# Testis-Specific and Sex-Associated Hexokinases in Drosophila melanogaster

Abstract. *Hexokinases of* Drosophila melanogaster *were investigated by starch-gel electrophoresis. A hexokinase is present in both sexes during earlier stages of development, but it persists only in male adults. In addition, in this species there is a testis-specific hexokinase which is first observed during the pupal period.*

Extensive study of the electrophoretic patterns of enzymes in a variety of organisms has revealed both genetic and ontogenetic variation. Examples in *Drosophila* include the esterases (*1*), alkaline phosphatases (*2*), and alcohol dehydrogenases (*3*) in which the electrophoretic patterns are autosomally inherited, as well as glucose-6-phosphate dehydrogenase (G-6-PD) (*4*) and 6-phosphogluconate dehydrogenase (6-PGD) (*5*) which are sex-linked.

Sex-associated forms of lactate dehydrogenase (LDH) have been localized to the mature testis of some mammals (*6, 7*) and pigeons (*8*). We now report some hexokinases in *Drosophila melanogaster* which include one form that is specific for the mature testis and another that is related primarily to the accessory gland of the male.

Single flies were etherized and ground in microhomogenizers containing 15 $\mu$l of cold (0° to 4°C) 0.25$M$ KCl buffered to $p$H 7.4 and containing $10^{-6}M$ diisopropylfluorophosphate, $10^{-3}M$ nicotinamide-adenine dinucleotide phosphate (NADP), and $10^{-3}M$ $\beta$-mercaptoethanol. Mass homogenates were prepared, 0.2 ml of the same buffer being used for every 50 flies. The homogenates were centrifuged at 9000 to 10,000 rev/min for 15 minutes, and the supernatant was removed for electrophoresis.

Starch-gel electrophoresis was performed according to a modification of the system described by Katzen and Schimke (*9*) by the discontinuous horizontal rather than the vertical method. A buffer of 0.02$M$ sodium barbital was used in the gel and 0.05$M$ barbital buffer in the electrode vessels. Starch gels were prepared according to the procedure described by Kristjansson (*10*). At the conclusion of the run, the gel was sliced horizontally, and the cut surface of the lower slice was stained with the reaction mixture described by Katzen and Schimke (*9*) with 0.1$M$ glucose. The mixture was applied in 10 ml of 0.75 percent agar (*11*). Bands began to appear within 20 minutes and were allowed to develop for approximately 2 hours. When the substrate was omitted from the reaction mixture, no bands appeared. With the described buffer system, we were unable to demonstrate G-6-PD or 6-PGD activity. Dissections were performed in drosophila Ringer solution, and dissected material was transferred immediately to cold homogenizing buffer with a micropipet. Fresh Ringer solution was used for each dissection to avoid contamination of the various tissue fluids. Protein was assayed by the method of Oyama and Eagle (*12*).

When individuals of a multiply-marked stock (*13*) were run on starch gels, and the gels were stained for hexokinase activity, several bands were obtained, including a slow-moving band present only in the homogenates from single male flies (Fig. 1). We refer to this band, the slowest-moving band in zone A, as Hex-1. To determine the generality of these findings, males and females of two other stocks (*14*) were tested. As in the first stock, Hex-1 appeared only and always in the male flies. In addition, gels were run on separate homogenates made from 50 males and 50 females from each of the three stocks. Although Hex-1 activity was seen in all of the homogenates of males, it was only seen in one out of six homogenates of females, in which case a faint band appeared (Table 1).

To test the possibility of a Y-linked gene, attached-X/Y females ($\overline{XX}$/Y) and XO males were tested. The Hex-1 band was observed in only a few females with Y chromosomes. All XO males tested showed the band, indicating that the activity was not controlled by a Y-linked gene. The fact that a few $\overline{XX}$/Y females showed the band suggested that two X chromosomes might be suppressing an autosomal gene. To test the latter possibility, intersexes were obtained from a triploid line. We thought that if three sets of autosomes were present, the proposed 2X suppression of an autosomal gene might not be effective. The results showed that the Hex-1 band was present in intersexes which were male-like in appearance and absent in those which more closely resembled females. Later experiments showed that Hex-1 activity was present in larvae, pupae, and newly hatched adults of both sexes. However, within 2 days after emerging, Hex-1 activity was no longer present in females, while its activity was increased in males. Since age was not taken into account in earlier experiments, an explanation of the appearance of occasional females with Hex-1 activity might be that they were less than 2 days old. Further experiments showed that females carrying the transformer gene (*tra/tra*) (*15*) also had Hex-1 activity; the experiments seem to indicate that only individuals which were male in appearance had this enzyme in observable amounts at maturity, while flies which were phenotypically female generally did not.

Dissections were performed in order to determine whether Hex-1 activity was present in the whole male fly or localized to a certain organ or organs. The Hex-1 appeared only in the rear half of the male. The testes, accessory glands, genital plates, and malphigian tubules were dissected and

Table 1. Presence (+) or absence (−) of Hex-1 and Hex-t hexokinase bands according to chromosomal constitution and stage of development.

| Chromosomal constitution | Sex | Adults | | Larvae | | Pupae | |
|---|---|---|---|---|---|---|---|
| | | Hex-1 | Hex-t | Hex-1 | Hex-t | Hex-1 | Hex-t |
| 2A;X;X | Female | − | − | + | − | + | − |
| 2A;XX;Y | Female | − | − | | | | |
| 2A*;XX;Y | Male | + | + | | | | |
| 2A;X;Y | Male | + | + | + | − | + | + |
| 2A;X;O | Male | + | + | | | | |
| 3A;X;X;Y or no Y | Intersex | +/− | | | | | |

* Phenotypically male due to the presence of the transformer gene.

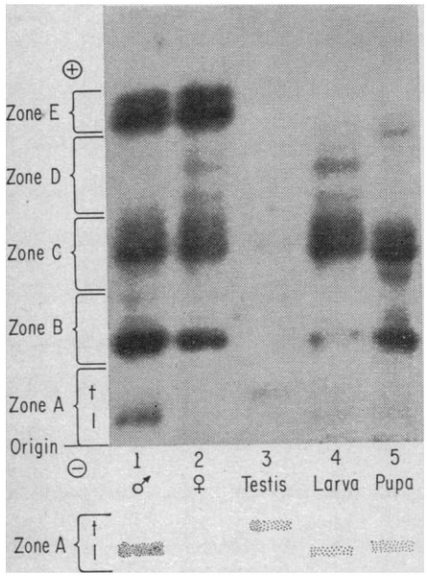


Fig. 1. Starch-gel electrophoretic pattern of hexokinases in homogenates of *Drosophila melanogaster* at different stages of development, and in testes of adult males. The diagram shows the positions of Hex-t and Hex-l bands present in zone A.

tested separately. At this point a new band was discovered which we call Hex-t. The newly discovered band, which migrated slightly faster than Hex-1 (Fig. 1), was found only in the testes, while the original sex-associated band was present only in the accessory gland. Testes from XO males and *tra/tra* females also had Hex-t activity. Ovaries concentrated from as many as 20 females showed neither Hex-t nor Hex-1 activity.

Larvae and pupae were tested to establish the time during development at which these two bands were first detectable. In homogenates of individual third-instar larvae and pupae, a weak Hex-1 band was present in both males and females. Females as well as males continued to show Hex-1 activity through the pupal period and as late as 2 days after hatching. However, the activity in larvae, pupae, and young adult females was never as great as that in adult males. Hex-1 activity could not be localized to a particular organ or region in larvae and pupae or in young, dissected females. Hex-t activity was not observed in whole larvae or in testes dissected from larvae, but was first observable in testes of individuals in the early pupal stage. The relative amounts of protein in larval and adult testes were determined, and the results indicated that more than enough material should have been present in the number of larval testes used to show activity if the specific hexokinase activities in larval and adult testes were comparable.

The occurrence of testis-specific LDH's in various organisms has been described (*6*) and the occurrence of a testis-specfiic hexokinase in the rat has been reported (*16*). The LDH testis-specific activity reported in mammals appears to be correlated in some way with the production of mature and functional sperm. Since it is known that sperm from XO males are not motile (*17*), the fact that XO males showed Hex-t activity would indicate that this band is not associated with sperm motility. Testes dissected from *tra/tra* females were examined and no mature sperm were found, indicating that testes do not need to contain mature sperm in order to show Hex-t activity. This does not rule out the possibility that the Hex-t band is in some way associated with spermatogenesis, perhaps at some earlier stage. Hex-t activity is first noted in the early pupal stage at approximately the same time as mature sperm appears. The band might also be related to the elongation of the testis which begins at this time.

During the course of this study, electrophoretic variation was noted in the Hex-1 band as well as in others. Homogenates of whole flies and testes of *D. pseudoobscura* were examined. The pattern of hexokinase bands was markedly different from that of *D. melanogaster*, and no sex-associated or testes-related variations were observed.

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**References and Notes**

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## Rabbit Lactate Dehydrogenase Isozymes: Effect of pH on Activity

Abstract. *The ability of lactate dehydrogenase isozyme five from rabbit skeletal muscle to catalyze pyruvate reduction is extremely sensitive to changes of* p*H within the range of 6.2 to 7.8. The activity of lactate dehydrogenase isozyme one from rabbit heart is virtually unaffected by changes of* p*H within the same range. Isozyme five is activated by low concentrations of oxalacetate and inhibited by higher concentrations, but other substrates of the citric acid cycle have no effect.*

The traditional interpretation of the effect of *p*H on enzyme activity is that a *p*H optimum is caused by ionization of a group in the region of the active center on the enzyme. If the enzyme has no *p*H optimum, it is assumed that no ionizable group is involved in its active center. Thus Winer and Schwert (*1*), in studying the influence of *p*H on the kinetics of lactate dehydrogenase from beef heart, concluded that the imidazolium ring of histidine must be protonated for the conversion of pyruvate to lactate and unprotonated for the conversion of lactate to pyruvate. A number of enzymes undergo changes in conformation when exposed to certain small molecules, and the manifestation of a *p*H optimum by an enzyme could be the result of such an exposure. In these cases, the active sites of the enzymes are not necessarily directly involved; for example, the catalytic site of bovine L-glutamate dehydrogenase has a *p*H optimum of around 9; this optimum is apparent only if the native conformation of the enzyme is stabilized by either methyl mercuric hydroxide or adenosine diphosphate (*2*). Interactions between subunits may be additional determining factors not directly involving the active sites, as is the case with hemoglobin and aspartate transcarbamylase (*3*).

I have studied the effect of *p*H on the activity of lactate dehydrogenase