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3-SUBSTITUTED ALANINES INACTIVATE γ-AMINOBUTYRIC ACID AMINOTRANSFERASE BY THE SAME MECHANISM AS DO 4-AMINO-5-HALOPENTANOIC ACID ANALOGUES

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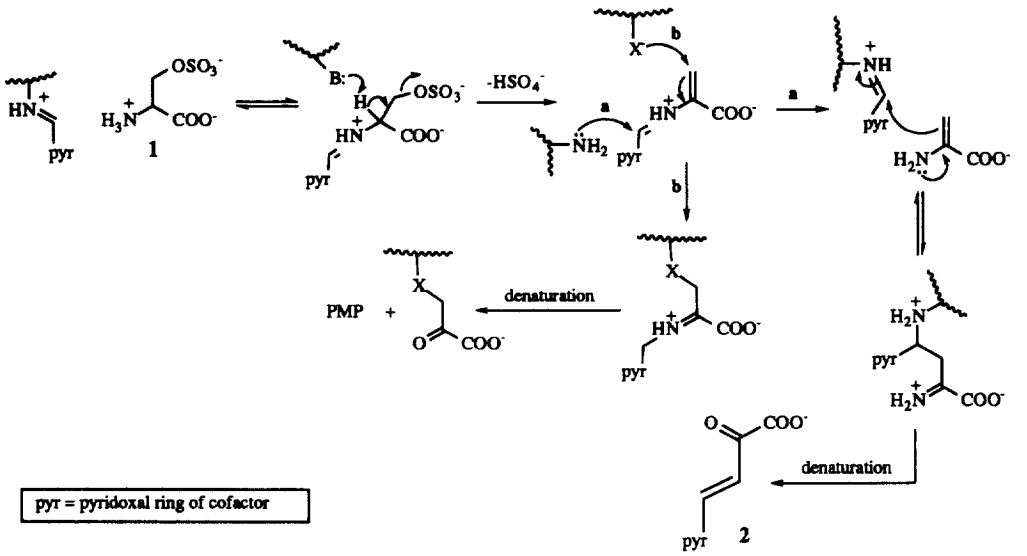
Abstract: L-Serine *O*-sulfate inactivates γ-aminobutyric acid aminotransferase (GABA-AT) by a mechanism that involves elimination of the leaving group and an enamine rearrangement, the same mechanism as that for the inactivation of GABA-AT by 4-amino-5-fluoropentanoic acid.

γ-Aminobutyric acid aminotransferase (GABA-AT, EC 2.6.1.19) is vital to the maintainence of the balance between GABA, the most abundant inhibitory neurotransmitter in mammalian brain,[1] and L-glutamate, an important excitatory neurotransmitter. When the GABA concentration diminishes, convulsions can arise.[2] An increase in the GABA levels can terminate the seizure.[3] Because GABA does not cross the blood-brain barrier, it is not useful as an anticonvulsant agent. One successful approach to increase brain GABA levels has been the use of mechanism-based inactivators[4] of GABA-AT.[5]

Although numerous β- and γ-amino acids are known to act as inactivators of GABA-AT,[4,5] only recently was it shown that certain substituted α-amino acids also irreversibly inactivate this enzyme.[6] Previously, we had investigated the mechanism of inactivation of GABA-AT by 4-amino-5-fluoropentanoic acid (4-fluoromethylGABA) and, at first, suggested that it proceeded by an elimination-Michael addition mechanism,[7] but later found that it followed an enamine rearrangement mechanism.[8] Given the potential different binding mode of the α-amino acid analogue relative to the corresponding γ-amino acid analogue, it was unclear as to which of these two mechanisms would be preferred. Here we describe our study of the inactivation mechanism of 3-substituted-α-amino acids toward GABA-AT using L-serine *O*-sulfate (1) as a representative member of the family. This compound could inactivate GABA-AT either by an enamine mechanism (Scheme 1, pathway a) or a Michael addition mechanism (Scheme 1, pathway b).

Scheme 1



Homogeneous GABA-AT,[9] reconstituted with [7-^{3}H]pyridoxal 5'-phosphate (1.0 mL, 0.45 mg/mL),[8] in 100 mM potassium phosphate buffer, pH 8.5 containing 3 mM α-ketoglutarate, 0.1 μL of β-mercaptoethanol, and 25 mM *L*-serine-*O*-sulfate was incubated, protected from light, for 20 h at 25 °C. Two controls containing 0.5 mL of [^{3}H]PLP-reconstituted GABA-AT with 0.1 μL of β-mercaptoethanol and either 3 mM of α-ketoglutarate (to show that the coenzyme remains as PLP) or 14 mM GABA (to show that the coenzyme is reduced to PMP) also were made. After 20 h, the pH of the solutions was raised to 12 using 1 M KOH and they were allowed to incubate for 1 h. The pH of the denatured enzyme solutions was then lowered to 2 with 1 M HCl and standards of PLP, PMP, and 4-[2-methyl-3-hydroxy-5-(phosphooxymethyl)-4-pyridinyl]-2-oxo-3-butenoic acid (**2**, Scheme 1),[10] which had been subjected to the same conditions as the enzyme solution, were added (25 μL each). The precipitated protein was pelleted by centrifugation and the supernatants were each injected into the HPLC, eluting with H_2O-0.1 % TFA at 0.5 mL/min for 25 min. At 25 min the flow rate was increased to 1 mL/min, and the solvent was switched to 100 % methanol over 15 min. During this time 1 min fractions were collected for the first 25 min with 2 min fractions collected after that. Scintillation cocktail (10 mL) was added to the fractions, and they were counted for radioactivity. As shown in Figure 1 almost all of the radioactivity comigrated with **2**. The amount of PLP and PMP shown in the figure is within experimental error; other runs gave no PLP or PMP. No radioactivity remained bound to the protein. These experiments are consistent only with the enamine mechanism shown in Scheme 1

(pathway a), the same mechanism that was demonstrated when 4-fluoromethylGABA inactivates GABA-AT,[8] and not with the elimination-Michael addition mechanism (Scheme 1, pathway b).

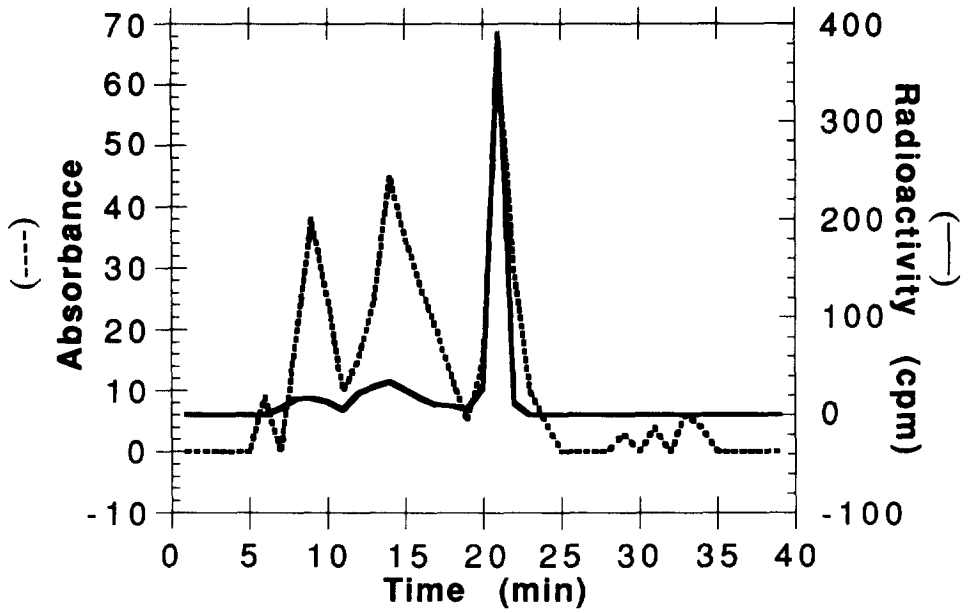


Figure 1. HPLC of Tritiated Products Following Inactivation of [^{3}H]PLP-reconstituted GABA-AT by *L*-Serine-*O*-sulfate. The solid line is the radioactivity; the dashed line is the absorbance of the standards that were injected with the [^{3}H]-labeled metabolites. PMP elutes at 8 min, PLP elutes at 14 min, and 4-[2-methyl-3-hydroxy-5-(phosphooxymethyl)-4-pyridinyl]-2-oxo-3-butenoic acid (**2**) elutes at 21 min under the conditions described in the experiment.

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