Inactivation of a Flavin-Linked Oxidase, *N,N*-Dimethylglycine Oxidase, *in Vivo* and *in Vitro*

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SUMMARY

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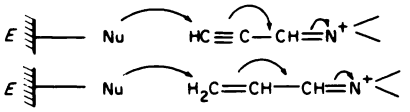
Attention is focused on mechanistic proposals concerning the unique inhibitory properties of *N*-allyl or *N*-propargyl substituents toward flavin-linked oxidases such as *N,N*-dimethylglycine oxidase. It is concluded that inhibitory substrates act through the formation of alkylating species, which can react directly with an enzyme site without the intermediacy of free aldehydes. These results extend and consolidate the concept of enzyme inactivation by inhibitory substrates or "suicide substrates." The activity displayed *in vivo* by the *N,N*-dimethylglycine oxidase inhibitory substrates demonstrates the practical potential of suicide substrates in molecular pharmacology and chemotherapy.

Previous studies (1, 2) have suggested that the concept of inhibitory substrates (*N*-allyl- and *N*-propargylglycine and *N*-allyl- and *N*-propargylsarcosine) is applicable to flavine-linked oxidases such as *N,N*-dimethylglycine oxidase and sarcosine oxidase.

The observation of significant kinetic isotope effects on the rate-limiting inhibitory reaction led us to suggest that the enzyme generates a reactive alkylating species which may react directly to form a covalent bond with an active site nucleophile (Nu) (Scheme 1) or, alternatively, generate propargylaldehyde or acrolein at the same site, followed by alkylation of a proximal nucleophile (Scheme 2).

SCHEME 1



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The present communication offers supplementary evidence and arguments strengthening the proposed mechanism of enzyme inactivation by inhibitory substrates and reports on the potent inhibition of *N,N*-dimethylglycine oxidase *in vivo* by candidate inhibitory substrates.

Experiments *in vitro* were performed with male Sprague-Dawley rats weighing 150–200 g. They were decapitated and their livers removed. The mitochondrial fraction was prepared as described earlier (2).

The effect of oxygen on the rate of dimethylglycine oxidase inhibition by inhibitory substrates was evaluated using an incubator equipped with inlets for N_2, air, and O_2. The rate of inactivation was determined by assaying for residual enzymatic activity (3). For experiments *in vivo* the inhibitory substrates were given orally (200 mg/kg) to male Sprague-Dawley rats. After a prescribed period of time the rats were decapitated, their livers were re-

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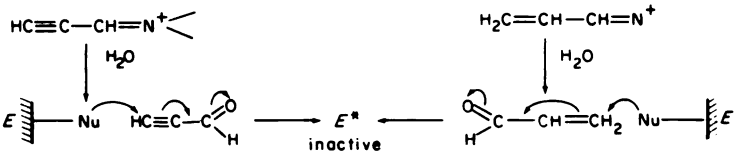
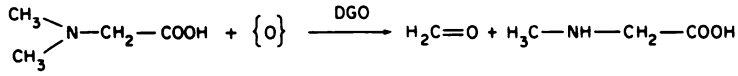
SCHEME 2



moved, and the degree of enzyme inactivation was measured by assaying for residual activity in the usual manner.

Figure 1 summarizes some typical results relative to the effect of oxygen on the rate of dimethylglycine oxidase inhibition by the inhibitory substrate *N*-methyl-*N*-propargylglycine. It is clear that the activity of the inhibitory substrate decreased markedly (80%) after incubation with the enzyme under a nitrogen atmosphere. MacKenzie and co-workers (4, 5) previously observed that the dimethylglycine oxidase-catalyzed demethylation of dimethylglycine to sarcosine has a similar requirement for oxygen.

Earlier studies have established that the metabolism of dimethylglycine in liver mitochondria proceeds by the following over-all reaction (where DGO denotes dimethylglycine oxidase):



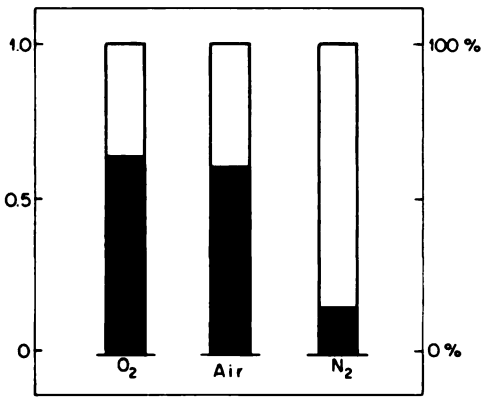
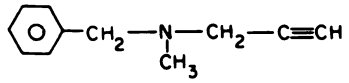


FIG. 1. *Effect of oxygen on rate of N,N-dimethylglycine oxidase inhibition by N-methyl-N-propargylglycine (inhibitory substrate)*

Enzyme solutions were degassed under a slow stream of N_2 gas for 5 min and then incubated with inhibitor (*N*-methyl, *N*-propargylglycine) under either nitrogen, air, or oxygen for 20 min at 37°C. Residual enzyme activity was determined by measuring formaldehyde production from *N,N*-dimethylglycine as previously described (3).

It was important to verify whether the intermediate suggested above (Scheme 1a) as an oxidation product of *N*-methyl-*N*-propargylglycine could generate propiolaldehyde at the active site of dimethylglycine oxidase after hydrolysis as shown in Scheme 2. The rate of inactivation by propiolaldehyde at 1 nM was found to be negligible, whereas under the same conditions *N*-methyl-*N*-propargylglycine caused 50% inactivation of the enzyme. To obtain the same rate of inactivation with propiolaldehyde, a 50,000-fold increase in concentration was required. Table 1 compares the rates of inhibition of dimethylglycine oxidase with propiolaldehyde and *N*-methyl-*N*-propargylglycine. Propiolaldehyde was less potent than *N*-methyl-*N*-propargylglycine by a factor of 5×10^4, indicating that neither propiolaldehyde nor hydrolysis of the hypothetical intermediate (Scheme 1a) is involved in the dimethylglycine oxidase inactivation mechanism.

It is known that pargyline (Eutonyl)



is a potent monoamine oxidase inhibitor *in vivo*. It is also an effective antihypertensive agent (7, 8), but an inconsistent relationship has been found between the ability of this agent to lower blood pressure and its ability to inhibit monoamine oxidase (9, 10).

It was of interest to verify whether various dimethylglycine analogues could inhibit dimethylglycine oxidase *in vivo* and whether the duration of action would bear

TABLE 1

Comparison of dimethylglycine oxidase inhibition by propiolaldehyde and N-methyl-N-propargylglycine

Inhibitor	Concentration	Amount added	Inhibition
	M	*μmoles*	%
$HC{\equiv}C{-}CH_2{-}\underset{\displaystyle CH_3}{\overset{|}{N}}{-}CH_2COOH$	4×10^{-7}	0.001	56
$HC{\equiv}C{-}\overset{\displaystyle O}{\overset{\|\|}{C}}{-}H$ (ref. 6)	4×10^{-7}	0.001	5
	2×10^{-2}	50	44

TABLE 2

Duration of inhibition of dimethylglycine oxidase in vivo by structural analogues of N,N-dimethylglycine

$$R{-}\underset{\displaystyle CH_3}{\overset{|}{N}}{-}CH_2{-}COOH$$

R	Residual enzyme activity after	
	1 day	14 days
	%	%
$HC{\equiv}C{-}CH_2{-}$	5	95
$CH_3C{\equiv}C{-}CH_2{-}$	5	25
$\phi{-}C{\equiv}C{-}CH_2{-}$	9	
$H_2C{=}CH{-}CH_2{-}$	15	97
$CH_3CH{=}CH{-}CH_2{-}$	15	97
$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!C{=}CH{-}CH_2{-}$	13	95
Pargyline[a]	7	93

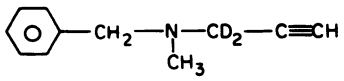
[a] Monoamine oxidase inhibition; data of Taylor *et al.* (7).

similarities to the effect of pargyline on monoamine oxidase. Table 2 shows that significant inhibition occurred *in vivo* for all the inhibitory substrates tested. Both the observed rate of inhibition and duration of the blockade are similar to the effect of pargyline on monoamine oxidase activity.

The above results establish that the inhibitory substrate *N*-methyl-*N*-propargylglycine requires oxygen to inactivate dimethylglycine oxidase. Analogously, the enzyme requires oxygen to transform dimethylglycine into sarcosine. The catalytic behavior of dimethylglycine oxidase toward its natural substrate, *N,N*-dimethylglycine, and the inhibitory substrate, *N*-methyl-*N*-propargylglycine, strongly suggests that in the first step the enzyme is unable to differentiate between the methylene group alpha to the acetylenic or olefinic function and the methyl group of the substrate. This conclusion is logical in view of our observation that the inhibitory reaction is characterized by a rate-limiting abstraction of an α-hydrogen from the allyl or propargyl group (1, 2). In other words, inhibition is formally associated with an oxidation reaction.

These results extend and consolidate the concept of enzyme inactivation by inhibitory substrates or "suicide substrates," which produce their effects through the formation of alkylating species such as 1a and 1b. Such species can react directly with an enzyme site without the intermediacy of free aldehydes, as was initially envisaged.

It should also be noted that we have observed a significant isotope effect on the rate of monoamine oxidase inactivation with the deuterated analogue of pargyline,



thus establishing that the latter is indeed a suicide substrate for monoamine oxidase (2).

The activity displayed *in vivo* by the dimethylglycine oxidase inhibitory substrates (Table 2) is parallel to the activity of pargyline toward monoamine oxidase. These results demonstrate the practical potential of suicide substrates in molecular pharmacology and chemotherapy.

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