O'Brien, S.J. Cornell University, Ithaca, New York. Functional and locational distinction between soluble and mitochondrial α-glycerophosphate dehydrogenase in D. melanogaster.

The α-glycerophosphate cycle of insects is critical in energy production, the intracellular NAD-NADH equilibrium, and in connecting carbohydrate and lipid metabolism (Sacktor in Physiology of Insecta, M. Rockstein, Ed., Academic Press, New York, ed. 2, 1965, p. 483). The two enzymes involved in the cycle in Drosophila have been the subject of investigation in our laboratory and this note is a preliminary report on our findings on the functional distinction between the soluble and mitochondrial α-glycerophosphate dehydrogenases.

Preparation of the soluble enzyme (αGPDH-1) involves mass homogenization of adults in .05 M Tris HCl pH 8.6 followed by precipitation of insoluble material by centrifugation at 30,000 g. The activity is recovered in the supernatant. Preparation of the particle associated enzyme (αGPDH-2) involves isolation of mitochondria by homogenization in .05 M phosphate pH 6.2 .001M EDTA, .38 M sucrose, followed by differential centrifugation between 500 g and 5000 g. The αGPDH-2 activity in the 5000 g pellet is particulate for the most part but can be solubilized by a variety of detergents, sonication, and enzymatic digestions. The most effective method is incubation of mitochondria with 1% Triton-X 100 for 2 hours, followed by centrifugation at 30,000 g. αGPDH-2 activity is found only in the supernatant after such treatment.

There are three general assays which we use to detect activity, (1) appearance of NAD at 340 nm, (2) reduction of PMS - INT read at 490 nm, (3) reduction of 2, 6-dichlorobenzenoneindophenole read at 600 nm. Qualitative electrophoretic detection employs only tetrazolium assays on cellulose acetate gels.

We can functionally distinguish between the soluble and mitochondrial enzyme by five different criteria. They are:

(1) Differential coenzyme specificity. αGPDH-1 shows a definite requirement for NAD in all assay procedures while αGPDH-2 show no activity dependence upon exogenous NAD. That the lack of coenzyme dependence does not depend upon mitochondrial impermeability to added NAD is demonstrated by identical independence of extracted "soluble" αGPDH-2 (see 2).

(2) Differential association of respective enzymes with the soluble and particulate fractions. Multiply washed mitochondrial preparations show no αGPDH-1 activity either spectrophotometrically (as determined by NAD stimulation) or electrophoretically (see 4). However, the soluble fraction always contains residual αGPDH-2 activity along with NAD stimulated αGPDH-1 activity (20x greater specific activity than αGPDH-2). This residual activity is presumably due to some αGPDH-2 which is normally soluble or solubilized by the isolation procedure.

(3) pH optimum - αGPDH-2 has a pH optimum between 6.1 - 6.4, while αGPDH-1 has an optimum above pH 9. These assays involve the oxidation of α-glycerophosphate.

(4) Electrophoresis - αGPDH-1 has a characteristic migration pattern which distinguishes electrophoretic variants on cellulose acetate strips in a .05 M Phosphate pH 7.4 system. Flies isolated in the absence of mitochondrial dissociating agents also show stain development of the origin independent of exogenous NAD. αGPDH-1 development depends on exogenous NAD. For a variety of reasons we think that the zone at the origin is the particulate αGPDH-2. Supernatant fractions show no development at the origin while mitochondrial preparations show only this development. Solubilized mitochondrial supernatant fractions show a variety of patterns depending upon the conditions of electrophoresis. None of these patterns which are seen in mitochondrial preparations correlate with those of the soluble enzyme. The former patterns can be detected in single flies and presumably represent αGPDH-2. The greatest homogeneity is detected with .05 M Acetate pH 4.8 at which pH there is inactivation of the αGPDH-1 enzyme.

(5) Presence of αGPDH-2 activity in αGPDH-1 deficient mutants. Four alleles of αGPDH-1 (O'Brien and MacIntyre DIS 43: 1968) which are deficient for αGPDH-1^B have been isolated by EMS mutagenesis. These have been tested and possess normal activity of αGPDH-2 as detected by electrophoresis and test tube assays.

Experiments designed to determine the genetic control of αGPDH-2 and to detect any genetic relationship between control of the enzymes are in progress.

This work was supported by Grant T1 GM 1035 from the National Institute of General Medical Sciences.