GENETIC VARIATION IN LIVER ACID PHOSPHATASES OF CHICKENS

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Studies for genetic variation of proteins were much stimulated by recent improvements in zone electrophoresis, such as starch gel electrophoresis (Smithies 1955). Furthermore, the combination of zone electrophoresis with appropriate histochemical staining methods has made it possible to study electrophoretic variants in various enzymes. Within the past few years, a number of enzyme variants, designated isozymes by Markert and Möller (1959), have been found in various organisms.

Genetic variants in acid phosphatases were reported in man (Hopkinson, Spencer and Harris 1964; Karp and Sutton 1967), silkworms (Yoshitake and Akiyama 1964), house flies (Ogita and Kasai 1935), Drosophila (MacIntyre 1966), and Tetrahymena (Allen, Misch and Morrison 1963). In chickens, Tanabe and Tamaki (1966) detected four zones of liver acid phosphatase activities by agar gel electrophoresis, and found that each phosphatase band was genetically dominant to absence of the band.

The present paper reports another new genetic variation in liver acid phosphatases of chickens.

MATERIALS AND METHODS

Chickens used in the studies were from two populations maintained at the Experimental Farm of Hokkaido University. One population was a White Leghorn strain which has been closed since it was introduced to this farm in 1957 from the Takikawa Livestock Breeding Station, Takikawa, Hokkaido. The other population was a composite of White Rocks × New Hampshires.

Liver, kidney and spleen extracts were prepared by grinding tissues in a glass homogenizer. A volume of deionized water equal to the weight of tissue was added before homogenation. The homogenate was frozen overnight at −20°C and at the following day it was centrifuged at 10,000 × g for 20 minutes at 2°C. The supernatant was stored at −20°C when it was not in use.

Horizontal starch gel electrophoresis was carried out, using a continuous buffer system. In the series of experiments, boric acid-Tris buffers in the pH range 6.7 to 8.3 were used for the bridge solution, and for the gel preparation the bridge buffer was diluted to the concentration of one eighth (the pH range was 7.5 to 8.5). For routine assay of acid phosphatases, a 0.3 M boric acid-Tris buffer containing 0.285 M boric acid and 0.015 M Tris(hydroxymethyl) aminomethane (pH 6.8) was used for the bridge solution, and the diluted buffer (0.038 M) for the gel (pH 7.6). Hydrolyzed potato starch
prepared by Connaught Laboratories was used at a concentration (12%) somewhat greater than that recommended by the manufacturer.

The extract, absorbed onto a small piece of filter paper (about 8 x 6 mm), was inserted in a slit in the starch gel. Electrophoresis was performed at a voltage gradient of 13-14 v/cm at the temperature of 7°C to 10°C. Electrophoresis was continued until the leading band of a sample dyed by Amido Black 10B, inserted as a marker, had migrated 8 cm or longer from the point of insertion. The total time of electrophoresis was approximately 6 hours. After the electrophoresis the gel was sliced horizontally into halves and stained for acid phosphatase, using the method described by Lawrence, Melnick and Weimer (1960). Fast Garnet GBC salt was used as a dye coupler. After staining, the gel was fixed in a mixture of ethanol, glacial acetic acid and water (5:1:5).

RESULTS

Description of zymograms

Liver acid phosphatases were, in a series of experiments, separated electrophoretically using boric acid-Tris buffers of different pH. The electrophoretic patterns of the acid phosphatase showed two distinct regions of activities which were designated as I and

![zymogram image]

Fig. 1. Liver acid phosphatase patterns obtained by electrophoresis of extracts in starch gel of pH 8.0. Acid phosphatase activity was detected in two regions designated I and II.
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II, in order of their proximity to the anodic end of the gel. A typical zymogram, subjected to electrophoresis in the gel of pH 8.0, is shown in Figure 1. Region I showed a single band with weak activity and was detected when the gel was incubated for about two hours at 37°C. Furthermore, when electrophoresis was carried out at a pH lower than 8.0, it was sometimes difficult to detect the band in Region I.

On the other hand, strong activity was found in Region II. The band appeared within 10 or 15 minutes of incubation at 37°C. When the samples were subjected to electrophoresis at a pH higher than 8.0, all of them also showed a single band in Region II. However, electrophoresis at a pH lower than that revealed variation between samples. Three distinctive acid phosphatase phenotypes were found in Region II, and the best resolution of the phenotypes was obtained when they were subjected to electrophoresis at pH 7.6 (the pH of the bridge solution was 6.8).

These three phenotypes, named A, B and AB, are shown in Figure 2. Phenotypes A and B are characterized by very intense single band patterns, fast and slow moving, respectively. The third pattern, phenotype AB, consists of somewhat paler fast and slow moving bands together with a heavier band midway between them. However, these three bands in phenotype AB could be separated only when they were run for longer than routine hours (the leading band migrated about 12 cm). In the routine assay (leading band 8 cm), it was observed as one wide band covering both A and B zones.

The same phenotypes of acid phosphatase were also found in the kidney and spleen, suggesting that the acid phosphatases in these organs are controlled by the same allele.

**Inheritance of the acid phosphatases**

The distribution of the three phenotypes A, B and AB in 283 offspring from the

![Fig. 2. Zymogram of the three phenotypic patterns of the acid phosphatase found in Region II. Starch gel prepared in a 0.038 M boric acid-Tris buffer, pH 7.6. A+B is a mixture of A and B types.]
nine possible kinds of matings is shown in Table 1. The results are in agreement with expectation based on a single pair of codominant autosomal alleles. The gene symbols \( Acp-2^a \) and \( Acp-2^b \) are proposed, since another gene-enzyme system may exist in Region I. One allele, \( Acp-2^a \), controls the synthesis of the fast moving acid phosphatase variant, and the other, \( Acp-2^b \), produces the slow moving variant. Heterozygotes \((A/B)\) produce a hybrid enzyme in addition to the fast and slow enzymes.

The presence of the hybrid enzyme in heterozygotes suggests that the molecular form of the acid phosphatase is at least a dimer. The general appearance of the heterozygote zymogram also agrees with the assumption that each allele forms a polypeptide subunit of the enzyme and the subunits are combined in random pairs. Specifically, in heterozygotes they appear to exist in a 1:2:1 ratio of fast : hybrid : slow enzymes. Sometimes, it has been reported that in vitro formation of hybrid enzymes was possible by mixing two forms of isozymes and freezing the mixture overnight in the presence of 1 M NaCl (Markert 1963; Hubby and Narise 1967). However, such treatment on the acid phosphatase was capable of forming no detectable hybrid enzyme.

**DISCUSSION**

In electrophoretically detected gene-enzyme systems in higher organisms, the types of inheritance fall into three classes. The first is codominance with hybrid band(s) such as red cell acid phosphatase in man (Hopkinson, Spencer and Harris 1960). The second is codominance without hybrid band(s) as in the case of esterase-6 in *Drosophila* (Wright 1963). The third type is complete or incomplete dominance. Examples are alkaline phosphatase in chickens (Law and Munro 1965) and esterase-D in mice (Oki et al. 1966).

The acid phosphatase reported here belongs to the first type. Tanabe and Tamaki (1966), however, reported another type (the third type) of inheritance of liver acid phosphatase of chickens by agar gel electrophoresis. The variation was characterized by the presence or absence of bands. The presence of a band was controlled by an incomplete dominant gene, showing reduced intensity of the band in heterozygotes and no band in recessive homozygotes. In the present experiments, chickens having no activity in Region II have not yet been found. Furthermore, in a preliminary analysis in

<table>
<thead>
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<th>Matings</th>
<th>No. of mating</th>
<th>No. of progeny</th>
<th>Distribution of phenotypes of progeny</th>
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<tr>
<td></td>
<td>Male Female</td>
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this laboratory, the agar gel electrophoresis was not efficient in detecting these individual differences in this region (unpublished data). Therefore, it seemed that Tanabe and Tamaki's population might have gene(s) differing from the alleles presented here.

Another point of interest is the distribution of progeny shown in Table 1. The observed numbers of heterozygotes were a little higher than the expected under the hypothesis, though the differences were not significant. It occurred in all of the matings producing both heterozygotes and homozygotes. This suggests that this polymorphism may be maintained by the superiority of heterozygotes. The work to test this assumption is under way.

SUMMARY

Two regions of acid phosphatase activity were resolved from liver extracts of chickens by starch gel electrophoresis. With respect to Region II, three phenotypes have been observed. These phenotypes were shown to be controlled by a pair of autosomal codominant alleles; $Acp-2^A$ producing a fast moving band and $Acp-2^B$ producing a slow band. Heterozygotes have, in addition to the fast and slow enzymes, a hybrid enzyme of intermediate electrophoretic mobility.

LITERATURE CITED


