Inactivation of Flavocytochrome b_2 from Bakers' Yeast with Phenylglyoxal†

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ABSTRACT: Flavocytochrome b_2 from bakers' yeast is inactivated by phenylglyoxal in phosphate buffer at pH 8. The reaction shows a biphasic time course. The rates of both phases increase linearly with reagent concentration. A saturable protection effect is afforded by D-lactate, a competitive inhibitor. Enzyme reduction also slows down both inactivation phases. Fluorescence monitoring indicates that flavin dissociates from the modified enzyme but more slowly than activity is lost. The order of the first inactivation phase with respect to reagent concentration is 1. The loss of activity as a function of [^{14}C]phenylglyoxal incorporation suggests that modification of one arginine leads to an alteration in flavin binding such that activity is decreased and the cofactor dissociates. It is proposed that the modified arginine may be responsible for stabilization of the flavin N(1) anion in the reduced enzyme.

Arginine-specific reagents have been widely used in recent years to identify anion-binding sites in proteins (Riordan et al., 1977; Patthy & Thész, 1980). We report in this paper results concerning the reaction between flavocytochrome b_2 from bakers' yeast and phenylglyoxal (Takahashi, 1968).

Flavocytochrome b_2 (L-lactate:cytochrome *c* oxidoreductase, EC 1.1.2.3) catalyzes the oxidation of lactate to pyruvate. It is a tetrameric protein of M_r 220000 (Jacq & Lederer, 1974). Each subunit carries one flavin mononucleotide (FMN) and one protoheme IX as prosthetic groups. The enzyme is thought to consist of several functional domains (Gervais et al., 1977; Mulet, 1976; Pompon & Lederer, 1978). The amino-terminal heme-binding domain has been well characterized and is known to be homologous to cytochrome b_5 (Guiard et al., 1974; Guiard & Lederer, 1976). Much less is known concerning the flavodehydrogenase part. Chemical modification experiments have singled out three cysteine residues in the sequence. Cysteine-233 (Ghrir et al., 1984) is thought to be located in the active site, and its modification by bromopyruvate, a substrate analogue, leads to enzyme inactivation (Alliel et al., 1982). Modification of flavin-free enzyme with 2-keto-3-butynoate leads to the loss of flavin-binding capacity. In the process, cysteines-200, -216, and -233 are each fractionally labeled and partially cross-linked. These experiments therefore suggest that cysteines-200 and -216 are implicated in flavin binding (Pompon & Lederer, 1982, 1983). We have now used phenylglyoxal in the hope of identifying the active site residue that attracts the substrate carboxylate group. The results may however be best interpreted as pointing to the role of an arginine residue in flavin binding.

Experimental Procedures

Materials. Intact flavocytochrome b_2 was prepared as described by Labeyrie et al. (1978). Phenylglyoxal (Fluka AG) was recrystallized in water. [2-^{14}C]Phenylglyoxal (25–35 mCi/mmol) was purchased from the Commissariat à l'Energie Atomique (Saclay) and was diluted according to need. Fresh stock solutions of phenylglyoxal were prepared daily in 0.1 M phosphate buffer–1 mM EDTA (ethylenediaminetetraacetic acid), pH 8. Their concentrations were determined spectrophotometrically by dilution of an aliquot in methanol, with ϵ_{247} = 11 300 M^{-1} cm^{-1} (Kohlbrenner & Cross, 1978).

Preparation and Assay of Enzyme Stock Solutions. Flavocytochrome b_2 was stored precipitated at 4 °C in 0.1 M phosphate buffer, 10 mM DL-lactate, 1 mM EDTA, and 1 mM PMSF (phenylmethanesulfonyl fluoride), pH 7, at 70% ammonium sulfate saturation. The suspension was centrifuged, and the pellet was resuspended twice in a few milliliters of 70% saturated ammonium sulfate in 0.1 M phosphate buffer–1 mM EDTA, pH 8.

This procedure resulted in residual lactate elimination and enzyme autoxidation. The pellet was then redissolved in the required amount of 0.1 M phosphate buffer–1 mM EDTA, pH 8 (incubation buffer), at 30 °C. The enzymatic activity was determined in 0.1 M phosphate buffer, 10 mM L-lactate, 1 mM ferricyanide, and 1 mM EDTA, pH 7 (standard assay solution). The enzyme concentration was expressed relative to one heme (ϵ^{ox}_{413} = 129 mM^{-1} cm^{-1} or ϵ^{red}_{423} = 183 mM^{-1} cm^{-1}, according to the case).

Inactivation and Incorporation Experiments. Incubations with phenylglyoxal were carried out at 32 °C in the pH 8 phosphate buffer. At suitable time intervals, aliquots were removed and assayed after immediate dilution in the standard assay solution. For the determination of ^{14}C-labeled reagent incorporation, separate samples were loaded onto columns (0.4 × 2 cm) of hydroxyapatite (Biogel HTP). These were washed at 4 °C with 0.1 M phosphate buffer, 10 mM DL-lactate, 1 mM EDTA, and 10 mM arginine, pH 7, until the excess radioactivity had been washed out. The modified enzyme was then eluted with 1 ml of 0.4 M phosphate buffer, 10 mM DL-lactate, and 1 mM EDTA, pH 7. These conditions were adapted from Pompon & Lederer (1982), and the whole procedure was complete in less than 1 h after incorporation was stopped. The protein concentration was then determined both spectrophotometrically and after amino acid analysis (see below). The radioactivity was determined by scintillation counting with ACS scintillation fluid (Amersham International) in an Intertechnique SL 30 scintillation counter.

Flavin dissociation was followed at 530 nm with a Perkin-Elmer LS-5 spectrofluorometer, with excitation set at 450 nm. For these experiments, a solution of 10 μM enzyme was incubated with phenylglyoxal under the usual conditions. Aliquots were withdrawn at various time intervals and either assayed for activity or diluted in the spectrofluorometer cuvette for immediate determination of flavin fluorescence. The latter was corrected for the low fluorescence of the control incubated without phenylglyoxal.

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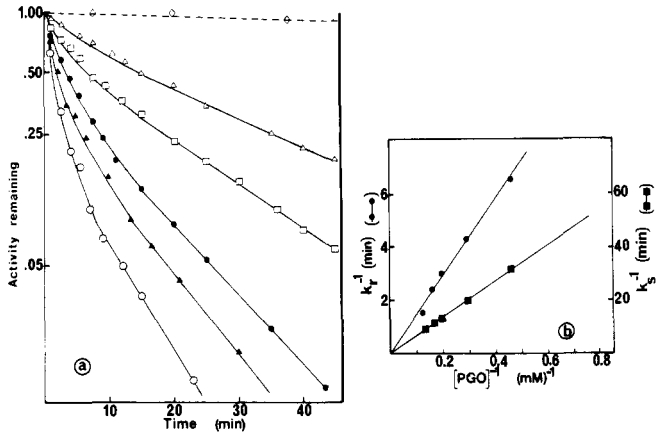

FIGURE 1: Inactivation of flavocytochrome b_2 by phenylglyoxal. (a) Time course of the inactivation at various reagent concentrations. Incubation conditions are described under Experimental Procedures. Enzyme concentration was 3.5 μM. Reagent concentration was 2.16 (△), 3.46 (□), 5.20 (●), 6.07 (▲), and 7.80 mM (○). Control without reagent (◇). (b) Influence of reagent concentration on the inactivation rate. The values of k_r (■) and k_s (○) were derived from the data in (a) as described under Results.

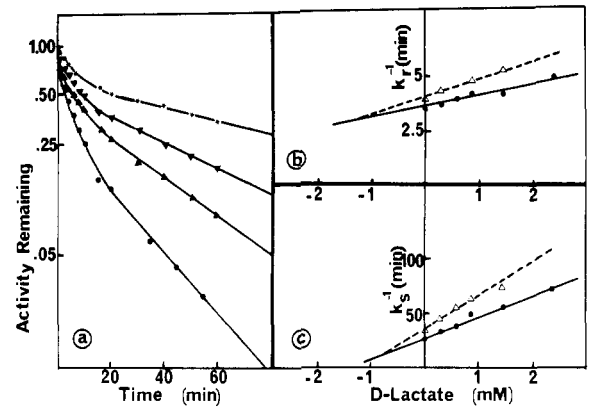

FIGURE 2: Effect of D-lactate on the inactivation by phenylglyoxal. (a) Time course of the inactivation in the presence of various D-lactate concentrations. Enzyme (13 μM) was treated with 5.35 mM phenylglyoxal in the presence of the following D-lactate concentrations: 0 (●), 0.59 (▲), 1.45 (▼), and 4.45 mM (*). (b) Dependence of k_r on D-lactate concentration. Phenylglyoxal concentration was 4.20 (△) and 5.35 mM (●). (c) Dependence of k_s on D-lactate concentration. The phenylglyoxal concentrations are the same as those for (b).

Protein Chemistry Methods. Amino acid analyses were carried out with an LKB 4400 amino acid analyzer as described by Pompon & Lederer (1983). Chymotryptic digestion was carried out at 0 °C as described in Pompon & Lederer (1976).

Sulfhydryl Group Content Determination. After modification with nonradioactive phenylglyoxal, excess reagent was removed by filtration on a column (1.2 × 19 cm) of Sephadex G-25 equilibrated in 0.1 M phosphate buffer, 10 mM L-lactate, and 1 mM EDTA, pH 7. Blocking of available free sulfhydryl groups was achieved by incubating the eluted protein in 29 mM bromopyruvate for 45 min at room temperature. Excess reagent was destroyed by addition of 20 μL of mercaptoethanol. The protein was then filtered on a column (1 × 17.5 cm) of Sephadex G-25 equilibrated in 0.1 M ammonium acetate, 10 mM lactate, and 1 mM EDTA, pH 7. After lyophilization, S-carboxymethylation under denaturing, reducing conditions was carried out under conditions adapted from Crestfield et al. (1963). The protein was dissolved in 0.4 mL of 0.5 M Tris/HCl [tris(hydroxymethyl)aminomethane hydrochloride] buffer, 0.2% EDTA, and 6 M guanidinium chloride, pH 8.4, containing 5 mg of dithiothreitol. After 4 h at room temperature under nitrogen, 10 mg of iodo[2-^{14}C]acetate (460 counts min^{-1} nmol^{-1}) was added. The reaction was terminated after 30 min by addition of 20 μL of mercaptoethanol. The protein was freed from excess reagents by filtration on a column (1 × 17.5 cm) of Sephadex G-25 equilibrated in 30% acetic acid. Radioactivity was determined as usual, and protein was quantified by amino acid analysis. In another experiment, inactivated enzyme was directly treated with iodo[2-^{14}C]acetate without pretreatment with bromopyruvate, under the conditions described above but in the absence of dithiothreitol.

Gel electrophoresis in a vertical flat-bed apparatus was carried out as described by Douglas et al. (1979).

Results

Kinetics of Inactivation with Phenylglyoxal. Inactivation experiments were carried out in 0.1 M phosphate buffer–1 mM EDTA, pH 8, because the enzyme was found unstable in borate, *N*-ethylmorpholine/acetate, and carbonate buffers.

Figure 1a shows that at all reagent concentrations tested the inactivation appeared biphasic. Both phases were dependent on reagent concentration. The experimental data could be satisfactorily analyzed as the sum of two exponential processes. Two series of pseudo-first-order rate constants were thus obtained, k_r for the faster phase and k_s for the slower one. A double-reciprocal plot relating the two rate constants to the reagent concentration showed two straight lines that intersected the origin (Figure 1b). It appeared therefore that the reagent had no affinity for flavocytochrome b_2, despite some similarity between phenylglyoxal and the keto acids derived from phenyl lactate (a substrate) and mandelate (a competitive inhibitor).

Protection against Inactivation. D-Lactate is known as a competitive inhibitor of the enzyme with K_i = 1.4 mM (Somlo & Slonimski, 1966). Addition of D-lactate to the incubation medium slowed down both inactivation phases (Figure 2a). A plot of the reciprocal of the rate constants vs. the competitive inhibitor concentration (Figure 2b,c) showed that the protection effect was saturable with respect to lactate, according to

$$E + PGO \rightarrow E_{inact}$$

$$E + I \underset{K_d}{\rightleftharpoons} E{\cdot}I$$

The K_d values obtained from these plots with k_r and k_s values were 1.2 ± 0.1 mM and 0.7 ± 0.2 mM, respectively.

Pyruvate and oxalate were also tried as protecting agents. At 8.6 mM phenylglyoxal, 3 mM pyruvate reduced k_r about 3-fold; at 5.3 mM phenylglyoxal, 1.1 mM oxalate decreased k_r about 1.5-fold. A complete concentration-dependence study of the protection effect was not attempted, because pyruvate and oxalate do not behave as simple competitive inhibitors of lactate oxidation (Lederer, 1978).

The effect of reduction on inactivation was tested next. It is known that, when L-lactate is added to the enzyme, the two prosthetic groups are immediately reduced, but the enzyme is then slowly autoxidized. Therefore, the experiments were carried out under argon. Figure 3 shows that, in the presence of either 0.45 mM or 1.8 mM L-lactate, the protection effect was the same within experimental error. Since a value of 0.4 mM has been determined both for the K_d of L-lactate at 4 °C (Pompon et al., 1980) and for its K_m at 30 °C (Jacq & Lederer, 1974), it appears that the protection effect is due to reduction and not to a physical protection afforded by the substrate at the active site.

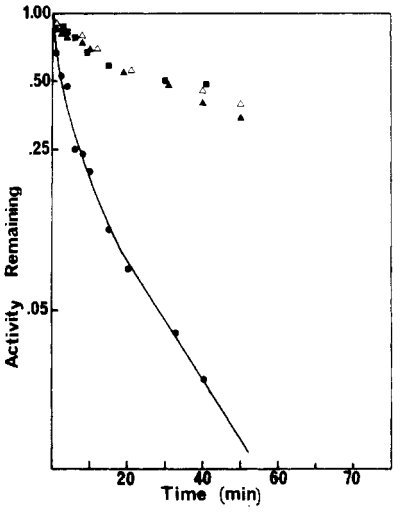

FIGURE 3: Effect of reduction on flavocytochrome b_2 inactivation by phenylglyoxal. Enzyme (9.7 μM) was incubated with 5.94 mM phenylglyoxal under argon after the following additions: none (●), 0.45 mM L-lactate (△), 1.8 mM L-lactate (▲), and 1.8 mM L-lactate + 7 mM pyruvate (■).

Table I: Comparison between Rates of Flavin Dissociation and Enzyme Inactivation by Phenylglyoxal[a]

phenylglyoxal concn (mM)	k_r (min^{-1})	k_s (min^{-1})	k_f (min^{-1})
2.6	0.15	0.018	0.03
5.25			
expt 1	0.25	0.04	0.09
expt 2	0.24	0.04	0.09

[a] All values except those for experiment 2 at 5.25 mM reagent are derived from the experiments presented in Figure 4.

The reduced enzyme has no affinity for L-lactate but can reduce pyruvate (Urban & Lederer, 1983). With the enzyme form used in this study, the K_m of pyruvate for this reaction is about 3 mM (P. Urban, personnal communication). When 3 mM and 7 mM pyruvate were added to the reduced enzyme, no significant increase of the protection was observed relative to the incubations with L-lactate alone (Figure 3).

Dithionite, a reducing reagent without affinity for the active site, also slowed down the inactivation, as expected from the experiment with L-lactate. It is however known that dithionite is in equilibrium with bisulfite (Mayhew, 1978), and bisulfite reacts with phenylglyoxal as it does with other carbonyl reagents. The effect of dithionite was therefore not studied further in view of the uncertainty introduced concerning the actual phenylglyoxal concentrations.

Flavin Fate during Inactivation. FMN loses its fluorescence upon binding to flavocytochrome b_2 (Iwatsubo & di Franco, 1965), as is the case with most other flavoproteins. Inactivated enzyme showed a strong fluorescence indicative of flavin dissociation. When the time course of the phenomenon was studied in parallel with inactivation, flavin dissociation was found to be monophasic. Its rate was dependent on reagent concentration and was lowered in the presence of protective agents like D-lactate or upon enzyme reduction. Table I shows that the value of the first-order rate constant for flavin dissociation, k_f, was intermediate between those of k_r and k_s, but closer to the latter. Some inactivation experiments were carried out in the presence of 66 μM FMN without any modification of the inactivation rate. When 10 μM FMN was added to the enzymatic assay solution, no activity increase was observed.

Labeling Stoichiometry. When the data of Figure 1 were replotted as log k_r against the log of reagent concentration,

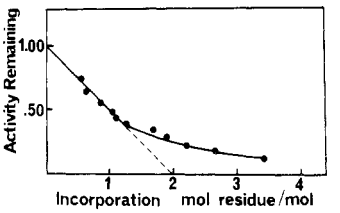

FIGURE 4: Incorporation of [^{14}C]phenylglyoxal into flavocytochrome b_2. Enzyme (54.9 μM) was treated with 5.3 mM [^{14}C]phenylglyoxal under the usual conditions. Separate aliquots were withdrawn for activity measurements or for determining the incorporation. The protein was separated from excess reagent on hydroxyapatite columns as described under Experimental Procedures.

the slope was found to be 0.98; for another experiment, it was found to be 1.08; this suggested that 1 mol of reagent only was responsible for the inactivation in the first phase. A similar plot for k_s gave a slope of 1.12.

Incubation with [^{14}C]phenylglyoxal led to the results presented in Figure 4. Flavocytochrome b_2 contains 18 arginine residues per subunit and about 44 lysine residues (Lederer & Simon, 1971; Ghrir et al., 1984; S. Coctial, A. M. Becam, and F. Lederer, unpublished experiments). Amino acid analyses after hydrolysis gave no reliable indication for arginine or lysine loss. This was expected since at the high sensitivity used we consider the experimental error to be on the order of 5–10%. The cysteine side chain has been found to disappear in the presence of high concentrations of phenylglyoxal, but the nature of the reaction product has not been determined (Takahashi, 1977a). Flavocytochrome b_2 contains seven cysteine residues (Lederer & Simon, 1971), and its activity is known to depend on the integrity of several of them, as exposed in the introduction. The possibility of their being modified in the inactivated enzyme was therefore investigated as described under Experimental Procedures. Incorporation of iodo[2-^{14}C]acetate under denaturing, reducing conditions, with or without prior blocking of available -SH groups by the more reactive bromopyruvate, was determined in order to differentiate between reversible and irreversible sulfhydryl group modification. No significant difference was observed between the control and the treated protein: for example, a sample of 70% inactivated enzyme contained 0.3 sulfhydryl group less than the control. In view of the selectivity of phenylglyoxal (Takahashi, 1968, 1977a,b), it appears therefore most likely that incorporation of phenylglyoxal took place on arginine residues.

Discussion

Inactivation Kinetics and Stoichiometry. Kinetic results that can be analyzed in terms of two exponential processes can correspond to many different kinetic schemes. In the case of enzyme chemical modifications, this problem has been discussed by Ray & Koshland (1961). Apparent rate constants and amplitudes will have a different physical meaning according to which scheme is considered. In the case of flavocytochrome b_2, the idea that k_r approximates a microscopic constant (namely, that for the modification of a particular arginine) is supported by two pieces of evidence: its order of 1 with respect to phenylglyoxal concentration and the Dixon plot of Figure 2b, which yields a K_d for D-lactate in keeping with its known K_i.

The reported stoichiometry for the phenylglyoxal–arginine adduct is 2:1 (Takahashi, 1977a). This value has been verified in a good number of cases. With this in mind, one could give the following interpretation of Figure 4: attack of a first mole of reagent would lead to a totally inactive subunit, and this would be followed rapidly by incorporation of a second mole

on the same arginine. Nevertheless, a few examples of 1:1 complexes formed in buffers other than borate have been published (Borders & Riordan, 1975; Philips et al., 1978, 1979). A second interpretation is therefore equally if not more plausible: the inactivation first phase would lead to a partially active enzyme species with incorporation of 1 mol of reagent/critical arginine.

Flavin release is clearly not the cause of activity loss since it is dissociated more slowly than activity is lost (Table I). Therefore, the primary modification event, which affects activity, results in an enzyme with decreased affinity for flavin. The simple scheme

$$\text{E·Fl} \xrightarrow[k_r]{\text{R}} \text{E'·Fl (partially active)} \xrightarrow[k_f]{} \text{E'} + \text{Fl (inactive)}$$

is attractive. If it were operative, k_f and k_s should be equal. Their determined values differ by a factor of about 2 (Table I), a difference that may not be much larger than could be accounted for by experimental error. If however the difference is real, the above scheme cannot be operative; an interpretation of the second inactivation phase, which is accompanied by additional—apparently limited—incorporation, then becomes difficult. The data at hand do not fit various simple kinetic schemes adapted from Ray & Koshland (1961).

Possible Role of the Arginine Modified in the First Inactivation Phase. The protection against modification afforded by the substrate competitive inhibitor D-lactate could indicate that this arginine lies at the substrate binding site; it could possibly form an ion pair with the substrate carboxylate. A similar role has been attributed to an arginine modified by butanedione and phenylglyoxal in D-lactate monooxygenase (Peters et al., 1981) and by phenylglyoxal in *p*-hydroxybenzoate hydroxylase (Shoun et al., 1980). Arginines were also suggested to be present at the active site of pig kidney general acyl-CoA dehydrogenase (Jiang & Thorpe, 1982) and of pyridoxamine-5′-phosphate oxidase from rabbit liver (Choi & McCormick, 1981). In none of those cases was any evidence for flavin dissociation obtained.

We want to suggest another role for the arginine modified in flavocytochrome b_2. If the protection afforded by D-lactate arose from a direct competitive effect, pyruvate would be expected to provide protection to the reduced enzyme; the rationale is that, being a substrate for the reduced enzyme (Urban & Lederer, 1983), pyruvate should interact with the same arginine in the reduced enzyme as substrates and inhibitors in the oxidized enzyme. The experiment does not suggest any influence of pyruvate (Figure 3). Therefore, the effect of D-lactate is probably indirect, and we want to suggest that the arginine modified in the rapid phase interacts with the flavin rather than with the substrate.

Recently, Fitzpatrick & Massey (1983) concluded that the arginine that is modified by cyclohexanedione in D-amino acid oxidase interacts with the flavin in the N(1)—C(2)=O region and not with the substrates, as originally thought (Ferti et al., 1981). It is interesting to note that, in that case also, a protection against modification was afforded by the competitive inhibitor benzoate, and the affinity for flavin was decreased. It may be that the arginine modified by phenylglyoxal plays in flavocytochrome b_2 the role attributed to the cyclohexanedione-modified residue in D-amino acid oxidase. Indeed, even though flavocytochrome b_2 belongs to the dehydrogenase–electron transferase class, it presents most of the properties described for dehydrogenases–oxidases, which presumably arise from the interaction of a positively charged group with the flavin N(1)—C(2)=O locus (Massey & Hemmerich, 1980). It forms a red semiquinone at neutral pH (Capeillère-Blandin et al., 1975), it reacts with sulfite (Lederer, 1978), and it stabilizes the blue benzoquinoid-type spectrum of 8-mercaptoflavin (D. Pompon, personal communication).

Another possibility is that the modified arginine interacts with the ribitol phosphate group. This seems however much less likely, since in that case one does not easily explain the rate difference between activity loss and flavin dissociation, considering the importance of the ribityl phosphate chain in binding.

Preliminary experiments indicate that most of the label is initially incorporated in the flavocytochrome b_2 proteolytic fragment α, which encompasses the first 300 residues of the 55-kDa subunit. Future efforts will be directed at identifying this arginine in the sequence.

Acknowledgments

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Registry No. Flavocytochrome b_2, 9078-32-4; arginine, 74-79-3; phenylglyoxal, 1074-12-0; D-lactic acid, 10326-41-7.

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Effect of α-Methylation on Inactivation of Monoamine Oxidase by *N*-Cyclopropylbenzylamine†

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ABSTRACT: Monoamine oxidase (MAO) was shown previously [Silverman, R. B., & Hoffman, S. J. (1980) *J. Am. Chem. Soc. 102*, 7126–7128] to catalyze the oxidation of *N*-cyclopropylbenzylamine (N-CBA) at two sites on the molecule. Oxidation at the benzyl methylene gave benzaldehyde and cyclopropylamine; oxidation of the cyclopropyl group, which involved cyclopropyl ring cleavage, led to inactivation of the enzyme. In this paper it is shown that methylation of the benzyl methylene dramatically alters this partition ratio in favor of enzyme inactivation. Contrary to a previous report [Alles, G., & Heegaard, E. V. (1943) *J. Biol. Chem. 147*, 487–503], it is shown here that α-methylbenzylamine *is* a substrate for MAO; consequently, *N*-cyclopropyl-α-methylbenzylamine (N-CαMBA) is a good candidate for mechanism-based inactivation. *N*-Cyclopropyl[7-^{14}C]benzylamine, *N*-cyclopropyl-α-methyl[*phenyl*-^{14}C]benzylamine, *N*-[1-^{3}H]-cyclopropylbenzylamine, and *N*-[1-^{3}H]cyclopropyl-α-methylbenzylamine are synthesized, and product formation following MAO inactivation is quantified. The results obtained with these compounds indicate that with N-CαMBA, α-methylbenzyl oxidation (which produces acetophenone and cyclopropylamine) is only 1% that of cyclopropyl oxidation (which gives enzyme inactivation), whereas with N-CBA the amount of oxidation at the corresponding sites is equal. It also is shown that the K_i values for (*R*)-(+)- and (*S*)-(−)-α-methylbenzylamine are similar, suggesting that dimethylation of N-CBA should not interfere with binding to MAO. *N*-Cyclopropyl-α,α-dimethylbenzylamine, which should be incapable of benzyl oxidation, is prepared and shown to be a mechanism-based inhibitor of MAO whose properties differ slightly from those of N-CBA and N-CαMBA. These results show that α-substitution of N-CBA has a profound effect on the partition ratio and present a new approach to rational drug design based on physical organic chemical principles.

Monoamine oxidase (MAO,[1] EC 1.4.3.4) is an important enzyme in the treatment of certain forms of depression; compounds that inhibit MAO exhibit antidepressant activity (Kaiser & Setler, 1981; Rowan et al., 1981; Baldessarini, 1977; Berger & Barchas, 1977; Tyrer, 1976). One of the earlier clinically used antidepressants, tranylcypromine, is a cyclopropylamine-containing MAO inhibitor. Recently, the mechanism of MAO inactivation by this drug was reported (Silverman, 1983), and it was shown to belong to the class of enzyme inhibitors known as mechanism-based inhibitors (Silverman & Hoffman, 1984; Walsh, 1982; Abeles & Maycock, 1976; Rando, 1974). A mechanism-based inhibitor is an unreactive compound that generally is converted to an activated form by an enzyme via its normal catalytic mechanism of action, and this altered form of the compound leads to inactivation of the enzyme. Therefore, a mechanism-based inhibitor initially acts as substrate for the enzyme, but instead of being converted into a product that is released from the enzyme, it is converted into a species that inhibits the enzyme without prior release from the active site. Numerous other cyclopropylamines, particularly the secondary amines, *N*-cyclopropyl-*N*-arylalkylamines, have been shown to be potent MAO inhibitors (Kutter & Hansch, 1969; Winn et al., 1975; Long et al., 1976; Murphy et al., 1978; Fuller et al., 1978; Silverman & Yamasaki, 1984; R. B. Silverman and M. L. Vazquez, unpublished results; Fuller et al., 1983). In our work with N-CBA (R. B. Silverman and M. L. Vazquez, unpub-

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[1] Abbreviations: MAO, monoamine oxidase; N-CBA, *N*-cyclopropylbenzylamine; N-CαMBA, *N*-cyclopropyl-α-methylbenzylamine; N-CααDMBA, *N*-cyclopropyl-α,α-dimethylbenzylamine; Cbz, carbobenzoxy group; KP_i, potassium phosphate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane.

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