Short Paper

Distribution and Some Properties of Tryptophan Hydroxylase from Liver in Several Fishes

Takeshi Nagai,^{*1,2} Moritsugu Hamada,^{*1} Norihisa Kai,^{*1} Yasuhiro Tanoue,^{*1} and Fumio Nagayama^{*2†}

^{*1}Department of Food Science and Technology, National Fisheries University, Nagata-honmachi, Shimonoseki, Yamaguchi 759-65, Japan

^{*2}Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan

(Received May, 9, 1994)

Key words: tryptophan hydroxylase, fish, liver, K_m value

Tryptophan hydroxylase [L-Tryptophan, tetrahydropyridine: oxygen oxidoreductase (5-hydroxylating) EC 1.14.16.4] catalyzes the conversion of tryptophan to 5-hydroxytryptophan. This enzyme is of great interest because it is one of the three known pterin-requiring aromatic amino acid hydroxylases and catalyzes the rate-limiting step in the biosynthesis of serotonin in the brain and other peripheral tissues.^{1–3} It was first discovered in mammalia,⁴ and purified from rat brain stem and mouse mastocytoma.^{5,6}

On the other hand, this enzyme in fish has not been studied in detail yet,⁷ and its distribution and characterization have not been clarified. As a part of studies on serotonin biosynthesis in fish, tryptophan hydroxylase activity in the liver of several fishes was surveyed in this paper.

Fish samples were purchased from the Tokyo Central Wholesale Market. Their livers were excised then stored at -80°C until use. Liver samples were homogenized with 3 volumes of 50 mM Tris-acetate buffer (pH 7.6) containing 2 mM phenylmethylsulfonyl fluoride (PMSF) and 4 mM 2-mercaptoethanol (2-ME) in a Potter-Elvehjem homogenizer with a motor-driven teflon pestle.

The homogenate was centrifuged at $100,000 \times g$ for 30 min. The supernatant was filtered through glass wool, and the filtrates were used as a crude enzyme solution. The reaction mixture contained 0.05 ml of 1 mM ferric ammonium sulfate, 0.57 ml of 1 M HEPFS buffer (pH 7.6), 0.025 ml of 20 mg/ml catalase, 0.05 ml of 5 mM D, L-6-methyl-5, 6, 7, 8-tetrahydropterine in 10 mM HCl, 0.05 ml of 10 mM L-tryptophan, 0.025 ml of 0.2 M 2-ME, 0.38 ml of H_2O , and 0.1 ml of the enzyme preparation. The reaction was started by the addition of D, L-6-methyl-5, 6, 7, 8-tetrahydropterine. The reaction mixture was incubated at 35°C for 30 min with mild shaking. The reaction was stopped by the addition of 0.125 ml of 40% perchloric acid. After the precipitated protein was removed by centrifugation, a 1.0 ml aliquot of the supernatant was withdrawn and mixed with 1.5 ml of 5 N HCl.⁸ The fluorescence of the solution was measured at an excitation wavelength of 295 nm and emission of 530 nm by the method of Friedman *et al.*² The enzyme activity was expressed as the activity which

produces 1 nmol of 5-hydroxytryptophan per min per g of tissue.

Table 1 shows tryptophan hydroxylase activity in the livers of 16 fish species. Skipjack, croaker, and sweetfish showed a considerably higher activity compared with grass carp, eel, and red sea bream. On the other hand, no activity was found in saury, horse mackerel, sardine, and Pacific cod. For comparison, some invertebrates such as arrow squid, swimming crab, and scallop were examined, but little or no activity was detected.

Optimum pH, optimum temperature, and K_m value are summarized in Table 1. The optimum pH for the enzyme reaction was found to be 7.0–8.2 which is similar to that for rat stem and mouse mastocytoma enzymes.^{5,6} Serrano and Nagayama⁷ reported that optimum pHs for the enzymes from tilapia, grass carp, common carp, and rainbow trout were 8.5, 7.0, 9.0, and 8.0, respectively.

The optimum temperature range was $30\text{--}37^{\circ}\text{C}$. The K_m values for L-tryptophan were 0.0042–0.92 mM; sweetfish

Table 1. Characterization of tryptophan hydroxylase in fish liver

Species	Activity*	Optimum pH	Optimum temp ($^{\circ}\text{C}$)	K_m (mM)
Skipjack	134	8.2	35	0.27
Croaker	129	7.4	37	0.39
Sweetfish	92	8.2	35	0.92
Japanese sea-bass	77	7.8	37	0.012
Rainbow trout	65	7.4	37	0.46
Autumn albacore	62	7.6	35	0.50
Chub mackerel	56	7.6	35	0.013
Yellowtail	51	7.4	37	0.34
Eel	22	7.6	30	0.21
Red sea bream	18	7.6	30	—
Grass carp	10	7.0	35	0.16
Three-line grunt	3	7.4	37	0.0042
Horse mackerel	ND	—	—	—
Pacific cod	ND	—	—	—
Sardine	ND	—	—	—
Saury	ND	—	—	—

* n mol product/min per g tissue at pH 7.6 and 35°C .

ND, not detected; —, not determined.

† Present address: School of Marine Science and Technology, Tokai University, Orido, Shimizu, Shizuoka 424, Japan

showed the highest value and three-line grunt showed the lowest value. The K_m values of Japanese sea-bass and chub mackerel enzymes were comparable to those obtained for rat brain and mouse mastocytoma.^{5,6)} In grass carp, the K_m value of the enzyme was reported to be 1.27 mM,⁷⁾ which is for higher than the value of (0.16 mM) in Table 1.

From the present results, the activity of tryptophan hydroxylase in fish liver did not seem to be species specific, and while it was detected in many species, it was not detected in saury, horse mackerel, sardine, and Pacific cod. The K_m values varied greatly among these fishes, but the enzyme from fish liver clearly had a considerably lower affinity for the substrate compared with that from mammals.

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education,

Science and Culture.

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