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α-AMINO ACID ANALOGUES AS MECHANISM-BASED INACTIVATORS OF γ-AMINOBUTYRIC ACID AMINOTRANSFERASE

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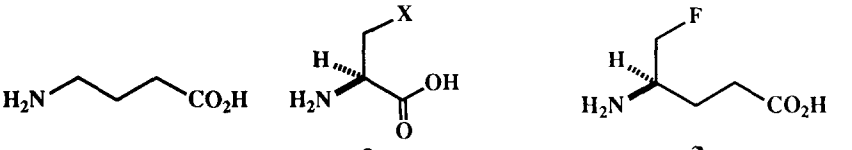
Abstract: γ- and β-Amino acids have been used previously to inactivate γ-aminobutyric acid aminotransferase. Here it is shown that β-halo-, β-phospho-, β-sulpho-*L*-alanine, and vinyl-*L*-glycine, all α-amino acids, are time dependent inactivators of purified γ-aminobutyrate aminotransferase. Observations that this inactivation is stereospecific and requires the formation of a Michaelis complex support a catalytic mechanism for this process.

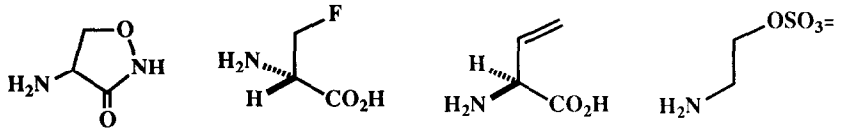
The pyridoxal 5'-phosphate-dependent enzyme, γ-aminobutyric acid aminotransferase (GABA-AT, EC 2.6.1.19), plays a central role in the catabolism of γ-aminobutyric acid (GABA, Chart 1, **1**), a major inhibitory neurotransmitter in the mammalian central nervous system.[1] Consequently, inactivation of this enzyme is a logical approach for the manipulation of GABA levels in brain and for modulating GABAergic neurotransmission. The concept of mechanism-based enzyme inactivation[2] has been exploited successfully in the design of irreversible inhibitors of GABA-AT.[3] For example, 4-amino-5-hexenoic acid,[4] which elevates whole brain GABA levels in rodents[5] and exhibits anticonvulsant activity in a variety of animal seizure models,[6] is now in clinical use as an anticonvulsant drug.

Chart 1. GABA and α-Amino Acid Inactivators of GABA-AT



1 **2** **3**

X
a; OSO_3^{-2} c; F
b; OPO_3^{-2} d; Cl

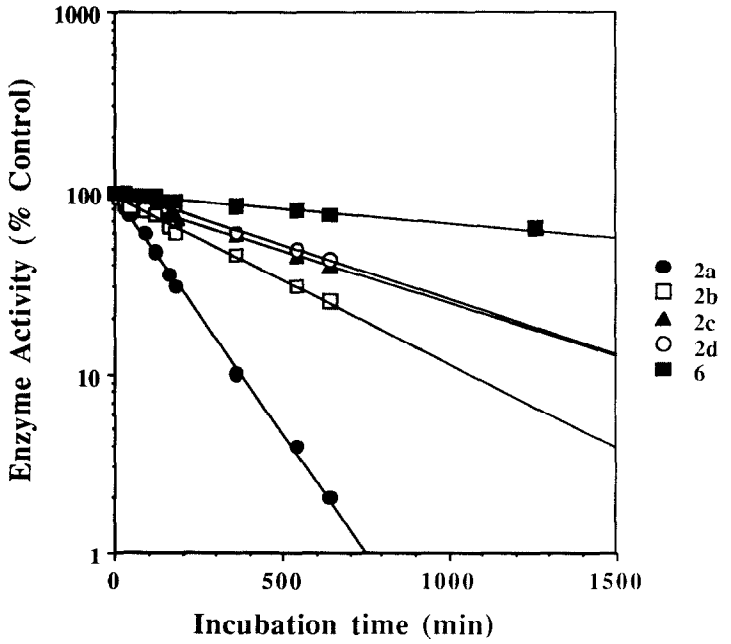


4 **5** **6** **7**

Although both γ- and β-amino acids act as substrates for GABA-AT, α-amino acids are known *not* to be substrates for GABA-AT.[7] Also, a variety of substituted γ- and β-amino acids have been shown to be inactivators of this enzyme.[2,3] It is believed that the mechanism of inactivation of GABA-AT by the halomethyl compounds is the mechanism that was proposed originally by Metzler and coworkers for the inactivation of aspartate aminotransferase[8] and glutamate decarboxylase[9] by serine *O*-sulfate (Chart 1, **2a**), by Walsh and coworkers for the inactivation of alanine racemase by β-fluoroalanine,[10,11] and by Silverman and Invergo[12] for the inactivation of GABA-AT by 4-amino-5-fluoropentanoic acid (**3**). However, because cycloserine (**4**) is really an analogue of an α-amino acid and it is known to inactivate GABA-AT,[13] we decided to investigate whether β-substituted alanine analogues in general could act as mechanism-based inactivators of GABA-AT. In this letter we show that a variety of substituted α-amino acids (**2a-e**, and **6**) are inactivators of GABA-AT, and we compare the effectiveness of inactivation as a function of β-substitution.

Purified GABA-AT[14] was incubated with compounds **2a-d, 5,** and **6**[15] at various concentrations. The remaining enzyme activity was measured[16] after different time intervals. In all cases the derivatives of L-alanine resulted in a rapid and irreversible loss of enzyme activity. As can be seen in Figure 1, the inactivation was progressive with time and followed pseudo first-order kinetics. The rate of inactivation increased with increasing

Figure 1. Inactivation of GABA-AT by **2a-d,** and **6**. Purified GABA-AT (0.2 unit) was incubated with 10 mM of inactivator in a total volume of 1.0 mL of 100 mM potassium pyrophosphate, pH 7.4. Aliquots were withdrawn at various time intervals and assayed for remaining enzyme activity as previously described.[16]



concentration of inhibitors. Furthermore, no activity was restored by exhaustive dialysis of the inactivated enzymes; therefore inactivation is irreversible. Since mercaptoethanol is routinely added to the incubation

medium, one can rule out that the inactivation of GABA-AT by these inactivators occurs through the formation of a diffusible alkylating species. These results, in combination with the fact that the inactivators also are competitive inhibitors, suggest that the inactivation is active-site directed. By plotting the half-life of enzyme as a function of the reciprocal of inhibitor concentration, apparent dissociation constants (K_I) and the rate constants for inactivation at infinite concentration (k_{inact}) were determined and are shown in Table 1. Saturation kinetics was observed with all inactivators.

Table 1. Kinetic Constants for Inactivation of GABA Aminotransferase by **2a-d, 6.**

Compound	K_I (mM)	k_{inact} (min^{-1})
2a	2.4	5.5×10^{-3}
2b	5.7	1.4×10^{-3}
2c	6.8	5.9×10^{-4}
2d	6.5	9.3×10^{-4}
6	12.8	8.5×10^{-5}

γ-Hydrogen abstraction of GABA by GABA-AT is stereospecific for the *pro-S* hydrogen.[17,18] The proton abstraction for inactivation by the α-amino acid analogues also was found to be stereospecific. The D-isomer of β-fluoro-alanine showed no inactivation of the enzyme even after 24 h at a potential inhibitor concentration of 100 mM.

In conclusion, as is evident from Table 1, irreversible inactivation of purified mammalian GABA-AT is stereospecific for *L*-alanine analogues. The β-leaving group appears to play a small role in the rate of inactivation. There are two potential effects that the leaving group could have. Increased electron-withdrawing ability of the leaving group would lower the basicity of the amino group, which would influence Schiff base formation between PLP and the inactivator. Also, as the electron withdrawing ability increases, the α-proton would become more acidic and hence its abstraction would be facilitated. This may change the rate-determining step from α-proton removal, which often is the slow step in amino acid inactivators of PLP enzymes in general,[2] to cleavage of the C-X bond, which depends upon the leaving group abilities. From Table 1 the rate constants are in the order **2a>2b>2d>2c>6**, corresponding to the leaving groups $SO_4^{=}>PO_3H^{=}>Cl^{-}>F^{-}>CH=CH_2$. This corresponds to neither the electron-withdrawing abilities nor the leaving group abilities of these groups, which is not surprising because these two effects are competitive. The notable increase in activity with the compounds containing β-anionic groups (sulfate, phosphate), however, may result from their more effective ability to mimic the carboxylate of the natural substrate and, therefore, they may have a different binding orientation than the compounds with halogen leaving groups. The potency of the best of these GABA-AT inhibitors, however, is still inferior to other GABA-AT inactivating homologues such as serine-O-sulfate,[19] possibly due to the poorer binding of the α-amino acids or to the increased difficulty for enamine condensation with the PLP, a step required by the previously proposed inactivation mechanism for β-fluoro amino acid derivatives,[8,9] as a result of the presence of the carboxylate group.

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15. Compounds **2a,b,d** and **6** were pruchased from Sigma Chemical Co.; **2c** and **5** were generous gifts from Dr. J. Kollonitsch of Merck, Sharp, & Dohme.

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