Genetic control of urea resistant esterase of liver extracts in chicken

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Summary

Starch gel electrophoresis according to Okada & Sasaki (1970) revealed six regions of esterase activity designated I to VI. Further genetic variation was found in esterase region III in this study. Two phenotypes, A and O, were observed by means of urea denaturation of chicken liver extracts. These were genetically controlled by an autosomal locus, designated as Es-9, with a completely dominant (Es-9^d) and a completely recessive (Es-9^o) alleles.

Es-9^d was the most frequent allele in White Plymouth Rock, New Hampshire and Australorp strains and rare in White Leghorn strains.

Introduction

Numerous esterase isozymes in chicken have already been reported by various workers. For example, two genetically controlled systems were found in blood serum: Es-1 (Csuka & Petrovský, 1968; Grunder, 1968; Kimura 1969a) and Es-2 (Kimura, 1969b), and another six genetically controlled systems were found in liver extract: Es-3 (Okada & Sasaki, 1970), Es-4 and Es-6 (Tanabe & Ise, 1972), Es-5 (Okada, 1973), and Es-7 and Es-8 (Asanoma & Tanabe, 1973).

This paper presents evidence for an additional genetically controlled system in chicken liver esterase isozyme, viz Es-9, and reports on a preliminary investigation on the difference of the phenotypic frequencies of the Es-9 locus among five strains.

Materials and methods

The five strains of chicken used in this study were obtained from the Experimental Farm of Hokkaido University. These were White Leghorns I, White Leghorns II (developed by the National Institute of Animal Industry, Chiba), White Plymouth
Rocks, New Hampshires, and Australorps (developed by the Takikawa Animal Husbandry Experiment Station). The chickens used for the analysis of inheritance were a triple-cross bred from White Leghorns I, White Plymouth Rocks, and New Hampshires.

In order to detect new variants of esterase, urea was incorporated into the chicken liver extracts (obtained by the methods of Okada & Hachinohe, 1968) so as to give a final concentration of 6 M. The samples in the presence of 6 M urea were left overnight at 4°C in a refrigerator before electrophoresis. Tris-borate continuous buffer system (pH 7.4) and staining techniques of esterase forms were carried out by the methods of Okada & Sasaki (1970).

To classify urea-resistant esterase, inhibition tests were carried out with various inhibitors, namely ethylenediaminetetraacetic acid (EDTA), eserine sulphate, acetazolamide, methyl parathion, and dimethyldichlorovinyl phosphate (DDVP). These were used in concentrations of 10⁻⁴ M according to the procedure of Okada & Sasaki (1970). Furthermore, in this experiment 1 M 2-mercaptoethanol was also used as the inhibitor. This was added to the samples together with urea prior to electrophoresis.
Table 1. Distribution of Es-9 phenotypes in chicken liver extracts.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype (presumed)</th>
<th>Number of matings</th>
<th>Number of progeny</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>phenotype</td>
<td>phenotype</td>
<td></td>
</tr>
<tr>
<td>A × A</td>
<td>AA × AA or AO</td>
<td>17</td>
<td>72</td>
<td>72 0</td>
</tr>
<tr>
<td></td>
<td>AO × AO</td>
<td>4</td>
<td>24</td>
<td>17 7</td>
</tr>
<tr>
<td>A × O</td>
<td>AA × OO</td>
<td>5</td>
<td>16</td>
<td>16 0</td>
</tr>
<tr>
<td></td>
<td>AO × OO</td>
<td>15</td>
<td>108</td>
<td>50 58</td>
</tr>
<tr>
<td>O × O</td>
<td>OO × OO</td>
<td>30</td>
<td>120</td>
<td>0 120</td>
</tr>
</tbody>
</table>

Results

Inheritance of urea resistant esterase

A complete reference to all combinations of mating are summarized in Table 1. The data confirms the theory of inheritance that are controlled by a single locus with a completely dominant active allele and a completely recessive null allele. According to the order of findings of the locus, we designated it as Es-9 with Es-9^A and Es-9^O, respectively. These phenotypes are shown in Fig. 1.

Characterization of urea-resistant esterase

The results of tests with various inhibitors are given in Table 2. The data indicated

Table 2. The effect of various inhibitors on esterase in region III.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Activity of urea-resistant esterase in region III¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-naphthyl acetate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>a-naphthyl acetate</td>
<td>EDTA</td>
<td>+</td>
</tr>
<tr>
<td>a-naphthyl acetate</td>
<td>Eserine sulphate</td>
<td>+</td>
</tr>
<tr>
<td>a-naphthyl acetate</td>
<td>Acetazolamide</td>
<td>+</td>
</tr>
<tr>
<td>a-naphthyl acetate</td>
<td>Methyl parathion</td>
<td>—</td>
</tr>
<tr>
<td>a-naphthyl acetate</td>
<td>DDVP</td>
<td>—</td>
</tr>
<tr>
<td>a-naphthyl propionate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>a-naphthyl butyrate</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Naphthol AS acetate</td>
<td></td>
<td>±</td>
</tr>
</tbody>
</table>

* + presence of high activity; ± presence of low activity; — absence of activity.

that urea resistant esterase in region III may be aliesterase (Augustinsson, 1961). The esterase in region III was presented as multiple characteristic esterase by Okada & Sasaki (1970). Chicken liver extracts in the presence of high concentrations of urea appeared only as simple characteristic esterase in region III, since they were completely inhibited by methyl parathion and DDVP. Eserine sulphate, EDTA and acetazolamide did not inhibit urea-resistant esterase in region III. 1 M 2-mercaptoethanol also completely inhibited urea-resistant esterase, but when it was merely added to the samples without 6 M urea, no inhibited esterases were seen in any of the regions.

**Gene frequencies**
The phenotypic frequencies of five strains are given in Table 3. The phenotype A was the most frequent type in New Hampshire and Australorp strains. White Plymouth Rock strain possessed A type in all individuals. Each strain of White Leghorn I and II was derived from different sources possessed high frequency of O type.

**Discussion**
Genetic variation of isozymes found by means of urea denaturation has not been reported with the exception of milk proteins presented by Wake & Baldwin (1961).

In this study, high concentrations of urea appeared to the difference of inhibition in esterase region III of liver in chicken among the individual fowls. The same results showing different activities in esterase region III in starch gel electrophoresis of samples previously treated with urea were obtained by using 6 M urea starch gel. After treatment of the samples with 6 M urea and 1 M 2-mercaptoethanol prior to electrophoresis, the activities of urea-resistant esterase were completely inhibited. These results show that the different activity of esterase in the presence of urea may be due to the different structure of enzyme molecules. High concentrations of urea and 2-mercaptoethanol are known to be reagents for the denaturation of
protein structure concerned with hydrogen bonds and disulphide bonds, respectively. Therefore, the genetic system of Es-9 locus may be controlled by structural genes.

Runnegar et al. (1969) reported that high concentrations of urea give rise to dissociation of enzyme molecules into two subunits in ox liver esterase. Also the increasing of molarity of urea concentration gradually inhibiting the enzyme activities was reported in human kidney alkaline phosphatase by Butterworth & Moss (1966), and in human placental alkaline phosphatase by Bahr & Wilkinson (1967), and Beratis & Hirschhorn (1972). However, as results from the present investigation, genetic difference was clearly seen in the urea inhibition of enzymes.

In Australorp strain some individuals showed a slightly different mobility of urea resistant esterase activity in region III. This result may indicate that another active allele is present at Es-9 locus, although family data were not available in Australorps.

A phenomenon similar to milk proteins was also observed in the regions VI (Es-3) and I (Es-5) of chicken liver esterase isozymes, that is to say, the diffusion and tailing of esterase zones disappeared from the electrozymogram, when low concentrations of urea were added to the starch gel or samples (unpublished).

These techniques utilizing the character of urea may reveal more polymorphic isozymes, especially in multiple characteristic isozymes of some animals.

References


