Changes in brain acetylcholinesterase activity in thermal acclimation in frog, *Rana tigrina*

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The behaviour of brain membrane-bound acetylcholinesterase of frog (*Rana tigrina*) during thermal acclimation was studied. The enzyme from warm-acclimated, cold-acclimated and control frogs had the highest affinity for substrate at the respective ambient temperature. Enzyme from cold-acclimated (15°C) animals had overall higher K_m while enzyme from warm-acclimated (37°C) animals had overall lower K_m compared to enzyme from control (28°C) animals.

POIKILOTHERMS have various strategies of respoding to variations in ambient temperature. Biochemical adaptations like enzyme reaction rate compensation, altered enzyme–substrate (E–S) affinity, altered protein synthesis, etc. have been extensively studied in fishes[1-5]. There are a few reports on the effect of temperature acclimation on enzyme systems in amphibians. The effect of temperature on lactate dehydrogenase in tissues of *Discoglossus pictus pictus* was studied by D'Costa *et al.*[6] Singh and Singh[7] reported variations in serum cholinesterase in *Rana tigrina* during cold acclimation. The effects of heat and cold exposures on acetylcholinesterase (AChE) activity in brain and spinal cord of *Rana ridibunda* were studied by Hussain *et al.*[8] The present paper deals with the response of brain membrane-bound AChE of *Rana tigrina* during thermal acclimation.

Male common Indian bullfrogs, *R. tigrina*, of approximately the same size were kept in large glass troughs partly filled with dechlorinated water and covered with wire mesh. The animals were occasionally fed pieces of earthworm and were acclimated to laboratory conditions for one week, after which they were divided into three groups of five each. One group were maintained at room temperature (28 ± 1°C), a second at 15°C in a refrigerator, and the third at 37°C in a laboratory incubator for 48 h. After 48 h, the animals were decapitated and the brain was immediately dissected out and washed with 0.9% NaCl solution containing 0.1% EDTA. Membrane-bound AChE was extracted by the method of Varela[9] and assayed by the method of Ellman *et al.*[10] The reaction mixture consisted of 2.2 ml of 0.1 M potassium phosphate buffer (pH 8.5), 0.1 ml of DTNB reagent, 0.1 ml of enzyme extract and 0.1 ml of 50 mm acetylthiocholine iodide. Absorbance at 412 nm was read on a Beckman spectrophotometer. Protein was estimated by the method of Lowry *et al.*[11]

In order to evaluate the effect of temperature on the behaviour of membrane-bound AChE, it is necessary to compare the K_m–temperature relationships for the enzyme from cold-acclimated, warm-acclimated and control frogs.

Table 1. Values of K_m of brain membrane-bound AChE of control, cold-acclimated and warm-acclimated frogs.

Assay temp. (°C)	K_m (mM of acetylthiocholine iodide)		
	Control (28°C)	Cold-acclimated (15°C)	Warm-acclimated (37°C)
5	2.3 ± 0.0	1.28 ± 0.1	0.385 ± 0.06
15	1.30 ± 0.08	0.81 ± 0.09	0.425 ± 0.03
22	0.42 ± 0.08	0.86 ± 0.09	0.180 ± 0.04
28	0.36 ± 0.08	1.06 ± 0.09	0.204 ± 0.03
37	0.62 ± 0.04	1.23 ± 0.2	0.158 ± 0.05
50	1.42 ± 0.08	2.0 ± 0.2	0.3 ± 0.04

Values are mean ± SE.

The K_m–temperature data (Table 1) show the effect of assay temperature on the K_m of the enzyme from frogs acclimated to different temperatures. A significant observation is that the enzyme from each group shows the least value of K_m, or the highest affinity for substrate, at an assay temperature identical with the ambient temperature for the group, i.e. the enzyme from control (28°C-acclimated) frogs showed highest affinity for substrate at 28°C, while enzyme from cold-acclimated (15°C) and warm-acclimated (37°C) animals showed highest affinity for substrate at 15°C and 37°C respectively. A similar observation was reported by Baldwin and Hochachka[1] for AChE from the brain of trout, *Salmo gairdneri*, acclimated to 2°C and 18°C.

Another significant feature observed is that the enzyme from cold-acclimated animals showed an overall higher K_m while the enzyme from warm-acclimated animals showed much lower K_m over the entire range of temperature studied compared to the K_m values for the enzyme from control animals. Such a response of an enzyme involved in neuromuscular activity is in agreement with the observation that the cold-acclimated (15°C) animals were far less active and the warm-acclimated (37°C) animals more active than animals maintained at 28°C.

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