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ASCORBIC ACID AND FAT CONTENT IN THE RED AND WHITE MUSCLES OF CARP, *CATLA CATLA*

R. V. KRISHNAMOORTHY and T. NARASIMHAN

Department of Zoology, Bangalore University, Bangalore 1, India

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Abstract—1. Ascorbic acid,* the degradation products of ascorbic acid and total fat content were analysed in the red and white muscles of lateral musculature of riverine major carp *Catla catla*.

2. The white muscles are characterized by low fat, high iodine number and high water content.

3. The white muscles contain 2·5–3 times more ascorbic acid; about twelve times less dehydroascorbic acid and one–two times less diketogulonic acid than the red muscles.

4. Post-mortem changes and cold storage of these muscles altered the relative proportions of the above substances in varying amounts.

5. The data on the relative ratios of ascorbic acid and its metabolites, as well as ascorbic acid oxidase activity, suggested that ascorbic acid oxidation is higher in red muscle than in white muscle.

INTRODUCTION

STRIKING differences exist in the biochemistry (Hamoir & Konusu, 1965; Syrovy *et al.*, 1970), anatomy, electrophysiology and pharmacology (Bone, 1966; Rayner & Keenan, 1967) of red and white muscles of fish. They also differ in post-mortem changes, rigor mortis (Buttkus & Tomlinson, 1966) and in nutritional biochemistry Braekkan, 1959). The red muscles are characterized by a higher myoglobin content, characteristic sarcoplasmic proteins (Hamoir & Konusu, 1965), higher succinic dehydrogenase and respiratory enzymes (Lawrie, 1953; Fakuda, 1958), greater fatty acid oxidation and lecithinase activity (Bilinski, 1963; Bilinski & Jonas, 1966), greater oxygen consumption (Gordon, 1968), less water content, and more glycogen and lipids (Wittenberger *et al.*, 1969). Braekkan (1956) has correlated the biochemical dissimilarities of red muscles to the metabolic role of the muscle. Bone (1966) and Rayner & Keenan (1967) have correlated the metabolic as well as structural differences to the functional differences of red and white muscles. According to the above authors, the red muscle provides the sole motive power for swimming at slow and cruising speeds by the fish. In this paper the ascorbic acid content of the red and white muscles is compared as it is known to

* Abbreviations used: I_2 number, iodine number; ASA, l hydroascorbic acid; DHA, dehydroascorbic acid; DKA, diketogulonic acid; ASA oxidase activity, ascorbic acid oxidase activity; TCA, trichloro acetic acid.

play a major role in oxidative metabolism (Fruton & Simmonds, 1961), and to act as an antioxidant to fat metabolism in vertebrate (Fruton & Simmonds, 1960) as well as invertebrate tissues (Krishnamoorthy, 1969a). Further, it is known that ascorbic acid influences many enzymes systems (Lloyd & Sinclair, 1953; Ayubkhan & Swami, 1966b; Krishnamoorthy, 1969b; Krishnamoorthy & Shakunthala, personal communication) besides being a nutritional factor (Mahler & Cordes, 1967).

MATERIALS AND METHODS

The riverine major carps, *Catla catla*, weighing 1–1·5 kg each, were purchased alive from local fish suppliers. Live fishes were dissected in the laboratory to isolate the red and white muscles of the lateral line musculature for biochemical analysis. The post-mortem changes in these muscles were studied 6–8 hr after death. For investigations on changes due to cold storage a few muscle slices isolated from the live fish were transferred to a refrigerator at 0°C, stored at that temperature for 1 week and then used. The following methods were employed for the analyses.

(a) *Water content*

A known amount of fresh tissue was dried to a constant weight in a hot air oven regulated at 100°C, and the percentage water loss on drying was calculated after determining the weight of the dried material in an electrical single pan balance.

(b) *Total fat content*

The material dried in the hot air oven as described above was transferred into a Soxhlet extraction thimble and the fat was extracted by the continuous boiling of a ether–chloroform (3 : 1) mixture for 8–12 hr in a Soxhlet apparatus. The amount of fat extracted was calculated by determining the weight of fat extracted after evaporating the solvent under reduced pressure.

(c) *Iodine number*

The fat as extracted above was dissolved in 5 ml chloroform and mixed with Hanus iodobromide solution (Winton & Winton, 1947). The amount of iodine taken up by the fat was determined iodometrically according to Winton & Winton (1947). The percentage iodine absorbed by the fat was presented as the iodine number.

(d) *ASA, DHA and DKA*

The muscles were excised, weighed and homogenized in ice-cold 5% meta-phosphoric acid containing 1% $SnCl_2$ (Glick, 1964). The homogenates were centrifuged at 2000 rev/min for about 20 min and the acid-soluble fraction was used for the assay of ascorbic acid; ASA, DHA and DKA were determined by the 2,4-dinitro-phenyl-hydrazine method of Roe & Keuther (see Glick, 1964).

(e) *Demonstration of ascorbic acid activity*

The muscles were homogenized in 0·1 M phosphate buffer, pH 6·8, and centrifuged at 5000 rev/min for 25 min. The supernatant was dialysed in 0·5 cm × 10 cm dialysis tubing against the phosphate buffer at 4°C for 3 hr. One ml of the dialysed supernatant was incubated at 30°C with 5 ml of the same buffer containing 5 mg of ascorbic acid (purchased from Merck Chemicals). After 1 hr, the medium was quantitatively analysed for DHA as described in the preceding paragraph. The protein in the homogenate was precipitated with cold 10% TCA and estimated by the Biuret method (Layne, 1957). The data were statistically (Croxton, 1953) analysed.

RESULTS

Table 1 shows the differences observed in the fat, water content and the iodine number of total lipids of red and white muscles of the carp *C. catla*. More fat (about threefold higher, $t = 5{\cdot}516$ $P < 0{\cdot}1$) and less water (about 2 per cent less, $t = 4{\cdot}413$, $P < 0{\cdot}1$) are extractable in red muscle. Though the fat content is greater (Table 1), red muscle lipids are more saturated as indicated by a much lower iodine number than white muscle lipids (Table 1) [a fivefold difference ($t = 8{\cdot}772$, $P < 0{\cdot}1$)].

TABLE 1—COMPOSITION OF LATERAL MUSCLES IN *C. catla*

Quantity	White muscle	Red muscle	Relative quantity (white/red)
1. Total fat content (on dry wt. basis(%)	5·36 ± 1·72 (5)	14·4 ± 2·79 (5)	0·39 ± 0·16
2. Water content (%)	80·2 ± 0·29 (5)	77·5 ± 1·19 (5)	1·022 ± 0·020
3. Iodine number	147 ± 27 (5)	28 ± 30 (5)	5·3 ± 0·37
4. Total ascorbic acid (mg/g wet wt.)	2·16 ± 0·34 (7)	2·41 ± 0·54 (7)	0·89 ± 0·28

Values are mean ± S.D.
Number in parentheses indicates number of experiments.

When presented on a wet weight basis the two muscles did not show a significant difference ($t = 0{\cdot}945$, $P < 0{\cdot}1$) in total ascorbic acid content (Table 1). The contents of individual metabolites such as dehydroascorbic acid (DHA), hydroascorbic acid (ASA) and diketogulonic acid (DKA) vary in these muscles (Table 2).

TABLE 2—ENDOGENOUS LEVELS OF ASCORBIC ACID AND ITS DEGRADATION PRODUCTS IN THE LATERAL MUSCLES OF *C. catla*

Product (mg/g wet wt.)	White muscle	Red muscle	Relative quantity (white/red)
1. ASA	1·62 ± 0·23 (7)	0·67 ± 0·13 (7)	2·49 ± 0·57
2. DHA	0·028 ± 0·008 (7)	0·36 ± 0·078 (7)	0·134 ± 0·023
3. DKA	0·51 ± 0·11 (7)	1·38 ± 0·35 (7)	0·40 ± 0·12

Values are mean ± S.D.
Number in parentheses indicates number of observations.

The white muscle is characterized by about a 2·5-fold ($t = 7{\cdot}75$, $P < 0{\cdot}1$) higher ASA and the red muscle is characterized by a thirteenfold higher DHA ($t = 11{\cdot}3$, $P < 0{\cdot}1$) and a fourfold greater ($t = 5{\cdot}812$, $P < 0{\cdot}1$) DKA (Table 2). These

variations in the endogenous levels of metabolites reflect the active ascorbic acid metabolism in the muscles. It is known that the hydroascorbic acid cyclically undergoes oxidation to form dehydroascorbic acid and the latter is irreversibly hydrolysed to DKA (Fruton & Simmonds, 1961). The results of Table 3 represent

TABLE 3—DISCRIMINATIVE RATIOS INDICATING THE ASCORBIC ACID OXIDATION AND HYDROLYSIS IN THE LATERAL MUSCLE OF *C. catla*

Ratio	Reaction	White muscle	Red muscle
1. ASA/DHA	Higher ratios indicate lesser oxidation	58·1 ± 22·3 (6)	1·91 ± 0·42 (7)
2. DHA/DKA	Higher ratios indicate lesser hydrolysis	0·057 ± 0·027 (7)	0·293 ± 0·198 (7)

Values are mean ± S.D.
Number in parentheses indicates number of observations.

the extent of these reactions in the lateral muscles. The higher ASA/DHA ratios indicate less oxidation of hydroascorbic acid in the white muscle to an extent of thirtyfold ($t = 5{\cdot}70$, $P < 0{\cdot}1$) when compared to red muscle (Table 3). On the other hand, the red muscle is characterized by about a fivefold greater ($t = 2{\cdot}896$, $P < 0{\cdot}1$) ratio of DHA/DKA indicating a lesser hydrolysis of dehydroascorbic acid. From the nutritional as well as the physiological point of view, the hydro- and dehydroascorbic acid prevent the oxidation of fats at double bonds and also have antiscorbutic properties (Fruton & Simmonds, 1960). The white muscles have a greater quantity of antiascorbutic molecules compared to the red muscles (see Table 2), but the white muscle is characterized by greater hydrolysis of DHA and the red muscle by greater oxidation of hydroascorbic acid.

Post-mortem changes and cold storage alter the composition of the muscle (Table 4) and bring about the same changes. Although these effects are the same, they are muscle specific. There is a considerable increase in the levels of water, DHA/DKA and the iodine number in both the muscles as a consequence of cold storage or post-mortem changes, but to varying degrees. The fat content increased in white muscle in contrast to red muscle. The total ASA content decreased in both muscles but decreased more in red muscle. ASA and the relative ratios of ASA/DHA decreased to variable degrees in both muscles (Table 4) due to the above effects. Further, the ratios of DHA/DKA contents increased in white muscle in contrast to a decrease in red muscle. The iodine number increased about three- to fourfold in red muscle whereas it changed insignificantly in white muscle.

The results presented in Table 5 obviously demonstrate that white muscle homogenate oxidizes ascorbic acid, which is added exogenously, at a slower rate than red muscle homogenate. Further, the homogenates of muscles have a reduced ascorbic acid oxidase activity from post-mortem effects and after cold storage.

TABLE 4—RELATIVE CHANGES IN THE MUSCLE COMPOSITION OF LATERAL MUSCLES ON POST-MORTEM AND DURING COLD STORAGE

Quantity	White muscle		Red muscle	
	Cold storage	Post mortem	Cold storage	Post mortem
1. Water content	102 ± 0·31	101 ± 0·28	102 ± 0·41	101 ± 0·31
2. Fat content	111 ± 3	109 ± 2·1	96 ± 4	95 ± 2·90
3. Total ascorbic acid (mg/g wet wt.)	68·7 ± 16·1	61·1 ± 14·2	101 ± 8	104 ± 9·2
4. ASA (mg/g wet wt.)	42 ± 3	40·8 ± 2·5	35 ± 14	37·3 ± 15·1
5. DHA (mg/g wet wt.)	389 ± 60	391 ± 52	129 ± 17	125 ± 18
6. DKA (mg/g wet wt.)	119 ± 6·2	117 ± 7·2	139 ± 19	132 ± 21·0
7. ASA/DHA (mg/g wet wt.)	13·2 ± 4·5	12·7 ± 3·2	31 ± 15	28·6 ± 11·2
8. DHA/DKA (mg/g wet wt.)	201 ± 18	295 ± 14·75	91 ± 25	86 ± 21·3
9. I_2 number	109 ± 9	105 ± 8·1	380 ± 92	360 ± 72

The composition in the live muscle is taken as 100 per cent.
Values are mean of four observations ± S.D.

TABLE 5—ASCORBIC ACID-OXIDASE ACTIVITY OF DIALYSED MUSCLE HOMOGENATES OF *C. catla*

	Muscle homogenate	DHA (mg) formed per hr per mg muscle protein
Live	White muscle	0·12 ± 0·03 (5)
	Red muscle	2·63 ± 0·58 (5)
Post mortem	White muscle	nil (4)
	Red muscle	nil (4)
Cold storage	White muscle	0·08 ± 0·03 (4)
	Red muscle	0·12 ± 0·06 (4)

Assay conditions: pH 6·8, temperature 30°C and 5 mg ascorbic acid.
Values are mean ± S.D.
Number in parentheses indicates number of observations.

DISCUSSION

The cyclic oxido-reduction of ASA and DHA is known to be catalysed by ascorbic acid-oxidase localized in the mitochondria (Fruton & Simmonds, 1960). These cyclic oxidoreductions play an important role in the energy metabolism of cells. DHA is irreversibly hydrolysed to DKA by a specific enzyme (Fruton & Simmonds, 1961). DKA is not considered as vitamin C, since it lacks anti-ascorbutic properties (Lloyd & Sinclair, 1953; Fruton & Simmonds, 1961).

Detailed analyses of ASA, DHA and DKA contents of vertebrate muscles are scanty (Graff *et al.*, 1965; Ayubkhan & Swami, 1966a, b; Krishnamoorthy & Satyam, 1969). On histochemical evidence Chinoy (1969) discussed whether a higher ascorbic acid content in the red flight muscles of birds might form a source of electron energy for metabolism. He did not mention whether a higher ascorbic acid content in red muscle included DKA. The present results illustrate that DKA occurs more in red muscle and ascorbic acid more in white muscle. White muscle contains more antiscorbutic vitamin which includes ASA and DHA while red muscle has a greater turnover of ASA oxidations (Table 5). The higher level of the total ascorbic acid content in red muscle may be due to larger amounts of DKA. It is suggested that due to high ascorbic acid–oxidase activity, red muscle has a greater potentiality towards oxido-reduction systems in order to control the aerobic metabolism of the muscle.

Correlations between a high ASA content and a high degree of unsaturation have been made (Fruton & Simmonds, 1961; Krishnamoorthy, 1969a), based on the antioxidant property of ascorbic acid. In the present study the same correlation holds good in the case of white muscle. Though red muscle also has a large amount of ascorbic acid, as the greater percentage of it is DKA, the above correlation may not hold good in this case. In both these muscles cold storage and post-mortem changes depleted the level of antioxidants like ASA and increased the DHA level to a varying degree. These changes cannot be correlated to the degree of unsaturation especially in the case of red muscle where the iodine number increases post mortem and with cold storage. As viewed from these results, it seems probable that the high ASA-oxidase activity of red muscle may account for the contribution of hydrogen atoms for the oxidation of fat at double bonds. Secondly, there is an increase in the iodine number when the enzyme is inactivated (see Table 5) in red muscle during cold storage and post-mortem. The changes in ascorbic acid post mortem and after cold storage suggest that the muscle loses its antiscorbutic value post mortem. Though the white muscle has more antiscorbutic vitamin and less unsaturated lipids, from a nutritional point of view white muscle is more significant than red muscle. Changes in the iodine number and ascorbic acid content post mortem have a great importance as a quality index (from the nutritional point of view) in these muscles.

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