MULTIPLE FORMS OF TREHALASE IN PHORMIA REGINA

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SUMMARY

Gel electrophoresis of soluble extracts from a number of tissues of Phormia regina indicates the presence of multiple forms of the hydrolytic enzyme, trehalase. An analysis of flight muscle in this species argues for the presence of both soluble and particulate forms of the same enzyme. A preliminary survey of other species suggests that the presence of trehalase isozymes may be a general phenomenon.

Published studies on trehalase (α,α' glucoside l-glucohydrolase, E.C. 3.2.1.28) in flight muscle of higher Diptera have followed a line similar to other investigations in which the function of a given enzyme cannot be easily ascertained due to its peculiar intracellular emplacement. It has been reported that trehalase is mitochondrial (1), and/or attached to myofibrillar membranes (2), and/or soluble (3). Its physiological function (the enzyme is responsible for hydrolysis of the major insect blood sugar, trehalose) has been modified each time a new area of subcellular localization has been described.

Recently (1), an intensive examination of the flight muscle of Sarcophaga bullata was concluded with the assertion that there is no soluble trehalase in this tissue and that it is probable that there is none in the muscle of Phormia regina. The explanation given for taking exception to other results (3) which had indicated the presence of a soluble form of the enzyme was based on the possibility of contamination of the muscle preparation with blood. Fortunately, we have been working

on an allied problem for the past few months (i.e., the origin of blood trehalase in Phormia), so we can put this conjecture to rest very quickly, and, at the same time describe some rather interesting findings which may, in future, have some taxonomic value. Although the experiments described below still leave us with the problem of function of particulate and soluble trehalases in muscle, it is possible to state definitely that in Phormia, at least, the last named form does not arise from blood contamination.

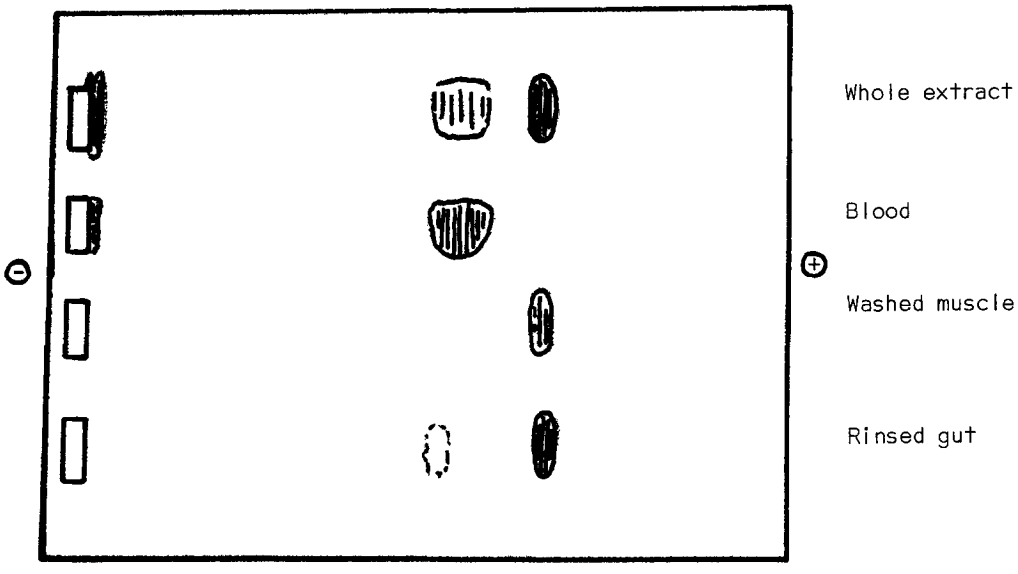

Figure 1

Horizontal slab gel electrophoresis of extracts of various tissues from adult Phormia regina. The slab, 9 x 12 cm x 3 mm thick was formed according to Davis (4) using 7% acrylamide. It was packed in ice and run for 2-2.5 hours at 30 ma, with the direction of current flow as indicated in the figure. The position of trehalase activity was established by incubating the gel after removal from the electrophoretic assembly for 3-3.5 hours in the dark at 37° C in 50 ml K-PO_4 buffer (0.1 M, pH 5.65) containing 320 mg trehalose, 600 units of glucose oxidase (Sigma Type V), 3 mg phenazine methosulfate, and 12 mg nitro blue tetrazolium. Extracts were the supernatants of material prepared as follows: Whole extract: 3 whole flies homogenized in 0.1 ml homogenization medium (see fig. 2) and centrifuged at 15,000g for 15 min; Blood: blood drawn from the neck region and used undiluted or diluted 1:3 with H_2O; Muscle: thoracic muscle washed as noted in the text, homogenized in homogenization medium and centrifuged at 400g for 5 min; Gut: mid and hind gut rinsed in homogenization medium, homogenized and centrifuged at 10,000g for 15 min.

EXPERIMENTAL

In order to establish the origin of blood trehalase, it has been necessary to either find a means of obtaining enough of the isolated enzyme to examine and compare its kinetic properties with trehalases from other tissues, or to find some property which is different enough from that of other trehalases to permit its identification in mixture. We have been able to accomplish the latter task, i.e., to establish the fact that there are several electrophoretically separable trehalases in Phormia, among them the blood and muscle enzymes. Applying extracts of whole flies, blood, washed muscle, and gut to a horizontal gel, and running and developing the gel as detailed in the legend for Figure 1

TABLE 1

Fraction	Trehalase Activity µM/ml/min	Protein mg/ml	SA µM/mg/min
A	410	2.21	185
B	1875	17.25	109
C	538	4.82	111
D	1540	17.1	90
E	1150	11.6	99
F	121	1.1	110
G	368	3.5	105
H	114	0.6	190
I	88	0.43	204
J	12.9	0.08	161
K	160	1.0	160
L	307	2.92	105

Activity determined using the coupled glucose oxidase-peroxidase-o dianisidine method as described by Friedman (5). Total volume of the assay mixture was 0.79 ml; final trehalose concentration was 6.3 mM. Protein determined according to Lowry, et al. (6).

produces the results visualized in the figure. (There is no similar activity with maltose, sucrose, or α-methyl glucoside as alternative

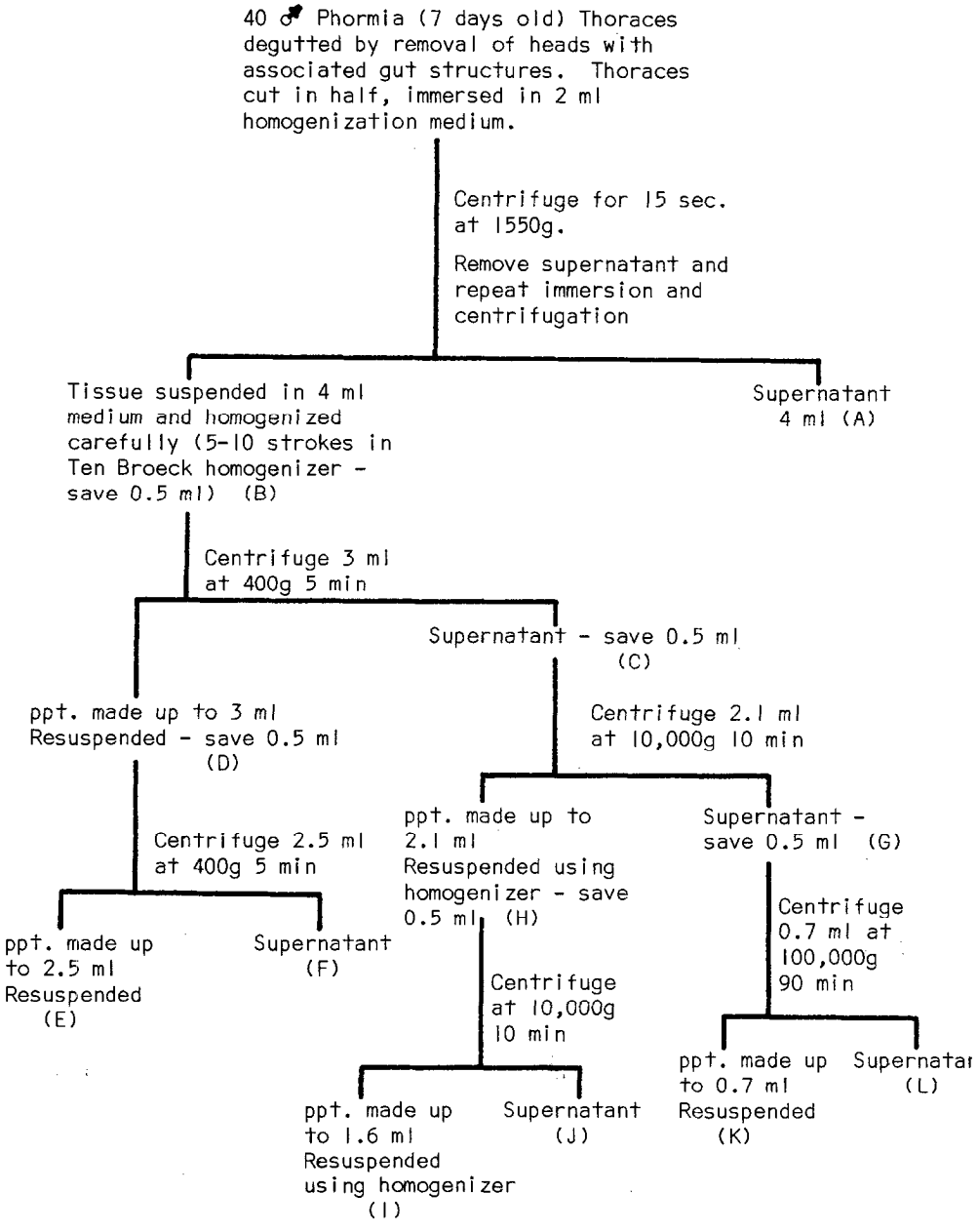

Figure 2

Flow chart for separation of particulate and soluble fractions from flight muscle of adult Phormia regina. The homogenization medium consists of 1 mM EDTA, 154 mM KCl, and 10 mM KH_2PO_4, pH 7.0.

substrates.) Moreover, the purification of muscle trehalase away from the blood enzyme can be electrophoretically followed. Using the isolation scheme indicated in the flow chart (Figure 2), it may be seen (Table I) that there is, indeed, a relatively large amount of enzymatic activity remaining in the soluble fraction tested after centrifugation of muscle homogenate at 100,000g for 90 minutes. It may also be seen that an ample quantity of activity is present in the wash which precedes homogenization. It would be expected that the wash contains the blood surrounding the muscle, since the mechanics of washing consists of centrifuging teased muscle through a relatively large volume of homogenization medium twice at 1550g. Figure 3 depicts the purification as far as it can be presently determined. The wash (FracA) contains trehalase activity moving in the gel at a rate equivalent to blood trehalase. Fraction C, the material from which myofibrils and some mitochondria have been removed contains material corresponding to soluble muscle

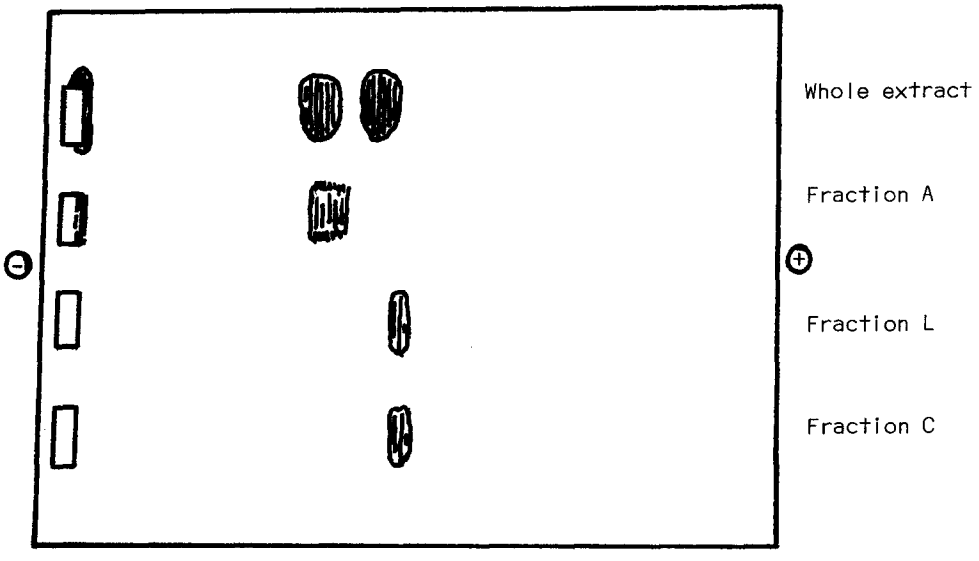

Figure 3

Horizontal slab gel electrophoresis of various fractions taken from the preparation described in the flow chart (fig. 2). The techniques used in making and developing the gel are described in Figure 1 as is the preparation of the whole extract.

enzyme plus a trace of blood enzyme. Fraction L, the soluble enzyme remaining after high speed centrifugation also corresponds to soluble muscle enzyme.

DISCUSSION

There is no question that the material described by others as soluble muscle trehalase derives from tissue other than blood. Nor do we believe that it is simply removed from mitochondria during preparation. We have sonicated mitochondria (Branson sonifier, maximum output, 1 min.) in thick saline suspension and have been unable to find an active band on the gel after subsequent centrifugation and application of the supernatant. We have also incubated washed mitochondria in saline for varying lengths of time and have been similarly unsuccessful in obtaining activity from the supernatant. (It must be recognized that the sieving properties of the gel are such that only protein in solution would be expected to pass through it.) It is our opinion that we shall probably have to rationalize the presence of two enzymes located in different parts of the flight muscle cell of Phormia, each capable of the hydrolysis of trehalose.

It is also of interest to note that a gel electrophoretic survey of representatives of a number of families of higher Diptera indicates the presence of trehalase isozymes in many species.

ACKNOWLEDGMENT

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