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MITOCHONDRIAL AND CYTOSOLIC ASPARTATE AMINOTRANSFERASE FROM CHICKEN: ACTIVITY TOWARD AROMATIC AMINO ACIDS

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SUMMARY. The mitochondrial and cytosolic isoenzymes of aspartate aminotransferase from chicken heart accept as substrates L-phenylalanine, L-tyrosine and L-tryptophan. The specific activities of the mitochondrial isoenzyme toward these substrates are between 0.1 to 0.5% of that toward aspartate and two orders of magnitude higher than that toward alanine. The specific activities of the cytosolic isoenzyme toward the aromatic substrates are 10 to 70% of the respective values of the mitochondrial isoenzyme. The activities of both isoenzymes toward aromatic amino acids are increased two- to threefold by 1 M formate. Larger increases by formate were observed for the alanine aminotransferase activity of both isoenzymes whereas their aspartate aminotransferase activity was inhibited by formate. The opposite effects of formate on the activities toward the aromatic and aliphatic monocarboxylic substrates on the one hand and the dicarboxylic substrate on the other are consonant with the notion of formate occupying the binding site of the distal carboxylate group of the substrate (Morino Y., Osman A.M., and Okamoto M. (1974) J. Biol. Chem. 249, 6684-6692). Apparently, in the ternary complex of aspartate aminotransferase with formate and aromatic amino acids, the aromatic rings of the latter bind to a site which does not overlap with the binding site for the distal carboxylate.

A less emphasized aspect of the specificity of aspartate aminotransferase (AAT)[2] is its activity for aromatic

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[2]Abbreviations: AAT, aspartate aminotransferase; mAAT and cAAT, mitochondrial and cytosolic AAT; tyrAT, pheAT, trpAT, aspAT, alaAT, aminotransferase activities of AAT toward tyrosine, phenylalanine, tryptophan, aspartate and alanine, respectively.

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amino acids. Earlier studies with AAT from two mammalian species, viz. pig (1,2) and beef (3) had shown that the mitochondrial isoenzyme (mAAT) and, to a lesser extent, the cytosolic isoenzyme (cAAT) display low but measurable activity toward tyrosine, phenylalanine and tryptophan. Correspondingly, the identity of the previously isolated and designated mitochondrial tyrosine aminotransferase from rat liver and phenylalanine aminotransferase from pig brain with mAAT had been established (2,4). The metabolic significance, if any, of such secondary activities is unknown (see ref. 4). Experimentally, these activities were recently exploited in x-ray crystallographic studies of mAAT from chicken by marking the catalytic site with phosphopyridoxyl-3-iodo-L-tyrosine, the heavy iodine atom being readily detectable in the difference electron density maps (G. Eichele et al., manuscript in preparation). The present communication reports on the activity toward aromatic amino acids of mAAT and cAAT from chicken.

MATERIALS AND METHODS. mAAT and cAAT were isolated from chicken heart as reported previously (5,6). L-Tyrosine, L-phenylalanine, L-tryptophan, and α-ketoglutaric acid were from Fluka, pyridoxal-5'-P from Merck, phenylpyruvate tautomerase (EC 5.3.2.1) from Sigma, malate dehydrogenase and lactate dehydrogenase from Boehringer. AspAT was assayed by the method of Karmen (7) as previously described (8). Activity toward aromatic amino acids was assayed by two methods. Assay A was that described by Diamondstone for tyrAT (9) and was carried out at 37° C, pH 7.3, in the presence of 1.5 mM α-ketoglutarate and the stated concentrations of amino acids. Absorptions of the aldehydes formed by decarboxylation of the keto acid product upon addition of NaOH were measured at 330 nm, 320 nm and 334 nm for tyrAT, pheAT and trpAT, respectively. The respective molar absorptivities were 19,900 $M^{-1}cm^{-1}$, 17,500 $M^{-1}cm^{-1}$ and 10,000 $M^{-1}cm^{-1}$ (10). Assay B was the continuous spectrophotometric method of Lin et al. (11) for aromatic amino acid aminotransferase in borate/phosphate (0.42 M sodium borate, 0.17 M sodium phosphate, pH 7.8) in the presence of 0.1 unit of phenylpyruvate tautomerase in a total volume of 1.0 ml at 37° C. Tautomerase was omitted in the assay of trpAT (11). The amino acid substrates were at the stated concentrations and α-ketoglutarate was 1.5 mM. Absorptions of the enol-borate complexes of the aromatic keto acid products were measured at 310 nm, 300 nm and 328 nm for tyrAT, pheAT and trpAT, respectively. The

Table I Specific activities of mAAT and cAAT toward aromatic amino acids as determined by assays A and B (see Materials and Methods section). The specific aspAT activity of mAAT was 169 U/mg and that of cAAT 199 U/mg.

Amino acid substrate	Conc. (mM)	Specific activity (U/mg) mAAT assay A	mAAT B	cAAT A	cAAT B
Tyrosine	6	0.33		0.06	
	3		0.10		0.02
Phenylalanine	100	0.91		0.10	
	30		0.32		0.03
Tryptophan	40	0.47		0.36	
	40		0.46		0.32

respective molar absorptivities used were 9,850 $M^{-1}cm^{-1}$, 5,800 $M^{-1}cm^{-1}$ and 14,000 $M^{-1}cm^{-1}$ (11). All reactions were linear with time after a lag of 8 to 10 min. AlaAT was assayed in 50 mM potassium phosphate, pH 7.5, in the presence of 40 mM L-alanine, 10 mM α-ketoglutarate, 0.3 mM NADH and 4 units lactate dehydrogenase.

RESULTS AND DISCUSSION. Tyrosine, phenylalanine, and tryptophan are all accepted as transamination substrates by the AAT isoenzymes from chicken (Table I). The specific activities range between 0.1 to 0.5% of that toward aspartate, those of cAAT being generally lower than those of mAAT. In all cases the amino acid concentrations used were below saturation; with both assays A and B first order kinetics were observed for up to 4 mM tyrosine (solubility limit), 100 mM phenylalanine, and 40 mM tryptophan. Double reciprocal plots (not shown) yielded straight lines passing very near the origin, thus not allowing accurate determination of K_m and V_{max} values. Aspartate (0.5 mM) inhibited all three activities by 40 to 50% when tyrosine, phenylalanine, and tryptophan were used in 3, 50, and 25 mM concentration, respectively.

The low activity of AAT toward alanine as compared to that toward dicarboxylic substrates has been interpreted as to

Table II Effect of formate on aminotransferase activities of mAAT and cAAT. Assay B was used and the amino acid substrate concentrations were: with mAAT tyrosine 3 mM, phenylalanine 25 mM, tryptophan 25 mM; with cAAT tyrosine 3 mM, phenylalanine 30 mM, tryptophan 20 mM. The concentrations of aspartate and α-ketoglutarate in the assay for aspAT were 120 mM and 3.6 mM, respectively.

Conc. of formate (M)	Activation factor (ratio of activities measured in the presence and absence of formate)				
	tyrAT	pheAT	trpAT	alaAT[a]	aspAT
			Mitochondrial AAT		
0.5	1.5	1.5	1.5		0.45
1.0	2.3	2.2	2.1		0.26
3.0				3.8	
			Cytosolic AAT		
0.5			1.2		0.91
1.0	3.3	3.2	1.7	14.5	0.62
3.0				37.6	

[a]The alaAT of mAAT and cAAT in the absence of formate was $2.1 \cdot 10^{-3}$ U/mg and $2.4 \cdot 10^{-3}$ U/mg, respectively.

indicate not only the existence of a special binding site for the distal carboxylate group but also the importance of its occupancy for effective catalytic function of the active site. Consonant with this notion, the marked increase of the activity toward alanine brought about by formate in high (molar) concentration is thought to be due to the binding of formate as a substitute of the distal carboxylate group (12-14). A rate-enhancing effect of formate has now also been found in the case of the aromatic substrates (Table II). The effect is less marked than for alanine; however, it seems to be similar in kind, cAAT (with the exception of trpAT) being more activated than mAAT. If the observed stimulation of activity were the result of formate binding to the distal carboxylate binding site (14,15), formate would be expected to inhibit aspAT and

this was indeed observed (Table II). The increase in activity toward aromatic amino acids by formate implies that formate and aromatic amino acids form a ternary complex with the enzyme. While formate and alanine may be accommodated together in the binding site for dicarboxylic amino acids (see ref. 12), simultaneous binding of formate and aromatic amino acids can only be explained by assuming that the aromatic rings bind to a site which does not overlap with the binding site for the distal carboxylate group.

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