STRUCTURAL-ANALYSIS OF ADHS ELECTROMORPH OF DROSOPHILA-MELANOGASTER

https://escholarship.org/uc/item/70n5p3hx

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 75(11)

0027-8424

FLETCHER, TS
AYALA, FJ
THATCHER, DR
et al.

1978

10.1073/pnas.75.11.5609

CC BY 4.0

Peer reviewed
Structural analysis of the ADH$^6$ electromorph of Drosophila melanogaster

(genetic variation/evolution/polymorphism/adaptation)

THOMAS S. FLETCHER*, FRANCISCO J. AYALA*, DAVID R. THATCHER†, AND GEOFFREY K. CHAMBERS*

* Department of Genetics, University of California, Davis, California 95616; and † Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland

Communicated by R. W. Allard, August 14, 1978

ABSTRACT Population geneticists have often determined the fitness differences that account for the dynamics of naturally occurring genetic polymorphisms. However, to understand causal aspects of evolutionary processes requires, in addition, investigation of the physiological and molecular structural differences underlying adaptively significant genetic polymorphisms. The characteristics of the alcohol dehydrogenase gene–enzyme system in Drosophila melanogaster make it well suited for this kind of study. Natural populations of this species are polymorphic for two electrophoretically detectable variants, ADH$^F$ and ADH$^S$, of the enzyme. Structural studies reported here reveal that the two variants differ by (at least) a single amino acid replacement, threonine in ADH$^F$ for lysine in ADH$^S$.

Genetic polymorphisms are of great interest to evolutionists because they permit investigation of the adaptive basis and of the dynamics of the processes of genetic change underlying adaptive evolution. At a first level of investigation, population geneticists are concerned with ascertaining whether or not alternative genotypes differ in fitness; fitness differences may lead to the evolutionary replacement of one allele for another but may also lead to equilibrium polymorphic situations with two or more alleles maintained at certain frequencies. The causal study of genetic evolution requires, moreover, investigation of the functional (physiological) and biochemical (molecular) bases of fitness differences. It is remarkable that more than a century after Darwin (and more than four decades after the birth of experimental population genetics), we know the physiological and biochemical bases of very few polymorphisms. The sickle-cell polymorphism in regions where malaria is rife represents the most familiar example. An urgent need exists for ascertaining the physiological and biochemical bases of the pervasive polymorphisms found in nature.

A polymorphism that has been much investigated in recent years involves the Adh locus and its gene product, alcohol dehydrogenase (ADH; alcohol:NAD$^+$ oxidoreductase, EC 1.1.1.1), in Drosophila melanogaster (1). Two electrophoretically distinguishable forms of the enzyme, one ("fast," ADH$^F$) migrating farther than the other ("slow," ADH$^S$), are found in natural populations throughout the world. A rare variant, termed "ultrafast" (ADH$^{UF}$), has been found in a Spanish population (2) and a variant (ADH$^{H}$) with the same mobility as ADH$^{UF}$ has been obtained through ethyl methanesulfonate treatment (3).

The adaptive basis of the ADH polymorphism has been extensively studied. Flies possessing the ADH$^F$ (or ADH$^{UF}$) form of the enzyme exhibit greater tolerance of environmental alcohol than do flies having the ADH$^S$ form (4–7). The physiological basis of this difference in alcohol tolerance has been inferred to be a difference in ADH activity, because ADH$^F$ flies generally exhibit higher ADH activity than do ADH$^S$ flies. This inference has been further corroborated by showing that, among flies that are all ADH$^S$, those more tolerant of environmental alcohol manifest greater ADH activity than do the less-tolerant flies. This was demonstrated in two lines fixed for ADH$^S$ and derived from the same original strain, one line having been selected for alcohol tolerance and the other, kept as a control (8). The difference in ADH activity between the selected and control lines was shown to be genetic but independent of the Adh locus. The Adh locus is located on the second chromosome of D. melanogaster, but regulatory loci modifying the levels of ADH activity exist on the third chromosome (9) as well as elsewhere in the genome (10, 11).

The biochemical properties of ADH$^F$ and ADH$^S$ have been investigated. Typically, the fast electromorph has higher specific activity but lower thermostability and isoelectric point than the slow electromorph; no differences in Michaelis constants for NAD$^+$ and ethanol are detectable (12–14). However, it may be that the most striking biochemical difference between the two electromorphs is the efficiency with which they can utilize NAD$^+$ analogs in alcohol oxidation (unpublished data).

Clearly, it is of great interest to investigate the structural basis of the different properties of the ADH$^F$ and ADH$^S$ electromorphs. The small subunit size (molecular weight, 24,000; ref. 15) of the dimeric ADH enzyme facilitates such investigation. It has been shown that the rare ADH$^{UF}$ and the ethyl methanesulfonate-induced ADH$^S$ each differ from ADH$^F$ by a single amino acid replacement (16, 17). We here report that ASH$^S$ differs from ADH$^F$ also by (at least) a single amino acid replacement.

MATERIALS AND METHODS

D. melanogaster flies were made isogenic for the whole second chromosome by crossing flies collected at MacDonald Ranch (Napa Valley, CA) with the SM5/Bl, L$^2$ balancer stock, as described (9). (The isogenic lines were kindly provided by Robert Seager.) The work reported here was performed with flies from one such isogenic line, M134, carrying the ADH$^F$ electromorph, but it deserves mentioning that three other ADH$^F$ isogenic strains produce peptide maps identical with the map of M134 (and thus may have ADH with the same primary structure as that of M134).

Larvae were raised by mass culture in a standard cornmeal/molasses/agar medium and then stored at –70°C. ADH was purified from larvae by reported methods (8) with the addition of an initial chromatography on hydroxylapatite. The purity of the protein was evaluated by means of native and sodium dodecyl sulfate/polyacrylamide gel electrophoresis (18, 19).

Abbreviations: ADH, alcohol dehydrogenase; >PhNCS, phenylthiohydantoin

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.
19). The enzyme was carboxymethylated (20), digested with 2% trypsin ( Worthington, TPCK-treated) in 100 mM ammonium bicarbonate for 6.5 hr at 37°C, and then lyophilized. Material insoluble in pyridine/ acetic acid pH 3.6 buffer (pyridine/ acetic acid/ water, 1:10:289 [vol/vol]) was removed by centrifugation.

Peptide mapping was performed according to Bennett (21). The first dimension was descending chromatography with n-butanol/ acetic acid/ water/ pyridine, 15:3:12:10 (vol/vol), and the second was electrophoresis at pH 3.6 in pyridine/ acetic acid buffer. Analytical maps (1-mg load) were stained with general and specific stains (22). The preparative map (9-mg load) was stained with fluorescamine (17); spots were then cut out of the sheet and eluted with 30% acetic acid. Aliquots for analysis were hydrolyzed with 6 M HCl in vacuo for 24 hr at 110°C. Amino acid analyses were done with a Durrum model D-500 amino acid analyzer.

The replacement peptide was sequenced on a Beckman model 890C sequencer using the DMAla Peptide Program (Beckman no. 102974). Acetylated cytochrome c (2.2 mg) was added to minimize extraction losses (23). Phenylthiohydantoin (>PhNCs) derivatives of amino acids were identified in three ways: by two-dimensional thin-layer chromatography (24), by gas/liquid chromatography, sometimes after trimethylsilylation (25), and by amino acid analysis after back-hydrolysis of >PhNCs derivatives, as described (26).

RESULTS

We have consistently observed that peptide maps of ADHs from isogenic lines of D. melanogaster exhibit one spot (yellow with cadmium/ acetic acid/ ninhydrin, tyrosine-negative, and Pauly positive) not present in maps of ADHs. Fig. 1 shows typical chromatograms of the tryptic peptides of ADHs (strain M26) and ADHs (strain M134). The maps are formally identical, except for peptide T20 showing in the ADHs but not in the ADHs peptide map.

The T20 peptide was eluted from a preparative map of ADHs from isogenic strain M134. Its amino acid composition (Table 1) does not correspond to any peptide isolated by Schwartz and Jornvall (17) from ADHs, ADHs, or ADHs (n1 is the product of “null” allele and exhibits virtually no ADH activity). However, the composition and sequence data of Schwartz and Jornvall amount to only about 50% of the total ADH sequence (assuming a subunit molecular weight of 24,000). The remaining 50% is insoluble in the buffers used for peptide mapping.

One of us (D.R.T., unpublished data) has isolated the insoluble tryptic peptides from ADHs and ADHs and has determined the sequence of one of the insoluble peptides from ADHs to be Thr-Thr-Leu-Val-His-Thr-Phe-Asp-Ser-Leu-Leu-Asp-Val-Glu-Pro-Gln-Val-Ala-Glu-Lys. The first six residues from the NH2 terminus of this peptide resemble the composition of T20, except that this peptide has one extra lysine and one less threonine. We therefore conjectured that the sequence of the T20 peptide is Thr-Thr-Leu-Val-His-Lys.

This conjecture was confirmed by obtaining the amino acid sequence of T20. For the results shown in Table 2, the following should be noted. The failure to identify threonine at position 2 by thin-layer chromatography can be attributed to the presence of an unidentified fluorescence-quenching impurity in the sample; we do not know why this threonine was not detected by back-hydrolysis. The presence of threonine at position 2 is, nevertheless, unambiguously established by its detection through the two gas/liquid chromatography techniques. Histidine at position 5 was established by back-hydrolysis; as expected, histidine is not detected by the other methods because its >PhNCs derivative is insoluble in the organic extraction buffers used. The low yield expected for the COOH-terminal amino acid of a peptide rarely allows its unambiguous identification; we were nevertheless able to do so with the thin-layer chromatography method.

Therefore, the sequence obtained agrees with our conjecture, confirming a substitution of lysine in ADHs for threonine in ADHs. It is possible that there may be additional substitutions that are not detected by our peptide mapping. However, we have analyzed six additional soluble tryptic peptides of ADHs from the M134 isogenic strain and found them identical in composition to the corresponding peptides from ADHs (17).

---

**Table 1.** Amino acid composition of T20 peptide present in the peptide map of ADHs but not in that of ADHs

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Ratio relative to His</th>
<th>Nearest integer of ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr</td>
<td>1.99</td>
<td>2</td>
</tr>
<tr>
<td>Val</td>
<td>1.01</td>
<td>1</td>
</tr>
<tr>
<td>Leu</td>
<td>1.10</td>
<td>1</td>
</tr>
<tr>
<td>His</td>
<td>1.00</td>
<td>1</td>
</tr>
<tr>
<td>Lys</td>
<td>1.11</td>
<td>1</td>
</tr>
</tbody>
</table>

Amino acids present in amounts less than 7% of His are not included.
DISCUSSION

The electrophoretic mobilities, charge changes, and sequence differences of the four ADH electromorphs are given in Table 3. The amino acid replacements account for the charge changes from ADHF to ADHF (threonine, neutral → lysine, positive) as well as for the other electromorph substitutions; the charge changes in turn account for the observed differences in electrophoretic mobility. The replacement of threonine by lysine in the ADHF electromorph results in the cleavage of an otherwise insoluble peptide, forming a soluble NH2-terminal portion (the T20 peptide) and a still insoluble COOH-terminal fragment and hence the appearance of one additional spot on the peptide map of ADHF compared to that of ADHF.

The number of peptides observed in our maps deserves comment. Assuming complete cleavage by trypsin, the number of peptides should be the sum of the numbers of arginine and lysine residues plus one. Our own amino acid analysis, as well as published ones (2, 15), indicates that there should be 22 peptides for ADHF; yet, we obtain 24 in our maps. A likely explanation for the extra peptides is that they are due to incomplete tryptic cleavages. This has been confirmed with respect to T13 and T18. The completely cleaved peptide is T13 [TN6 of Schwartz and Jörnvall (17)], which has the sequence Glu-Leu-Leu-Lys. T18 (TB5 of Schwartz and Jörnvall) is a corresponding incompletely cleaved peptide with the sequence Glu-Leu-Leu-Lys-Arg. The sequences obtained for ADHF by Schwartz and Jörnvall (17) for these two peptides are consistent with the results of our amino acid analysis of the same peptides from ADHF.

The more-negative ADHF electromorph exhibits higher specific activity but lower thermostability than the more-positive ADHF. We do not know why the single amino acid replacement involved has these effects, nor why it affects the activity of the enzyme with NAD+ analogs. We can only hope that the explanations will become apparent when the full amino acid sequence of the enzyme is known. Another unsolved question is why the more negatively charged, more active, and more thermolabile molecules (ADHF and ADHF') are present in the flies in greater concentrations than ADHF.

A single base substitution in the second position of the codon-mRNA, AGA→Lys—can account for the amino acid replacement between ADHF and ADHF. It is interesting to note that the other naturally occurring variant, ADHF', is also due to a second-position substitution of adenine for cytosine. Whether this intriguing correlation is a mere coincidence or has biological significance remains to be ascertained.

The development of a chemical screening technique has allowed the isolation of several null mutants at the Adh locus (28); most, perhaps all, of these nulls appear to be missense mutants within the structural gene (29). Ashburner and his collaborators (11) have mapped genetically the position of several null mutation sites, as well as that of the known electrophoretic variants, and of a single regulatory site (L) that maps outside but close to the right-hand end of the structural region of the Adh locus. Knowing the location of the ADHF replacement in the amino acid sequence will help to establish the orientation of the Adh gene with respect to other second chromosome markers and, hence, the direction of transcription of the Adh gene in the genetic map of D. melanogaster.

We thank Vivian McCarthy-Walker for raising the larvae, Al Smith for valuable help with the sequencing, and Katherine Kanagaki for the amino acid analyses. This work was supported by National Institutes of Health Grant 1 PO1 GM 22221 and by Contract PA 200-14 Mod 4 with the U.S. Department of Energy.