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Studies on Aminotransferase Enzymes in Fish and Shellfish

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The distribution of aspartate aminotransferase (AAT) and alanine aminotransferase (ALAT) activities in the skeletal muscle of several fish species is reported. AAT activity is found higher than ALAT activity and that the red muscle has higher aminotransferase activity than the white muscle. It is observed that 2-oxoglutaric acid has a wider scope as an amino group acceptor than pyruvic acid in the skeletal muscle of fish. The significance of transaminations in fish is discussed.

Aminotransferases are a group of enzymes that catalyse the process of biological transamination. The transamination reactions involve the transfer of the amino group of an amino acid to a keto acid with the formation from the latter of an amino acid and the generation of a new keto acid. These reactions represent a prime mechanism for the synthesis and deamination of various amino acids. The amino acids found in the tissues are mainly aspartate aminotransferase (AAT, 1-aspartate: 2-oxoglutarate aminotransferase E.C. 2.6.1.1) and alanine aminotransferase (ALAT, 1-alanine: 2-oxoglutarate aminotransferase, E.C. 2.6.1.2) (Braunstein, 1960; Meister, 1962).

AAT catalyses the reaction:

1-aspartate + 2-oxoglutarate $\rightleftharpoons$ 1-glutamate + oxaloacetate, whereas ALAT catalyses the reaction:
1-alanine + 2-oxoglutarate $\rightleftharpoons$ 1-glutamate + pyruvate

In fish, the transamination reactions probably play significant role at some stage in autolytic degradation of muscle proteins (Siebert & Schmitt, 1965). It is probable that the pool of free amino acids present in the fish muscle is utilized by transaminations to produce a variety of keto acids, some of these keto acids could be volatile and in addition some might be decarboxylated and contribute a variety of aldehydes to the pool of carbonyl compounds which probably determine the overall flavour of fish (Murray & Burt, 1974). Bell (1968) discussed the practical value of AAT estimations in serum of salmon for the distinction of apparently healthy fish from those treated with the hepatic poisons or those affected by bacterial kidney disease. Mounib & Eisan (1969) observed that the differences in the activity of aminotransferases among fish and also between cells (eggs or sperm) and the suspending medium (egg fluid or seminal plasma) may be useful in tracing evolutionary differences in fish.

The present investigations report the distribution of aspartate and alanine aminotransferases and the scope of apparent transaminations in several fish and shellfish species.

Materials and Methods

Samples of different marine fish, shellfish and freshwater fish were obtained fresh for the analysis. The fish were beheaded, gutted and thoroughly cleaned with water. Unless otherwise specified, only white skeletal muscle was taken for analysis. At least five or more individual numbers of each species were analysed. The total aminotransferase activity was extracted by homogenizing the tissue with chilled 0.1 M phosphate buffer, pH 7.6 using a Torry-Brown homogenizer. The final proportion of tissue to buffer was 1:10. The homogenate was centrifuged at 10,000 *g* for 10 minutes in cold. The supernatant was

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dialysed overnight in cold against frequent changes of 0.1 M phosphate buffer, pH 7.6 containing 10^{-3} g/l pyridoxal phosphate. It was noted that the loss in activity during dialysis could be prevented by incorporating pyridoxal phosphate in the dialysing medium. The dialysate, free from endogenous amino acids and keto acids was appropriately diluted for the assay of AAT and ALAT activities. AAT and ALAT activities were determined by the coupled enzyme reaction systems according to Bergmeyer & Bernt (1965) using Carl-Zeiss PMQ II spectrophotometer. The reaction mixture for the assay of AAT activity contained 2.3 ml of phosphate-aspartate solution (0.1 M phosphate buffer, pH 7.6, 2.5 x 10^{-1} M l-aspartate), 0.1 ml of 0.2 M 2-oxoglutarate, 0.5 ml of the tissue homogenate, 0.05 ml of 1.2 x 10^{-2} M NADH (reduced nicotinamide adenine dinucleotide-disodium salt) and 0.05 ml of malate dehydrogenase (0.5 mg protein/ml) previously dialysed against phosphate buffer to remove ammonium sulphate. For ALAT assay, the reaction mixture contained 1.8 ml of phosphate-alanine solution (0.1 M phosphate buffer pH 7.6, 0.1 M DL-alanine), 0.1 ml of 0.2 M 2-oxoglutarate, 1 ml of the tissue homogenate, 0.05 ml of NADH and 0.05 ml of lactate dehydrogenase (0.5 mg protein/ml) free from ammonium sulphate. One unit of enzyme activity is defined as that activity which catalyses the oxidation of one micromole of substrate per minute at 30° C. The scope of transaminations to keto acids such as 2-oxoglutaric acid and pyruvic acid with various l-amino acids in the tissue homogenates of several fish species was investigated following the paper chromatographic method according to Rowsell (1962). The ascending development of the paper was performed using n-butanol/acetic acid/water (4:1:5) and n-propanol/water (4:1).

Results and Discussion

Table 1 reports the AAT and ALAT activities in the white skeletal muscle of several fish species. The AAT activity varied between 1.15 (Bombay duck) and 11.92 (horse mackerel). The ALAT activity varied from 0.35 (shark) to 2.26 (pomfret). The level of activities among individuals of the same species varied considerably. It is evident from the results that the level of AAT activity is comparatively higher than that of ALAT activity. It is seen that aminotransferase activity is relatively higher in some varieties such as horse mackerel, Indian mackerel, seer fish and cat fish. It is significant to note that enzyme activities, particularly AAT, are significantly greater in the red muscle of mackerels.

Table 1. *Distribution of aspartate and alanine aminotransferases in the white skeletal muscle of different fishes*

U/g average (± S. E.)

Species		AAT	ALAT
Megalaspis cordyla (Horse mackerel)	i) White muscle	11.92 (2.3)	0.37 (0.07)
	ii) Red muscle	40.76 (5.5)	1.75 (0.5)
Rastrelliger kanagurta (Indian mackerel)	i) White muscle	8.89 (0.82)	0.95 (0.18)
	ii) Red muscle	49.25 (4.20)	2.52 (0.8)
Scomberomorus guttatus (Seer fish)		5.40 (1.4)	2.15 (0.7)
Pampus argenteus (Pomfret)		3.88 (0.68)	2.26 (0.74)
Otolithus argenteus (Croaker)		3.50 (1.65)	0.85 (0.62)
Psettodes erumei (Flat fish)		3.00 (1.1)	—
Trichiurus savala (Ribbon fish)		4.70 (1.8)	1.53 (1.3)
Trachysurus dussumieri (Cat fish)		5.50 (2.0)	1.30 (0.91)
Harpodon nehereus (Bombay duck)		1.15 (0.8)	0.49 (0.14)
Scoliodon sorrakowah (Shark)		3.59 (2.15)	0.35 (0.25)
Cyprinus carpio (Common carp)		2.98 (1.47)	—
Penaeus monodon (Prawn)		2.31 (1.17)	2.92 (1.82)

Hamm *et al.* (1969) noted that AAT and ALAT activities of bovine and porcine muscle were similar to the activities observed in the skeletal muscle of rat, rabbit and man. It is seen from the present studies that aminotransferase activity in fish muscle is relatively lower than the activity observed in other mammalian tissues.

The higher level of AAT activity seems to be associated with high myoglobin content of muscle (red muscle). Narawane (1967) obtained higher level of AAT and ALAT activities in lateral red muscles of two freshwater species of fish. Hamm (1969) observed a highly significant correlation between either AAT or ALAT activity and the amount of pigments in bovine and porcine tissues. Similarly, the red muscle of the carp showed higher activity than the white muscle (Masic & Hamm, 1971).

The extent of apparent transaminations in the skeletal muscle of seven species of fish and a crustacean using 16 different amino acids and 2-oxoglutaric acid as the amino group acceptor is presented in Table 2.

The results indicate that fish muscle catalyse transamination of several l-amino acids such as leucine, valine, ornithine, tyrosine, tryptophan, phenylalanine, methionine and lysine. In addition, some species show positive reaction utilising arginine, histidine and proline, though in traces. Transaminations involving cystine, glycine, serine, threonine and B-alanine could not be detected. Table 3 reports the occurrence of transaminations to pyruvic acid as the amino group acceptor. It is evident from the results that fish muscle catalyse the transaminations to pyruvic acid with very few amino acids. The possibility of coupling two different transamination reactions to indicate 'apparent' reaction (Guirad & Snell, 1964) is unlikely in the present studies since there were no chromatographically detectable amino acids or keto acids in the dialysed tissue homogenates. The study of Siebert *et al.* (1965) indicated that transaminations to 2-oxoglutaric acid were prominent in cod muscle as compared to pyruvic acid as the amino group acceptor. Creach (1967) detected transaminations to 2-oxoglutaric acid with leucine, isoleucine, valine, tyrosine, ornithine, phenylalanine and arginine in addition to aspartic acid and alanine in the tissues of carp. A survey of apparent transaminations in dialysed homogenates of brain, heart, kidney, liver and muscle of salmon indicated that AAT and ALAT were active in all tissues and

Table 2. *Transaminations to 2–oxoglutaric acid in the skeletal muscle of different fishes and crab*

Amino acids	Pomfret	Croaker	Flat fish	Horse mackerel	Shark	Rohu	Mrigal	Crab
Leucine	+ +	+ + +	+ +	+ +	+ +	+ +	+ +	+ +
Valine	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Ornithine	+ + +	+ + +	+	+ +	+	+	+	+ +
Methionine	+	+	+	+	(t)	(t)	(t)	+
Tyrosine	+	+	(t)	+	(t)	(t)	(t)	+
Tryptophan	+	+	+	+	(t)	(t)	+	+
Phenylalanine	+ +	+ +	+	+	+	+	+	+
Lysine	(t)	(t)	(t)	(t)	(t)	(t)	(t)	(t)
Arginine	(t)	+	(t)	+	(t)	(t)	—	—
Histidine	(t)	(t)	+	(t)	(t)	—	—	—
B–alanine	—	—	—	—	—	—	—	—
Proline	(t)	(t)	—	—	—	—	—	(t)
Cystine	—	—	—	—	—	—	—	—
Glycine	—	—	—	—	—	—	—	—
Serine	—	—	—	—	—	—	—	—
Threonine	—	—	—	—	—	—	—	—

\+ present, (t) traces, — absent

Table 3. *Transaminations to pyruvic acid in the skeletal muscle of different fishes and crab*

Amino acids	Pomfret	Croaker	Flatfish	Horse mackerel	Shark	Rohu	Mrigal	Crab
Leucine	+	+	—	—	+	—	—	+
Valine	+	+	+	—	+	—	—	+
Ornithine	+	+	+	—	—	—	—	—
Methionine	—	+	—	—	—	—	—	—
Tyrosine	—	+	—	+	—	—	—	—
Tryptophan	+	+	+	—	+	—	—	+
Phenyl alanine	+	+	+	+	+	—	—	+
Lysine	+	+	+	+	—	—	—	—
Arginine	—	—	—	—	—	—	—	—
Histidine	—	—	—	—	—	—	—	—
B–alanine	—	—	—	—	—	—	—	—
Proline	—	—	—	—	—	—	—	—
Cystine	—	—	—	—	—	—	—	—
Glycine	—	—	+	+	—	—	—	—
Serine	—	—	—	—	—	—	—	—
Threonine	—	—	—	—	—	—	—	—

\+ present, — absent

that 2-oxoglutaric acid had wider scope as the amino group acceptor (Bell, 1968). This is in agreement with Cammarata & Cohen (1950) and Cohen & Sallach (1961) on other animal tissues.

The presence of several transamination mechanisms in fish probably account for the significant role played by these reactions in the biosynthesis or transformation of a number of amino acids and keto acids in fish *post-mortem* and in the metabolism of live fish. Siebert *et al.* (1965) observed that cod muscle contained active proteolytic enzymes capable of breaking proteins to yield free amino acids. It is probable that several interconversions of amino acids take place during storage and eventual spoilage of fish. Murray & Burt (1974) observed that transamination reactions were responsible for marked changes in the levels of aspartic acid, glutamic acid, alanine, valine, leucine and isoleucine in the extracts of cod muscle.

Chhatbar & Velankar (1977) noted that freezing and thawing of fish resulted in the release of mitochondrial isozyme of AAT, based on which an objective test to distinguish fresh, unfrozen fish from frozen and thawed fish was suggested. A critical study of various transamination reactions in different fish species is probably of significance from the aspects of *post-mortem* handling and preservation of fish.

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