

A Putative Sugar-binding Protein in the Chemosensory Organ of the Fruit Fly, *Drosophila melanogaster*

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A single chemosensillum usually includes five sensory cells, and one of them is traditionally called as the sugar receptor cell. We have collected the legs and wings of the fruit fly, *Drosophila melanogaster*, using several grades of sieve and here demonstrate the presence of the putative pyranose-site protein by a two-dimensional affinity electrophoresis method.

KEYWORDS: sugar-binding protein, fruit fly, *Drosophila melanogaster*, affinity electrophoresis, P-site

Introduction

Flies have chemosensilla on the labellum, tarsi (the most peripheral part of legs) and wings. By their shape and a small opening to the external world, these chemosensilla are regarded as taste organs (Sturckow *et al.*, 1967). A single sensillum includes five sensory cells: one mechanoreceptor and four contact chemoreceptors (Dethier 1955). One of the latter is traditionally called as the sugar receptor cell and is considered to include the detection of chemical information about a wide variety of nutritious substances. Since the pioneering work on taste in the blowfly (Dethier, 1955), the presence of functionally different receptors has often been discussed in the sugar receptor cell.

Shimada *et al.* (1974) provided electrophysiological evidence for the existence of two kinds of sugar receptor sites on a single sugar receptor cell in the fleshfly. One is P-site for pyranose sugars and another is F-site for furanose sugars. In the labellar extract of the blowfly, the putative proteins for the both P- and F-sites appeared on two-dimensional affinity electrophoretic gels, in which antagonistic polysaccharides starch and levan, were used as affinity ligands to bind P- and F-site proteins, respectively (Ozaki, 1988; Ozaki *et al.*, 1993).

There are several mutants concerning with sensory systems in the fruit fly, *Drosophila melanogaster*. Recently, a few taste mutants in this animal were also reported (Rodrigues *et al.*, 1981, Tanimura *et al.*, 1988). In spite of its small size there is an advantage to use for the taste study, because of the existence of several mutants. We could collect the legs and wings of the fruit fly as separately fractions, using several grades of sieve and are here able to demonstrate the presence of the putative P-site protein according to the Ozaki's (1988) method.

Materials and Methods

Flies

A wild-type strain of *Drosophila melanogaster*, Canton-S, was used in this experiment. Flies were reared in vials under a 12-hour light: dark cycle at 25°C and fed with ordinary yeast and corn standard medium. Two-to-four days old adult flies were collected and used in the same day, using both males and females.

Two dimensional affinity electrophoresis

Sample preparation

About 500 animals (as estimated by weight, since a fly is ca. 1 mg) were used. The head, wings and legs including some other hair were separated from the bodies by three cycles of freezing and vortex (S-100, TAITEC) agitation at liquid nitrogen temperature, and passage through two kinds of sieves (IIDA testing sieve aperture 710 μm , wire diameter 450 μm : aperture 425 μm , wire diameter 290 μm). Legs and wings pass through these two sieves, and were collected and ground with a pestle and mortar for 10 min at liquid nitrogen temperature. The homogenate was transferred into an Eppendorf tube, suspended in 30 μl sample buffer (4.65 mM sodium barbiturate-HCl, 2% Triton X-100, 10% glycerol, pH 6.8) and incubated at 5°C for an hour. It was then centrifuged at 5,000 rpm at 5°C for 10 min, and the supernatant was used. An extract of 10 fly bodies without legs, wings and heads was prepared by the same procedure as a control.

Gel preparation and affinity electrophoresis

The first and the second electrophoretic systems were constructed under the same conditions except for the affinity ligand. The stacking gels (4.5% acrylamide in stacking gel buffer: 9.3 mM sodium barbiturate-HCl, pH 6.7) and running gels (7.5% acrylamide in running gel buffer: 91.1 mM sodium barbiturate-HCl, pH 8.3) both contained Triton X-100 (2% final concentration). Tris buffers were avoided because they are known to inhibit the sugar responses (Kuwabara *et al.*, 1973). Each gel was $130 \times 115 \times 1$ mm and the electrode buffer was 41.1 mM sodium barbiturate-glycine (pH 8.3). Since the binding constant between the ligand and the receptor depends on temperature the gel was maintained at 22–24°C in an incubator. On the first run, the electric current was 20 mA for 4 hours; and On the Second Run, 40 mA for 2 hours, because the second run is not actually the affinity electrophoresis. Under the condition of 40 mA for 2 hours, the buffer temperature was about 20–30°C but it did not affect the results.

About 40 μ l of sample extract was applied per lane of the first dimensional gel containing 0.5, 1.0 or 1.5% starch. After the first run, each lane was cut into strips and shaken in the sample buffer for 1 hour at room temperature (20–25°C). Each gel strip were then loaded onto the slab gel for the second dimension. Since the amount of sugar-binding proteins was expected to be very small, we stained the gel using the high sensitive silver staining method (Oakley *et al.*, 1980).

Results and Discussion

As there are many different kinds of proteins present in the legs and wings of the fly, it is difficult to detect a minor protein such as the sugar-binding protein in one-dimensional electrophoresis. We therefore used two-dimensional polyacrylamide gel electrophoresis, in which the affinity ligand was present only in the first dimension. Polysaccharides are large molecules with no net electric charge, so they are useful as immobile affinity ligands when mixed in a polyacrylamide gel. Figure 1 illustrates the principle of the affinity electrophoresis. Proteins that do not interact with the polysaccharide migrate at the same speed in both the first and the second electrophoresis, and so are collected along a diagonal within the second gel (Fig. 1(a)). Proteins that interact with the polysaccharide are retarded in the first dimensional run but their migration speed is normal in the second electrophoresis. Proteins that interact with the polysaccharide are detected as spots separated from the diagonal line (Fig. 1(b)). The affinity ligand, starch, was chosen as this polysaccharide is known to be a competitive inhibitor for the P-site of the blowfly (Ozaki, 1983).

Figure 2(a) is a control gel without starch in the first run, in which all proteins migrated onto a diagonal line. Figure 2(b) and 2(c) show test gels with 0.5% starch and with 1% starch in the first run respectively. A putative

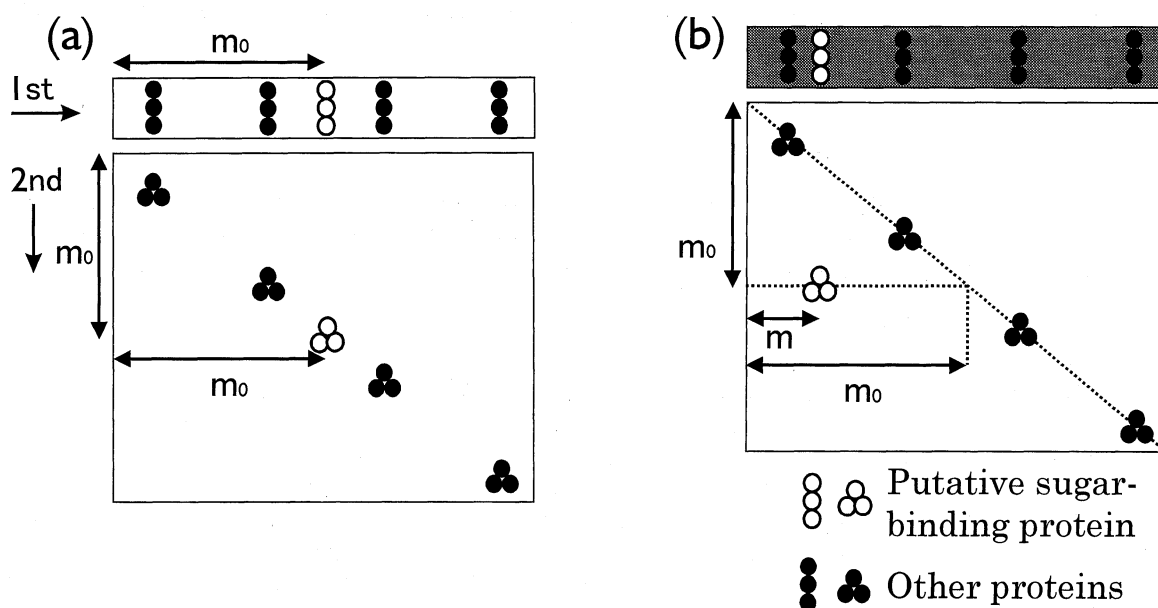


Fig. 1 A schematic drawing of the two-dimensional affinity electrophoresis diagonal method.

(a) In the absence of starch, every protein migrates at its individual migration speed in both the first and the second dimensions, such that the proteins form a diagonal line when stained. (b) When the first dimensional gel contains starch (shadow), any protein reacting the starch should show a slower migration speed in the first run, and also be separated from the diagonal line.

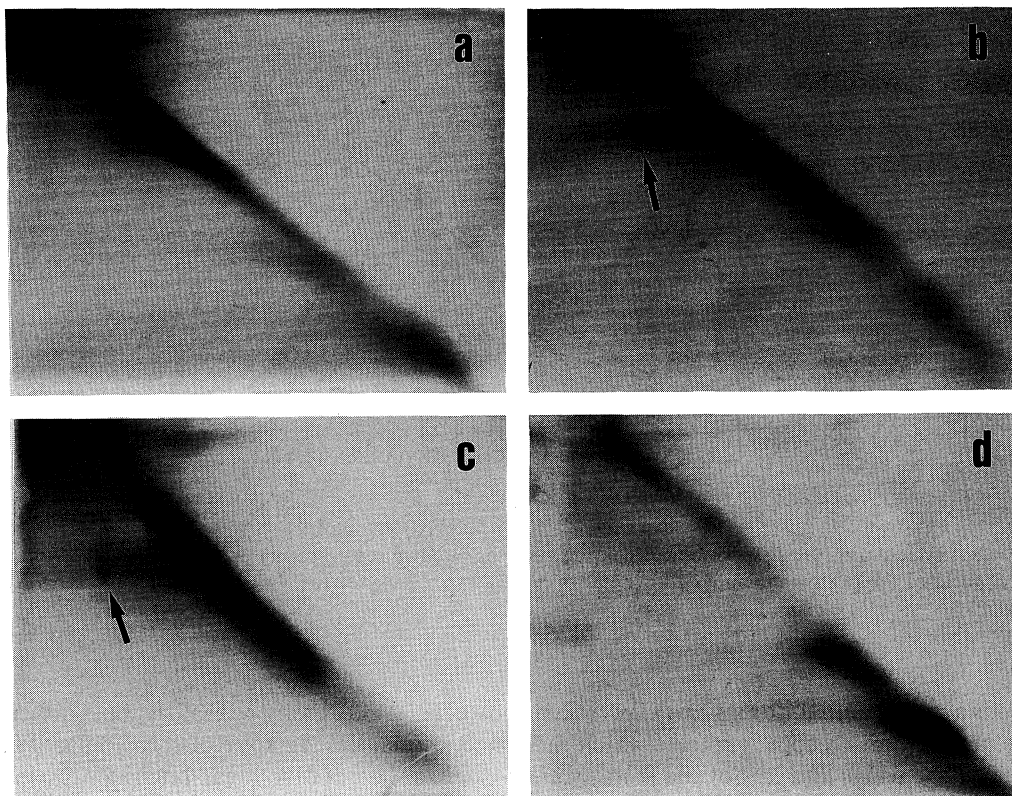


Fig. 2 Two-dimensional affinity electrophoresis of leg- and wing-extract of the fruit fly. The origin of electrophoresis is the upper-left corner and the proteins migrate to the right in the first dimension and to the bottom in the second dimension. (a) 0% starch in the first dimensional gel, (b) 0.5% starch in the first dimensional gel. (c) 1% starch in the first dimensional gel, (d) control (sample from body without legs, wings and heads). Arrows indicate the spot of the putative sugar-binding protein in each gel.

sugar-binding protein appears as an isolated spot, off the diagonal line because of its interaction with starch (arrows) in each gel. The distance of the spot from the diagonal line in the 0.5% starch gel (Fig. 2(b)) was shorter than that in the 1% gel (Fig. 2(c)). From the same experiment with the body (without legs, wings and heads) extract, this isolated spot on the gel was not detected (Fig. 2(d)).

The relative mobility, m , of the putative sugar-binding protein in the starch-containing first dimensional gel to the relative mobility, m_0 , in the starch-free gels can be obtained as follows: on the two dimensional gel (see Fig. 1), m is the horizontal distance from the origin to the isolated spot of the protein; m_0 is that from the origin to the crossing point of the horizontal line passing the spot and the diagonal line of all other proteins. If the mobility, m_0 , of the receptor protein decreases to m in the presence of starch of concentration $[I]$ (w/v%), the protein-starch interaction can be expressed by the following equation (Takeo, K., *et al.*, 1972, Takeo, K., *et al.*, 1978):

$$m_0/m = 1 + [I]/K_i,$$

where K_i is the dissociation constant between the receptor and starch. The concentration of starch, K_i or $[I]$ is represented by w/v%. We changed the concentration of starch added to the first dimensional gel to be 0.5, 1.0 and 1.5% and investigated 11 samples in total.

Figure 3 shows the plot of m_0/m against $[I]$. The line is the best fit for the data, based on the method of least squares. The value of $-K_i$ was obtained as the concentration at the intersection of the line and the abscissa. The K_i value of 0.7% is in good agreement with that reported for the blowfly (Ozaki, 1988), that is the P-site proteins in both the fruit fly and the blowfly show similar affinity to the common site specific inhibitor, starch.

This fly strain, Canton-S is a common wild-type stock of *Drosophila melanogaster* but the taste response of this strain is known to have unusually high trehalose sensitivity, while sensitivities to other mono- and di-saccharides are similar to other wild-type strains (Tanimura *et al.*, 1982). "Tre", the gene related with sensitivity to trehalose, is located on the X chromosome (Tanimura *et al.*, 1982). Canton-S flies have the Tre^+ allele, which accounts for their high sensitivity to trehalose. Thus, significant properties of the taste receptor protein for trehalose would be appeared by comparing Tre^+ with Tre^- flies with this two dimensional affinity electrophoretic method.

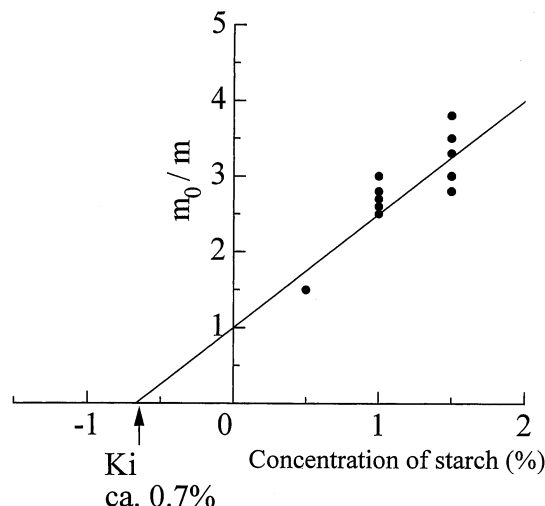


Fig. 3 Determination of the dissociation constant between the putative sugar-binding protein and starch. Plot of the reciprocal of the relative mobility of the putative protein m_0/m , against the concentration of the starch affinity ligand.

Drosophila is a useful animal for several experiments, however, it may easily be realized how difficult to collect and visualize one kind of receptor proteins which this small animal has. In spite of these difficulties, we could effectively collect the wings and legs of the fruit fly and visualize the putative sugar-binding protein as reported in the bigger fly. The breeding of the fruit fly is able to make a large number of animals easily. According to the establishment of the separation of the putative sugar-binding protein from the fruit fly, we can gather the significant volume of the protein to be able to investigate by other analyzing methods such as immunology and molecular biology, combining with convenient taste mutants.

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