Biphasic expression and function of glucose dehydrogenase in Drosophila melanogaster

(six-limited gene expression/eclosion/copulation/ejaculatory duct/lethal phase)

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Communicated by Adrian M. Srb, July 7, 1983

ABSTRACT Glucose dehydrogenase (GO) was found to be expressed during the pupal stage in both sexes of Drosophila melanogaster, but is limited to the male ejaculatory duct at the adult stage. During copulation GO is transferred from males to females. Mutational analysis of the Go locus indicates that a single structural gene encodes the pupal and ejaculatory duct GO. Thus an example of an enzyme structural gene switching from non-sex-limited to sex-limited expression has been found. Go mutants are recessive lethals exhibiting a late pupal effective lethal phase. These mutants can be rescued by excising the anterior end of the pupal case 0–2 days prior to the normal adult emergence time. It appears that the function of GO in pupae is to aid in the degradation of the puparium cuticle in preparation for the eclosion of the adult.

In higher eukaryotes many enzymes must function in more than one tissue and during more than one developmental stage. As a consequence of the biochemical and genetical complexities intrinsic to this requirement, two general evolutionary solutions have occurred. One solution has been the development of genetically independent isozymes via gene duplication (1). The lactate dehydrogenase isozymes in vertebrates and the hexokinase isozymes in Drosophila are the classic examples of such systems (2, 3). Alternatively, many single genes are expressed in diverse tissues and developmental stages, after which they are post-transcriptionally modified in a tissue-specific manner (4). Inasmuch as sexual dimorphism presents a complex regulatory problem, it is not surprising that genetically independent isozymes constitute all known sex-limited enzyme systems. Thus testis-specific lactate dehydrogenase X in mammals (2) and testis-specific hexokinase I in Drosophila (3) are thought to be coded by structural genes that are independent of other non-sex-limited isozymes of lactate dehydrogenase and hexokinase, respectively. We report here a case of an enzyme structural gene exhibiting a non-sex-limited expression at one developmental stage and then switching to a male-limited expression at a latter stage. This gene is the structural gene for glucose dehydrogenase (GO) in Drosophila melanogaster.

Three distinguishable enzymes catalyze the oxidation of glucose to gluconolactone/gluconic acid: glucose oxidase (EC 1.1.3.4), NAD(P) glucose dehydrogenase (EC 1.1.1.47), and FAD glucose dehydrogenase (EC 1.1.99.10) (5, 6). Biochemical evidence (unpublished) from crude extracts of D. melanogaster GO indicates that the enzyme reported herein is a FAD glucose dehydrogenase rather than a glucose oxidase as previously reported (7). D. melanogaster GO will utilize both anomers of D-glucose as substrates, does not generate hydrogen peroxide as a product, and does not require a pyridine cofactor. Although these data appear to only be consistent with the known characteristics of FAD glucose dehydrogenase, the identity of D. melanogaster GO must await verification from studies on the purified enzyme.

GO was originally identified as a male-limited ejaculatory duct enzyme in D. melanogaster, and the structural gene was mapped to a position of 48 centimorgans on the third chromosome with the use of isozymes by Cavener (7). To our knowledge this is the only report of GO expression in the reproductive organs of any species. We report herein the non-sex-limited expression of GO in the pupal stage. Using mutational analysis, we (i) demonstrate that the expression of the pupal and ejaculatory duct GO is coded for by a single gene and (ii) elucidate the developmental function of pupal GO.

MATERIALS AND METHODS

Notation. Go and GO are symbols for the glucose dehydrogenase structural gene and enzyme, respectively. Go/Go, Go/Go, and Go/Go are the three wild genotypes. The Go/Go and Go/Go genotypes exhibit the GO and GO electrophoretic allozymes, respectively. The Go/Go heterozygotes exhibit Go, Go, and a Go hybrid allozyme. The three-band allozyme pattern of heterozygotes suggests that GO is a dimeric molecule.

Electrophoretic Assays. Crude extracts of one or more Go/Go individual organisms were subjected to 6% polyacrylamide gel electrophoresis in a 0.1 M Tris borate, pH 9.2, buffer system or to starch gel electrophoresis (7). The gels were histochemically stained for GO by the methods of Cavener (7).

GO Activity Profiles. For each developmental stage and postcopulation time point three or four replicates of six Go/ Go individuals were homogenized in 0.1 M Tris-HCl, pH 7.0, and the extracts were centrifuged for 10 min at 12,000 X g and 4°C. GO activity was determined spectrophotometrically with the following assay mixture: 100 μl of the supernatant from the centrifuged homogenate and 2.9 ml of 0.1 M Tris-HCl/76 mM β-d-glucose/48 μM 2,6-dichloroindophenol, pH 7.0. Reduction of dichloroindophenol was monitored at 600 nm for 10 min at 22°C.

Genetic Strains. The genetic strains used in the mutagenesis experiment include the following: Go/Go, a wild-type strain free of lethal mutations on the third chromosome; TM1, Me and TM3, Sb, third-chromosome balancers (8) with the dominant markers more eyes and stubble bristles, respectively; and Df(3R)Antp84B1-84D12, a deficiency (84B1.2–84D11.12) in which the Go locus has been deleted (unpublished data).

Isolation of Go Mutants. Go/Go males were starved for 1 day, then fed 0.023 M ethyl methanesulfonate in a 1% sucrose solution for 24 hr (Fig. 1). These mutagenized males (Go/Go) were mass mated to TM1, Me/lethal virgin females (Fig. 1, line

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RESULTS AND DISCUSSION

The complete developmental profile of GO (Fig. 2) demonstrates that GO first becomes detectable in male and female late third instar larvae and exhibits a peak during the middle of pupal development. Between 24 and 48 hr after eclosion of the adults, GO is no longer detectable in virgin females by electrophoretic techniques even when female tissues are concentrated 10-fold. Spectrophotometric assays indicate an apparent low level of GO activity in virgin females older than 2 days (Figs. 2 and 3). Because our electrophoretic GO assay has generally proven to be more sensitive than our spectrophotometric GO assay, we believe that these low activity levels in the latter assay are due to the reduction of dichloroindophenol by other enzymes in the crude homogenate. Often GO activity measurements in mature females are not significantly different from the control assays without glucose. In contrast, GO rapidly increases in virgin males and remains relatively constant for their entire life.

Two GO isozymes are observed throughout the pupal stage of inbred strains. The more cathodal isozyme is coincident with the single isozyme exhibited in male adults. The more anodal isozyme exhibits a sequential decrease in mobility as a function of time through the 5- to 6-day pupal period (Fig. 2a). Inasmuch as putative point mutations at the Go locus block the expression of GO at all developmental stages (see below), we propose that the ontogenic heterogeneity in electrophoretic mobility is due to differential modification of GO mRNA or GO protein. In addition to these developmental isozymes, two electrophoretically distinguishable Go alleles (Go<sup>5</sup> and Go<sup>6</sup>) exist in natural populations (9). The Go<sup>5</sup>/Go<sup>5</sup> and Go<sup>6</sup>/Go<sup>6</sup> genotypes both exhibit corresponding isozymic patterns during development.

During the pupal stage, GO is widely distributed in the head,
macIntyre performed by using a modification of the techniques of reduce mutations in the Go to obtain GO activity that was previously absent. Males of the male GO activity is lost during mating, whereas females to corroborate this suggestion, we measured GO activity in fe-
tative *gos/GoF that copulation induces the expression of the Go gene in fe-
t males and females. Under survival selection, we found less than 1% of these lethals: (i) developing larvae were fed 5% (vol/vol) glu-
conic acid (the presumed product of the GO-catalyzed reaction) and (ii) the anterior end of the puparium (operculum) was ex-
cised 0–2 days before ecosion normally occurs. Of these two procedures only the technique of operculum excision was ef-
fective in rescuing the GO-deficient flies. This technique can rescue more than 90% of these mutants. The ability to rescue Go mutant flies has allowed us to determine that GO activity is completely absent in these mutants in all developmental stages, demonstrating that a single structural gene is responsible for both the pupal and male adult GO activities.

Go mutant pupal cases are unusually resilient to mechanical pressure. In contrast, light lateral pressure applied upon the operculum seams of wild-type pupae cases easily breaks them open. We hypothesize that the function of GO at the pupal stage is to aid in the catabolism of the puparium cuticle in prepar-
for adult eclosion (emergence). This hypothesis assumes that GO is secreted by the pharate adult into the intercellular space between the imago and the pupic cases. Thus, GO may be secreted at both the pupal and male adult stages. Consistent with this notion is the fact that GO is a glycoprotein at both developmental stages (unpublished). We speculate that the sex-
limited expression of GO at the adult stage is directly related to the presence of the ejaculatory duct in males and its absence in females. Under this hypothesis, the GO gene might respond to unique regulatory signals in the ejaculatory duct in a cell-autono-
monous manner. Alternatively, the ejaculatory duct may respond to a specific circulating hormone to illicit the expression of GO.

The metabolic, physiological, and developmental functions of GO and the molecular basis of the genetic control of the unusual developmental expression of Go remain to be investiga-

We thank T. C. Kaufman for providing many of the D. melanogaster strains used in this study. This work was supported by National Re-
search Service Award GM08333-01 (to D.R.C.) and National Institutes of Health Grant AG01811-02 (to R.J.M.).

sophila melanogaster (Carnegie Institute of Washington, Wash-
ington, DC).