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PRELIMINARY COMMUNICATIONS

MECHANISM-BASED INACTIVATION OF GABA AMINOTRANSFERASE BY 3,5-DIOXOCYCLOHEXANECARBOXYLIC ACID

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Inhibitors of gamma-aminobutyrate aminotransferase (EC 2.6.1.19) are of interest for anticonvulsant and other pharmacological purposes (1-4). Several analogs of GABA have thus been devised as so-called "suicide" inactivators of this pyridoxal phosphate-dependent enzyme (1,4-6). Such analogs of GABA inhibit the degradation and thereby elevate levels of that neurotransmitter in the central nervous system. However, analogs of GABA also tend to inhibit glutamate decarboxylase (EC 4.1.1.15) and thus inhibit the synthesis of GABA (4,7). The amino acid analogs of GABA condense with the formyl group of the pyridoxal cofactor of either enzyme to yield a Schiff base. However, the cofactor of glutamate decarboxylase is expected to remain in the pyridoxal state (8) whereas that of GABA aminotransferase cycles between the pyridoxal and pyridoxamino states. We have thus sought an oxo analog of succinic semialdehyde to inactivate GABA aminotransferase in a suicide reaction initiated by condensation with the amino group of the cofactor in the pyridoxamino state. In this communication, we report that the pyridoxamino but not the pyridoxal form of GABA aminotransferase from Pseudomonas fluorescens and from porcine brain is subject to inactivation by 3,5-dioxocyclohexanecarboxylic acid, a structural analog of succinic semialdehyde. To our knowledge, a suicide substrate active against a vitamin B_6-dependent enzyme in the pyridoxamino state has not been previously described.

MATERIALS AND METHODS

3,5-Dioxocyclohexanecarboxylic acid was synthesized by reduction of 3,4,5-trimethoxybenzoic acid (Aldrich Chemical Company) by lithium in ammonia (9). Mammalian GABA aminotransferase was isolated from porcine brain (10). Partially purified pseudomonal GABA aminotransferase was obtained from the Sigma Chemical Company. Aminotransferase activity was spectrophotometrically assayed by a dehydrogenase-coupled method (10). The so-employed dehydrogenase was a partially purified preparation from P. fluorescens in which contaminating aminotransferases had been inactivated

by sodium borohydride (6). One unit of aminotransferase was taken to be that which generates one micromole of NADPH per min in a reaction solution containing 20 mM GABA, 10 mM 2-oxoglutarate,0.50 mM NADP, 100 mM potassium phosphate,0.10 mM EDTA and excess succinic semialdehyde dehydrogenase at 25° and pH 7.2.

RESULTS AND DISCUSSION

As illustrated in Figure 1, no inactivation of either aminotransferase occurs during incubation with the diketone when the enzyme has been fully oxidized to the pyridoxal state by pre-treatment with 2-oxoglutarate.

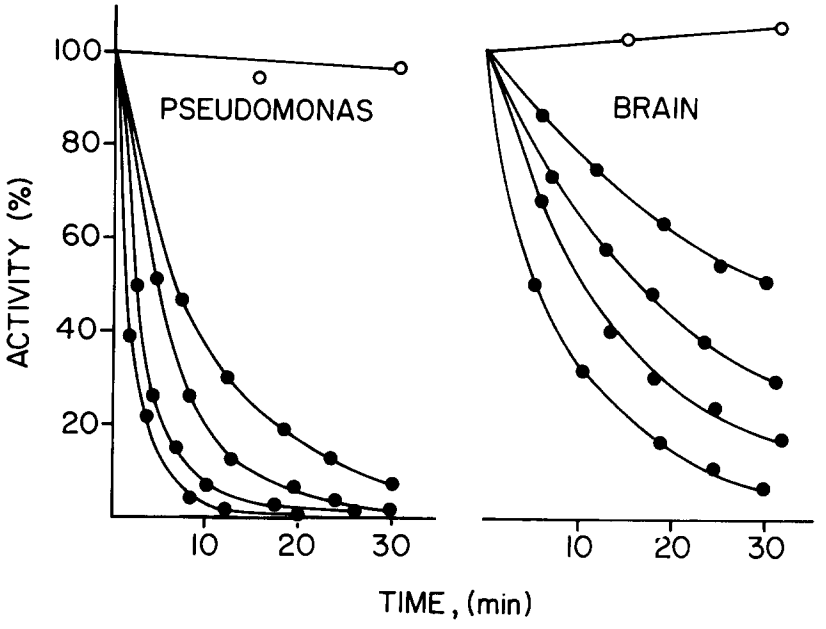

Figure 1. Inactivation of pyridoxamino but not pyridoxal GABA aminotransferase by 3,5-dioxocyclohexanecarboxylate. The microbial enzyme (770 unit/l) or brain enzyme (320 unit/l) was incubated with the inhibitor (2.5, 5.0, 10 or 20 mM) for the indicated periods of time in the presence of 1.0 mM GABA,0.50 mM NADP, excess succinic semialdehyde dehydrogenase,0.10 mM EDTA and 100 mM potassium phosphate at 25° and pH 7.2. The aminotransferase activity remaining was measured by the rate of NADPH accumulation upon dilution (200-fold in the case of the microbial enzyme and 66-fold in the case of the brain enzyme) of an aliquot of the incubation mixture into the standard assay mixture. The open circles represent experiments in which the incubation with 20 mM inhibitor occurred in the presence of 5.0 mM 2-oxoglutarate instead of 1.0 mM GABA. Addition of fresh samples of aminotransferase to the reaction solutions established that the dehydrogenase did not become rate-limiting.

As expected, the enzyme remains protected against the diketone when the 2-oxoglutarate is removed from the enzyme by dialysis before incubation with the diketone (not shown). The enzyme can be fully reduced to the pyridoxamino state by GABA in the presence of NADP and succinic semialdehyde dehydrogenase. The pyridoxamino enzyme remains fully active for more than 30 min in phosphate buffer at 25° and pH 7.2 in the absence of the

diketone, but is subject to inactivation by the diketone under these conditions.

The hypothesis which prompted this investigation is outlined in Figure 2. Since the pyridoxal (λ_{max} = 412 nm) and pyridoxamino (λ_{max} = 330 nm) states of the enzyme differ spectrally, the prediction that the inactivated enzyme is a pyridoxamine derivative was tested with the purified brain enzyme. In the absence of succinic semialdehyde dehydrogenase and NADP, only a fraction of the enzyme is converted to the pyridoxamino state during equilibration with 1.0 mM GABA. Addition of the diketone to pre-equilibrated solutions of the enzyme and GABA converts more of the enzyme to a 330-nm absorbing species at rates and extents in good agreement with those expected from the experiments shown in Figure 1. In contrast, 2-oxoglutarate drives the pyridoxal/pyridoxamino equilibrium in the opposite direction. The native pyridoxamino enzyme is almost fully oxidized to the pyridoxal state by 5.0 mM 2-oxoglutarate in less than 1.0 s. However, the 330 nm-absorbing species formed by reaction of the GABA-reduced enzyme with the diketone is re-oxidized by 2-oxoglutarate with a half-time of greater than 50 min. Thus, the inactivated enzyme bears a substituted pyridoxamine 5'-phosphate cofactor. Pyridoxamine 5'-phosphate itself and derivatives similar to that shown in Figure 2 are known to bind avidly to the apoenzyme (7).

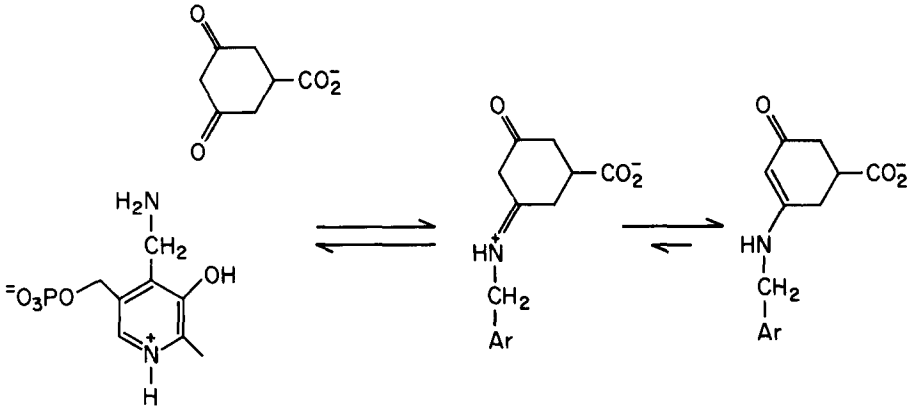

Figure 2. One plausible mechanism for the inactivation of GABA aminotransferase by 3,5-dioxocyclohexanecarboxylate. The free inhibitor is depicted as its diketone tautomer but may react as the predominant enol tautomer. Rather than bind to the pyridoxamine phosphate cofactor, the inhibitor may prove to covalently modify the active-site lysyl residue which is capable of engaging in Schiff base formation with the pyridoxal phosphate cofactor. Other mechanisms are also plausible.

1,3-Dioxo compounds are known to tautomerize to highly stable conjugated enamines upon Schiff base formation with amines (11,12). In general, enzymes are not readily inactivated by 1,3-dioxo compounds (13,14) because the rate of spontaneous enaminone formation in dilute solution is slow with respect to spontaneous enaminone hydrolysis. However, some enzymes with active-site lysyl residues that ordinarily engage in Schiff base formation with substrates have been found to be highly sensitive to 1,3-dioxo analogs of substrates. Thus, clostridial acetoacetate decarboxylase (EC 4.1.1.4) is inactivated by 2,4-pentanedione (15,16) whereas mammalian delta-aminole-

vulinate dehydratase (EC 4.2.1.24) is inactivated *in vivo* by 4,6-dioxoheptanoic acid (17).

3,5-Dioxocyclohexanecarboxylic acid and other analogs of succinic semialdehyde may prove to elevate levels of GABA in the central nervous system. Furthermore, 1,3-dioxo analogs of substrates may prove generally useful as selective inactivators of pyridoxamine phosphate-dependent enzymes. Similarly, appropriate amino ketones may prove generally useful as inactivators of pyridoxal phosphate-dependent enzymes.

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