetic constants for inactivation of MAO A and MAO B by 1-PCPA, 1-BCPA, and *N*-CαMBA is shown in Table I. Since 1-PCPA and *N*-CαMBA are derivatives of benzylamine, an excellent selective substrate for MAO B, it is not surprising that the K_I values are approximately 50 times higher for MAO A than for MAO B. The inactivation rate constants at saturation, k_{inact}, however, are similar. The semilogarithm plot for inactivation of MAO A by 1-PCPA showed an upward deviation from linearity that is reminiscent of what was observed for the inactivation of MAO B by 1-PCPA (Silverman & Zieske, 1985a). In the latter case this was explained as the result of two different processes, one involving attachment to an active site amino acid residue which then spontaneously decomposed (fast rate), and the other involving an irreversible attachment to the flavin (slower rate). In the case of MAO A there is no evidence that reversible attachment to an amino acid residue occurs. Gel filtration immediately following inactivation resulted in no return of enzyme activity. However, since it requires approximately 12 molecules of 1-PCPA to inactivate MAO A completely (Figure 1), it is possible that attachment to an amino acid residue does occur, but spontaneous decomposition of this adduct occurs much more rapidly than with MAO B and, therefore, it is not observed. The reaction of 1-PCPA with MAO B (Silverman & Zieske, 1985a) requires eight molecules of 1-PCPA, an average of seven of which are converted to product prior to enzyme inactivation by the eighth molecule. The molecules acting as substrates become transiently attached to a cysteine residue, as determined by DTNB titration (Silverman & Zieske, 1986), and the inactivator molecule becomes irreversibly bound to the flavin. In the case of MAO A inactivation cysteine titration with DTNB showed no difference in the number of sulfhydryl groups (ca. 8) before and after 1-PCPA inactivation (Table II). Furthermore, inactivation resulted in reduction of the flavin, which remained reduced even after denaturation (Figure 2). All of these results are consistent with attachment to the covalently bound flavin coenzyme rather than to an amino acid residue.

Although there are various similarities to the inactivation of MAO A and MAO B by 1-PCPA, the active site environments of the two enzymes must be different, since no amino acid adduct could be isolated with MAO A but one could be with MAO B. One obvious difference in the two isozymes is their total cysteine content; MAO A has 8 cysteine residues and MAO B has 6 (Silverman & Zieske, 1986).

The kinetic constants for inactivation of MAO A and MAO B by 1-BCPA are quite similar (Table I). The rate of inactivation of MAO A, however, was even slower than with MAO B, and consequently, it was impractical to continue studies with this compound.

The inactivation of MAO A by *N*-CαMBA is nearly identical with the corresponding reaction with MAO B (Silverman, 1984; Vazquez & Silverman, 1985). The partition ratio for *N*-CαMBA with MAO A or MAO B is about 2. From the optical spectrum (Figure 3) it is apparent that during inactivation the flavin is reduced, indicating that electrons are transferred in the process. Flavin reduction could be the result of either attachment of the inactivator to the flavin or attachment near the flavin such that reoxidation by O_2 is blocked. Denaturation of inactivated enzyme, however, results in reoxidation of the flavin, thus supporting the latter possibility. An alternative explanation for flavin reoxidation upon denaturation is that the flavin adduct decomposes upon denaturation, consequently leading to flavin reoxidation. This explanation was invalidated by experiments with *N*-[1-^{3}H]-CαMBA. Incubation of MAO A with increasing amounts of *N*-[1-^{3}H]CαMBA resulted in an increasing loss of enzyme activity with concomitant incorporation of tritium (Figure 4) to a maximum of 1.2 equiv after dialysis. Denaturation under the same conditions as those used in the spectral studies gave enzyme still labeled with 1.1 equiv of tritium. These results are essentially identical with those obtained with MAO B (Vazquez & Silverman, 1985). Therefore, the inactivator is not bound to the flavin. Cysteine titration of native enzyme and inactivated enzyme corroborated this suggestion; attachment of the tritium is to a cysteine residue (Table II). The proposed mechanism for inactivation is the same as that proposed by Silverman (1984) and Vazquez and Silverman (1985) for *N*-CαMBA inactivation of MAO B. This adduct is expected to be the same as that produced from *N*-cyclopropylbenzylamine inactivation of MAO B (Vazquez & Silverman, 1985), which was shown to undergo decomposition with enzyme reactivation in the presence of a variety of primary and secondary amines (Yamasaki & Silverman, 1985). Similar results were obtained with *N*-CαMBA-inactivated MAO A. As in the case with MAO B, there was no correlation between the substrate activity of the amine and this reactivation capability. When *N*-[1-^{3}H]CαMBA-inactivated MAO A was partially reactivated with benzylamine, there was a correlation between the amount of enzyme reactivated and the amount of tritium released from the enzyme. This is consistent with the reactivation mechanism proposed by Yamasaki and Silverman (1985) for MAO B.

Unlike inactivation of MAO A by 1-PCBA and 1-BCPA, inactivation by *N*-CαMBA appears to proceed the same with both MAO A and MAO B and produce a cysteine adduct. This is the first case where an inactivator of these two isozymes reacts with an amino acid residue rather than with the flavin, and it provides an approach to study active site peptides other than the pentapeptide which includes the active site flavin that was previously isolated (Yu, 1980; Nagy & Salach, 1981).

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Registry No. MAO, 9001-66-5; 1-PCPA, 41049-53-0; 1-BCPA, 27067-03-4; *N*-CαMBA, 51586-25-5; cysteine, 52-90-4.

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Rapid Attractant-Induced Changes in Methylation of Methyl-Accepting Chemotaxis Proteins in *Bacillus subtilis*†

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ABSTRACT: In *Bacillus subtilis*, addition of chemotactic attractant causes an immediate change in distribution of methyl groups on methyl-accepting chemotaxis proteins (MCPs), whereas in *Escherichia coli*, it causes changes that occur throughout the adaptation period. Thus, methylation changes in *B. subtilis* are probably related to excitation, not adaptation. If labeled cells are exposed to excess nonradioactive methionine, then attractant causes immediate 50% delabeling of the MCPs, suggesting that a flux of methyl groups through the MCPs occurs. Methanol is given off at a high rate during the adaptation period and probably reflects demethylation of some substance to bring about adaptation. The fact that many radioactive methyl groups are lost immediately from the MCPs but only slowly arise as methanol is consistent with the hypothesis that they are transferred from the MCPs to a carrier from which methanol arises. Demethylation of this carrier may cause adaptation.

Chemotaxis is a primitive sensory mechanism whereby bacteria are able to sense their environment and migrate toward favorable conditions. Chemoattractants cause a period of smooth swimming before the cells return to prestimulus behavior (Berg & Tedesco, 1974; Ordal, 1975). In *Escherichia coli*, it has been shown that during this period of swimming attractants cause a cessation of methanol production and a progressive increase in the level of methylation of the specific methyl-accepting chemotaxis proteins (MCPs) that act as the receptors (Springer et al., 1977; Toews et al., 1979). Conversely, repellents cause augmented methanol production and demethylation of the MCPs during the period of induced tumbling (Goy et al., 1977; Toews & Adler, 1979). It is generally accepted that methylation changes of MCPs are central to adaptation (Goy et al., 1977). In *Bacillus subtilis*, it has been shown that attractants cause methylation changes in various MCPs and cause increased methanol production

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