Esterase Variability in the Different Tissues of Farm Fowl

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Abstract: Studies were carried out on some selected digestive tissues for different age groups of farm fowl (Gallus sp.). The tissues were liver, crop, gizzard, small intestine & large intestine. Polyacrylamide gel electrophoresis was carried out to analyze the distribution of esterase isozymes in different tissues of Gallus sp. Maximum number of bands was found in the large intestine and the tissues of alimentary canal show a great variation in the expression of bands. Altogether five esterase bands were revealed using Polyacrylamide gel electrophoresis (7.5%) in 1 hour and 45 minutes running time. Both alpha & beta napthyl acetates were used in the gel electrophoresis. The esterase bands were recognized as Est-1, Est-2, Est-3, Est-4 and Est-5 in an increasing numbers based on the decreasing mobility from the origin. Variation was found in the number of bands in the different tissues of alimentary canal. The variation in the intensity of bands in the tissues of alimentary canal is thought to be related with the dissimilar actions of esterase in those tissues. The tissues of gizzard, small intestine and large intestine showed a great variation in the number of bands with their increasing ages.

Key words: Esterase - PAGE - Farm Fowl

INTRODUCTION

The first genetically accepted descriptive term for the existence of different molecular forms of proteins with the same enzymatic specificity was introduced by Market and Mollar [1], who coined the word isozymes to describe this phenomenon. According to them, isozymes may be defined as multimolecular forms of enzymes which are derived from the same organ or tissue and has at least one substrate as common. Different investigators have defined the term in different way, but the original definition by Market and Mollar [1] has been widely accepted. The group of genes which determine the structures of families of isoenzymes, can represent several different phenomena: the existence of multiple gene loci, the occurrence as the result of mutation of pairs of unlike genes (alleles) at the same locus, on the post translational modification. Isozymes which are the product of allelic genes are distributed in the population according to the laws of Mendelian inheritance. It has also been shown that isozymes are species-specific. Esterase isozyme is one of the lipid hydrolyzing enzyme which has a great significance in field of genetics. Extensive electrophoratic studies have been done on the various tissues of different animal and plant species, from which it has been revealed that multiple molecular forms esterases do exist. The electrophoratic methods can be successfully used for the identification fowl species. Esterase can be classified as arylesterase, carboxylesterase, acetylcholineesterase, (acetylcholine acyl hydrolase) cholinesterase (acetylcholine acyl hydrolase).The acetylcholineesterase is a characteristic of nervous tissue and the cholinesterase of serum. There is a great variation in esterase activity in different organs of various fowl
species. Many researchers have reported polymorphism of esterase isozymes loci in fowl species. Polymorphism or many forms in serum esterases of the fowl species has been found to be a profitable study. The biochemical and physiological properties of esterase have been previously studied by Heal et al. [2] and Oakesshott et al. [3]. The esterase pattern are usually highly variable in both intraspecies and interspecies [4]. The origin of such variability may have been constructed by divergence induced in terms of gene duplication [3, 4]. On the other hand, stage-specific and tissue specific expression patterns have also been observed in studies of esterase bands or isozymes [5-8]. These esterase enzyme is very interesting enzyme because it is implicated in synaptogenesis and involved in neurodegeneration in adult tissue. We thus conducted in this study a survey of farm fowl at esterase variably in the different tissues to compare the banding pattern of esterase.

**MATERIALS AND METHODS**

The experimental part of our study on the esterase isoenzymes to know the banding pattern was done in the Genetics and Molecular Biology Laboratory” of Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh. The samples were collected from local market (New Market). For the purpose of the study on the esterase isoenzyme of farm fowls was used the polyacrylamide slab gel with continuous buffer, non dissociating buffer system as described by Hames, [9] with a few modifications. Esterase were identified in the gels following basically the technique described by Johnson et al. [10] by using the mixture of both alpha and beta-naphthyl acetates. The tissues as liver, crop, gizzard, small intestine and large intestine were subjected to electrophoresis. Each sample of Gallus was taken into an eppendorf tube. Each liver, crop, gizzard, small intestine and large intestine were squared in 20µl Tris-Borate EDTA buffer. After squashing, 2X TBE-Bromophenol blue solution was added to each sample as the amount of 2X Tris-Borate EDTA that means the same amount of marker dye (Bromophenol blue) in TBE buffer was added to the respective squashed samples. The samples were then centrifuged at 12000 rpm for 5 minutes at 4°C. The supernatants were applied to electrophoresis. 7.5ml acrylamide and bisacrylamide were used in the ratio of 30: 0.8; 6.0 ml of TBE (5X) buffer, which contain 54g tris, boric acid 27.55g, 20ml of 0.5 M EDTA (Ethylendiamine Tetra acetic Acid); 0.140ml TEMED (Tetramethylethylene diamine); 0.150ml of 10% AMPS(Amonium Persulfate) and 16ml of distilled water were used for gel preparation. Gels were pre run at 120 volts for about 30 minutes. The sample (10µl) was then carefully loaded onto the wells using a micropipette, one sample to one well. Electrophoresis was done at 120 volt (constant voltage). In this condition, the electrophoresis apparatus was kept undisturbed for at least 1 hour and 15 minutes, until the tracking dye (bromophenol blue) comes out from the sandwich. The staining solution for esterase was contained 1.32g of 0.2M monobasic sodium phosphate and 0.5362g of 0.2 M dibasic sodium phosphate and the substrate were α and β naphthyl acetates. Running gel was kept in this mixture for 15 minutes at 25°C. After 15 minutes, substrate mixtures were poured out and the gel was immersed in staining mixture. Staining mixture was prepared by dissolving 0.02g Fast Blue RR salt in 120ml of water every day just before use. Then gel was stained at 37°C incubation for 25 minutes. After staining, the Fast Blue staining solution was drained out and the gel was washed with distilled water. The stained gel was photographed after decolorizing for 30 minutes. Esterase bands were scored from the stained gel as recorded previously [11-13].

**RESULT AND DISCUSSION**

Polyacrylamide gel electrophoresis was carried out to analyze the distribution of esterase isozymes in different tissues of Gallus sp. of fowl. Five tissues were taken for esterase analysis. Of these one is liver and other four belong to alimentary canal. To detect the isozyme banding pattern in liver and different tissues of the alimentary canal, 7.5% Polyacrylamide gel electrophoresis method was adopted in the present study. For this endeavor, altogether five different tissues were taken from the different age group of fowl sp. in mini slab gel Polyacrylamide gel electrophoresis with 7.8cm × 10.5cm gel size. When samples of different stages were subjected to PAGE and subsequently stained for esterase activity a number of electrophoretic bands appeared. The banding pattern observed in the selected age group of fowl samples are shown in the following plates. Individual sample from stages were tested for study of esterase isozyme variability. Following Webb [14] the esterases were assigned in increasing numbers based on the decreasing mobility from the origin, for example, EST-1 was referred to the band with the highest mobility. After electrophoresis and staining for esterase activity of three esterase bands were detected according to their relative mobility in the gels. All the three bands were not present in all the tissues taken in the experiment. The bands showed different staining pattern on the basis of the substrates on the gels, some were more intense compared to others.
Table 1: Showing the intensity variation of esterase isoenzyme bands in different gut tissues and liver tissue of one day chick.

<table>
<thead>
<tr>
<th>Esterases</th>
<th>liver</th>
<th>crop</th>
<th>gizzard</th>
<th>small intestine</th>
<th>large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est-1</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Est-2</td>
<td>+++++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Est-3</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Est-4</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Est-5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*+++++, very deep stained, ++++, Deep stained, ++, Light stained; +, Medium light stained

Table 2: Showing the intensity variation of esterase isoenzyme bands in different gut tissues and liver tissue of one day chick.

<table>
<thead>
<tr>
<th>Esterases</th>
<th>liver</th>
<th>crop</th>
<th>gizzard</th>
<th>small intestine</th>
<th>large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est-1</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Est-2</td>
<td>+++++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*+++++, very deep stained, ++++, Deep stained, ++, Light stained; +, Medium light stained

All mentioned esterase bands had tissue and substrate specific expression. Tissue specific expression of esterases was also found in sword tail fish (xiphophorus helleri) [15]. The bands also showed an intensity variation among different tissues. Based on staining intensity, esterase isozyme bands could be describe in the four categories such as- (a) very deep stained, (b) deep stained and (c) light stained d) medium light stained (Table 1, 2). Debnath [16] classified the intensity of esterase isozymes in to five categories among three fresh water fish species. Those were (a) very intense (b) intense (c) fairly intense (d) weak and (e) very weak.

Twenty one different esterase bands were found in Drosophila melanogaster when α and β naphthyl acetate used as substrate, eighteen of them were black and thus classified as α-acetate, two of them were red, classified as β-acetate [17] (Taskin). But in our study both α and β naphthyl acetate were used together and only α-acetate activity were showed in the figure 1 and 2.

**One Day Chicken:** When gels were stained with alpha and beta naphthyl acetates the five different tissues taken in the experiment showed various number and intensified bands. Maximum five bands namely Est-1, Est-2 and Est-3 Est-4 and Est-5 were found. The esterase band Est-2 is deeply stained while Est-3 and Est-4 are moderately stained and Est-5 is slightly stained. The intensity of staining is directly related with the expression of their activity. Similarly crop showed three esterase bands and the band Est-1 is highly stained. The esterase band Est-3 is faintly stained expressing the lower activity in the crop region. The tissue Gizzard showed two esterase bands and an anomaly is found here. The two bands have nearly same expression in this region. Small intestine showed three esterase bands. In this case, the difference of relative mobility between Est-1 and Est-2 is very low. Large intestine showed maximum five number of bands of which Est-2 and Est-3 are deeply stained and Est-4 and Est-5 are lightly stained.

**Five Days Chicken:** Same result as the previous one day chicken was found in this experiment. Liver showed three esterase bands in which Est-2 is very deeply stained. Crop showed two esterase bands and Est-2 is deeply stained.
Fig 2: Esterase banding pattern with the relative mobilities of different tissues of alimentary canal and liver tissue of farm fowl specie stained in \( \alpha \) naphthyl acetates by using 7.5% polyacrylamide gel electrophoresis (PAGE) (Slot 1. Liver 2. Crop 3. Gizzard 4. Small intestine 5. Large intestine

Gizzard showed two esterase bands and both the esterase bands are deeply stained. Small intestine showed three esterase bands in which Est-2 and Est-3 are deeply stained. Large intestine showed four esterase bands and Est-2 and Est-3 are deeply stained.

**Ten Days Chicken:** A great variation of the expression of bands was found in this experiment. Here maximum number of bands is two which is shown by liver tissue. The gizzad tissue showed a very faintly stained band. All the tissues except liver showed only esterase-2.

**Thirty Days Fowl:** Nearly similar result as the previous one was found in this experiment. The result for different tissues used for esterase electrophoresis from thirty days old chickens are presented in Figure 2.

Esterase activity were showed in different tissues may be due to different physiological function in contrast with digestion and metabolism. High esterase activity was found in the liver and blood plasma of a range of mammalian species [18], similar data were showed in the figure 1 & 2. In our study we showed Est 2, Est-3, Est-4 & Est-5 were showed in the figure in one & five days chicken. Cousin *et al* showed maximum number of bands were observed in liver, stomach and intestine of fish species.

But the numbers bands decrease in day ten chickens and also in thirty days fowl showed in figure 2. With the increasing of ages esterase shows the lower activity that means decreasing the number of bands, Luis [19] reported that isozymes have been used widely as tools to probe the mechanistic basis of evolutionary adaptation.

**CONCLUSION**

Electrophoretic techniques were employed to investigate the variation of esterase banding pattern in the different tissues of farm fowl. Changes are found in the number and intensity of bands during their growth stage. Five types esterase bands revealed in this attempt which were designated Est-1, Est-2, Est-3, Est-4 and Est-5 based on their decreasing relative mobility and Rm values of these bands were 1, .56, .35, .2 and .07 respectively. It is thought that each band is represented by a single allele. The least staining intensity of a band indicates the lowest activity of that enzyme. The liver tissue of chicken aged 1 to 30 days expressed Est-1 but the other tissues did not show same number of bands with the increasing ages indicating the lower and higher activity of the esterase enzymes.

**REFERENCES**


