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EFFECT OF SOME DICARBOXYLIC ACIDS ON SOYBEAN SEEDS ASPARTATE AMINOTRANSFERASE ISOENZYMES

BAZI DİKARBOKSİLİK ASİDLERİN SOYA FASULYESİ TOHUMLARININ ASPARTAT AMİNOTRANSFERAZ İZOENZİMLERİNE ETKİLERİ

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Bu çalışmada, bazı dikarboksilik asidlerin soya fasulyesi tohumlarından elde edilen iki aspartat aminotransferaz izoenziminin (AAT_1 ve AAT_2) aktivitesi üzerine etkileri araştırıldı. Ayrıca, aminotransferazları enzimin kofaktör kısmı ile birleşerek inhibe ettiği bilinen D-sikloserinin de izoenzimlerin aktivitelerine etkisi incelendi.

AAT_1 ve AAT_2 üzerine, kullanılan dikarboksilik asidlerden yalnız maleik asidin, 40 mM konsantrasyonda, istatistik olarak anlamlı bir inhibitör etki gösterdiği ve maksimum inhibisyonun 80 mM maleik asid konsantrasyonunda gerçekleştiği saptandı. D-sikloserinin her iki izoenzimi de anlamlı olarak inhibe ettiği bulundu. Bu bulgulara ve literatür bilgilerine dayanılarak, dikarboksilik asidlerin soya fasulyesi aspartat aminotransferaz izoenzimlerine etkilerinin, enzimin kofaktör kısmı ile değil, apoenzimin protein konformasyonu ile ilgili olabileceği ileri sürüldü.

Several studies have been carried out in order to find the factors contributing to the inhibition of aspartate aminotransferase, a pyridoxal phosphate (PLP)-dependent enzyme that catalyzes reversible transamination between aspartate and 2-oxoglutarate. Dicarboxylic acids structurally are among the simplest aminotransferase inhibitors known (1-4).

The present study was undertaken to show the effect of various dicarboxylic acids on two isoenzymes of aspartate aminotransferase (AAT_1 and AAT_2) isolated previously in our laboratory from soybean seeds (5). In addition, the action of D-cycloserine, which inhibits aminotransferases by combining with the cofactor of the enzyme (6, 7), was also examined.

Material and method

Chemicals. L-Aspartic acid (Merck 129), 2-oxoglutaric acid (Merck 5194), ß-nicotinamide adenine dinucleotide disodium salt reduced (NADH, Fluka 43420), malate dehydrogenase (1500 U/mg, Serva 28338), pyridoxal-5' -phosphate (Fluka 82870) were used in enzyme assays. Oxalic acid (Merck 492), malonic acid (Fluka 63290), succinic acid (Fluka 14079), glutaric acid (Fluka 49660), adipic acid (Merck 90), maleic acid (Fluka 63180), fumaric acid (Fluka 47900) and D-cycloserine (Fluka 30020) were used in order to check their effect on enzyme activity. All other chemicals were also analytical reagent grade.

Enzyme preparations. Soybean seeds aspartate aminotransferase isoenzymes desig- nated AAT_1 and AAT_2 were isolated as described previously by means of ammonium sulphate fractionation (0.25 and 0.45 saturation), hydroxylapatite and DEAE-cellulose column chromatographies (5). Further chromatography of AAT_2 on DEAE-cellulose (step 5) and preparative polyacrylamide gel electrophoresis (step 6), which afforded additional purification but very low yield and decrease in stability, were ommited. Enzyme solutions (in phosphate buffer 5 mM, pH 7) were diluted in order to obtain approximately 100 U/ml activity before each determination.

Enzyme assay. Aspartate aminotransferase activity was measured with a 3.0 ml reaction mixture which comprised of 104 mM, pH 7.8 Tris HCl buffer at 25 °C with 45 mM L-aspartate, 0.75 units malate dehydrogenase, 0.05 mM NADH, 0.0275 mM pyridoxal-5' -phosphate, 3.75 mM 2-oxoglutarate and 0.25 ml of the diluted enzyme solution. The oxaloacetate produced was detected by the methods of Karmen (8) and Rej et al. (9), the details of which have been previously described (5). The reaction was initiated by the addition of 2-oxoglutarate and the decrease in absorbance at 339 nm was followed for five minutes. The enzyme activity was expressed as the absorbance decrease of 0.001 per ml of the sample per minute. All spectrophotometric measurements were carried out with a Perkin-Elmer Coleman model 55 UV-VIS spectrophotometer, in 1 cm light path cells.

Effect of dicarboxylic acids and D-cycloserine on the activity of AAT_1 and AAT_2. The dicarboxylic acids used were oxalic, malonic, succinic, glutaric, adipic, maleic and fumaric acids. Dicarboxylic acid solutions were prepared in 5 mM phosphate buffer (pH 7) and neutralized with concentrated sodium hydroxide. D-cycloserine solutions were prepared daily in the same manner.

0.4 ml enzyme-dicarboxylate mixture contained 0.1 ml dicarboxylate solution and 0.3 ml diluted isoenzyme solution in order to give the proper concentration of the dicarboxylate in the final reaction mixture. After preincubation at 25 °C for 10 minutes, the enzyme assay was performed with 0.25 ml of the mixture. Enzyme-D-cycloserine mixture was treated similarly. Control experiment, the activity of which was taken as 100 %, contained 0.3 ml enzyme solution and 0.1 ml 5 mM phosphate buffer.

AAT_1 was experimented with 10, 20 and 40 mM dicarboxylic acids or D-cycloserine concentrations, furthermore the action of 60 and 80 mM maleate on

AAT_1 was investigated. Inhibition tests with AAT_2, because of its low yield and unstability during purification, were carried out only at 40 mM dicarboxylic acid or D-cycloserine concentrations.

For each experiment a minimum of five replicates were performed for the different concentrations. Mean value and standart deviation (± S.D.) were calculated and significance evaluated by Student's t test.

Results

Increasing concentrations of oxalic, malonic, succinic, glutaric and fumaric acids have no inhibitory effect on the activity of AAT_1 (Table 1). Among the dicarboxylic acids used, only maleic acid showed a weak but statistically significant inhibitory effect (Table 1) on both AAT_1 and AAT_2 (17.6 % and 13.3 % respectively) at 40 mM concentration. For this reason AAT_1 was assayed with higher maleate concentrations 60 and 80 mM, the remaining enzyme activity was 82.25 ± 5.03 % and 74.11 ± 5.56 % respectively. According to these findings maximum inhibition (25.9 %) was obtained at 80 mM maleate concentration. Further increase in concentration couldn't be accomplished because of the insolubility of maleic acid at neutral pH.

Oxalic acid produced a slight but statistically significant increase on the activity of AAT_1, 6.6 % at 10 mM and 10.9 % at 20 mM, as for adipic acid 8.2 % at 10 mM (Table 1). These increases were not observed at higher concentrations.

D- Cyloserine, a known inhibitor of PLP-dependent enzymes, singificantly inhibited both isoenzymes, AAT_1 seems a little more sensible to inhibition (55 %) than AAT_2 (51.7 %) and as seen in Table 1, the extent of AAT_1 inhibition increased with increasing concentration of the inhibitor.

Discussion

It is known that transaminases are inhibited by substrate analogues that are incapable of undergoing transamination (10). Several such compounds have been examined by Jenkins et al. who suggested by spectroscopic studies that the inhibitor combines with the same groups which normally combine with the substrate (1). Although malonate was found by some investigators to be completely inhibitory on pig heart aspartate aminotransferase at 12.5 mM concentration (11), our findings agree with the results concerning that malonate produced no inhibition at 40 mM concentration on the same enzyme (1). On the other hand, succinate (1-3, 11), glutarate, adipate (1,2,11) were found inhibitory at different extents on aspartate aminotransferases from some mammalian sources, although no inhibition for soybean seeds isoenzymes was detected (Table 1). Only maleic acid inhibited both AAT_1 and AAT_2 (Table 1), but much less than pig heart aminotransferases (1,2). The fact that soybean seeds aspartate aminotransferase isoenzymes were refractory to inhibition by some dicarboxylic acids, except maleic acid, as well as the slight activation by oxalate (10 and 20 mM) and adipate (10 mM), may be explained by the differential catalytic sites

Substance added	% Enzyme activity			
	AAT_1			AAT_2
	10 mM	20 mM	40 mM	40 mM
None	100	100	100	100
Oxalate	106.64 ± 4.26*	110.94 ± 8.15*	101.34 ± 4.00	96.48 ± 2.99
Malonate	100.36 ± 4.55	99.11 ± 7.40	99.4 ± 10.28	98.17 ± 1.76
Succinate	104.46 ± 10.64	98.72 ± 5.74	96.68 ± 6.56	97.97 ± 3.57
Glutarate	104.23 ± 5.87	102.63 ± 4.28	106.28 ± 4.00	96.95 ± 3.50
Adipate	108.23 ± 4.05*	104.24 ± 4.26	97.37 ± 6.94	99.60 ± 1.60
Maleate	97.1 ± 8.16	98.51 ± 7.93	82.39 ± 10.91*	86.69 ± 7.24*
Fumarate	101.34 ± 4.78	107.46 ± 7.07	—— **	—— **
D-cycloserine	81.12 ± 5.08*	67.57 ± 6.04*	44.96 ± 6.60*	48.29 ± 6.53*

(*) Statistically significant values
(**) Added substance insoluble at this concentration at neutral pH.

Table 1. Effect of some dicarboxylic acids and D-cycloserine, at different mM concentrations and pH 7, on the activity of aspartate aminotransferase isoenzymes from soybean seeds.

of the plant isoenzymes due to different protein conformation in comparison with the mammalian ones.

The PLP-cofactor and active site residues make aminotransferases susceptible to various type of inhibitors (12). One well known such inhibitor is cycloserine, an antibiotic (13) which inhibits transaminases by binding to pyridoxal phosphate (6, 7). We used D-cycloserine in order to control our inhibition experiments and found increasing inhibition with increasing concentrations of D-cycloserine (Table 1). These results may suggest that the effect of dicarboxylic acids on the activity of two isoenzymes of soy- bean seeds aspartate aminotransferases is not concerned with the cofactor PLP, but with the apoenzyme conformation as mentioned above.

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