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Sugar-hydrolysing enzymes in gustatory mutants of *Drosophila*

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Abstract. The distribution of the isozymes of α-glucosidase in different parts of *Drosophila melanogaster was* examined. Two of the eight glucosidase bands are associated with tarsi. The gustatory mutations in the genes *gust*B and *gust*C greatly reduce tarsal glucosidase activity, but the enzyme deficiency is not correlated with inability to taste sugars.

Keywords. Neurogenetics; glucosidase; taste receptors; gustatory mutants.

Introduction

Dipterans possess a number of sugar hydrolysing enzymes. The α-glucosidases (EC. 3.2.1.20) cleave the 1, 4α-glucosidic linkage, β-glucosidase (EC. 3.2.1.21) attacks β-glucosidic disaccharides and trisaccharides, and trehalase (EC. 3.2.1.28) cleaves the α-1,1 glucose-glucose bond. The glucosidases act on a broad-range of substrates although trehalase is relatively specific. Dethier (1955) found that glucosidase activity was present in the tarsal sensilla of blowflies. Hansen (1969) proposed that α-glucosidases act as receptor proteins for sugars. Although Hansen's working hypothesis has been entertained by several investigators, the arguments in its favour are mainly circumstantial (see review by Hansen, 1978). There is indeed little direct evidence to illuminate the exact physiological role of glucosidase in chemosensory reception.

We have obtained *gustatory* mutants in four different X-linked genes of *Drosophila melanogaster,* unable to taste sugars or salts (Rodrigues and Siddiqi, 1978, 1981). Some of these mutations specifically alter the electrophysiological responses of the taste hairs, which prompted us to examine the glucosidases of the gustatory mutants. In this paper we describe the tissue distribution of glucosedase activity in normal and mutant flies. In two of the *gust* mutants the sugar-hydrolysing activity, specific to tarsal hairs, is greatly reduced but glucosidase deficiency is not necessarily correlated with inability to taste sugars.

Materials and methods

Strain

The wild type strain was Canton Special (CS). The *gust* mutants are its derivatives (Siddiqi and Rodrigues, 1978). Flies were cultured on standard cornmeal medium.

Abbreviations used: CS, Canton Special; DTT, dithiothreitol; PMSF, phenyl methyl sulphonyl fluoride.

Enzyme preparation

Two to five days old flies were left in dry bottles to clean themselves of adhering food, then transferred to vials and frozen in liquid nitrogen. The heads and legs were separated by vigourous shaking followed by sieving through 0.8 mm nylon mesh. Legs, antennae and heads were spread on blotting paper; legs and antennae stick to the paper while the heads can be easily rolled off. Ten g of flies yield 1 g of heads and 50 mg of legs.

The legs (20 mg), heads (50 mg) and whole flies (100 mg) were separately homogenised in 1 ml of 0.05 M potassium phosphate buffer (pH 7.2) containing 0.1 M KCl, 0.4 mM dithiothreitol (DTT), 1 mM EDTA and 0.5 mM phenyl methyl sulphonyl fluoride (PMSF) and a mixture of protease inhibitors. Homogenisation was carried out on ice, with 100 up-down strokes in a motor dri-
ven Potter-Elvehjem homogeniser. The homogenate was stirred for 2 h at 4°C and centrifuged for 1 h at 27,000 *g*. The supernatant was used as a source of enzyme.

Soybean trypsin inhibitor (1 mg/ml), pepstatin (1 µg/ml) leupeptin (1 µg/ml), antipain hydrochloride (1 µg/ml), benzamidine HCl (1 µg/ml), and EGTA (0.1 mM) were added to the homogenisation buffer to inhibit protease activity.

Non-denaturing gels

Enzyme extracts were electrophoresed in 7.5% acrylamide gel with 0.2% bis-acrylamide. The resolving gel was polymerised in 0.03 M imidazole-HCl buffer (pH 7.2). The stacking gel (3.8% acrylamide with 0.1% bisacrylamide) was polymerised in 0.03 M imidazole HCl (pH 6.2). The cathode buffer contained 0.016 glycylglycine-imidazole (pH 6.7) and the anode buffer 0.03 M imidazole-HCl (pH 7.2). Electrophoresis was carried out at a constant current of 20 mA.

The enzyme was assayed as described by Tanimura *et al.* (1979). The gels were incubated in 0.5 M sucrose (or other sugars as required) in 100 mM sodium citrate NaH_2PO_4 (pH 5.5) for 30 min at 37° C and washed in distilled water, then soaked in 0.1% triphenyl tetrazolium chloride in 0.5 N NaOH at 30° C for 5 min. The stained gels were dried between two sheets of cellophane paper and scanned in an analytrol densitometer

Chemicals

Acrylamide, bisacrylamide, phenyl methyl sulphonyl fluoride (PMSF), soybean trypsin inhibitor, leupeptin hemi sulphate, antipain hydrochloride, benzamidine hydrochloride and EDTA were from Sigma Chemical Co., St. Louis, Missouri, USA. Sucrose and triphenyl tetrazolium chloride were from British Drug House, England.

Results

The Polyacrylamide gel profiles of the glucosidases of *Drosophila melanogaster* were examined by Tanimura *et al.* (1979). They found 7 isozyme bands; 3 of the bands which were prominent were designated I, II and III. Extracts from entire flies, in our hands yield 8 bands of α-glucosidase activity whose (table 1) positions

Table 1. α-Glucosidase activity in limb-specific bands in normal and mutant flies.

	Activity (%) in D and E*
Canton Special (C.S.)	40
*gust*A	48
*gust*B	5
*gust*C	6
gust D	33

* Expressed as fraction of total activity in the gel.

are comparable with Tanimura's bands except that band E is not present in his gels. We have designated these bands A-H as shown in figure 1. Bands A, B and F in our gels correspond with Tanimura's I, II and III. When the gels are stained with raffinose, only one band appears near the top of the gel; this is β-fructofuranosidase (Huber and Mathison, 1975). Staining with trehalose gives a single rapidly

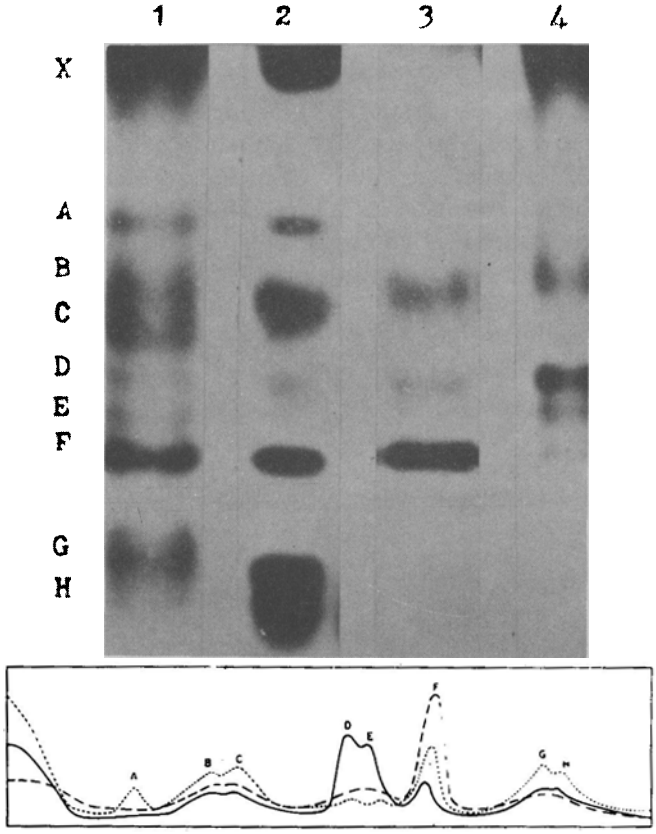

Figure 1. Electrophoretic species of glucosidase in normal *Drosophila* and its parts: 1. whole fly, 2. abdomen and thorax, 3. head and 4. leg. The band X near the top is a β-fructofuranosidase and strains with raffinose; A-H are α-glucosidases.

Densitometric tracing shows the profile of glucosidase isozymes in normal *Drosophila:* whole fly (·····); head (·····); legs (——).

migrating band of activity. Trehalose is not hydrolysed by α-glucosidase (Marzluf, 1969).

In order to examine the tissue distribution of the isozymes, extracts from separate parts of the fly, head, body and legs were electrophoresed. Figure 1 shows the glucosidase profile of different parts. The relative amount of activity in each band was estimated from densitometric scan of stained gels (figure 1). These are semi-quantitative estimates prone to errors arising from non-linearity of staining specially when the bands are too faint or too dense. Our estimates are averages of several gels. The standard error of these estimates ranges from 20 to 30%.

The band A is present in the body but absent from head and leg. The molecular weight of this band, 250,000 against 200,000 reported by Tanimura *et al.* (1979). The bands D and E are greatly enriched in the legs; the two bands together account for about 40% of the activity in the limbs D and E thus, seems to be the glucosedases associated with the tarsal sensilla. The molecular weight of these enzymes is 120,000 daltons. About 12% of the activity in the legs is contributed by a 75,000 dalton species designated as F; this is the predominant band in the head and is most likely the same as band III of Tanimura.

Glucosidase in gustatory mutants

Extracts from legs and heads of four different gustatory mutants *gust*A, *gust*B, *gust*C and *gust* D were examined. The glucosidase profiles are shown in figure 2. The mutants *gust*A and *gust* D are very similar to wild type but *gust* B and *gust* C show greatly reduced enzyme activity in the limb specific bands D and E. The proportion of total glucosidase activity in bands D and E in the four mutants, estimated by densitometric scanning of stained gels is given in table 1. In *gust*B, a prominent rapidly migrating band of activity as well as a band close to C appears in extracts of leg and head (figure 3) .

In order to decide whether the enzyme deficiency in the mutant strains is specifically related to the mutation in question, we isogenised the *gust* stocks by repeated back-crossing to wild type, followed by selection for the *gust* phenotype for 3 generations. A number of lines from these crosses, segregating for *gust*B and *gust* C, were then examined for limb-specific glucosidases. The glucosidase deficiency was inseparable from the gust mutation (table 2).

Only one allele of *gust*C was available but two independent alleles of *gust*B showed similar enzyme deficiency.

Discussion

The taste sensilla of *Drosophila* are present on its proboscis and tarsi. The neural organisation of the labellar and the tarsal hairs is similar. A typical gustatory hair contains five sensory neurons four of which are chemosensory. One of these neurons called *S* responds to sugars while two others *(L_1* and *L_2)* respond to salts. The gustatory mutations studied by us affect the labellar and the tarsal responses alike as judged by the proboscis extension test and electrophysiology (Rodrigues and Siddiqi, 1978). We have examined mainly the glucosidase of tarsal hairs.

The isozymes, D and E appear to be associated with the tarsal sensilla. This assumption is reinforced by the fact that two of the gustatory mutants are deficient in these two bands. The enzyme deficiency of the mutants, however, does not pro-

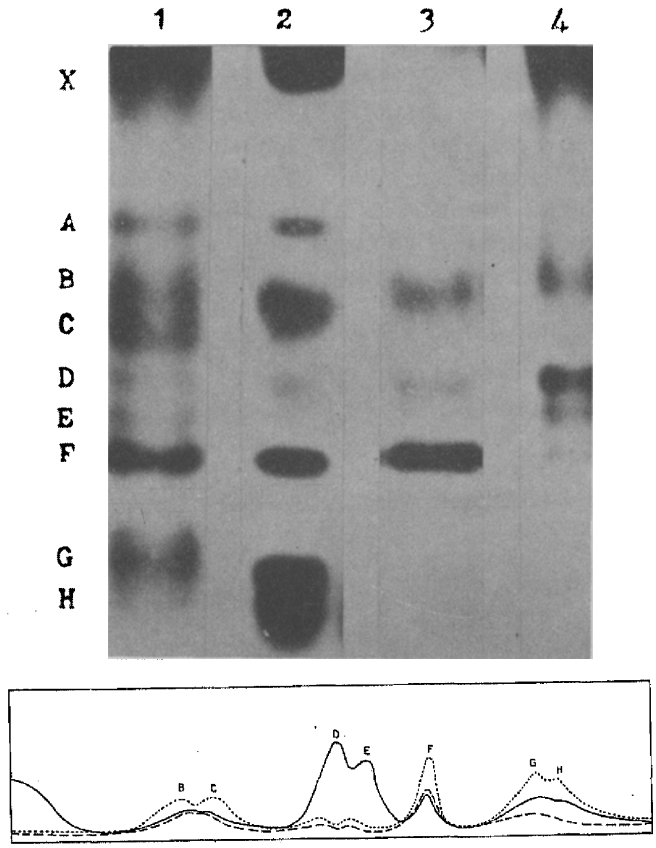

Figure 2. The pattern of glucosidase activity in the tarsi of gustatory mutants: 1. wild type, 2.*gust*B, 3.*gust*Cand4.*gust*A. Bands D and E are greatly reduced in *gust*B and *gust*C but *gust*B has increased activity at unusual positions.
Tracings of glucosidase profile of gustatory mutants: Leg extracts of *gust*A (——); *gust* B (·····) and *gust*C (-------).

Table 2. α-Glucosidase activity in recombinant progeny from crosses segregating for *gust*B and *gust* C.

	Recombinants			Lines examined	Activity in D and E bands
1.	*y*	*cv*	*gust*B	5	Absent
	y	*cv*	+	3	Present
	*gust*B	*v*	*f*	2	Absent
	+	*v*	*f*	2	Present
2.	*y*	*cv*	*gust*C	3	Absent
	y	*cv*	+	2	Present
	*gust*C	*v*	*f*	2	Absent
	+	*v*	*f*	1	Present

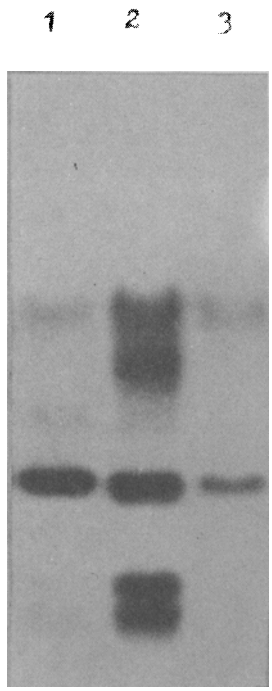

Figure 3. Glucosidase activity from head of normal and mutant flies: 1. wild type (CS), 2.*gust*B and 3. *gust*C. Note the presence of abnormal bands in *gust*B.

vide any clue to the functional involvement of glucosidase in chemoreception. The mutant *gust* A which is specifically blocked in behavioural and electrophysiological responses to pyranose sugars has a normal glucosidase profile; *gust* B and *gust* C which lack the limb-specific glucosidase, on the other hand, show apparently normal sugar responses; these mutants respond abnormally to salts. The simplest predictions of Hansen's hypothesis, that glucosidase itself acts as the receptor for sugars, are therefore, not borne out.

The fact that two different salt-insensitive mutations affect glucosidase levels in the sensilla is somewhat of an enigma. It is possible that the genes *gust*B and *gust*C are not directly concerned with glucosidase structure; rather an alteration in the normal organisation of the tarsal sensilla affects the accumulation or stability of the enzyme. Recent electrophysiological and behavioural experiments in our laboratory show that *gust*B responds to salts in an unusual way. Instead of being repellants NaCl and KCl act as attractants, at the same time eliciting greatly enhanced firing from sensory neurons (Rodrigues, Arora and Siddiqi, unpublished results). An interesting possibility is that *gust*B causes the appearance of salt acceptor sites on the *S* neuron, normally responsive to sugars alone. At any rate there is evidence of peripheral changes in the sensory receptors in this mutant. The same cannot be said of *gust*C, which has apparently normal firing responses to sugars and salts.

Lastly, although our results fail to provide any support for Hansen's hypothesis, they do not, strictly speaking, exclude the involvement of glucosidase in sensory reception. Some glucosidase activity appears in unusual position in the gels of

*gust*B. The mutants, although deficient in the bands D and E, do not altogether lack glucosidase activity in their sensilla.

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