Mechanism-Based Enzyme Inactivation via a Diactivated Cyclopropane Intermediate

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Mechanism-based enzyme inactivation is an important tool for the study of enzyme mechanisms and in the design of new classes of drugs.[1] For enzymes that catalyze oxidation reactions the standard approach to mechanism-based inactivation has been to design a compound which when oxidized becomes highly electrophilic, such as allylic or propargylic alcohols being converted to α,β-unsaturated ketones. These reactive species then react with active-site nucleophiles, generally via Michael addition reactions.[1] Cyclopropanes are similar to alkenes in that they are electron-rich species unless electron-withdrawing substituents are appended, in which case they can act as electrophiles.[2-4] There are only a few examples of cyclopropane-containing mechanism-based enzyme inactivators in which the target enzyme activates the cyclopropane for nucleophilic addition as a result of oxidation of an appended group to a carbonyl or related species.[5,6] These examples are cyclopropylmethanol analogues which inactivate the nicotinamide- and zinc-dependent enzymes, horse liver alcohol dehydrogenase and lactate dehydrogenase, and function by forming highly electrophilic, monoactivated cyclopropanes.[2] There are several other examples of monoactivated cyclopropanes that act as inactivators, but these compounds do not require oxidation for activation.[7-9]

We have been interested in the design of new inactivators of the enzyme monoamine oxidase (EC 1.4.3.4; MAO) for many years.[10] Some of our earlier inactivators of this enzyme were cyclopropane analogues,[11-21] but one of the cyclopropane compounds that we synthesized as a potential inactivator, (1-phenylcyclopropyl)methylamine (**1**), was found to be a very good substrate, being converted into 1-phenylcyclopropane-1-carboxaldehyde (**3**), without exhibiting any inactivation properties (Scheme I).[17] Compound **1** was designed to inactivate MAO by oxidation to the iminium ion (**2**) which, as a monoactivated cyclopropane, would react with an active-site nucleophile. However, the electrophilicity of cyclopropanes adjacent to just one anion-stabilizing group is not very great[2-4] and apparently, **2** is not reactive enough to cause enzyme inactivation. The lack of reactivity of monoactivated cyclopropanes has been observed with other enzymes as well.[4,7b,9,22]

Diactivated cyclopropanes, on the other hand, are considerably more electrophilic than monoactivated ones;[2-4] consequently, we sought to design a compound that could undergo mechanism-based inactivation of MAO via enzyme-catalyzed conversion to a diactivated cyclopropane. To the best of our knowledge there are no mechanism-based inactivators of any enzymes that have succeeded by this inactivation approach. Fraser et al.[22] designed a diactivated cyclopropane that inactivated dihydroorotate dehydrogenase, but this compound is an affinity labeling agent, not a mechanism-based inactivator.[1]

On the basis of the substrate activity of **1** with MAO, benzyl 1-(aminomethyl)cyclopropane-1-carboxylate (**4**)[23] was designed to act as a mechanism-based inactivator of MAO via oxidation to the diactivated cyclopropane **5** (containing both a carbonyl and an iminium ion attached to the cyclopropane) which could inactivate the enzyme to produce a covalent adduct (Scheme II; **6** may not be the final structure). Incubation of purified MAO-B from beef liver[26] with **4** led to a pseudo-first-order time- and concentration-dependent loss of enzyme activity. From a replot of the half-lives for inactivation ($t_{1/2}$) versus the inverse of the inactivator concentrations ($1/[I]$)[27] were obtained K_I and k_{inact} values of 240 μM and 0.092 min^{-1}, respectively. Room temperature dialysis at pH 7.40 for 24 h with several buffer changes did not restore any enzyme activity. The substrate 2-phenylethylamine was shown to protect the enzyme from inactivation,[28] indicating that inactivation is active-site directed. Glutathione (2 mM) did not have any effect on the rate of inactivation, suggesting that a reactive electrophile is not released from the enzyme that returns to inactivate the enzyme; inactivation, therefore, occurs at the active site prior to release of the reactive species. Compound **4** is a good substrate for MAO as well as an inactivator, having a K_m of 150 μM and a k_{cat} of 11.6 min^{-1}. The partition ratio, a measure of the production of product per inactivation event, can be calculated from the ratio k_{cat}/k_{inact} to

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(23) Benzyl 1-(aminomethyl)cyclopropane-1-carboxylate (**4**) was synthesized from the known compound 1-cyanocyclopropane-1-carboxylic acid.[24] Esterification of the cyano acid with benzyl alcohol under Dean-Stark conditions gave benzyl 1-cyanocyclopropanecarboxylate in 55% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (bs, 5 H), 5.27 (s, 2 H), 1.73 (m, 2 H), 1.67 (m, 2 H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 168.04, 135.19, 129.13, 129.06, 128.62, 118.95, 68.62, 19.60, 13.87; HRMS calcd for $C_{12}H_{11}NO_2$ 201.0790, found 201.0802; MS (EI) 201 (M^+, 54.9), 182 (10.2), 155 (27.7), 104 (47.2), 91 (100.0), 77 (16.9), 65 (26.7), 51 (12.4), 39 (24.7). Anal. Calcd for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.96. Found: C, 71.64; H, 5.61; N, 6.89. Selective reduction of the cyano group by the method of Satoh and Suzuki[25] gave **4** in 35% yield: mp 127-128 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.41 (bs, 5 H), 5.17 (s, 2 H), 3.18 (s, 2 H), 1.43 (bs, 2 H), 1.10 (bs, 2 H); ^{13}C NMR (300 MHz, D_2O) δ 174.92, 135.50, 128.95, 128.82, 128.31, 67.59, 43.26, 21.59, 15.42; HRMS calcd for $C_{12}H_{16}ClNO_2$ (-Cl) 206.1181, found 206.1189; MS (EI) 206 (1.8), 190 (18.4), 132 (6.0), 114 (30.3), 91 (100.0), 69 (16.1). Anal. Calcd for $C_{12}H_{16}ClNO_2$: C, 59.63; H, 6.67; Cl, 14.70; N, 5.80. Found: C, 59.47; H, 6.73; Cl, 14.83; N, 5.74.

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(28) The $t_{1/2}$ for inactivation of MAO with 0.25 mM **4** was 17 min and with 0.25 mM **4** in the presence of 0.5 mM 2-phenylethylamine (K_m 75 μM) was 80 min.

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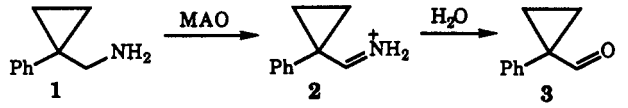
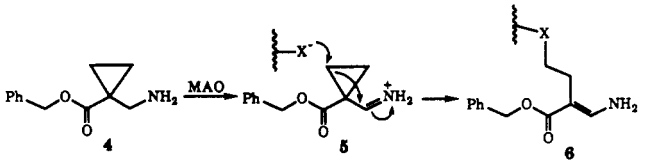
Scheme I



Scheme II



be 126. The fact that the flavin spectrum is converted to its reduced form concomitant with inactivation indicates that **4** is not acting simply as an affinity labeling agent; it requires activation by oxidation in order to be effective and, therefore, is a mechanism-based enzyme inactivator. Denaturation of the inactivated enzyme (5% $NaDodSO_4$), however, did not lead to reoxidation of the reduced flavin cofactor, suggesting that the inactivator becomes attached to the flavin (Scheme II, X^- = reduced flavin).

Given that **1**, which leads to a monoactivated cyclopropane intermediate, is a good substrate that does not undergo ring cleavage and is not an inactivator of MAO, and that the difference in reactivity between diactivated and monoactivated cyclopropanes is considerable, the mechanism for the inactivation of MAO by **4** shown in Scheme II is reasonable. If that is the case, then, to the best of our knowledge, this is the first successful application of the diactivated cyclopropane approach to mechanism-based enzyme inactivation. This should be a useful general approach to the inactivation of enzymes that catalyze oxidation reactions.

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Supplementary Material Available: Kitz and Wilson plot of the data for the inactivation of MAO by **4** and UV–visible spectra before and after inactivation of MAO by **4** and after denaturation of the inactivated enzyme (2 pages). Ordering information is given on any current masthead page.