CHAPTER 6

LIPASE AND SUCCINIC DEHYDROGENASE ACTIVITIES IN THE PARTICULATE FRACTIONS OF THE FISH RED MUSCLE

Studies on the fuel reserves and energy metabolism in organisms constitute a major field in biochemical research. The role of lipase which is a fat splitting and esterifying enzyme is of special significance in the light of the recent findings that fatty acids form the chief energy fuel for prolonged muscular activity (George, 1964). A high concentration of this enzyme has been shown to be present in the pectoralis muscle of pigeon (George and Talesara, 1961) and other highly active muscles such as the breast muscle of bats (George, Susheela and Scaria, 1958) and the flight muscles of insects (George, Vallyathan and Scaria, 1958; George and Bhakthan, 1960 a & b). Similarly, the red muscle of fish (George, 1962) and the vertebrate heart muscle (George and Scaria, 1957; George and Iype, 1959) which utilize fat for energy are known to be well equipped with a high concentration of lipase. Therefore the presence of considerably high lipase activity in these muscles is of special significance.

Succinic dehydrogenase (SDH) which is an important enzyme in the oxidative pathway in muscle metabolism is also known to be present in significant amounts in the red muscle of fish (George, 1962), in the breast muscle of flying birds (George and Talesara, 1961 a), in mammalian diaphragm (George and Susheela, 1961) and in insect muscle (Bhakthan and George, 1963).

In the recent studies on the lipase activity in the particulate fractions of the pigeon breast muscle (George and Talesara, 1962 b) and of the pigeon heart muscle (George and Iype, 1963), the importance of this enzyme in splitting neutral fats or triglycerides into fatty acids and glycerol as the first step in the utilization of fat is now well recognized. Further light on this subject was thrown by studies carried out on the lipase and SDH levels in the particulate fractions of the breast muscle of the migratory starling, Sturnus roseus (George and Vallyathan, 1964) and of the rat diaphragm (George and Susheela, 1964).

In spite of the vast amount of experimental work carried out on the cell particulate fractions of the various metabolically active tissues, relatively few studies have so far been made on skeletal muscle, and as far as we are aware, none on fish muscle. Since the white muscle of fish contains negligible amounts of lipase and succinic dehydrogenase, only the red lateral line muscle has been investigated in the present study.

MATERIALS AND METHODS

Two species of the common Indian carp (Labeo fimbriatus, and Cirrhina mrigala stocked in open air tanks were used. The fish was decapitated and skinned. A piece of the red muscle along the lateral line was removed rapidly and homogenized in a chilled mortar with ice cold isotonic 0.025 M sucrose solution. A 15% homogenate was prepared, an aliquot of which

was preserved at 4°C for estimating the lipase and SDH activities till the various fractions were isolated. Particulate fractions of the muscle homogenate were isolated from a known volume of the original homogenate by differential centrifugation methods adopted from the methods of Hogeboom, Schneider and Palade (1948); Schneider (1948), Schneider and Hogeboom (1956), Chappell and Perry (1953) and Harman and Osborne (1953). A known volume of the original homogenate (10 ml) was centrifuged in cold (0°-4°C) for 5 minutes at 600 x g in a "MSE Superspeed 25" refrigerated centrifuge. The sediment which consisted mainly of myofibrils, nuclei and cell debris was washed thrice with 0.25 M sucrose, centrifuged each time and finally suspended in isotonic sucrose making the final volume (equal to the volume of the homogenate used). Thus a 10% myofibrillar fraction was obtained. The total supernatant collected was then centrifuged for 15 minutes at 7000 x g. The mitochondria thereby isolated were dispersed in sucrose solution and the volume was made to half of the initial homogenate. This fraction was microscopically examined to test the purity of the mitochondria. The supernatant was further spun at 40,000 x g for one hour and the brownish jelly like residue obtained was suspended with sucrose and the volume was made to half of the original homogenate. This was labelled the microsomal fraction. The clear supernatant was also diluted to twice the volume of the original homogenate and used as the soluble fraction.

Lipase : Lipase activity was determined quantitatively according

to the method adopted by George et al. (1958) using the Warburg manometric system. The enzyme activity is expressed as µl CO_2 evolved per mg protein per hour at 37°C.

Succinic dehydrogenase (SDH) : SDH activity was assayed according to the method of Kun and Abood (1949). The activity of the enzyme is expressed as µg formazan formed per mg protein per hour at 37°C.

Both the enzyme assays were made at 37°C so that the results could be compared with those obtained for other vertebrate skeletal muscle fractions by earlier workers.

Protein was estimated in duplicate samples by the Biuret method (Gornall et al. 1949).

RESULTS

The results obtained are presented in Tables 1 & 2. In both the fishes studied the maximum yield of lipase was obtained for the soluble fraction. The enzyme activity in the order of predominance was found to be in the microsomal, mitochondrial and myofibrillar fractions respectively. As regards SDH however, in Cirrhina mrigala the enzyme activity was found to be high in the mitochondrial fraction and low in the microsomal and soluble fractions; the myofibrillar fraction contained one third that of the mitochondrial. In Labeo fimbriatus on the other hand, the soluble fraction showed highest SDH activity. The yield in the mitochondrial and microsomal fractions was nearly similar, and equal to

Table I

The specific activity of lipase and succinic dehydrogenase in the original homogenate and various particulate fractions of the red muscle of Labeo fimbriatus

Fraction	Lipase activity μl CO_2/mg protein/hr.	SDH activity μg formazan/mg protein/hr
Homogenate	58.64 (50.89-66.67)	7.39 (7.21-7.58)
Myofibrils (600 X g)	22.00 (20.37-24.75)	2.34 (2.01-2.66)
Mitochondria (7,500 X g)	17.89 (15.58-20.33)	6.55 (6.42-6.68)
Microsomes (40,000 X g)	86.03 (74.35-97.89)	6.84 (6.21-7.46)
Soluble	186.03 (174.8-206.5)	15.71 (15.62-15.83)

Figures presented are the average values of 3 sets of experiments.

Table II

The specific activity of lipase and succinic dehydrogenase in the original homogenate and various particulate fractions of the red muscle of Cirrhina mrigala

Fraction	Lipase activity µl CO_2/mg protein/hr	SDH activity µg formazan/mg protein/hr
Homogenate	55.66 (54.12 -57.39)	2.41 (2.07-2.87)
Myofibrils (600 X g)	24.11 (20.29-27.97)	0.89 (0.51-1.33)
Mitochondria (7,600 X g)	25.25 (25.11-25.49)	2.83 (1.97-3.57)
Microsomes (40,000 X g)	25.94 (22.99-28.68)	-
Soluble	122.38 (106.4 - 138.8)	-

Figures presented are the average values of 3 sets of experiments.

about half of that in the soluble fraction. The myofibrils showed least activity.

DISCUSSION

From the results given in Tables 1 & 2 it is evident that the red muscle of Labeo fimbriatus and Cirrhina mrigala which belong to the same family (Cyprinidae), shows significant enzymic differences. In L. fimbriatus, lipase activity was found to be much higher in the soluble as well as the microsomal fractions as compared to the mitochondrial fraction where it was rather low. In C. mrigala on the other hand, although considerable lipase activity was obtained in the soluble fraction, the activity in the microsomal fraction was consistently lower than in L. fimbriatus. The high concentration of the enzyme in the soluble fraction which is separated at a higher centrifugal force, suggests that lipase which is a water soluble enzyme can be readily extracted in this fraction from the mitochondria and microsomes. The soluble lipase present in this fraction is therefore partially mitochondrial in origin. These results were compatible with those obtained in the pigeon breast muscle (George and Talesara, 1962 b); in the beetle muscle (George and Bhakthan, 1963); in the vertebrate heart (George and Iype, 1962); in the breast muscle of the migratory starling, Sturnus roseus (George and Vallyathan, 1964) and in the rat diaphragm (George and Susheela, 1964).

The high lipase activity in the microsomal fraction is

ascribed to the fact that microsomes are the sites of lipid synthesis in the cell (Green, 1960). Further evidences in favour of the role of microsomes in fatty acid synthesis have been provided by Borgstrom (1953) and Jedeikin and Weinhouse (1954) who have shown that the esterification of neutral fats from fatty acids takes place by the reverse action of lipase. Stein et al. (1957) have reported the synthesis of neutral fats from labelled palmitate. They have suggested that the microsomes may bring about the initial steps while the mitochondria catalyze the final step in the conversion of fatty acids to triglycerides. Neptune et al. (1963) have also demonstrated the synthesis of glycerides in the microsomes by using labelled acetate. Lorch et al. (1963) have shown that the microsomes convert malonyl-Co A to complex lipids (phospholipids). They further observed that the addition of mitochondria to the supernatant or to the supernatant plus the microsomal system invariably resulted in a decrease in C^{14} labelled fatty acids yield from C^{14} acetate. The stimulation of fatty acid synthesis by microsomal fractions has also been reported by Lachance et al. (1958) and Matthes et al. (1960). The role of lipase in the microsomal fraction of the pigeon heart (George and Iype, 1963) as well as breast muscles (George and Talesara, 1962 b) has been described as for the esterification of fatty acids as well as for the hydrolysis of fat to fatty acids, so as to make the fatty acids available to the mitochondria for oxidation. From the studies of Senior and Isselbacher (1962), it appears that the enzymes needed for lipid synthesis are associated with the endoplasmic reticulum. They

have also shown that the endoplasmic reticulum or microsomal fraction is instrumental in carrying out the transport of lipid. The red muscle of fish contains a large amount of both extra- as well as intracellular fat. It is possible that a large part of this fat is synthesized by the microsomes in the muscle and stored extracellularly to provide the fuel for contraction of this muscle.

Mitochondria are known to be the well established sites of fatty acid oxidation (Kennedy and Lehninger, 1949; Green, 1954). The red muscle of fish possesses large amounts of fat and lipase, and its capacity to oxidize fatty acids is also great. Thus, when active fatty acid oxidation takes place, the activity of lipase is suppressed. This accounts for the low concentration of lipase in the mitochondrial fraction in comparison with that in the microsomal and soluble fractions. Histochemically, when Tween 85 which was shown to be a specific substrate for "true lipase", was used, lipase was found to be localized at the mitochondria (Chapter 7). It was suggested by George (1964) that there are two lipases - one capable of acting on glyceride esters of long chain fatty acids and the other on those of short chain fatty acids, the former as largely located in the mitochondria, and the latter, in the extramitochondrial region of the muscle cell such as the microsomes.

The activity of lipase in the myofibrillar fraction may either be due to some mitochondria, cell debris or traces

of microsomes which may not have been completely isolated from this fraction, thereby increasing the enzyme concentration than what is actually present.

Succinic dehydrogenase activity was found to be relatively lower in C. mrigala than in L. fimbriatus. In the former, almost a negligible amount of enzyme activity was found in the microsomal and soluble fractions, whereas highest concentration was obtained in the mitochondria. The high concentration of SDH in the mitochondria indicates a high potentiality for oxidative metabolism. George and Iype (1960) reported that SDH activity is more in the sites where fatty acid oxidation takes place. Therefore the high concentration of SDH in the mitochondria is to meet the increasing demands for oxidative metabolism.

The presence in the myofibrillar fraction, of a detectable amount of SDH activity was perhaps due to contamination by the mitochondria and microsomes.

In the case of L. fimbriatus, the soluble fraction contained maximum SDH activity. This result could probably be attributed to the rupture of the mitochondria into smaller particles in the course of homogenization, and the consequent dissociation of SDH which is released in the soluble form, or it may be that the SDH here is largely extramitochondrial. Van Wijhe et al. (1962) showed a stimulation of sarcoplasmic dehydrogenases in sections as well as in homogenates, on the addition of phenazine methosulphate (PMS). Cytoplasmic (non-mitochondrial) lactic and succinic dehydrogenase activity was

also demonstrated histochemically in the red and white fibres of the pigeon breast muscle (George, Susheela and Vallyathan, 1963) using PMS and testosterone.

The microsomal fraction of L. fimbriatus also displayed considerable SDH activity. As mentioned previously, the microsomes are known to be the sites of lipid synthesis in the muscle. Recent studies of George and Vallyathan (1964) on the lipase and SDH activity in the particulate fractions of the pectoralis muscle of a migratory starling carried out during the pre- and postmigratory periods, denote that there is an increased synthesis of fat during the premigratory period. They obtained a high activity of both lipase and SDH in the early mornings and suggested that synthesis of fat took place at night during rest.