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EXPRESSION OF ADH SYSTEM IN *DROSOPHILA BUZZATII*

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RESUM

El locus que codifica per a l'alcohol deshidrogenasa-NAD dependent a *D. buzzatii* està duplicat. En aquest estudi es determina l'expressió d'aquest sistema al llarg del desenvolupament de l'individu.

Els dos gens estudiats, *Adh-1* i *Adh-2*, presenten un sistema de regulació que permet l'expressió d'ambdós en les fases de larva de tercer estadi i pupa. En les primeres fases del desenvolupament —larva de primer estadi— únicament s'expressa l'*Adh-1* i en l'estadi d'adult és l'*Adh-2* l'únic gen actiu.

També s'ha estudiat l'efecte del 2-propanol sobre el sistema *Adh*, així com una nova manera per a diferenciar entre un patró electroforètic de tres bandes produït per interconversió del que s'obté per formació d'heterodímer i dos homodímers, en un sistema com l'Adh que està constituït per dos gens d'estructura dimèrica. Concentracions del 2 % d'isopropanol produeixen la inactivació de l'*Adh-2* i l'activació en els adults de l'*Adh-1*. Aquests resultats s'interpreten en base a un canvi en el sistema de regulació i no com a conseqüència d'una interconversió de bandes.

RESUMEN

El locus que codifica para el alcohol deshidrogenasa-NAD dependiente en *D. buzzatii* se encuentra duplicado. En este trabajo se determina la expressión de este sistema a lo largo del desarrollo del individuo.

Los dos genes estudiados, *Adh-1* i *Adh-2*, presentan un sistema de regulación que les permite expresarse en las fases de larva de tercer estadio y de pupa. En las primeras fases del desarrollo (larva de primer estadio) únicamente se expresa el *Adh-1*, mientras que en la fase de adulto el único gen activo es *Adh-2*.

También hemos estudiado el efecto que produce el 2-propanol en el sistema *Adh* así como una nueva manera de diferenciar entre un patrón electroforético de tres bandas que se ha originado por interconversión de las mismas, del producido por formación del heterodímero y los dos homodímeros, en un sistema de dos genes de estructura dímera como es el sistema Adh. A concentraciones del 2 % de isopropanol se produce la inactivación del *Adh-2* y la activación del *Adh-1* en los adultos. Estos resultados se interpretan en base a un cambio en el sistema de regulación y no como consecuencia de una interconversión de bandas.

ABSTRACT

The locus encoding the NAD dependent alcohol dehydrogenases in *Drosophila buzzatii* is duplicated. In this study we report the expression throughout development

for this *Adh* system, the effects that environmental 2-propanol has on it, and a new way for distinguishing a three banded zimogram pattern produced by an interconversion phenomenon than another produced by the assembly of monomers encoded by different loci. Our results indicate that there is a differential expression of both loci during development, that 2-propanol produces the inactivation of one of them and the activation of the other, and that this is not the result of a *melanogaster*-like interconversion.

Key words: *Drosophila buzzatii*; alcohol dehydrogenase; developmental expression; isozymes; 2-propanol; adaptation.

INTRODUCTION

Many genes in higher organisms are subject to developmental regulation, so that their products appear at specific concentrations in different stages. Developmental patterns are of interest both in apigenetic and evolutionary studies, because regulatory changes may affect the phenotype fitness and is on phenotype differences where natural selection can act (Valentine and Campbell, 1975; Wilson, 1976; Hedrick and McDonald, 1980; MacIntyre, 1982).

Adh is the structural gene for alcohol dehydrogenase (Alcohol: NAD oxidoreductase, EC 1.1.1.1) in *Drosophila*, and its gene-enzyme system is ideally suited for both regulatory and evolutionary studies. Detailed analyses have been done on different aspects of this system in *D. melanogaster* over the past years, ranging from molecular to population level. The structural gene and adjacent regions have been cloned (Goldberg, 1980; Benyajati *et al.*, 1980) and sequenced (Kreitman, 1983), and its mRNA characterized showing that genotypes differ by some insertions, and that there are two *Adh* transcripts throughout development differing in their 5' ends and in the promotor utilized (Benyajati *et al.*, 1983). The origin of three bands in the zimogram has been also uncovered (Schwartz *et al.*, 1979) resulting from a post-translational modification. *Adh* activity, *Adh* cross reacting material (CRM) and mRNA levels in different stages have been described, together with the action of modifier loci (McDonald and Ayala, 1978; Anderson and McDonald, 1981, 1983). Effect of environmental alcohols has also been studied (Schwartz and Sofer, 1976; Schwartz *et al.*, 1979; Papel *et al.*, 1979; Anderson and McDonald, 1981) resulting in an interconversion of bands and a depression in *Adh* activity and *Adh* CRM levels. Population and ecological studies have finally showed the physiology of alcohol metabolism (David *et al.*, 1976; David *et al.*, 1978) and the correlation between molecular phenotype and survivorship in alcoholic environments (Van Delden *et al.*, 1975; Caverner and Clegg, 1978).

D. buzzatii belongs to the *buzzatii* cluster, *mulleri* complex, *mulleri* subgroup, *repleta* group of *Drosophila* (Wasserman, 1982). Its habitat is restricted to *Opuntia* rotted pads and fruits. Mulley (1975) first postulated a two loci system in order to explain the three banded zimogram and its inheritance. Dakeshott *et al.* (1982) found two recombinants between the two postulated loci among 478 individuals, thus giving a distance of 0.4 ± 0.3 cm, and Sánchez (unpublished data) found a more accurate distance of 0.3 ± 0.1 cm screening 3129 individuals. The fact that larvae and adults differ in their electrophoretic patterns, has been used to infer duplication in other species with a *buzzatii*-like zimogram (Batterham *et al.*, 1984). Fontdevila *et al.* (1980) found a genotype-isopropanol interaction at the *Adh* locus and a *melanogaster*-like interconversion of bands in *D. buzzatii*.

Here we report the complete developmental pattern of ADH in *D. buzzatii*, the effects of environmental 2-propanol, one of the most abundant alcohols in Cactaceae rotted tissues (Fontdevila, 1980), and a new way for distinguishing a three banded pattern originated by a post-translational modification from another produced by a two loci *Adh* system.

MATERIALS AND METHODS

Stocks. Fly stocks were homozygous for both *Adh* loci, and the nomenclature and origin of the five genothypes used are as follows: *Adh* 1-100; 2-96 from Mogan (Canary Islands), *Adh* 1-92; 2-100, *Adh* 1-100; 2-100 and *Adh* 1-108; 2-100 from San Luis (Argentina), and *Adh* 1-100; 2-99 from Australia. These genotypes differ by the mobility of ADH dimers in starch gel electrophoresis, so that for example in *Adh* 1-100; 2-96 ADH 2 homodimer moves 4 mm less to the cathode than in *Adh* 1-100; 2-100, and in *Adh* 1-108; 2-100 ADH 1 homodimer moves 8 mm more to the anode than in *Adh* 1-100; 2-100.

Five developmental stages were used: first instar larvae, third instar larvae, early pupae, young adult and mature adult.

Stocks were maintained in standard food (David *et al.* 1959) at 25 C. Where indicated 2 % 2-propanol by volume was added to the food.

Enzyme methods. Single individuals were ground in small volumes of double distilled water. Extracts were applied to small filter papers (Whatman # 3) and these inserted in slots made at 4 cm from the cathodal end of 12 % horizontal starch gels. Buffer system was discontinuous and consisted of 76 mM Tris, 5 mM citric acid (pH 8.6) for gels, and 300 mM boric acid, 60 mM sodium hydroxide (pH 8.1) for electrodes (Poulik, 1957). Where specified 1 mM NAD was added to the gel mixture just before pouring. Gels were poured onto refrigerate plates maintained at 4 C to prevent overheating during running. Electrophoresis was perfomed at a maximum of 100 V for 4 h. Staining mixture consisted of 50 mM Tris-HCl buffer (pH 8.6), 200 μg of nitro-blue tetrazolium per ml, 150 μg of NAD

per ml, 16 μg of phenazine methosulfate per ml, and by volume 2 % 2-propanol. Gels were stained at 37 C for 1 h, and fixed in 1:5:5 acetic acid, methanol, water (Ayala *et al.*, 1972).

RESULTS

Developmental expression. Results indicate that in all genotypes assayed there is only activity in the *Adh* 1 homodimer zone when using first instar larvae. Sometimes, an additional weak activity can be seen in the heterodimer zone between the products of the *Adh* 1 and *Adh* 2 loci, but there is never activity for the *Adh* 2 homodimer. In third instar larvae and in early pupae three zones of activity are developed, *Adh* 1 homodimer, interloci heterodimer, and *Adh* 2 homodimer. Almost the same pattern is detected in young adults, except that *Adh*1 homodimer becomes generally fainter. In mature adults an specular image of that recorded for first instar larvae is observed, that is activity for ADH 2 homodimer and sometimes weak activity for inter loci heterodimer, which disappears in older individuals.

Addition of NAD to the composition of gels has two consequences, first all the pattern displaces to the anode, and second bands have better definition and are more conspicuous, so that any subbanding phenomenon is avoided.

Comparing the behavior of *Adh* pattern in NAD containing gels for *D. buzzatii* and *D. melanogaster*, which also has a three banded zimogram but resulting of a well known post-translational modification (Schwartz *et al.*, 1979), we have observed that in the first species there is no change but the displacement to the anode just described, nevertheless in *D. melanogaster* it results in an interconversion of *Adh*-5 and *Adh*-3 bands to the more cathodic *Adh*-1, and this can be observed anytime in the development. When using heterozygotes in NAD gels, up to ten bands can be seen in *D. buzzatii*, depending on the cross, but only three bands appear in *D. melanogaster*.

Effect of environmental alcohol. Electrophoresis of individuals reared in 2-propanol food show a dramatic change in *Adh* pattern throghout development. In first instar larvae *Adh* 1 homodimer increases its activity and two more cathodic subbands appear. In third instar larvae, early pupae and young adults, *Adh* 2 homodimer disappears and there is an increase in activity for the interloci heterodimer and for *Adh* 1 homodimer, appearing again the two more cathodic subbands. For mature adults a still more drastic change is observed, in as much as the only band in non threated flies, *Adh* 2 homodimer, disappears while non active bands appear, those corresponding to interloci heterodimer and *Adh* 1 homodimer plus the two more cathodic subbands mentioned before. Sometimes a subband between interloci heterodimer and *Adh* 1 homodimer may also appear. All these changes can be also achieved by maintaining mature flies in 2 % 2-propanol food for only 24 h. prior to experiment.

When using NAD in the composition of electrophoresis gels, a displacement of the pattern toward the anodal end can be observed, as described in the previous section, but there is not any additional subband, neither those migrating more to the anode than *Adh* 1 homodimer nor that between dimers, so that only interloci heterodimer and *Adh* 1 homodimer are visible.

In *D. melanogaster* there is the same interconversion pattern in NAD gels for flies treated with 2-propanol than for flies not treated, thus it is only visible the *Adh*-1 band being indistinguishable by means of electrophoresis the treatment done.

DISCUSSION

Differential expression of *Adh* system throughout development may be of adaptative significance in the different environments to which larvae and adults are confronted in cactophilic species. In effect, larvae are submerged in an alcoholic environment and its feeding activity is large in order to support the extensive growth to what they are subjected to. Thus the ingestion of alcohols is very important, while adults inhabit only in surface and eat considerably less. This has a correlation with the developmental pattern found in *D. buzzatii, Adh* 1 products are present since hatching from egg until little after emerging from pupae, *Adh* 2 products, on the other hand, are present since third instar larvae up to death Changes in *Adh* activity and in *Adh* cross reacting material have also been studied, changing also throughout development (Alberola, 1984). A similar developmental pattern has also been found in *D. mojavensis* (Batterham *et al.*, 1983), being postulated a two loci system in order to interpret such a temporal variation in gene products.

The presence of environmental 2-propanol, the major alcohol in rotted *Opuntia* (Fontdevila, 1980) and in many other cacti (Heed, 1978; Fogleman, 1982), produces a drastic change in *Adh* zimogram, disappearing *Adh* 2 homodimers, when active, and increasing activity for *Adh* 1 homodimers. Furthermore, in mature adults where there is no trace of *Adh* 1 homodimer, nor of interloci heterodimer, its activation can be induced by environmental 2-propanol concomitantly with *Adh* 2 homodimer desactivation. By using NAD in the composition of gels, we have showed that there is not a *melanogaster*-like interconversion phenomenon, as it was first postulated (Fontdevila *et al.*, 1980). This differential activation in front of environmental alcoholic composition is still more interesting if we consider that is accompanied by a depression in *Adh* activity (Alberola, 1981), resulting thus in less 2-propanol metabolized to acetone which is more toxic than it. Then we can suggest that *Adh* 1 products are directed to act in environments with high concentrations of 2-propanol, like larvae habitats, while *Adh* 2 products would be used with little 2-propanol or in a more inespecific way in low concentrations of environmental alcohol, like adults habitat.

The presence of three bands in larvae and one band in adults has been used to assign a duplicated *Adh* system in a wide number of species of the *mulleri* subgroup, however the only species in which recombinants have been found is *D. buzzatii* (Dakeshott *et al.*,1982; Sánchez, unpublished data), thus such a pattern could be also formaly explained in other ways, for example a developmental gradient of a substance producing interconversion. Gels containing NAD may be very useful in distinguishing different mechanisms for the presence of three bands in *Adh* zimograms, since species known to have a *melanogaster*-like system experiment an interconversion phenomenon resulting in the appearence of only one band also described by other authors (Dickinson and Carson, 1979; Kreitman, 1980; Dickinson *et al.*, 1984). Using this finer method we have found a duplicated *Adh* loci in species of the *mulleri* subgroup (in preparation), futhermore hybrids between *D. buzzatii* and its sibling *D. serido* (Data not shown) have an interspecies heterodimer, thus indicating structural homology between monomers, as expected if the two loci arised from a duplication event.

We are sure that the study of such suplicated systems may be very important in increasing our knowledge on regulation and evolution.

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