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Aspartate Aminotransferase Activities in the Tissues of *Tilapia zillii*

Muneo SAMESHIMA*1 and Hiroyasu NAKASHIMA*1,*2

Abstracts

GOT, GDH and SDH activities in various tissues of *Tilapia zillii* were measured. The activity of the GOT in heart was the highest, followed by liver, brain and intestine. This tendency was very resemble to GOT activities in tissues of *Tilapia nilotica* [1] (recently it has been called *Sarotherodon niloticus*). In cases of GDH and SDH, the activities were the highest in intestine.

Zymography for the GOT isozymes in the tissues of *T. zillii* was performed on starch gel at pH 8.4. The cytosol fraction made two distinctive bands and one light band on anode side of the zymogram, whereas the mitochondrial fraction yielded two clear bands on the cathodal side.

According to the results of subcellular fractionation, total GOT activities in liver or intestine of *T. zillii* was distributed in cytosol and mitochondria as form of s-GOT and m-GOT in the ratio of 4 : 6, respectively.

GOT activity is closely related to physiological status of animals[2]. It is well known that two isozymes of GOT exist in animal cells[3,4]. One of them is contained in the cytosol and other one exists in the matrix of the mitochondria. The two isozymes from any tissue differ in chemical, physical and immunochemical properties, but it has recently been shown that the primary structures of the isozymes from pig heart are homologous[5]. The physiological functions of aspartate and malate are to make malate-aspartate shuttle by MDH isozyme and GOT isozyme. After oxaloacetate in the mitochondria is converted to aspartate by mitochondrial GOT, it diffuses across the mitochondrial membrane into the cytosol, where it is converted to oxaloacetate again by cytosol GOT. Mitochondrial GOT contributes to the conversion of aspartate to glutamate with the aid of a mitochondrial GOT shuttle. On the other hand, it is said that GOT controls the flow of aspertate into nucleic acid metabolism[6]. A relationship has

*1 Laboratory of Marine Biochemistry, Faculty of Fisheries, Kagoshima University.
*7 Present address: Kumamoto Fisheries High School, Reihoku-Cho, Kumamoto, 863-25 Japen.

Abbreviations: In this paper, following abbrevitions are used.

GOT	: Aspartate aminotransferase (EG. 2. 6. 1. 1)
GDH	: Glutamate dehydrogenase (EC. 1. 4. 1. 2)
SDH	: Succinate dehydrogenase (EC. 1.3. 99. 1)
m-GOT	: Mitochondrial GOT
s-GOT	: Cytosol GOT
MDH	: Malate dehydrogenase (EC. 1. 1. 1. 37)
LDH	: Lactate dehydrogenase (EC. 1. 1. 1. 27)
Tris	: Tris (hydroxymethyl) aminomethane

been reported between the activity of this enzyme and growth of young chick[7], lake chubsucker (*Erimyzon sucetta*)[8] and green sunfish (*Lopomis cyanellus*)[8].

Previous paper[1] has shown the activity of GOT in various tissues of *T. nilotica*. In order to more fully chracterize the GOT of tilapia tissues, this paper concentrated upon aspects of the isozymes of GOT prepared from the subcellular fractions of the tissues of *T. zillii*.

Materals and Methods

Reagents: NADH (grade 3, from yeast), MDH and LDH were obtained from Sigma Chemical Co.. L-Aspartic acid, 2-oxoglutaric acid, pyridoxal-5′-phosphate, sucrose, Triton X-100 and specially prepared reagents for electrophoresis were obtained from Nakarai Chemicals Ltd., Kyoto. Potato starch was purchased from Wako Chemical Industries Tokyo.

Animals: *T. zillii*, which had a body length of 15 to 20 cm, were purchased from Kagoshima Prefecture Fisheries Experimental Station, Freshwater Branch at Ibusuki. Eel (*Anguilla japonica*) was purchased from a local fish market. Liver of pig was supplied from the Municipal Butchery of Kagoshima. Tilapia were cultured in separate tanks, and the water temperature was maintained between 25 and 27℃.

Preparation of tissues and subcellular fractionation[4,9,10,11]: Fishes were killed by decapitation. The excised internal organs were washed in isolation medium [sucrose (250 mM), EDTA (1 mM), pH 7-8] and were blotted the excess water with filter paper and were weighed. The liver was finely chopped with scissors in isolation medium, washed several times, and then homogenized in 40 ml of the same medium whth a Potter-Elvehjiem Homogenizer having a Teflon pestle. The homogenizing procedure was performed in iced water bath.

Centrifugal procedure was conducted at 4℃ using Centrifuge Kubota KC-70 D and Kubota KR-180 B. But for the sedimentation of the heavy and light microsomal fractions and cytosol fraction, Ultra Cantrifuge IEC 60 B was used at 4℃. The separated pellets were suspened in distilled water by stirring for 10 seconds with Ultra Turrax Homogenizer (IKA-Germany). The fractionation procedure of liver is summarized in Fig.1.

Measurement of protein concentration: The protein concentration was measured by LOWRY'S method[12].

Enzyme assays: All spectrophotometric measurements were performed with Hitachi 101 Spectrophotometer, with cell housing regulated at 30℃, or Shimadzu UV-200 S Double Beam Spectrophotometer with U-125 MU Recorder. Stock solution of substrates were adjusted to pH 7.5 with KOH. Each enzyme activities were expressed as μ moles of product formed per minute per g of the fresh tissues.

GOT[3,4,13,14] The enzyme activity was assayed a modified method of KARMEN[13]. The assay solution contained 1.5 ml L-aspartate (240 mM, pH 7.4), 1.5 ml potassium phosphate buffer (200 mM, pH 7.5), 0.2 ml NADH (approximately 0.1 mM), 10 μl MDH (750 units, 5 mg protein/ml), the enzyme sample solution (10-100 μl) to be assayed and 0.2 ml of 10% Triton X-100. After

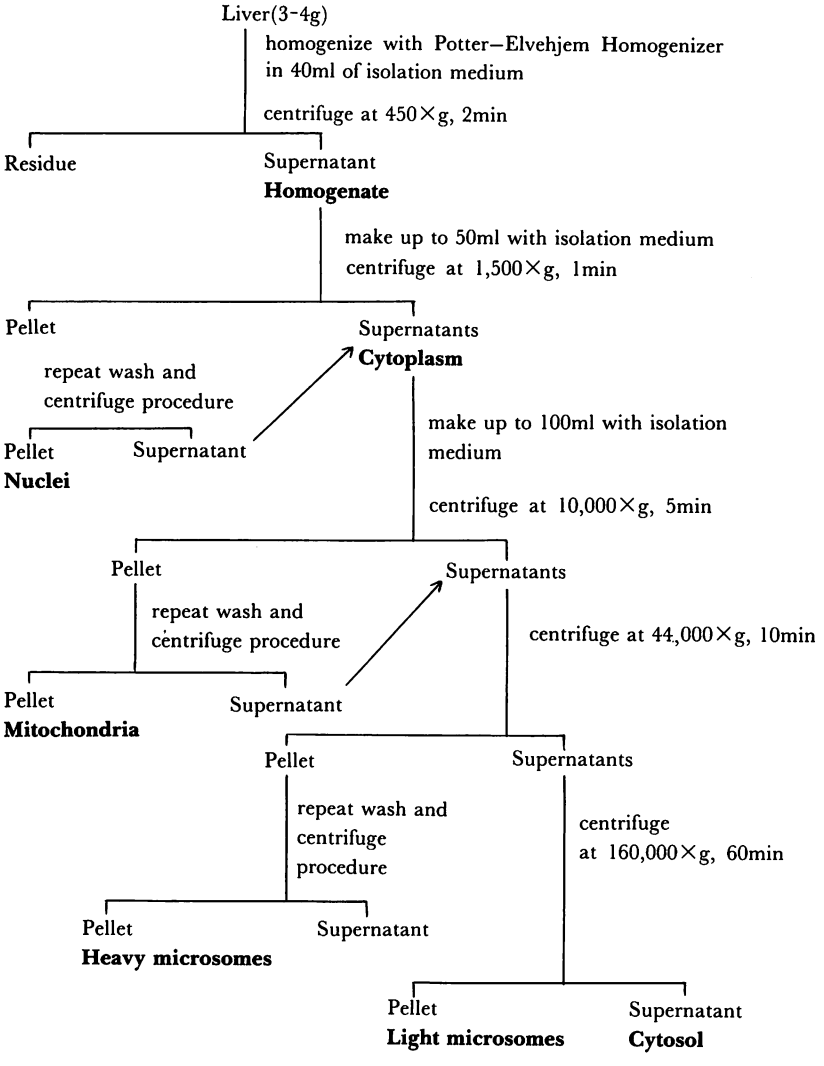

Fig. 1. Procedure for Subcellular Fractionation of Tilapia Liver.

incubation in the cell containing the mixed solution for 1 minute, reaction was initiated by addition of 0.2 ml of 2-oxoglutarate (300 mM) and the decrease in O.D. at 340 nm was measured.

GDH[4,15,16] The assay solution consisted of 3 ml potassium phosphate buffer (100mM, pH 7.5) containing NH_4Cl (200 mM), EDTA (0.1 mM), 0.2 ml NADH (approximately 0.1 mM), 0.15 ml L-leucine (5 mM), 10 μl LDH (550 units, 10 mg protein/ml), the sample solution (0.1-0.3 ml) to be assayed, and 0.2 ml of 10% Triton X-100. After incubation in the cell apploximately 1 minute at a temperature of 30℃, the reaction was initiated by addition of 0.2 ml of 2-oxoglutarate (300 mM), and the decrease in O.D. at 340 nm was recorded.

SDH[17] The assay solution contained 0.75 ml of potassium phosphate buffer solution (200 mM, pH 7.5), 0.1 ml of potassium cyanide (45 mM), 0.2 ml of succinic acid (600 mM, pH 7.5), 0.1 ml of 2,6-dichlorophenolindophenol (1.5 mM), 0.3 ml 1% crystalline bovine serum albumin solution, 0.2 ml of phenazine methosulfate (9 mM), 0.2 ml of 10% Triton X-100 solution and 1 ml of water. The reaction was initiatiated by addition of 0.5 ml of the enzyme solution. The decrease in O.D. was recorded at 600 nm.

Starch gel electrophoresis[4,18,19,20,21] Partially hydrolyzed potato starch, prepared by method of POULIC *et al.*,[18] (14 g) was suspended in 100 ml of gel buffer solution and was heated to make a gel plate. The gel buffer is consisted of 90 parts of 16 mM-Tris and 3.3 mM-citric acid, and 10 parts of 20 mM-lithium hydroxide and 76 mM-bolic acid[20]. Electrophresis was performed at 4℃ for 4 hours at 400 V. The gel was stained for GOT-protein by incubation in 50 ml of Tris-HCI buffer (100 mM, pH 7.4) containing L-aspartate (219 mg), 2-oxoglutarate (131 mg) and fast blue B salt (250 mg).

Results

GOT, GDH and SDH activities in several tissues of *T. zillii*: Table 1 shows GOT, GDH and SDH activities in several tissues of *T. zillii*. Prior to measurements of the enzymatic activities, the sample tissues were homogenized with distilled water by Turrax Homogenizer for 5 seconds.

Table 1. GOT, GDH and SDH Activities in Tissues of *T. zillii*.

	GOT	GDH	SDH
Muscle	11.4	20.3	—
Heart	416.4	41.0	2.6
Eye	19.5	—	—
Brain	109.9	7.8	0.1
Intestine	82.6	73.3	21.2
Gill	55.5	26.6	—
Liver	223.1	29.5	1.8
Spleen	50.5	24.2	—
Kidney	130.1	30.9	1.2

Enzyme activities: μ moles of products/g of fresh tissues/min

The GOT activities of heart, liver, brain and intestine were 416.4, 223.1, 109.9 and 82.6 μ moles of products/g of fresh tissues/minute, respectively. The activity of GOT in heart was the highest, followed by the liver, brain and intestine in decreasing order. In the case of GDH, the activity was the highest in intestine, followed by heart, kidney and liver. In the case of SDH, intestine had the highest activity among the tested tissues.

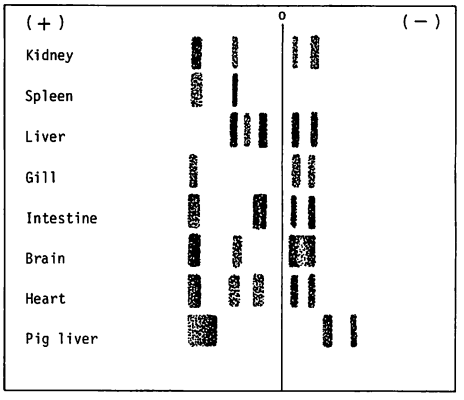

Fig. 2. Starch Gel Electrophoresis of Several Tissue Homogenates from *T. zillii* and Pig.

Fig. 2 shows the results of starch gel electrophoresis of the same samples. Electrophoresis was carried out at 4℃ for 3.5 hours at 400 V. The gels were staind for GOT active proteins. The electrophoregrams gave two distinctive cationic bands and two or three anionic bands. Pig liver was used as a reference.

Effects of pH value and temperature on the enzyme reaction: The effects of pH and temperature on activity of the GOT prepared from *T. zillii* liver were measured. Fig. 3 shows the effects of pH on the enzyme reaction. The buffer systems used were sodium acetate buffer (for pH 5.4), potassium phosphate buffer (for pH 6.5-8.0) and Tris-HCl buffer (for pH 8.0-9.0). The optimal pH value of the GOT on the enzyme reaction was about pH 8.0. Fig. 4 shows the relation between progress of enzyme reaction and temperature. It was said the GOT was relatively stable to higher temperature.

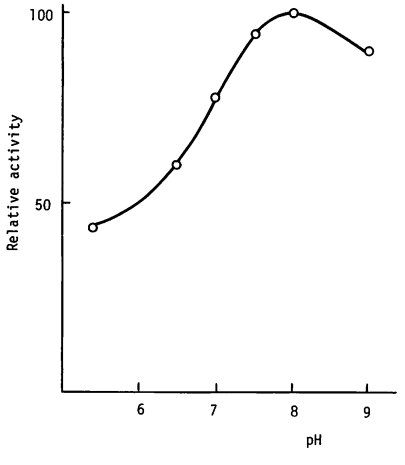

Fig. 3. Effect of pH Value on the Activity of GOT from *T. zillii* Liver.

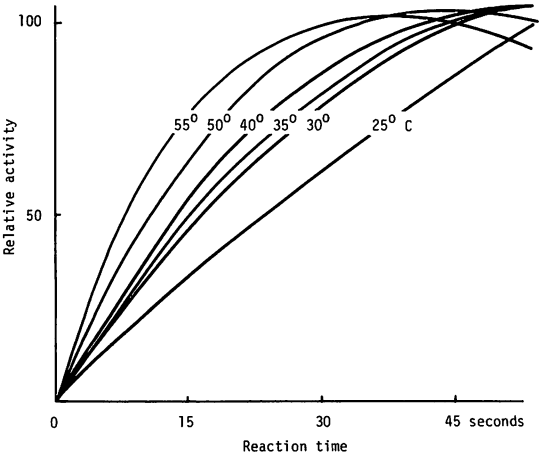

Fig. 4. Effect of Temperature on the Activity of GOT from *T. zillii* Liver. (pH value of reaction mixture: 8.0)

Distribution of GOT and GDH activities in subcellular fractions from liver and intestine of *T. zillii*: Liver of *T. zillii* was fractionated by the method explained in Fig. 1. The procedure was designed to obtain a high yield of mitochondria and to minimize contamination of cytosol components from particulate fractions. Triton X-100 was used in the assys for GOT and GDH in all fractions. The results shown in Table 2 are protein concentration in the original

Table 2. Protein Concentration of Enzyme Solution.

	T. zillii		Eel	Pig
	Liver	Intestine	Liver	Liver
Homogenate	205.6 μg/ml	150.9 μg/ml	237.2 μg/ml	268.8 μg/ml
Nuclei	27.1 13.2%	33.8 22.4%	44.8 18.9%	33.3 12.4%
Mitochondria	28.8 14.0	19.3 12.8	40.1 16.9	57.5 21.8
Heavy microsomes	17.3 8.4	14.3 9.5	20.2 8.5	31.4 11.7
Light microsomes	20.3 9.9	8.9 5.7	28.2 11.9	14.8 5.5
Cytosol	101.8 49.5	70.5 46.7	103.4 43.6	130.4 48.5
Recovery(%)	95.0	97.1	99.8	99.5

homogenates and fractions. Total activities of the enzymes in the homogenate and subcellular fractions are shown in Table 3. Percentage values of the activities are added in parentheses of the Table. In Tables 2 and 3, livers of eel and pig are used as control. It is evident that for GOT and GDH, the original activity could be accounted for by subsequent fractionation. Recoveries of enzyme activity and protein of liver and intestine of *T. zillii* were average of about 95%. GOT activity in the liver of the three species, tilapia, eel and pig, and in the intestine of *T. zillii* was higher than the GDH activity. In the case of liver and intestine of *T. zillii*, contamination of GDH activity in the cytosol fraction was found to be 3.5 and 5.4%, respectively. Generally speaking, GDH does not exist in the cytosol fraction, therefore the existance of GDH in the cytosol fraction was considered to be due to contamination by mitochondrial components.

Starch gel electrophoretic analysis of cytosol and mitochondrial fractions were performed by the method of BARRET *et al.*,[19] The gels were stained for GOT activity. A tipical example was shown in Fig. 5. The cytosol fraction yielded two distinctive bands and one of light band on the anode side of the zymogram, whereas the mitochondrial fraction yielded two distinctive bands on the cathode side. There were no cathodal band in the cytosol fraction nor anodal band in the mitochondrial fraction.

Table 3. Distribution of Enzyme Activities in Subcellular Fractions Prepared from Tissues of Tilapia, Eel and Pig.

	T. zillii			
	Liver		Intestine	
	GOT	GDH	GOT	GDH
Homogenate	257.7(100.0)	79.5(100.0)	77.4(100.0)	68.3(100.0)
Nuclei	22.9(8.9)	9.0(11.4)	5.0(6.5)	4.8(7.0)
Mitochondria	93.2(36.1)	51.8(65.6)	24.3(31.4)	35.7(52.3)
Heavy microsomes	18.9(7.3)	10.3(13.0)	10.4(134)	16.1(23.6)
Light microsomes	1.7(0.7)	1.3(1.6)	2.0(2.6)	1.5(2.2)
Cytosol	112.0(43.5)	2.8(3.5)	31.2(40.3)	3.7(5.4)
Recovery (%)	(96.5)	(95.1)	(94.2)	(90.5)

	Eel		Pig	
	Liver		Liver	
	GOT	GDH	GOT	GDH
Homogenate	431.9(100.0)	78.4(100.0)	130.6(100.0)	99.5(100.0)
Nuclei	39.6(9.2)	12.9(16.5)	13.8(10.6)	21.3(21.4)
Mitochondria	158.2(36.6)	50.0(63.8)	57.1(43.7)	65.5(65.8)
Heavy microsomes	24.9(5.8)	5.9(7.5)	4.6(3.5)	1.4(4.4)
Light microsomes	6.2(1.4)	0.4(0.5)	2.2(1.7)	1.6(1.6)
Cytosol	162.1(37.5)	1.8(2.3)	50.8(38.9)	0.9(0.9)
Recovery (%)	(90.5)	(90.6)	(98.4)	(94.1)

Enzyme activities: μ moles of products/g of fresh tissues/min

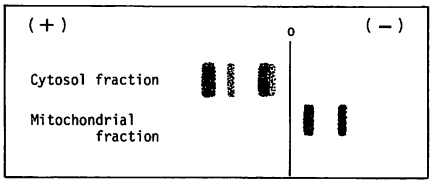

Fig. 5. Starch Gel Electrophoresis of Cytosol and Mitochondrial Fraction prepared from *T. zillii* Liver.

Discussion

The results in Table 1 indicate that quantitative activities of GOT, GDH and SDH differ in various tissues. Intestine and liver had high activities of GOT, GDH and SDH. The results in Fig. 2 show that the electrophretic pattern differs with various tissues in the anionic bands. It is considered that the anionic bands were subforms of the supernatant isozymes and cationic bands were subforms of the mitochondrial isozymes based on the report of BAUMER *et al.*,[4] and the data in Fig. 5. GDH is an enzyme restricted to the mitochondria matrix, and this enzyme was selected as a reference enzyme since it is established as a latent mitochondrial enzyme[22].

According to the results shown in Tables 2 and 3, it may be suggested that s-GOT prepared from the intestine and liver of *T. zillii* were cotaminated with 3-6% and 4-6% of m-GOT, respectively, during the fractionation procedure. Therefore, in the cytosol fractions, the activities of GOT may be 41-44% and 35-42% in liver and intestine, respectively. BOYD[3] made similar measurements of GDH in his studies of the distribution of GOT activity in rat liver. The remaining GOT (56-59% and 58-65% in the liver and intestine, respectively) and all of the GDH and SDH were distributed among the particulate fractions with the highest activities in the mitochondrial fraction. Table 3 shows the percentage values of the total activities in the particulate fractions.

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