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Further Evidence of Thermostability Variation Within Electrophoretic Mobility Classes of Enzymes

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Two Drosophila melanogaster *strains, each heterozygous for "fast" and "slow" alleles at the* Adh *locus, and each having balanced second chromosomes, were found to differ in the apparent thermostability of the slow allozyme. The two strains were crossed, and F_1 heterozygotes were separated on the basis of the origin of the slow allele. After electrophoresis, the cellulose acetate strips were treated $1\frac{1}{2}$ min at 35 C. The putatively more sensitive allozyme showed a strikingly greater response to heat. These findings further support the conclusion that electrophoretically cryptic allelic differences exist which are expressed in thermostability differences. Further application of this approach has revealed one similar sensitive slow allozyme and three cases of a relatively resistant fast ADH allozyme in wild-caught flies.*

KEY WORDS: allozymes; thermostability; alcohol dehydrogenase; *Drosophila melanogaster.*

INTRODUCTION

Following the demonstration of abundant genic polymorphism through the use of the electrophoretic analysis of enzymes (reviewed by Lewontin, 1973), the further resolution of allozymic variation was attempted by means of comparisons of thermostability. Evidence of thermostability variation was quickly obtained (Bernstein *et al.*, 1973; Singh *et al.*, 1974), although thorough genetic analysis was not immediately performed. More recently, Singh *et al.* (1975) reported findings which strengthen the conclusion that the observed differences in thermostability stem from hitherto undetected allelic variation at the same loci previously implicated in electrophoretic poly-

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morphism. Some doubt remained, however, on two grounds, among others:

1. High-resolution genetic analysis had still not been performed.
2. Heat inactivation studies were performed without purification of the enzymes. Thus a variety of small molecules with effects on thermostability might have been present in concentrations characteristic of the genotype at a large number of loci.

I therefore undertook to treat alcohol dehydrogenase after electrophoresis, which represents considerable purification. The results indicate clear-cut differences between "slow" allozymes of different origins.

MATERIALS AND METHODS

Various stocks of *Drosophila melanogaster* with marked, balanced second chromosomes were examined electrophoretically. Four stocks were heterozygous at the *Adh* locus: *Cy/Pm D/Sb*, 241, 303, and 711, the numbers being those of the Caltech stock list.

Each of these stocks carries *Cy* (curly wings) and Adh^F together on one second chromosome. Stock 771 carries S^2 and *E*(*S*) on the *Cy* chromosome; these alleles are associated with small, rough eyes. On the other second chromosome, Adh^S is associated with *Bl* (short bristles) in stock 241. Thus the offspring of crosses between 241 and 771 carry three phenotypes: curly wings, small rough eyes, and short bristles (Adh^S from 241); curly wings only (Adh^S from 771); and short bristles only (Adh^S from both parents). The last-named flies were used in other experiments.

Flies from these stocks were treated in teabags in a water bath at 45 C and then electrophoresed. Strain 771 appeared to have a particularly sensitive slow band. Of the other three, strain 241 seemed to have the most resistant slow band, although it was not certain that the three strains really differed. Strains 241 and 771 were crossed, so that the F_1 overall genotypes were largely mixed, but the markers permitted the distinction of flies as to the origin of their *Adh* alleles, as previously noted. Two classes of "fast"/"slow" heterozygotes were collected: those with "slow" alleles contributed by the 241 parent and those with "slow" alleles from the 771 parent. The fast alleles came from the other parent, of course. These flies were then subjected to electrophoresis on cellulose acetate for 45 min at 210 V in a Gelman system using the Adamkewicz multiple applicator. After electrophoresis, half of the strips were stained immediately, and the others were first placed in a Saran envelope and treated in a water bath at 35 C for $1\frac{1}{2}$ min. (The enzymes are, not surprisingly, more sensitive to heat under these abnormal conditions. Thus the most informative treatment utilized a lower temperature than in the case of intact flies.)

Flies from the original four strains were subsequently examined in the same way.

RESULTS

While the fast allozymes' thermostabilities were apparently all alike, the slow allozymes clearly differed from one another. Those from the sensitive parent were inactivated to a far greater extent than those from the resistant parent. Table I summarizes the observations. While the "resistant" allozyme's average activity in untreated samples was somewhat greater than that of the "sensitive" allozyme, the overlap in individual samples was very great, so that nonlinearity of staining intensity vs. activity cannot account for these results. Specifically, numerous cases were observed where the untreated sensitive bands were darker than nearby resistant bands, while

Table I. Frequencies of Staining Intensities of Slow ADH

	Untreated		Treated	
Rating[a]	S[b]	T[b]	S	T
0	2	1	170	1
1	93	35	23	71
2	106	129	0	110
3	12	51	0	15
4	1	0	0	0
Average	1.61	2.06	0.12	1.71
N	214	216	193	197
	Treated/untreated ratio		0.07	0.83

[a] 0, Absent; 4, darkest.
[b] S, *Adh*^{s} from 771; T, *Adh*^{s} from 241. See text.

Table II. Staining Intensities of Slow ADH Bands in Four Strains

	Average intensity rating		Treated/untreated ratio
Strain	Untreated[a]	Treated[b]	
771	1.46	0.05	0.03
241	2.44	2.09	0.86
303	2.27	1.91	0.84
Cy/Pm D/Sb	1.71	1.49	0.87

[a] *N* for each strain = 48.
[b] *N* for each strain = 43.

only the sensitive bands disappeared after treatment. Strains 241, 303, and *Cy/Pm D/Sb* do appear similar, as shown in Table II, while 771 shows sensitivity comparable to that of the 241/771 hybrid with the slow allele from 771.

DISCUSSION

Schwartz *et al.* (1974) have concluded that "multiple electrophoretic species are formed" from *Drosophila* alcohol dehydrogenase "by differential binding of a heat-stable substance probably containing nicotinamide." In the course of various experiments, I have observed that a faster band associated with the regular Adh^F band is much more thermostable than the regular band. While small molecules are unquestionably capable of altering both thermostability and electrophoretic mobility in a protein, how might they account for polymorphism in thermostability? When the evidence of polymorphism is the distinction among inbred lines, or differences among F_2 flies, then its genetic basis *might* lie in the control of the nature and quantity of certain small molecules, rather than the primary structure of the enzyme. The present technique makes primary structure differences a more likely explanation, although not certain.

Bonnie Sampsell and I are now surveying laboratory and newly established strains by a similar method. Individual males are crossed to a balanced marker stock, and individual F_1 males are backcrossed to the marker stock. This method has revealed, among 176 wild lines, one sensitive slow ADH allozyme similar to the one described and three cases of a relatively resistant fast ADH allozyme. The latter may be similar to that localized and reported by Thörig *et al* (1975); we, too, have localized the basis of the thermostability difference. After the analysis of 206 flies, including 104 recombinants for a 9-unit region of the second chromosome, we conclude that the indicated position (50.75) implicates the *Adh* locus. No *α-Gdh* thermostability variants have been detected in 136 lines.

We have only recently learned of the pioneering work of Wright and MacIntyre (1965) on the post-electrophoretic treatment of Esterase-6 allozymes and the tentative localization of an electrophoretically indetectible thermostability variant.

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