BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN FISH

Part VIII.—Acetylcholinesterase Activity in the Fresh and the Decomposed Fish Tissue and its Relationship with the Spoilage Characteristics

M. Qudrat-i-Khuda, H. N. De and N. M. Khan

Food and Fruit Research Division, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

(Received November 27, 1961)

The acetylcholinesterase activity of fifteen species of small and bigger size fish was estimated and the value QAChE ranged from 6.02 to 42.35 mg. per g. per hour. Some fish, specially the smaller size species showed an increase in the value on spoilage whereas the bigger species showed a decrease in the value. Bele (*Gobioides rubicandus*) fish maintained constant activity of nearly 12 mg. on storage. The logarithms of the percent increase and decrease in the activities plotted against the original values give a curve which shows that below 6 and above 17 mg. of activity (QAChE) in the fresh tissue there is no further change in the percent increase or decrease in the activities due to decomposition. At a normal level of nearly 12 mg. of QAChE there is no further change in the activity due to storage. The significance of these results in the evaluation of their spoilage characteristic as related to the muscular activity and tissue survival has been discussed.

Introduction

The role of acetylcholinesterase in cellular function is now well established because of its importance for the immediate removal of the load of acetylcholine generated in the conduction process of parasympathetic postganglionic and autonomic postganglionic (synaptic) impulses and also of the nerve impulse along the axon across the synapse and from motor nerves to striated muscles. This enzyme is present in remarkably high concentration in all types of conducting tissues, nerves and muscles (vertebrate and invertebrate), fibres and in the red blood cell, and a large amount of work has so far been recorded in the literature on the various aspects of its activity in cellular function.[1–5]

The maximum activity of the enzyme so far reported[6,7] is concentrated in the electric organs of some fish like *Electrophorous electricus*, *Melapterus electricus* and *Torpedo marmorala* and this accounts for the high bioelectric potential generated in these organs. Some other species of fish studied[4] also indicated the presence of this enzyme in organs like brain, peripheral nerve fibres, although in lower concentration.

Sufficient quantity of fish of various species are caught from the inland and marine water sources of East Pakistan but there is no information as to the activity of the acetylcholinesterase and other enzymes in the different organs and tissues. In the efforts relating to the enzymatic study of the fish tissues, Qudrat-i-Khuda, De, and Khan[8] reported the dehydrogenase activity of the tissue of some fish in their fresh and decomposed condition and found that air breathing species like Koi (*Anabas testudenius*) and Singi (*Heteropneustes fossilis*) possessed comparatively some more activity in fresh condition but on decomposition there was less generation of activity as compared to others, and this kept relationship with the *less* fall in titratable acidity in such case as reported previously by Qudrat-i-Khuda, De and Debnath.[9] The differential behaviour of these two species of fish as compared to carps and others was suggested as being due to their longer survival and continuous muscular activity in the process of struggling. Since the muscular movement is indirectly controlled by acetylcholinesterase by its participation in the maintenance of the flow of acetylcholine by utilising the same after completion of the conduction, it will be worthwhile to study the activity of the above enzyme in some species of fish of this region. The investigation in this line will also supplement the information already collected in the evaluation of the characteristic difference in the mechanism of spoilage in various species of fish.

Experimental

Samples of fifteen species of fish listed in the table were collected from the market in the early morning after 4 hours from the period of catch. The fleshy portion of the muscles of the dorso-ventral region between the pectoral and ventral fins under epidermis and epithelial layers of the skin were immediately dissected out. One g. sample of their flesh was then ground with quartz sand in buffer solution of pH 7.8, centrifuged and made upto 10 cc. with the same buffer solution. The acetylcholinesterase activity of this extract was then measured by the electrometric titration method of Stedman et al.[11] and Glick[12] as adopted by De[13] by using acetylcholinchloride (ACh) as the substrate. Twenty cc. of the solution

containing 50 mg. of the above substrate was first adjusted to pH 8.0 (approx.) and after bringing it to 37°C. was quickly added to the above fish extract after previously adjusting this to the same pH by addition of 0.02 N sodium hydroxide if required. The whole was then incubated at 37°C. for a period of 20 minutes and the amount of 0.02 N sodium hydroxide required to neutralise the liberated acetic acid for each 5-minute period was then recorded. From these values of acetic acid liberated, the amount of acetylcholinchloride in mg. hydrolysed by 1 g. of tissue during 1 hour was then calculated and this represents the activity QAChE as formulated by Nachmansohn and Lederer.[14] For each tissue of any particular species of fish replicate observations were made and these showed very little variation. For the study of the effect of storage and decomposition, similar fish of the same size from each species was stored for 24 hours at the room temperature of 80-85°F. and after the above storage period, the acetylcholinesterase activity of their muscles was determined in the same manner as before.

Results and Discussion

From the results presented in the table it would appear that there is a great variation of the AChE activity (QAChE) in the muscles of different species of fresh fish, ranging from 6.02 to

TABLE 1.—THE ACETYLCHOLINESTERASE ACTIVITY IN FRESH AND DECOMPOSED FISH MUSCLE.

Zoological name	Local name	Average size in mm.	Acetylcholinesterase activity (QAChE) expressed in mg. per g. tissue per hr.			
			In fresh	After 24 hrs	Total increase (+) or decrease (−) due to storage	Percent increase (+) or decrease (−) due to storage
GROUP A						
1. *Heteropneustes fossilis*	Singhi	15.2	6.02	15.74	+9.72	+161
2. *Ophicephalus punctuatus*	Leta	14.7	9.13	23.52	+14.39	+136
3. *Nandus nandus*	Meni	13.6	9.72	25.25	+15.53	+160
4. *Anabas testudinius*	Koi	14.7	10.30	15.82	+5.52	+53
5. *Callichrous pabda*	Pabda	15.7	11.44	13.80	+2.36	+20
6. *Gobioides rubicandus*	Bele	17.1	12.20	12.39	+0.19	+1.55
7. *Labeo rohita*	Ruhee	19.0	12.84	11.35	− 1.49	− 11
8. *Catla catla*	Katla	25.2	13.21	9.62	− 3.59	− 27
9. *Wallagu atu*	Boal	27.3	14.77	11.29	− 3.48	− 26
10. *Cirhinna mrigale*	Mrigale	22.8	17.54	10.49	− 7.05	− 40
11. *Notopterus notopterus*	Foli	21.7	17.88	12.05	− 5.83	− 32
12. *Labeo calbasu*	Kalibaus	22.3	29.52	17.10	− 12.42	− 44
13. *Ophicephalus marulius*	Gazar	27.5	40.35	22.48	− 17.87	− 44
14. *Ophicephalus striatus*	Shoul	30.5	42.76	22.93	− 19.83	− 46
15. *Mystus aor*	Air	26.8	43.35	22.74	− 20.61	− 47

43.35 mg. per g. per hour hydrolysis. This variation seems to relate proportionately to the size of the fish. It may be mentioned in this connection that the activity of the above enzyme of any tissue determined at any particular period does not represent the real quantity synthesised but the amount which is left as residue or as a reserve after utilisation in the functional activities in conduction process. On storage, some of the fish specially those belonging to the smaller species and size (group A) show increase in the activity of the enzymes by 2.36 mg. to 15.53 mg., i.e. 20 to 161 %, while the others of group B belonging to bigger species show a depression by 1.49 to 20.6 mg. or from 11 to 47 % from the original value. Bele fish stands between the above two groups and shows almost constancy in the above activity with a slight increase by only 0.19 mg. or 1.55% from the original value. This peculiar, but independent, behaviour of the Bele fish was also observed in the previous investigation by Qudrat-i-Khuda, De and Debnath.[9]

The peculiar findings as to the increase of the values in some fish, and decrease in others, due to storage may be better explained after careful scrutiny and analysis of the data. The general survey of the total increase or decrease of the activities due to storage as presented in the table does not apparently indicate any relationship with the original value but on expression as percent values, they seem to correlate with the activities of the fresh tissues. By plotting the logarithms of the percent increase and decrease of the activities due to storage against their normal contents before storage a reverse 'S' type graph is obtained which indicates that below the original activity equivalent to 6 mg. and above the range of 17 mg., there is no further perceptible increase or decrease of the values after storage. It is, further, noted that at a level of nearly 12 mg. as elaborated by Bele fish, there is no change in the activity due to storage. It may thus be postulated that in this fish the proportional synthesis and utilisation remain almost unchanged even during storage thus leading to a constant reserve or balance of enzyme equivalent to 12 mg. in both fresh and decomposed stages. Taking 12 mg. as the balancing level of enzyme activity in fresh fish, the values higher than the above level as elaborated by the fish of group B indicate the probability of less proportionate utilisation of the enzyme above this balancing level over the quantity synthesised in these fish. Reverse is the case in the fish of group A where the utilisation in normal course in fresh condition seems to be proportionately higher than the rate of synthesis of the enzyme. This does not seem to be unreasonable in view of the fact that the number of end plates and fibre surface where the release of acetylcholine and utilisation of acetylcholinesterase for immediate hydrolysis of acetylcholine takes place, is greater per g. of tissue, in the case of small fish than in bigger species. Further, in small fish the muscular activity due to struggling efforts is also high for which more enzyme is being constantly utilised.

The peculiar behaviour of the fish of group A showing the increase of the activity on storage may be explained now as probably due to survival of the tissues of these fish for a prolonged period on storage even when the muscular activity slows down; as a result of which the synthetic mechanism for esterase formation remains in operation whereas its utilisation for impulse conduction process decreases. The bigger species of fish of group B, on the contrary, perhaps die very quickly for which the enzyme synthesis mechanism in their tissues becomes inoperative. These observations are quite in agreement with our previous findings[10] that air breathing small species of fish like Koi and Singi and also Pabda, Meni Tengra etc. because of their similar ecological behaviour, are apt to less spoilage on storage as compared to carps and other bigger species of fish.

References

1. D. Nachmansohn, *Vitamins and Hormones*, **3**, 337 (1945).
2. D. Nachmansohn, *Currents in Biochemical Research* (Interscience, New York, 1946), pp. 335-336.
3. K. Angustinnson, *The Enzymes* (Academic Press, New York), **1**, (Pt. I), 444 (1950).
4. D. Nachmansohn, *Advances in Enzymology*, **12**, 259-334 (1957).
5. D. Nachmansohn, *Chemical and Molecular Basis of Nerve Activity* (Academic Press, New York, 1959).
6. A. Marnay, Compt. rend. soc. biol., **126**, 573 (1937).
7. D. Nachmansohn, Yale J. Biol. and Med., **12**, 565 (1940).
8. M. Qudrat-i-Khuda, H.N. De and N.M. Khan, Pakistan J. Sci. Ind. Research, **3**, 79 (1960).
9. M. Qudrat-i-Khuda, H. N. De and J.C. Debnath, *ibid.*, **2**, 217 (1959).
10. M. Qudrat-i-Khuda, H. N. De and M.A.H. Sharif, *ibid.*, **3**, 187 (1960).
11. E. Stedman, L. Stedman and L.H. Easson, Biochem. J., **26**, 2056 (1932).
12. D. Click, Gen. Physiol., **21**, 289 (1938).
13. H. N. De, Indian J. Med. Research, **43**, 71 (1955).
14. D. Nachmansohn and L. Lederer, Compt. rend. soc. biol., **130**, 231 (1989).