Molecular Heterosis for Heat-Sensitive Enzyme Alleles

(Drosophila pseudoobscura/electrophoretic alleles/classical and balance hypotheses)

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ABSTRACT Heat denaturation studies were carried out and revealed hidden genetic variants with the same net charge at the Octanol dehydrogenase-1 locus in Drosophila pseudoobscura. Studies of several genetic crosses between strains with different heat-sensitivity alleles showed that the F1 retained more in vitro enzyme activity after being heat-treated for a specified amount of time at a given temperature than the heat-resistant parent. We call this phenomenon “heterosis for heat-stability of enzyme activity” and discuss its possible molecular mechanism, its relation to maintenance of genetic variation in natural populations, and its bearing on the “classical” and “balance” hypotheses.

It has been suggested that electrophoretic techniques reveal only part of the genetic variation at polymorphic loci (1, 2) and, thus, an electrophoretic allele may consist of several genetic variants of the same net charge (3, 4). Recently, Bernstein, Throckmorton, and Hubby (5) reported different heat-sensitivity alleles in each electrophoretic class at the Xanthine dehydrogenase locus in 11 species of the virilis group of the genus Drosophila. In our recent survey for such hidden genetic variation through heat-denaturation studies, we have discovered several different heat-sensitivity alleles at the Octanol dehydrogenase-1 (Odh-1) locus in Drosophila pseudoobscura. (Octanol dehydrogenase is EC 1.1.1.73.) These heat-sensitivity alleles share the same electrophoretic mobility. During the course of a genetic study of these alleles at the Odh-1 locus, we have observed results which bear directly on the two major hypotheses proposed to account for causal mechanisms of heterosis. Under the classical hypothesis, different chromosomes carry deleterious alleles at different loci and when two such chromosomes are brought together in a heterozygous condition, the deleterious genes on one chromosome fail to express themselves because of dominant genes on the other chromosome, and thus the heterozygote shows a higher fitness than either homozygote. On the other hand, the balance hypothesis states that heterosis is an intrinsic property of the individual heterozygous loci themselves (6).

In this report, we show that, when different lines homozygous for different heat-sensitivity alleles at the Odh-1 locus in D. pseudoobscura are crossed, the individuals from the F1 generation show more heat-stability for this enzyme than either parent. This finding supports the causal mechanism of heterosis as expected under the balance hypothesis.

MATERIALS AND METHODS

In D. pseudoobscura a total of five electrophoretic alleles are revealed at the Odh-1 locus, and one of them, Odh-1<sup>1</sup>-<sup>B</sup>, is the most common allele in all populations surveyed (7).

From a preliminary study of a number of lines homozygous for this allele, we categorized several of them as having different heat-sensitivities. For this study, we chose two strains, to be called R<sub>1</sub> and R<sub>2</sub>, to represent an allele which retains much of its activity after it has been heat-treated for 5 min at 60°, and three strains, to be called S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub>, to represent another allele which retains very little or no activity at all after it has been heat-treated for 5 min at 60°. In the following, these two types of alleles will be referred to as “resistant” and “sensitive,” respectively.

A total of eight crosses, including some duplicates and reciprocals, were made among these five lines using a single male and female for each cross (Table 1). Three of these crosses were between the strains which had been classified as having the “same” heat-sensitivity allele. These were to be used as controls for genetic studies. Electrophoresis was done using the method of Prakash et al. (8) with the modifications that 6% acrylamide gel was used instead of 5% and 1 ml of octanol instead of 0.5 ml was used for staining due to the larger size of the gel (old gel size: 15 × 12 cm; new gel size: 22 × 18 cm).

For heat treatment, we used a constant temperature bath (Chicago Surgical and Electrical Co.) filled with water. However, less fluctuation in temperature can be obtained by using light petroleum oil rather than water. The temperatures used for treatment were 55°, 58°, and 60°, with an error of approximately ±1/4°. These temperatures were chosen from previous empirical studies with these strains.

For preparation of the samples, we began by grinding individual flies for each treatment, but due to body size differences it was difficult to obtain uniform results. We then pooled several flies and, after grinding and centrifuging, we distributed the supernatant equally among the treatments. For this study, six flies were ground together in 0.09 ml of 0.1 M Tris–borate buffer, pH 8.9, containing 15 mM ethylene-diaminetetraacetate and the resulting suspension was centrifuged in a 0.5-ml centrifuge tube at 21,500 × g for 3 min. Ten-microliter aliquots of the supernatant were placed into each of four 0.1-ml centrifuge tubes. Of these four tubes, one was kept at 0° (control) and, of the remaining three, one was heat-treated for 5 min, one for 10 min, and one for 15 min, at 55°. After being subjected to heat-treatment for the specified length of time, the tubes were quickly returned to the ice bath. Then the extracts were placed into the pockets in the gel and electrophoresed. The same procedure was followed for the heat-treatment at 58° and 60°. At each temperature similar samples were electrophoresed at least twice to confirm the results.
RESULTS

Fig. 1 illustrates the effect of heat-treatment of 58° on parents and their F1's from two crosses. There is no difference between the enzyme activity of the parents and their F1's in untreated (control) samples. However, in the heat-treated samples, the F1 retains more enzyme activity and thus shows a relatively higher optical density than the heat-resistant parent. We call this phenomenon “heterosis for heat-stability of enzyme activity.”

The cross R1 (♂) × S1 (♀) shows heterosis at all three treatments (5 min, 10 min, and 15 min), while cross S2 (♂) × R1 (♀) shows heterosis only at two treatments (5 min and 10 min).

The first cross shows a stronger difference between parents and F1's than the second cross. This difference is quite clear in the 10-min treatment. Fig. 1 also shows bands for xanthine dehydrogenase and tetrazolium oxidase. Since the two parents in both crosses are homozygous for two different electrophoretic alleles at the Xdh locus, the F1, being heterozygous, possesses a dark wide band and thus confounds the effect, if there is any, of heterosis in the F1. Also, the two parents in both crosses appear to be similar in their heat-sensitivity for this enzyme at this temperature. However, the tetrazolium oxidase does show heterosis in the F1's of both crosses in the 5-min treatment and in the F1 of only the first cross in the 10-min treatment. It appears that at this locus parents of these two crosses were homozygous for alleles sharing the same electrophoretic mobility, but the alleles were incidently different in their heat-sensitivity and show heterosis when brought together in the F1.

Table 1 shows the octanol dehydrogenase heat-sensitivity of the F1's as compared to that of the heat-resistant parent in each cross. The F1's from all eight crosses were studied at 58° and seven of them were also studied at 60°. Since the 55° temperature did not reveal any differences between the two parents, only a few F1's were tested at this temperature and the results are not reported here. In the 60° temperature treatment, only in a few cases was there activity left after 5 min. At 58°, all except two crosses show heterosis at 5-min treatment and all except one cross in the 10-min treatment. Two crosses show heterosis also after 15 min of treatment, although in this treatment complete inactivation of the enzyme occurred in most of the cases.

In Table 1 some crosses show stronger heterosis in the 5-min treatment and some in the 10-min treatment. What we mean by stronger heterosis in one treatment than in the other can be illustrated by Fig. 1. The difference between the parents and their F1 is larger in both crosses in the 10-min treatment than in the 5-min treatment. Thus, for these two crosses, the heterosis in the 5-min treatment is denoted by one plus, while in the 10-min treatment by two pluses. We can see an inconsistency in the data presented in Table 1. The duplicate crosses R1 (♂) × S1 (♀) do not behave similarly and also there is a difference between this cross and its reciprocal, S1 (♂) × R1 (♀). Similarly, there is a difference between the duplicate crosses S2 (♂) × R1 (♀). These differences between duplicates and reciprocals may be due to experimental error.

It is interesting to note in Table 1 that the F1's of the three crosses in which both parents had been classified as having the ‘‘same’’ heat-sensitivity allele nevertheless show heterosis. This means that even heat-denaturation is not revealing all the hidden genic variants at Odh-1 locus.
DISCUSSION

For a study designed to examine if the heterozygous state at a given locus provides the individuals with some property in quality or quantity not possessed by individuals which are homozygous for this locus, three requirements must be satisfied: (1) the genic variants to be examined should be true alleles at a given locus, (2) the gene products of this locus or some other property of the organism solely determined by this locus should be examined in vitro and in vivo in heterozygous and homozygous individuals at this locus, and (3) the differences, if any, between the homozygotes and heterozygotes observed in (2) should be related to the functional differences between these genotypes which affect their fitnesses.

In our study the first two requirements are fully satisfied and the third one, we think, is at least partly satisfied. As stated above, although it was difficult to obtain uniform results with individual flies due to differences in their body size, nevertheless the individual flies were assayed from several F1's and their respective F2 populations. With extra precaution it was possible to get uniform results in F1. The details of the genetic crosses will not be published here, but it should suffice to say that the two heat-sensitivity alleles in this study were recovered in F1 populations and thus behaved in a Mendelian fashion. Since we are examining the gene product (protein) directly, the second requirement is satisfied. The third requirement is satisfied to the extent that we are examining the property of these protein molecules with respect to temperature, which is an important component of all organisms' environment. However, we do not know what relation these high temperatures used in our study have to the physiological functioning of the organism. In a recent study, Pandey (9) has shown a correlation between the heat-sensitivity of certain esterase isozymes at 50° and the degree of self-compatibility in six species of Lilium longiflorum. It may well be that the heterosis observed in our study is related to the physiology of the individuals and it will be interesting to examine if this heterosis can be reproduced by heat treatments for a relatively longer time but at lower, physiological, temperatures, thus meeting the third requirement.

What is the explanation for this heterosis? Appearance of a third band at an intermediate position in individuals heterozygous for two different electrophoretic alleles at Odh-1 locus suggests that this enzyme is at least a dimer. Thus, the individuals which are heterozygous for different heat-sensitivity alleles must also be forming dimers. If the dimerization is at random, we expect half of the protein molecules from this locus to be the heteromer. Since enzymes in this study have the same electrophoretic mobility, we cannot visually differentiate between these different dimer molecules on the gel as we can in case when an individual is heterozygous for two electrophoretically different forms of the enzymes. If the heat-sensitivity of the heteromer is intermediate between those of the two homomers, we expect to find in F1 a band of intermediate intensity between the two parents. Since this did not occur, the simple hypothesis which can explain the observed results is that the heteromer is more heat-resistant than either of the homomers. What could cause this? Formation of additional quaternary bonds may be the answer. What kind of bonds we do not know; hydrogen bonds, ionic bonds or van der Waals forces are, of course, the most likely. In any event, if the monomers produced by each allele allowed in the heteromer the formation of one or more such quaternary bond, then the heteromer would be more heat stable.

It is expected that in vitro biochemical study of enzyme molecules, as attempted in this study, will help our understanding of the functional basis of the differences among the genic variants at a given locus, and, possibly, of their maintenance in natural populations.

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