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INACTIVATION OF γ-AMINOBUTYRIC ACID AMINOTRANSFERASE BY (*Z*)-4-AMINO-6-FLUORO-5-HEXENOIC ACID: IDENTIFICATION OF AN ACTIVE SITE RESIDUE

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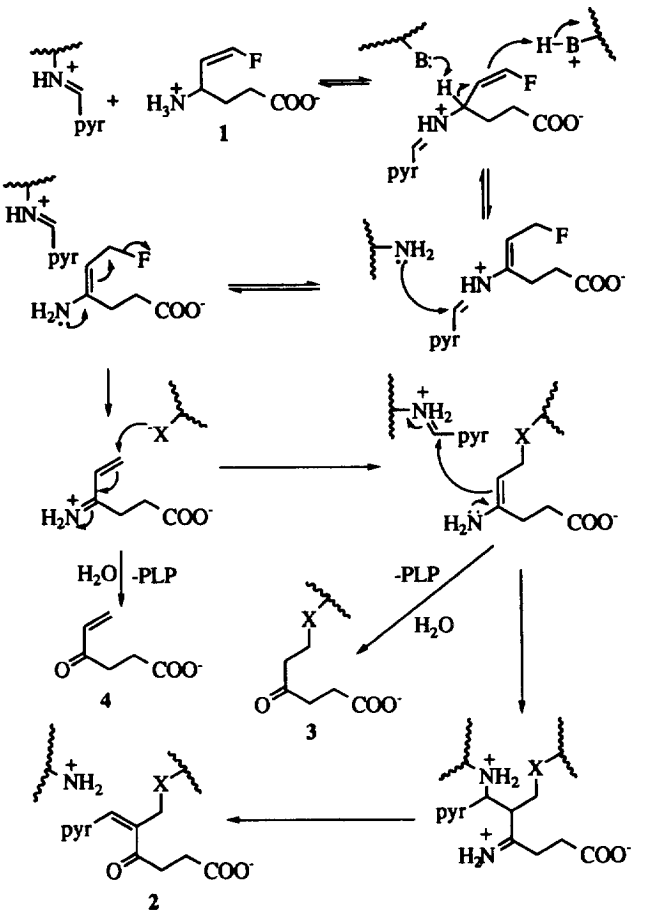
Abstract: (*Z*)-4-Amino-6-fluoro-5-hexenoic acid is a mechanism-based inactivator of pig brain γ-aminobutyric acid (GABA) aminotransferase. An active site residue to which this inactivator attaches is Cys-294. This is the first time a residue other than the lysine to which PLP is bound has been implicated in active site labeling.

γ-Aminobutyric acid (GABA) aminotransferase (EC 2.6.1.19), a pyridoxal 5'-phosphate (PLP)-dependent enzyme from pig brain, was shown to undergo time-dependent irreversible inactivation by the mechanism-based inactivator[1] (*Z*)-4-amino-6-fluoro-5-hexenoic acid (**1**).[2] Two inactivation pathways accounted for >95% of the inactivation and are shown in Scheme 1. With the use of tritium-labeled PLP and tritium-labeled **1** and UV-visible spectroscopy, evidence for the formation of **2** and **4** was obtained. Following inactivation by [2-^{3}H]-**1**, dialysis to remove noncovalent products, and denaturation, a small amount of radioactivity was released as **4**, suggesting that the active site nucleophile X is a good leaving group and that **3**, at least partially, degraded to **4**. Peptide mapping of the labeled enzyme was carried out, and the results of those studies are reported here.

GABA aminotransferase (0.32 mg)[3] was inactivated with [2-^{3}H]-**1**[2] and dialyzed exhaustively against 0.4 mM ammonium bicarbonate, pH 8.0 containing 8 M urea. Dithiothreitol was added to 4 mM, the protein was incubated at 50 °C in the dark for 15 min, cooled to room temperature, and iodoacetamide was added to 8 mM concentration. After 15 min, trypsin (100 μg) was added, and the sample was incubated in the dark at 37 °C for 24 h. The tryptic digest was analyzed by HPLC using a Vydac C_{18} protein and peptide column (0.46 x 25 cm, 5 μ). The peptides were eluted with a gradient of two solvents: solvent A was 0.06% trifluoroacetic acid in water and solvent B was 0.056% trifluoroacetic acid in 80% acetonitrile/20% water. The gradient was started as 98% solvent A and then changed to 63% solvent A over 63 min. At 63 min the gradient was changed to 25% A over the next 32 min and then 2% A over the next 10 min.

Scheme 1



The flow rate was 0.5 mL/min and detection was at 214 nm. Fractions were collected every min for 100 min. Peptide fractions containing the most radioactivity were concentrated to approximately 100 μL with a Speed Vac concentrator. The sample was analyzed by electrospray mass spectrometry (Figure 1).[4] This spectrum shows the presence of a major peptide with a molecular mass of 4547.4 Da and a minor peptide with a molecular mass of 4605.9 Da. The major peptide was subjected to gas phase sequencing,[5] and the results are shown in Table 1 (only the first 15 cycles are shown). The peptide sequence determined by gas phase sequencing matches a peptide of GABA aminotransferase from amino acid 291 to amino acid 305 in the cDNA-derived sequence of GABA aminotransferase.[6] The blank at cycle four during gas phase sequencing corresponds to the cysteine at position 294 in the sequence. The molecular mass of the peptide that extends from Lys-291 to Lys-330 in the GABA aminotransferase sequence plus 128 is 4547.8 Da (accounting for the loss of a proton from the cysteine when the adduct becomes

Figure 1. Electrospray Mass Spectrum of the Peptide Isolated from the Tryptic Digest

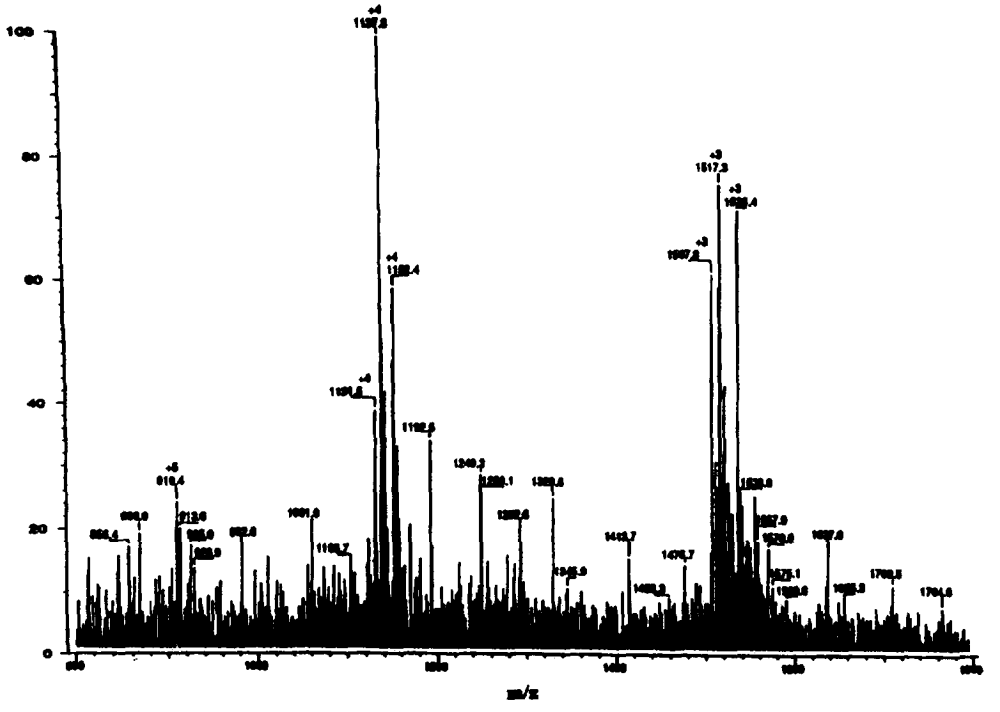
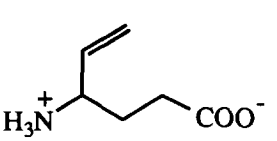


Table 1. Sequence of Major Peptide Isolated from Tryptic Digest

Cycle	Residue		pmole	Abs	Bkg	Lag%
1	Lys	K	3.1	3.1	0	4.7
2	His	H	1.2	1.5	0.3	1.5
3	Gly	G	7.3	10.9	3.7	9.8
4		-	0	0	0	na
5	Ala	A	4.6	7.3	2.7	0.9
6	Phe	F	4.5	5.9	1.4	3.9
7	Leu	L	4.7	7.2	2.5	3.1
8	Val	V	4.4	5.7	1.3	6
9	Asp	D	2.8	3.6	0.8	6.3
10	Glu	E	1.7	2.5	0.8	6.3
11	Val	V	1.6	3.9	2.2	5.9
12	Gln	Q	1.3	1.8	0.4	5.4
13	Thr	T	1.1	1.6	0.5	3.3
14	Gly	G	1.3	3.3	2	na
15	Gly	G	2	4.1	2	

bound), which compares favorably to the peptide mass of 4547.4 Da isolated from the tryptic digest. The additional mass of 128 Da corresponds to the mass of the inactivator adduct attached to the cysteine (Scheme 1, **3**, X = S). The active site lysine residue that binds PLP is Lys-330.

The epilepsy drug vigabatrin (**5**) was shown to inactivate human liver GABA aminotransferase and label the active-site lysine residue to which PLP is bound in the native enzyme.[7] Evidence for attachment of vigabatrin[8] and another anticonvulsant agent, γ-ethynyl GABA (**6**),[9] to an activ e site lysine residue in the pig brain enzyme also was reported. Here we show for the first time that a residue other than the lysine to which PLP binds can be involved in reactions of mechanism-based inactivators. These results also suggest that cysteine-294 may be included in the active site of GABA aminotransferase.



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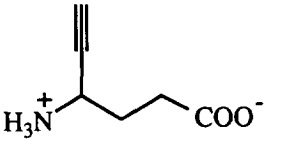

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