CHAPTER - I

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Amphibians are well studied animal group for their reproductive processes. The reproductive processes in seasonally breeding animals are influenced by the environmental factors such as light, temperature, availability of food etc. The phenomenon of hibernation is observed in most of the seasonally breeding species. Thus the processes become more interesting as well as complicated when considered in relation with each other.

During hibernation the gonads including ovary are at sexually quiescence. At the onset of the prebreeding period the gonads spring in the development which is followed by the growth of the oocytes. In breeding period the copulatory activities are coupled with ovulation and spawning. The successive period is of post-breeding where postovulatory ovary enters in regression followed by the sexual quiescence.

All the above processes involve many physiological, cell biological and biochemical alterations. In that interest the present problem is very small attempt to study some biochemical

characters of Rana cyanophlyctis (skipper frog) ovary.

Before going into details of the problem it is appropriate to review the relevant literature revealing some important physiological, cell biological and biochemical events concerned with the present work.

Review of the literature :

Acid phosphatases [EC 3.1. 3.2] are a group of widely distributed enzymes of very broad specificity differing somewhat with the source of the enzyme. They all act on a wide range of monoesters of orthophosphoric acid both aliphatic such as glycerol-1-phosphate and glycerol-2-phosphate and aromatic such as 4-nitrophenyl phosphate (Fernley, 1971; Hollander, 1971; Morton, 1955). They do not act on phosphoric diesters or triesters. There is a major distinction between the two groups with regard to sulphur containing esteres (Hollander, 1971). Acid phosphatase can hydrolyse O-substituted monoesters of phosphorothioic acid such as O-4-nitrophenyl thiophosphate.

Non-specific acid phosphatase [E.C.3.1.3.2] is first time shown to present in lysosomes (de Duve et. al., 1951) of rat. On further studies it was shown in the lysosomes of almost all cells with few exceptions (Dingle and Fell, 1969; Holtzman, 1976). It s physiological role is described in manyphysiological processes and pathology (Dingle and Fell, 1969; Holtzman, 1976). In recent years its presence has been shown in endoplasmic reticulum and

the Golgi apparatus intracellularly in addition to lysosomes in various animals (Ide and Fishman, 1969; Dauwalder et. al., 1969; 1972; GoldFischer etal., 1973; Nyquist and Mollenhauer, 1973; Holtzman, 1976).

But in early years acid phosphatase activities in mammals were studied mostly as compared to submammalian vertebrates.

In amphibians the ovary is studied for its histochemical and biochemical contents and other components including acid phosphatase enzyme activities.

Histochemically the acid phosphatase activity was located in the theca externa and theca interna in Rana temporaria (Przelecka et. al., 1962), in Rana pipines (Kessel and Decker, 1972) and in Xenopus laevis (Jared et. al., 1973; Deroly, et. al., 1979; Dehn, et. al., 1973). Dhange (1981) showed presence of acid phosphatase activity in the ovary of Bufo melanostictus histochemically. He reported the enzyme activity in the germinal epithelium, cytoplasm of developing oocytes, in cortical granules of developing oocytes, matured oocytes and atretic follicles.

There is hardly any attempt made on the amphibian ovarian acid phosphatase isolation and characterization.

Kanamori (1963) examined acid phosphatase activity in ovarian egg homogenates of frog.

Dhange (1981) studied acid phosphatase activitiess in various reproductive conditions of ovary of Bufo melanostictus. He reported highest activity in post breeding condition and lowest activity in breeding condition. Moderately high activity was reported in the prebreeding condition.

Mester, et. al,(1985) isolated acid phosphatase which was associated with the acid phosphocasein phosphatase, on Sephadex G-100 and resolved the bulk phosphatases into 3 major peaks, all active with p-nitrophenyl-phosphate and casein. Both the enzymes showed the same mobility on gel electrophoresis with same molecular weight. Both the enzymes were inhibited by NaF, molybdate, adenine nucleotides and divalent cations (Mn^{++}, Ca^{++}, Zn^{++}).. They differ in sensitivity to ATP, tartarate and Zn^{++}.

Significant attempts were made on the isolation and characterization of acid phosphatase from amphibian liver.

Seasonal variations were observed in liver acid phosphatase activity showing peak activity from October to August (Kamyk et. al,, 1985).

Multimolecular forms of acid phosphatase were reported in Rana esculenta. They differ in their thermal stability, substrate specificity and activator specificity. Acid phosphatase I and II were sialoglycoproteins (Kubicz , et al, 1981).Mester et. al.,(1982) isolated acid phosphatase from nuclear fraction of

liver of Rana ridibunda L. The enzyme activity was inhibited by ATP, ADP, AMP. But the activity was resistant to cAMP, tartarate molybdate. Janska, et. al., (1989) further showed that in Rana esculenta liver acid phosphatase III and IV were the major enzyme forms of the low molecular weight acid phosphatase. Both of them showed a single protein band on SDS-PAGE corresponding to a molecular weight of about 35000 D. Thus they are monomeric proteins showing the same protein molecule.

Both the enzymes differ in their thermostability and the activating effect of concavilin A binding. They are considerably activated by Dithiothretol but they differ markedly by the extent of activation. It also indicated changes in pH activity curves. Possibly the low molelcular weight forms may represent a set of glycoforms, whose different biochemical activity may be determined by the redox states of their essential cystein residues.

The subcellular localization of Acid phosphatase I, II (. Kubicz[illegible], et. al., 1981), III and IV (Janska, et. al., 1989) from liver of Rana esculenta was studied further by Panara, et. al., (1989). Acid phosphatase I, II, III and IV were associated with the microsomes, mitochondria, lysosomes and nuclear and soluble fractions respectively. All had same 'Km' values for p-nitrophenyl-phosphate and showed apparent molecular weights of 240,000 D; 110,000 D; 38,000 D; and 17,000 D respectively. Acid phosphatase

I, II and III hydrolysed most of the common phosphate esters but Acid phosphatase IV hydrolysed effectively only p-nitrophenyl phosphate, phenyl phosphate and flavin mononucleotide.Acid phosphatase III and IV were tartarate resistant. Temperature inhibited acid phosphatase I, II and IV but it activated acid phosphatase III.

From the comparative studies of hepatic acid phosphatase I from Rana esculenta and Cyprinus carpio (Kubicz , et. al,, 1981) it was revealed that both the enzymes differed in substrate requirements; some activation and inhibition properties by some compounds as well as thermostability. But they were shown tobe sialoglyccproteins varying in their sialic acid content.

The skipper frog used for the present studies was Rana cyanophlyctis. The ovary of this frog was well studied for the other enzymes except acid phosphatase. Most of this work is histochemical.

Saidapur and Nadkarni (1972 a,b) showed $^{4}\Delta^{5}$ - 3β-hydroxysteroid dehydrogenase, glucose - 6 - phosphate dehydrogenase and NADH diaphorase in the post ovulatory follicles in the hypertrophied granulosa cells only.

The other endocrinological work on the ovary of the Rana

cyanophlyctis is also reviewed in the following paragraphs.

17-α-hydroxy corticosterone, deoxy-corticosterone, progesterone induced ovulation and oviposition (Ramaswami and Lakshman, 1960). (Ramaswami, 1962, a, b, c).

The ovulation process and the selection of oviduct by the eggs from the same side was observed in Rana cyanophlyctis (Suvarnalata and Sarkar, 1972).

In hypophysectomized frogs the ovarian recruitment and the development was observed in response to homoplastic pituitary parsdistalis homogenate and PMSG, HCG, GH, and 17-β-estradiol-combinations. It showed the recruitment and growth of the oocytes (rapid) in prebreeding condition than in post-breeding condition. In frog seasonal variations in the ovarian responsiveness were also observed (Panchratna and Saidapure, 1985; [illegible]). Melatonin showed inhibitory effect on growth and recruitment of oocytes when given in photophase but if continuously given the effect was antigonadal (Kupwade and Saidapur, 1986).

Reasons to select the problem :

Thus on the basis of the survey of the available literature it can be revealed that the ovarian acid phosphatase

of amphibians still remains to be studied for its isolation, characterization and its functions also.

Because the amphibians are the first tetrapodes, any specificities possibly observed in the ovarian acid phosphatase characters may be revealing some evolutionary significant points of biochemical interests.

Kubicz, et. al., (1981) reported some specificities in the hepatic acid phosphatase characterization as compared to fish hepatic acid phosphatase.

For the reasons discussed above the present problem was selected and worked out within the laboratory availabilities.

Reasons to select the animal :

For the study of isolation and characterization of the acid phosphatase the large tissue is needed; therefore, large number of animals are required and since the skipper frogs are easily available in surrounding area, they are selected for the study.

The animals were collected for the studies during prebreeding period [March and April (Saidapur, 1986)]. The maturation of oocytes was observed at its peak during the prebreeding condition (Wossermand and Smith, 1978; Saidapur,

1986). Therefore this period was selected so that the enzyme/s involved in the maturation of oocytes could be isolated for the studies.

The kinetic studies on acid phosphatases revealed three different enzymes of varied characteristics.For the presentation of this data the present work is arranged in VII chapters in this thesis.

Ist Chapter - **Introduction**

This chapter deals with the review of literature, reasons to select problem and reasons to select the animal.

IInd Chapteer - **Material and Methods**

IInd chapter deals with the different methods and materials used for the present work.

IIIrd Chapter - **Enzyme I**

This chapter includes Kinetic studies ofEnzyme I with 3.7 pH optimum.

IVth Chapter - **Enzyme II**

This chapter is based on the Kinetic studies ofEnzyme II with 4.4 pH optimum.

Vth Chapter - **Enzyme III**

This chapter includes Kinetic studies ofEnzyme III with 5.00 pH optimum

VIth Chapter - **Discussion**

In this chapter the results of Chpater III, IV and V are discussed to reveal the significance of the present work.

VIIth Chapter - **Concluding Remarks**

Under this chapter the remarks on the present work and future plans in this line of work are included.