Proc. Indian natn. Sci. Acad. **B52** No. 5 pp 637–641 (1986)

Tissue Distribution of Alanine and Aspartate Aminotransferases in an Indian Air-breathing Fish, *Channa punctatus* (Bloch)

SUDIPTA DAS, KASTURI SAMANTARAY and ASIT BARAN DAS
Laboratory of Environmental Biochemistry, Department of Zoology, School of life Sciences, Visva-Bharati University, Santiniketan 731235

(Received 27 December 1985)

The maximal catalytic activity (V_{max}) of AsAT in any tissue of *Channa punctatus* is remarkably higher than that of AlAT. Even the substrate affinity (as measured by the $1/K_m$ value) of AsAT for aspartate is higher than that of AlAT for alanine. The activities of both the cytosolic transaminases decrease in different tissues in the following order:

for AlAT { Specific activity : *liver >red muscle >brain = kidney = white muscle>gill*
Tissue activity : *liver>red muscle = kidney brain>white muscle>gill*

for AsAT { Specific activity : *red muscle = liver>brain>white muscle = gill = kidney:*
Tissue activity : *liver>red muscle>kidney = brain>gill>white muscle*

However, the apparent K_m values of both the enzymes are higher in liver, white or red muscle than in kidney, brain or gill. The SGOT and SGPT levels of *Channa* are strikingly higher than those of other teleostean fishes. Although the values of V_{max} of the two enzymes are lower in mitochondria than in cytosol of liver, the substrate affinities ($1/K_m$ values) of mitochondrial enzymes seem to be higher than their cytosolic counterparts.

Key Words: Aminotransferase, Aspartate, Alanine, Teleost

Introduction

Assay of serum transaminases for detection of the site and the extent of organ injury, as in human clinical biochemistry, has recently emerged as an important diagnostic procedure in the environmental toxicology of fishes. Serum and tissue levels of transaminases have been investigated in a number of teleosts and lungfishes, quite often under varied environmental conditions (Janssens 1964, Ogino 1965, Kulikova 1966, Creach 1967, Johnson 1967, Bell 1968). However, in most of these reports enzymatic activities have been measured by fixed time colorimetric assay procedures and expressed as total or tissue activities (units/weight of tissue or volume of homogenate). Besides, little attention has been paid to the Indian air-breathing teleosts in this respect (except Tandon & Chandra 1978 and Mukhopadhyay et al. 1982). No information is available in the relative levels of the two common transaminases concerned with non-essential amino acid metabolism and gluconeogenesis, viz. glutamate pyruvate transaminase (GPT) or alanine aminotransferase (AlAT) and glutamate oxaloacetate transaminase (GOT) or aspartate aminotransferase (AsAT) in the snake-headed murrels (*Ophiocephalus* or *Channa* species). This air-breathing teleost occurs abundantly in freshwater bodies of the swampy areas of eastern and southern India along with other air-breathing fishes. Not until recently the pisciculturists of this country have paid enough attention to the culture of air-breathing teleosts in addition to intensifying the breeding programmes of the major carps. Hence, kinetic assays of catalytic activities (both specific and total activities) of the two aminotransferases have been conducted in this preliminary study in different tissues of *Channa punctatus* (Bloch).

Materials and Methods

Tissues like liver, white and red muscles, brain and kidney, removed from a single fish weighing 25–40 g and collected from local ponds, were frozen immediately at −15°C for 20–30 mins. Blood collected directly from the heart of a live fish was centrifuged (700 × g) in a cold room for obtaining the serum. While the storage of tissue homogenates (5% for liver or kidney, 10% for brain and gill and 7.5% for muscle), prepared in ice-cold 0.25 M sucrose (in 0.05 M potassium phosphate buffer, *p*H 7.5) for 24 hr at −15°C did not result in appreciable change of enzyme activities, storage of blood resulted in remarkable increase in activities of both AlAT (50-fold) and AsAT (8-fold), probably due to leakage from erythrocytes, as evident from the data in table 1. Complete sedimentation of mitochondrial pellet from the 800 × g supernatant of a tissue homogenate, could be achieved at 2500 × g in a Beckman preparative ultracentrifuge (L-50B), as monitored by the assay of the mitochondrial marker enzyme, succinate dehydrogenase (Srikantan & Krishnamurthi 1955). Appreciable levels of AlAT could be detected in the mitochondrial fraction of liver-approximately 1.5–2.0% of the total activity. The mitochondrial level of AlAT activity in rat is about 8% of the total tissue activity (Swick et al. 1965). No attempt was made to record the mitochondrial enzymatic activities of muscle, brain or kidney in *Channa*, because of the extremely low levels in contrast to the cytosolic activities.

Table 1 *Effect of cold storage on the levels of AlAT and AsAT activities in serum*

	Units of serum	
	AlAT	AsAT
Control	47.90 ± 13.900(4)	401.90 ± 87.000(4)
Stored at 0°C	1716.60 ± 682.500(5)	1555.40 ± 188.200(5)
P	<0.05	<0.001

Table 2 *Effect of solubilizing medium on the specific activities of hepatic mitocondrial AlAT and AsAT*

Solubilizing medium	μ moles of NADH oxidized/ min /mg protein	
	AlAT	AsAT
Deoxycholate (0.25%)	0.09	0.79
Lubrol (0.5%)	0.12	1.03
Triton X-100 (0.5%)	0.14	1.07
Glycerol and buffer alanine	0.18	1.62

Solubilization of hepatic mitochondrial AsAT and AlAT was accomplished by treatment with glycerol and subsequent extraction with 0.05 M phosphate buffer (*p*H 7.5), containing 0.025 M L-alanine and 0.002 M cysteine according to the procedure adopted with minor modifications from Swick et al. (1965). Attempts were also made to release the mitochondrial enzymes by treatment of mitochondrial pellet with Triton X-100, Lubrol and deoxycholate. However, treatment with glycerol and subsequent solubilization with phosphate buffer and amino acid solution was found to be the most effective (vide table 2).

AsAT (EC 2.6.1.1) and AlAT (EC 2.6.1.2) activities were assayed kinetically at 25°C and *p*H 7.5 following the UV-spectrophotometric method (Karman 1955, Segal & Matsuzawa 1965); after ensuring the linearity of assay with respect to enzyme concentration and time of incubation. Enzymatic activities under substrate saturating conditions (V_{max}) were expressed both as total activity (tissue activity) in units (μmoles of NADH oxidized/min) per g wet wt of tissue, as well as specific activity in units per mg protein (Lowry et al. 1951). Serum enzyme level was expressed as units per litre. The apparent K_m values were determined from Lineweaver-Burk plots.

Results and Discussion

In contrast to most of the earlier studies of tissue distribution of AlAT and AsAT activities in mammmals (Cornelius et al. 1959, Herzfeld & Greengard 1971, Zinkl et al. 1971) and fish (Janssens & Cohen 1968, Wittenberger & Giurgea 1973, Nemcsok et al. 1981, Waarde 1981) in which data were expressed in units per g tissue (tissue activity), the data on *Channa* have been tabulated in the present work as both tissue activity and specific activity (table 3) as was also done by Wilson (1973) on the American channel catfish, *Ictalurus punctatus*.

It is quite evident (table 3) that the level of AsAT activity in any tissue (even serum) is remarkably higher (5–20 fold) than that of AlAT activity ($p<0.005$ to 0.001). Similar, but lesser degrees of difference between the two enzymatic levels have been detected in the tissues and serum of *Ictalurus* (Wilson 1973) and *Cyprinus*, the common carp (Nemcsok et al. 1981). Even the apparant K_m value of AsAT for asparate is significantly lower than that of AlAT for alanine in any tissue of *Channa*, the data for any other fish being unavailable. However, the relative levels of activities of these two enzymes in fish seem to depend on their nutritional status, starvation causing an augmentation of AlAT in muscle but a diminution in liver (the reverse changes for AsAT) in the carp (Wittenberger & Giurgea 1973). Although in mammals, the transaminase participating in hepatic gluconeogenesis during starvation is

Table 3 *Tissue distribution of AlAT and AsAT activities**

Tissue	AlAT				AsAT			
	Specific activity (units/mg protein)	Tissue activity (units/g wet weight of tissue or 1 serum)	Apparent K_m (mM)		Specific activity (units/mg protein)	Tissue activity (units/g wet weight of tissue or 1 serum)	Apparent K_m (mM)	
			Alanine	α-keto-glutarate			Aspartate	α-keto-glutarate
Liver cytosol	0.26 ± 0.022 (7)	17.92 ± 630 (7)	5.5–7.0	0.18–0.20	1.68 ± 0.086 (7)	115.06 ± 10.650 (7)	2.0–2.6	0.30–0.34
Liver mitochondria	0.12 ± 0.013 (4)	0.32 ± 0.003 (4)	4.4–4.6	0.14–0.18	1.18 ± 0.075 (5)	3.05 ± 0.195 (5)	1.3–1.5	0.22–0.30
Red muscle cytosol	0.18 ± 0.010 (4)	5.86 ± 0.470 (4)	3.0–3.5	—	1.99 ± 0.17 (4)	66.04 ± 7.330 (4)	1.8–2.0	—
White muscle cytosol	0.03 ± 0.003 (7)	1.20 ± 0.106 (7)	3.0–3.5	—	0.36 ± 0.028 (7)	12.45 ± 0.943 (7)	1.8–2.3	—
Kidney cytosol	0.04 ± 0.005 (6)	5.00 ± 0.251 (6)	2.5–2.7	—	0.24 ± 0.034 (7)	28.82 ± 2.764 (7)	1.0–1.2	—
Brain cytosol	0.05 ± 0.015 (2)	2.18 ± 0.150 (2)	2.3–2.6	—	0.74 ± 0.102 (5)	27.37 ± 4.014 (5)	2.2–2.4	—
Gill cytosol	0.01 ± 0.002 (4)	0.61 ± 0.095 (4)	—	—	0.27 ± 0.014 (4)	17.65 ± 1.260 (4)	1.8–2.0	—
Serum	—	47.90 ± 13.900 (4)	—	—	—	401.90 ± 87.000 (4)	—	—

*Mean ± SE

Note: The figures in parentheses indicate the number of fish

the AlAT, the relative roles of AlAT and AsAT in gluconeogenesis of starving fish tissues are still debatable (Wilson 1973, Wittenberger & Giurgea 1973). This calls for a comprehensive investigation on the effect of varying diet on gluconeogenesis and the activities of AlAT and AsAT in fish tissues.

The results of the present investigation reveal that the lateral red muscle of *Channa*, in its AlAT and AsAT activities is more akin to the hepatic tissue than to the white muscle of the fish. The maximal catalytic activities (V_{max}) of the two cytosolic enzymes decrease in the following order:

for AlAT { Specific activity : L>R>B=K=W>G
Tissue activity : L>R=K>B>W>G

for AsAT { Specific activity : R=L>B>W=G=K
Tissue activity : L>R>K=B>G>W

where L = liver, R = red muscle, B = brain, K = kidney, G = gill and W = white muscle

The significance of difference between any two tissues has been accepted at $P<0.05$ (between 0.05–0.001). A comparison of the apparent K_m values of the two enzymes in different tissues shows the highest values for liver. Hence it is evident that the catalytic activities of both AlAT and AsAT at physiological substrate concentrations in kidney, brain and gill cytosol of the fish are quite significant, although the activities of the enzymes under substrate saturating conditions (V_{max}) in these tissues are lower than those of liver or red muscle. It is interesting to note that both red and white muscle exhibit similar values of apparent K_m for their AlAT and AsAT although the apparent enzymatic levels (V_{max} being a kinetic index of this level) are remarkably higher (5 times) in the former. This confirms the earlier observation regarding the sharp difference in this respect between lateral red and white muscles of the common carp (Wittenberger & Giurgea 1973), although in goldfish the level of AsAT of dorsal white muscle is 4–5 times higher over that of lateral red muscle (Waarde 1981)

The levels of SGOT (Serum AsAT) and SGPT (Serum AlAT) in *Channa* are surprisingly higher than those recorded in other teleostean fishes (5–30 units/l) (Tandon & Chandra 1978, Nemcsok et al. 1981, Mukhopadhyay et al. 1982, Nemcsok et al. 1982, Tandon & Chandra 1980) or human beings (Bergmeyer & Bernt 1974). Although the levels of mitochondrial activities of AlAT and AsAT are no doubt lower than the cytosolic activities of the enzymes in liver of *Channa* (as is true in mammals), the apparent K_m values of mitochondrial enzymes (with respect to both α-ketoglutarate and aspartate/alanine) are lower than those of their cytosolic counterparts. However, more data are required to confirm if the activities of the transaminases in the two subcellular fractions represent two isozymes (Swick et al. 1965).

Acknowledgement

This work was supported by financial assistances from the University Grants Commission.

References

Bell G R 1968 The relative susceptibilities of some species of freshwater fish to poisons. I. Ammonia; *Water Res.* **1** 767–775

Bergmeyer H U and Bernt E 1974 glutamate-oxaloacetate transaminase and Glutamate pyruvate transaminase; in *Methods of Enzymatic Analysis* pp 731 & 756 ed. G U Bergmeyer (Weinheim: Verlag Chemie)

Cornelius E E, Bishop J, Switzer J and Rhode E A 1959 Serum and tissue transaminase activities in domestic animals; Cornell. Vet. **49** 116–126

Creach Y 1967 Transamination entre less acides amines et lacide-cetoglutarique Chez la carp; *Arch. Sci. Physiol.* **21** 443–448

Herzfeld A and Greengard O 1971 Aspartate aminotransferase in rat tissues: Changes with growth and hormones; *Biochem. Biophys. Acta* **237** 88–98

Inui Y and Yokote M 1974 Gluconeogenesis in the eel. I. Gluconeogenesis in the fasted eel; *Bull. Fresh Fish Res. Lab., Tokyo* **24** 33–45

Janssens P A 1964 The metabolism of the aestivating African lung fish; *Comp. Biochem. Physiol.* **11** 105–117

—— and Cohen P P 1968 Nitrogen metabolism in the African Lung fish; *Comp. Biochem. Physiol.* **24** 879–886

Johnson C I 1967 Progress in sport fishery research; *U S Wild life Serv. Res. Pub.* **39** 100–101

Karmen A 1955 A note on the spectrophotometric assay of glutamic-oxaloactic transaminase in human blood serum; *J. Clin. Invest.* **34** 131–133

Kulikova N A 1966 Activity of blood and tissue transaminases during muscular activity of various durations; *Ukr. Biokhim. Zh.* **38** 247–251

Lowry Off, Rosenborough N J, Farr A L and Randall R J 1951 Protein measurement with Folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Mukhopadhyay P K, Mukherji A P and Dehadrai P V 1982 Certain biochemical responses in the airbreathing cat fish *Clarias batrachus* exposed to sub lethal carbofuran; *Toxicology* **23** 337–345

Nemcsok J, Benedecyky I, Boross L, Asztalos B and Orban L 1981 Sub-cellular localization of transaminase enzymes in fishes and their significance in the detection of water pollution; *Acta Biologica. Szeged* **27** 9–15

——, Gyore K, Olah J and Boross L 1982 Effect of NH_3 on blood glucose level, GOT, GPT, TDH enzyme activity and respiration of three fish species; *Aquaculte Hung. Szarvas* **111** 63–68

Ogino C 1965 As quoted by Tandon R S and Chandra S 1978 Effect of asphyxiation stress on serum transaminases (GOT and GPT) levels of fresh water cat fish, *Clarias batrachus; Z. Tierphysiol. Tierernahrgu. Futtermittelkde.* **40** 34–38

Segal H L and Matsuzawa T 1968 L-Alanine Aminotransferase (Rat Liver); in *Methods in Enzymology* **A 17** 151–159 eds S P Colowick and N O Kaplan (New York: Academic Press)

Srikantan T N and Krishnamurti C R 1955 Tetrazolium test lor dehydrogenases; *J Sci. Ind. Res.* **140** 206–207

Swick R K, Barnstein P L and Stange J L 1965 The metabolism of mitochondrial protein. I. Distribution and characterization of the isozymes of alanine aminotransferase in rat liver; *J. Biol. Chem.* **240** 3334–3340

Tandon R S and Chandra S 1978 Effect of asphyxiation stress on serum transaminases (GOT and GPT) levels of fresh water cat fish, *Clarias batrachus; Z. Tierphysiol, Tierernahrg U. Tuttermittelkde* **40** 34–38

—— and —— 1980 Ecophysiology of fishes; Changes in serum glutamic oxaloacetic transaminase levels of fresh water fish. Wullago attu during varied conditions of life; *J. Adv. Zool.* **1** 28–32

Waatde A van 1981 Nitrogen metabolism in gold fish *Carassiu auratus* (L): Activities of transamination reactions, purine nucleotide cycle and glutamate dehydrogenase in gold fish tissues; *Comp. Biochem. Physiol.* **B68** 407–413

Wilson R P 1973 Nitrogen metabolism in Channel cat fish, *Ictalurus punctatus* L tissue distribution of aspartate and alanine aminotransferases and glutamic dehydrogenase; *Comp. Biochem. Physiol.* **B46** 617–624

Wittenberger C and Giurgea R 1973 Transminase activities in muscle and liver of the carp; *Rev. Roum. Biol.-Zoologie Tome* **18** 441–444

Zinkl J G, Bush R M, Cornelias C E and Freedland R A 1971 Comparative studies on plasma and tissue sorbitol, glutamic, lactic and hydroxybutyric dehydrogenase and transaminase activities in the dog; *Res. Vet. Sci.* **12** 211–214