CHAPTER - VI

DISCUSSION

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From the results obtained by the Kinetic studies of isolated and partially purified enzyme; it is revealed that skipper frog (Rana cyanophlyctus) ovary contained three species of acid phosphatase each acting at different pH and differing in its characteristic. They are designated as Enzyme I, Enzyme II and Enzyme III in present description. Their characters are summed up in Table 29.

Enzyme I acts at lowest pH 3.7 while Enzyme II acts at 4.4 pH and Enzyme III at 5.00 pH. The peak activities observed at 3.7, 4.4 and 5.00 pHs indicate presence of the three enzyme-substrate complex for three protonic states and pK values indicating three species of enzymes (Tiplon and Dixon, 1979).

Optimum temperature of incbation for Enzyme I, II and III is 30^{0}C which is common to all the three enzymes though they differ in their optimum pH. The optimum incubation time is also common for the three enzymes I,II and III.

Kms for substrate P-nitrophenyl-phosphate are also different for Enzyme I, II and III. They are 20.8mM, 34mM, 30.4mM respectively for Enzyme I, II and III.

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TABLE - 29

	Enzyme-I pH - 3.7	Enzyme-II pH - 4.4	Enzyme-III pH - 5.0
km (mM)	20.8 mM	34 mM	30.4 mM
Mg^{++}	+	+	-
Mn^{++}	+	+	+
Cu^{++}	+	-	+
Ca^{++}	↓	↓	↓
Citrate	+	↓	↓
Glycerol	+	-	-
EGTA	+	-	-
EDTA	-	-	-
EGTA + + Mg^{++}	-	-	-

\+ - Activation

\- - No effect

↓ - Inhibition

The study on the effects of the divalent ions reveal that Enzyme I is activated by Mg^{++}, Mn^{++} and Cu^{++} while Enzyme II is activated by Mg^{++} and Mn^{++} and Enzyme III is activated by Mn^{++} and Cu^{++}. Enzyme I, II and III are inhibited by Ca^{++}. Enzyme III is not influenced by Mg^{++}.

Enzyme I is specifically different from Enzyme II and III since it is activated by citrate and glycerol. Remaining two enzymes Enzyme II and III are not influenced by glycerol but are inhibited by citrate.

All the three enzymes are totally inhibited by 1% of formalin, methanol, ethanol, acetone, Triton-X-100 and Sodium fluoride.

The observations of the Figures 3, 13 and 20 which show effects of temperature on enzyme activity indicated high temperature upto 40^{0}C for incubation have no inhibitory effects on Enzyme I, II and III.

Ten minutes of incubation time for optimum activity has been selected for all the three enzymes from the figures 4, 14 and 21 which indicate effects of incubation time on the activities of enzymes. This time interval is selected so that activation and inhibitory effects can be studied and compared.

From the figures 5, 15 and 22 which show effects of substrate concentration on enzyme activities, it is indicated that the small concentrations of the substrate are not showing any enzyme activities but from the concentrations of 10mM in case of Enzyme I, 12mM in case of Enzyme II and 20mM in case of Enzyme III evoke the enzyme activities.

In case of Enzyme I the activities increased smoothly from 15mM concentration of substrate to 30mM concentration of the substrate to achieve maximum activity. In case of Enzyme II steady increase is observed from 15mM of substrate concentration to 35mM concentration to achieve maximum activity. Enzyme III is expressed after 20mM concentration of the substrate and smoothly increases the activity upto 35 mM concentration of the substrate to achieve maximum expression of the enzyme activity.

The period of early concentration of nil activity is generally referred as 'initial lag' (Dixon and Webb, 1979). This type of enzymes showing 'initial lag' periods are termed as 'hysteretic enzymes' by Frieden (1970) which is accepted as general term for such initial lag showing enzymes (Dixon and Webb, 1979). These observations fall in the category of model suggested by Richard et. al.,(1974). This model suggests that the free enzymes is capable of existing in two conformations

(represented by □ and O), both of which can bind substrate but with different affinities, substrate binding induces a third conformaticn (Δ) which can only revert to one of the two free enzyme forms when the produt is released. Frieden (1970) has considered this mechanism making the assumptions that the substrate binding steps are rapid and remain in equilibrium. The reaction is irreversible and is not inhibited by the products.Frieden (1970) has also suggested that such effects may be important in the regulation of metabolic pathways in which several enzymes compete for the same intermediate. Hysteretic effects of this type have been observed with the phosphorylase from frog muscle (Frieden, 1970). Many more complicated mechanisms are known in which there are slow transitions which can lead to hysteretic effects (Ainslie et. al.,1972; Frieden, 1973 and Smith and Perry, 1973) and a number of cases have been observed in which they are due to slow association or dissociation of the enzyme e.g. α -N-acetylgalactosaminidase (E.C. 3.2.1.49) (Wang and Weisman, 1971; Kurganov et. al., 1976 .

The studies on effect of divalent ions on enzyme activities indicate that Enzyme I and II are activated by Mg^{++} just similar to phospholipase (Banghaus and Dawson, 1962). Enzyme III is not influenced by Mg^{++} as phospholipase B (Fairbairm, 1948) but any of the tthree enzymes is not inhibited by Mg^{++}. Still

pyrophosphatase is shown to be inhibited by Mg^{++} completely (Fernley and Walker, 1967). Enzyme III is activated by Mn^{++} and Mg^{++}, such activation effect with less expressions is also observed in case of phospholipase C (Zamicnek et. al., 1947).

None of the Enzymes I, II and III activated by Ca^{++} similar to the enzyme phospholipase B(Fairbairm, 1948) but phospholipase C was activated by Ca^{++} (Macfarlane and Knight, 1942).

Among the other characteristics of the enzymes, Enzyme I is specifically activated by glycerol and citrate; while Enzymes II and III are inhibited by citrate. Inhibition by citrate is also observed in case of phospholipase C (Macfarlane and Knight, 1942) but it is Ca^{++} activated system.

All the three enzymes i.e. Enzyme I, II and III are inhibited by formalin, Triton X-100, Methanol, Ethanol, acetone and NaF. All the three enzymes were not affected by tartarate. In similar type of studies in Rana esculenta Panara et. al., (1989) have sh own that hepatic microsomal acid phosphatase and mitochondrial acid phosphatase are inhibited by NaF and tartarate but are not sensitive to formaldehyde. While hepatic lysosomal acid phosphatase and nuclear acid phosphatase are inhibited by Triton X-100 and formaldehyde, but they are resistant to tartarate

and nuclear acid phosphatase is also resistant to NaF. Among the above four hepatic acid phosphatases lysosomal enzyme is activated by temperature but not the remaining.

Frog liver also contains tartarate sensitive high molecular weight acid phosphatase (140,000 D) which is a dimeric glycoprotein (Janska et. al., 1988). NaF sensitive and tartarate resistant and thermostable acid phosphatase of 38000 D molecular weight is also reported in liver of frog (Kubicz et. al., 1981). All the three enzymes presently reported in Rana cyanophlyctis ovary are also NaF sensitive, tartarate insensitive and thermostable. Similar characters showing enzyme is reported by Kubicz et. al., (1989) which has 38000 D molecular weight.

Therefore it can be suggested that ovarian acid phosphatases (Enzyme I, II and III) may be of low molecular weight belonging to lysosomes since they show similar other properties exhibited by the hepatic lysosomal enzyme reported by Kubicz et. al., (1978, 1981) and Panara et. al., (1989) in Rana esculenta. But their lysosomal origin has to be confirmed, similarly the hepatic enzyme may not be necessarily similar to the ovarian enzymes though it exhibits some enzymatic characteristics common.

Jan ska et. al., (1989) and Panara et. al., (1989) have

studied the thermal effect on the hepatic acid phosphatase. Jan ska et. al., (1989) assay enzyme activities on heat treatment given upto 56°C to acid phosphatase III and IV (low molecular weight and monomeric). The results show that Acid phosphatase IV is more sensitive to heat treatment than Acid phosphatase III. The pronounced differences are observed after 45 minutes of preincubation. They also report that concavalin A binding activated both the enzymes. Acid phosphatase III is activated significantly. Similarly Panara et. al., (1989) also observe thermosensitivity of acid phosphatases. They find that lysosomal acid phosphatase is activated by prior treatment of temperature upto 60°C. The microsomal, mitochondrial and nuclear acid phosphatases were inhibited by thermal pretreatment.

Similar type of thermostability of enzymes is also studied in present investigation. All the three species of enzymes; Enzyme I, II and III show activation by prior temperature treatment; which is retained at its maxima by prior treatment of the 60°C. The effect of varied time intervals shows that the prior temperature treatment (at 60°C) for 10 minutes slowly evoke the enzyme activities showing recognizable bursts in enzyme activity at 15' of prior incubation, in case of Enzyme I (3.7 pH) and Enzyme III (5.0) but incase ofEnzyme II (pH 4.4) the increase in enzyme activity is linear to the enhancement of time reaching

at its maximum at 25 minutes of prior incubation.

These results differ from what is observed by Janska et. al., (1989) and Panara et. al.,(1989) incase of hepatic acid phosphatases. The similarity being that prior treatment of temperature of 60°C is needed for the full expression of enzyme activities; and onlylysosomal enzyme is thermoactivated. The Enzyme II which islinearly thermoactivated, activated by Mg^{++}, Mn^{++}, not influenced by tartarate, glycerol and is inhibited by citrate, NaF, formaldehyde, ethanol, methanol, acetone and Triton X-100 is similar in the thermoactivation to lysosomal enzyme described by Janska et. al., (1989) and Panara et.al., (1989).But though these properties are similar the ovarian lysosomal enzymes may have different identity and function though it shares some properties with that of hepatic acid phosphatases.

The other microsomal, mitochondrial and nuclear enzymes are thermosensitive (Janska et. al., 1989; Panara et. al., 1989) which is not true in case of ovarian enzymes. The ovarian acid phosphatases I, II and III are thermoactivated and the activation is significantly evoked at 20' of preincubation in case of I and II enzyme.

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