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Propargyl amine-induced irreversible inhibition of non-flavin-linked amine oxidases

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THE INHERENT specificity of enzymes is primarily based on the k_{cat} term(s). Although many molecules may bind at the active site of a particular enzyme, only a very limited number will actually serve as substrates, and notions such as induced-fit, non-productive binding, and the "rack" mechanism all are intended in one way or another to rationalize this fact.[1] This being the case, irreversible enzyme inhibitors whose

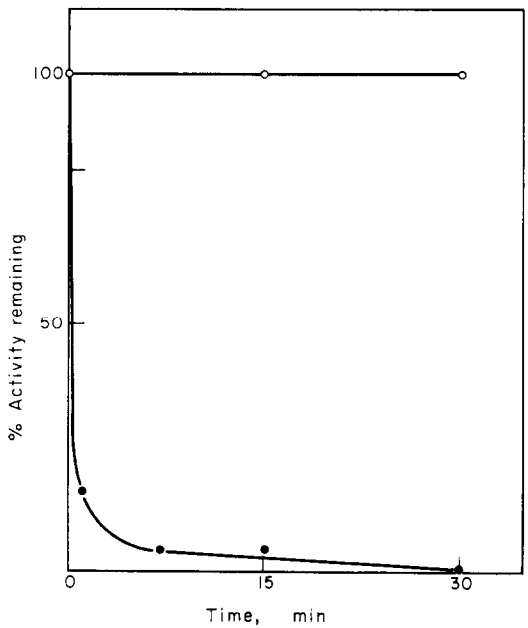


FIG. 1. Irreversible inhibition of plasma monoamine oxidase by propargyl amine. (○) = control, (●) = 3 mM propargyl amine. Ten units of enzyme (sp. act. = 200 units/mg)[4] was incubated in 0·2 M phosphate buffer, pH 7·5, with 3 mM freshly distilled propargyl amine (Aldrich Chemical Co.) along with a control. The remaining activity was measured with time by diluting the enzyme 50-fold in 3·3 mM benzyl amine and measuring the increase in O.D. at 250 nm (benzaldehyde λ_{max} = 250 nm).[4] The activity of the inhibited enzyme could not be restored in the slightest by continued dialysis against the phosphate buffer.

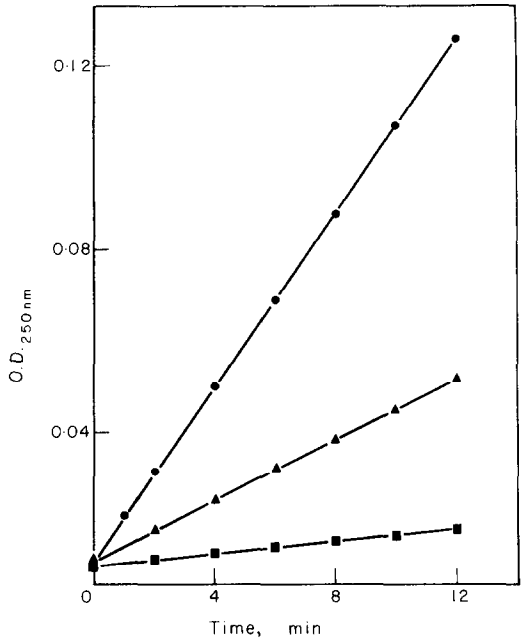


FIG. 2. Substrate protection against irreversible inhibition. (●) = control, (▲) = 10 mM benzyl amine + 1 mM propargyl amine (■) = 1 mM propargyl amine. Ten units of the enzyme were each preincubated with the above solutions for 5 min. Aliquots were then removed and assayed by the standard benzyl amine assay.[4]

selectivity is based on the k_{cat} term rather than the K_s term, as is the case with conventional affinity labeling agents,[2] ought to show exceedingly high specificities. Inhibitors of this kind should possess a chemically unreactive functional group which becomes reactive at the active site when acted upon by the enzyme. The reactive moiety, once generated, ought then to engage in a chemical reaction with a propinquous active-site residue, thus irreversibly inhibiting the enzyme. This kind of inhibitor would lead the enzyme to commit "suicide" by its specific mode of action. The paradigm of this kind of inhibitor is found in studies on the irreversible inhibition of β-hydroxydecanoyl thioester dehydrase by 3-decynoyl *N*-acetylcysteamine.[3] In this instance, the unconjugated acetylenic substrate is isomerized to the highly reactive conjugated allene which alkylates an active-site histidine residue. In this report, we should like to describe the irreversible inhibition of non-flavin-linked plasma monoamine oxidase and kidney diamine oxidase by the acetylene, propargyl amine (1-amino-2-propyne).

Plasma monoamine oxidase oxidatively deaminates a series of aliphatic and aromatic amines to yield the corresponding aldehydes after hydrolysis.[4] The enzyme has two prosthetic groups, and the first has been unequivocally characterized as being cupric ion.[5] A less definite statement can be made about the second, however, but it is certainly not a flavin, as in the mitochondrial monoamine oxidase. It is likely a pyridoxal derivative, although unequivocal evidence for this has yet to be secured.[6] Importantly though, a Shiff base is formed between the substrate and second cofactor which allows one to understand why the enzyme can be inhibited by carbonyl reagents.[4]

Incubation of plasma monoamine oxidase with propargyl amine led to the immediate inactivation of the enzyme as shown in Fig. 1.* Enzyme activity could not be recovered by prolonged dialysis, attesting to the enzyme's being irreversibly inactivated. If propargyl amine is active-site directed, one should be able to protect against its action by adding substrate to the medium. This is demonstrated in Fig. 2 where the substrate, benzyl amine, is shown to protect against enzyme inactivation by propargyl amine. If cupric ion is removed by chelating agents, the enzyme loses its activity, although it still maintains its structural integrity. The enzyme's activity can be regained by removing the chelating agent and adding back cupric ions.[5] This being the case, we would expect enzyme inactivated in this fashion not to be affected by added propargyl amine, simply because the enzyme can no longer catalyze the formation of the inhibitory sub-

* This observation has also been independently made by Prof. R. H. Abeles of Brandeis University (unpublished).

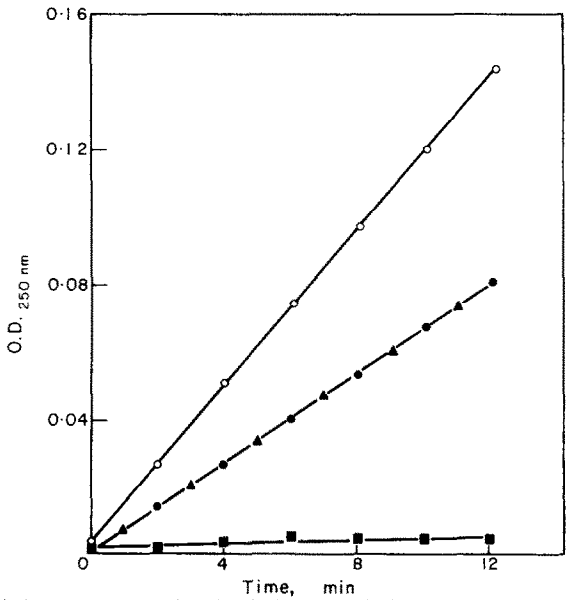
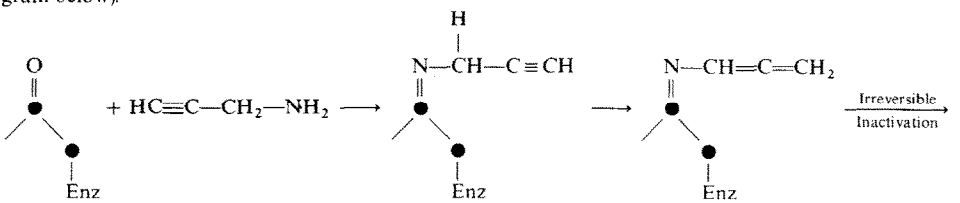


FIG. 3. Effect of cupric ion on propargyl amine-induced inhibition of plasma monoamine oxidase. (○) = control. (●) = reactivated enzyme after treatment with propyl amine – Cu^{+2}, (▲) = reactivated enzyme + propargyl amine – Cu^{+2}, (■ = enzyme – Cu^{+2}. Copper was removed from monoamine oxidase (3 ml of 376 units/ml, sp. act. = 200) by dialyzing against 0·1 M diethyldithiocarbamate after the published procedure.[5] Excess chelating agent was removed, at which point the enzyme was totally inactive.[5] One ml of the enzyme was then incubated with 3 mM propyl amine (control) and two 1-ml samples were each incubated for 0·5 hr with 3 mM propargyl amine. The amines were removed by exhaustive dialysis and the enzymes were then reactivated by adding back the copper.[5] The propargyl and propyl amine-treated samples had exactly the same activity by the standard benzyl amine assay.[4] The upper curve (○) refers to the activity of untreated enzyme, and the lowest curve (■) the activity of enzyme – Cu^{+2}.

stance at its active site. Thus, we would expect copper-deficient enzyme exposed to propargyl amine to regain its activity when the copper is added back after removal of the propargyl amine by dialysis. This is precisely what happens, as shown in Fig. 3. Note then the striking difference in behavior of this kind of inhibitor as compared to what one would expect with a conventional affinity labeling agent.

Since diamine oxidase (histaminase) appears to contain the same cofactors as does the plasma monoamine oxidase, and since it oxidatively deaminates short chain aliphatic amines (although not nearly as well as diamines), we might expect propargyl amine to inactivate this enzyme also.[7] That this is the case is shown in Fig. 4.

The precise mode of irreversible inhibition in these cases is as yet unknown. The acetylenic drug pargyline (*N*-methyl, *N*-benzyl-2-propynyl amine) irreversibly inactivates flavin-linked monoamine oxidase as does cis-3-bromoallyl amine, a vinyl bromide.[10,11] In addition, 2-hydroxy-3-butynoic acid irreversibly inactivates flavin-linked lactate dehydrogenase.[12] In these cases, it is assumed that an enzyme-mediated double bond migration precedes the actual irreversible inhibition. Thus the unreactive acetylenes are converted into the highly reactive allenes, and the unreactive vinyl bromide is converted into the highly reactive allylic bromide. These reactive intermediates then engage in chemical reactions with the enzymes leading to their irreversible inactivation. It is tempting to speculate that an acetylene to allene interconversion mediates the propargyl amine-induced irreversible inhibition of the mono- and diamine oxidase (see diagram below).



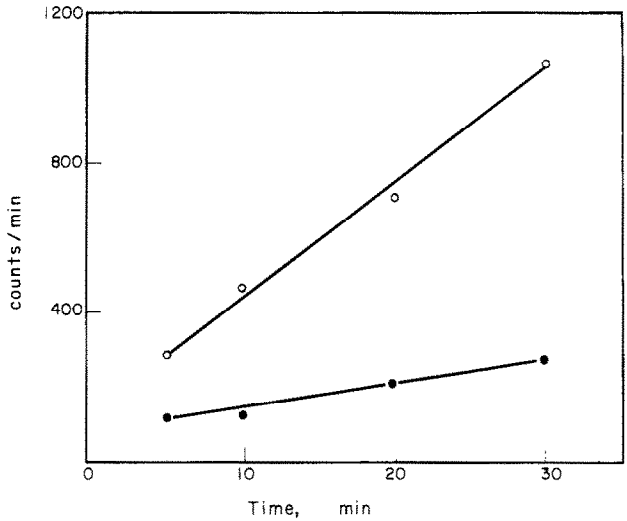


FIG. 4. Irreversible inhibition of hog kidney diamine oxidase by propargyl amine.[8] (○) = control, (●) = enzyme treated with propargyl amine. One unit hog kidney diamine oxidase (Sigma Chemical Co.) (sp. act. = 0·04 units/mg, 2 units/ml) in 0·1 M potassium phosphate buffer, pH 7·6, was incubated with 1 mM freshly distilled propargyl amine for 15 min at 37°. A control containing 1 mM propyl amine was incubated simultaneously under the same conditions. The samples were thoroughly dialyzed against the phosphate buffer at 4° to remove the amines. The enzymatic activities were then assayed by the standard ^{14}C-cadaverine assay.[9] Thus the ordinate gives a measure of the amount of ^{14}C-cadaverine (1,5-diaminopentane) converted into 5-aminopentanal.

The observations that these enzymes, which may be pyridoxal-5-phosphate linked, can be irreversibly inhibited by these acetylenes leads to the hope that other enzymes of this type could be irreversibly inhibited when challenged with the appropriate "suicide-inducing" substrates. Reagents of this kind would be exceedingly useful because of the great specificity implicit in their mechanism of action. Furthermore, this mechanism does not appear to be limited to just one type of enzyme; examples now exist for flavin-dependent enzymes and non-cofactored enzymes, in addition to that reported here.

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Department of Pharmacology,
Harvard Medical School,
Boston, Mass. 02115, *U.S.A.*

ROBERT R. RANDO
JUAN DE MAIRENA

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Action of anti-inflammatory steroids on the lytic action of phospholipase C and 2,4,6-trinitrobenzene sulphonic acid on lysosomes

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THE UNDERLYING biochemical mechanism of the action of anti-inflammatory steroids on lysosomal membranes is not known but work with model systems clearly indicates that steroid–phospholipid interactions are important.[1] It is less clear why the stabilising action of anti-inflammatory steroids on the membranes should fall off at high concentrations but there is some evidence that steroid–protein interactions may be involved.[2] In order to gain information on the mechanisms underlying the action of anti-inflammatory steroids on lysosomes we have investigated their effect on the action of phospholipase C (PLC) and the protein denaturing agent 2,4,6-trinitrobenzene sulphonic acid (TNBS) on lysosomes.

Lysosome-containing suspensions in 0·05 M Tris–acetate buffered sucrose (0·25 M, pH 7·4) were prepared from rabbit liver as previously described.[2] Portions (5 ml) were incubated at 37° for 90 min in 50 ml conical flasks with 100 μl of various concentrations of PLC and TNBS added in aqueous solution, or with distilled water as controls. After the incubation period the suspensions were centrifuged and the amount of lysosomal enzymes released (acid phosphatase and β-glucuronidase) determined by methods previously described,[2] using *p*-nitrophenyl phosphate and phenolphthalein glucuronide as substrates for acid phosphatase and β-glucuronidase, respectively. The results are shown in Figs. 1 and 2.

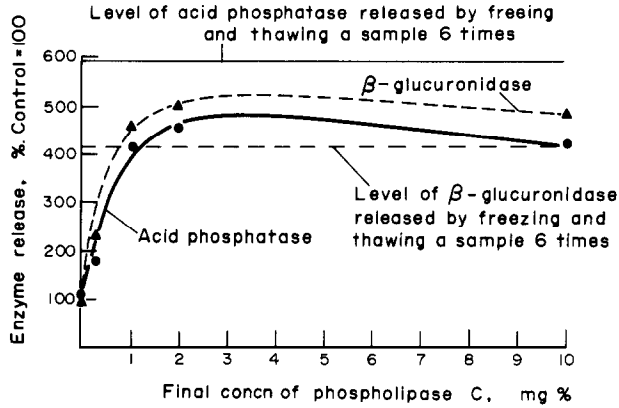

FIG. 1. Effect of phospholipase C on lysosomal enzyme release. The total amount of "releasable" enzyme was determined by freezing and thawing a lysosomal suspension six times, centrifuging and measuring enzyme concentrations in the clear supernatant. All results shown are expressed as a percentage of the controls, i.e. no PLC added. (▲) β-glucuronidase; (●) acid phosphatase.