STUDIES ON CERTAIN ASPECTS OF BIOCHEMICAL GENETICS IN SELECTED SPECIES OF FROGS FROM NORTH-EASTERN REGION OF INDIA

ABSTRACT

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The transition from water to land is a very remarkable step in the phylogenetic history of vertebrates. This conquest of land was initiated by the primitive amphibians in the Devonean period. In the course of their evolutionary history amphibians have become extraordinarily modified to lead a dual life. Their life cycle involves a drastic metamorphosis. They are cold blooded and so hibernate during winter months.

They are world wide in distribution and as many as 2600 species of anurans, the largest group of amphibians have been reported. In India annurans are represented by about 165 species of frogs and toads belonging to 6 families. Out of the 55 species of anurans reported from North-Eastern region of India 40 belong to the hills of Meghalaya. In spite of this richness genetic studies with these anurans are very limited. The present investigation is thus carried out to evaluate the electrophoretic pattern of multilocus isozyme systems. Seven species of frogs belonging to three families were analysed from different populations to study the isozyme pattern. The results are recorded in chapter one. In chapter two the developmental genetic aspect of the isozymes during tadpole growth and metamorphosis has been studied for one commonly available species.

In each of these chapters we have included the pattern of gene expression of four dehydrogenases (viz. Lactate dehydrogenase, Malate dehydrogenase, Alcohol dehydrogenase and Glucose-6-phosphate dehydrogenase). Seven tissues of adult frogs were analysed to study the isozyme pattern. Tadpole tail muscle, head region and liver tissues were used for similar study at different stages of development in chapter two. Related work has been recorded in introductory section of each chapter.

Live specimens were sacrificed in the laboratory and fresh tissues viz. gonad, kidney, heart, brain, eye, liver and skeletal muscle were used. Isozymes were separated on 7.5% polyacrylamide rod gel according to the standard disc-electrophoretic procedures. Gels were subjected to specific staining solution to visualize the isozyme bands.

Lactate dehydrogenase (LDH) is a tetrameric enzyme catalyzing the interconversion of lactate to pyruvate. In most vertebrates the enzyme is coded by two codominant loci(A and B). Random association between the products of these genes yield five electrophoretically distinguishable isozymes in vertebrates. In some vertebrates a third locus designated C codes for highly tissue specific LDH. The presently studied frogs revealed a similar pattern of the LDH isozyme phenotypes as observed in other vertebrates. The

pattern manifests itself in predominance of acidic isozymes of heart and predominance of basic ones in skeletal muscle. Activity of the locus A was found to be more than that of locus B. The expression of locus B showed a restriction in some of the tissues of certain species. The heteropolymeric isozyme A_1B_3 also showed a restricted assembly in certain species. Allelic variant of the locus A was detected in one species resulting into a twelve-banded heterozygotic expression of LDH. The observed number and their Hardy-Weinberg expectations for the polymorphic loci support the validity of the proposed model and indicate that the samples were collected from a single Mendalian population. Further in two species tissue specific expression of the isozyme in eye and liver were observed. The isozyme showed a different electrophoretic characteristics comparable to the C4 LDH observed in other vertebrates.

Malate dehydrogenase (MDH) is dimeric in nature and catalyzes the interconversion of malate to oxaloacetate in Krebs cycle. The cytosolic malate dehydrogenase (s-MDH) is encoded by two loci in most vertebrates. In the present study both the loci have been found to be active.

Alcohol dehydrogenase (ADH) in most vertebrates is the product of a single locus and is primarily a liver specific

enzyme. It catalyzes the interconversion of many alcohols to their corresponding aldehydes and ketones. A single banded pattern of the enzyme could be resolved in liver and kidney tissues of the frogs analysed here. However, additional bands of low staining intensity has also been observed in some species. These may be due to allelic variants in the population of the species.

Glucose-6-phosphate dehydrogenase(G6PDH) is a key enzyme in pentose phosphate shunt. It is dimeric in nature and exist in two forms (A and B), without exhibiting heterodimeric forms. A single form corresponding to the form A was resolved in most of the tissues of the frogs examined in the present study. However, liver, gonad and eye tissues revealed additional band corresponding to form B in some cases. Skeletal muscle tissue did not show any activity for G6PDH enzyme indicating little or no pentose phosphate shunt.

Analysis of the tadpole tissues at various stages of development showed a distinctive isozyme pattern. Tadpole tail, head region and liver tissues exhibited predominance of LDH polypeptides at the various developmental stages. Expression of the B locus showed a restriction, Malate dehydrogenase(MDH) showed a less complex isozyme pattern. Both the locus were equally active. However there was a

restriction in the heteropolymeric assembly. A remarkable variation in the expression of alcohol dehydrogenase gene was noted in the liver tissue of the tadpoles. During early stages of development multiple of ADH bands were resolved. Subsequently there was reduction in the number of isozymes with the progression of development. Glucose-6-phosphate dehydrogenase (G6PDH) revealed a single banded phenotype in the tail, a two-banded phenotype corresponding to A and B form of the isozyme were observed in the head region. Liver tissue exhibited a differential repression activation phenomenon of the genes coding for G6PDH isozymes. During early metamorphic stages both the form showed higher concentration of the enzyme. Subsequently with the progression of growth and development the isozyme form A was predominantly synthesized.

The isozyme pattern obtained for the four dehydrogenases in the adult specimens of the seven species and the tadpole tissues of one frog have been correlated with the local ecological conditions as well as with the physiological states of the frogs. However, our presumption of the various correlates with the observed isozyme pattern need further research to draw a final conclusion.