Melezitase and Maltase from the Midgut of *Sesamia inferens* Walker (Lepidoptera: Insecta)

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Summary. The activity of melezitase and maltase was optimal at pH 6.2 and temperature 35–40°C and inhibited by the end-products. Melezitase activity was affected by K^+, Li^{++} and *Tris* ions and was reduced by dialysis.

Very little is known about the properties of insect melezitase and maltase; therefore their properties were studied.

Materials and methods. Midguts of the larvae were homogenized, the homogenate was centrifuged at 3000 *g* for 30 min and the supernatant was used as the enzyme extract. The reaction mixture contained 0.2 ml of substrate solution, 0.2 ml of buffer and 0.2 ml of enzyme extract. The experiments were carried out at 37 °C (except for temperature experiments). The reaction of melezitase was stopped by adding 3,5-dinitrosalicylic acid reagent[2], then the colour was developed by heating the reaction mixture in a boiling water-bath; after cooling, the volume of the mixture was adjusted to 5 ml by adding distilled water. The amount of reducing sugars formed was estimated with the help of Baush and Lomb 'Spectronic 20' colorimeter at 540 nm. The reaction of maltase was stopped by immersing the reaction mixture in a boiling waterbath for 10 min, and the amount of glucose formed was estimated by the method described by YANG and DAVIES[3].

The enzyme activity was estimated at several pH values ranging from 3.0 to 9.0 using different buffer systems (0.1 *M* sodium citrate-HCl buffer for pH 3.0–5.0; 0.1 *M* phosphate buffer for pH 5.5–7.5; and NaOH-glycine buffer for pH 8.0–9.0). Effects of all other factors were studied at the pH at which optimal activity was observed. The temperature effect on the activity of the enzyme was studied by incubating the reaction and control mixtures at various temperatures ranging between 5 and 70 °C. To study the effects of incubation periods, a pool of reaction mixture was incubated at 37 °C, and the samples were taken out at different intervals of time for the assay of reducing sugars formed. For substrate concentration effect studies, different concentrations of substrate were used varying from 0.5 to 5.0%. To find out the effects of enzyme concentration, different concentra-

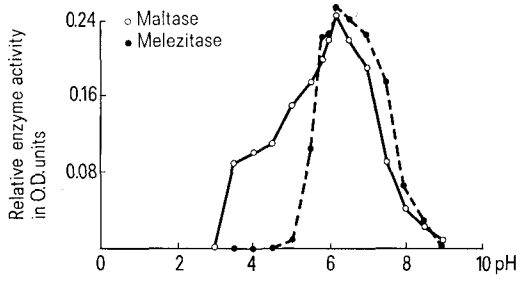

Fig. 1. pH-enzyme activity curves of the larvae of *S. inferens*.

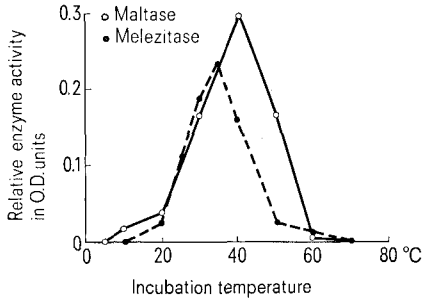

Fig. 2. Temperature-enzyme activity curves of the larvae of *S. inferens*.

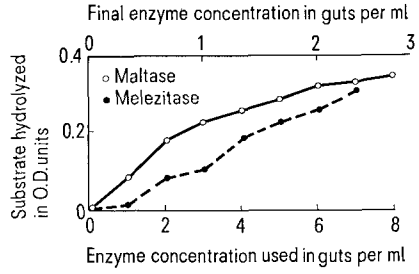

Fig. 4. Enzyme concentration-substrate hydrolysis curves of the larvae of *S. inferens*.

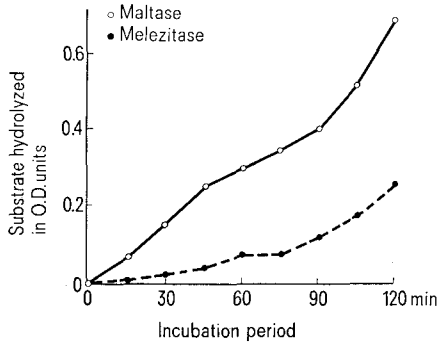

Fig. 3. Incubation period-substrate hydrolysis curves of the larvae of *S. inferens*.

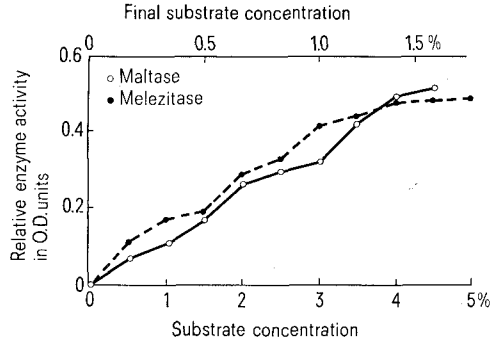

Fig. 5. Substrate concentration-enzyme activity curves of the larvae of *S. inferens*.

tions of the enzyme ranging from 1 midgut per ml to 8 midguts per ml were used in the reaction mixture. To see the effect of end-products, corresponding sugars were added to the reaction mixtures; in controls they were added after stopping the reaction. The effect of dialysis on the melezitase activity was observed after dialyzing the enzyme extract against distilled water at 4 °C for 24 h. To see the effect of ions, different concentrations of KCl, $LiCl_2$ and *Tris* salt solutions were added to the reaction mixtures so as to bring their strength in different mixtures from 0.001 *M* to 1.025 *M*; and then relative melezitase activity was determined.

Results and discussion. The optimum activity of melezitase and of maltase from midguts of *S. inferens* was at pH 6.2 (Figure 1). KRISHNA[4] found 2 pH optima, 4.8 and 6.0 in case of melezitase from the gut of the larva of *Trogoderma*, while maltase from different insects showed optimal activity between pH 5.2–6.8[5–8]. The pH range of the midgut of *S. inferens* is 7.9–8.2. At this pH the activity of melezitase and maltase will be very low, that is only 20–26% and 13–18% of the optimum respectively.

Melezitase and maltase showed optimum activity at temperature of 35 and 40 °C respectively (Figure 2). As the larvae were reared at 32 °C, at this temperature the activity of melezitase and maltase will be 87.5% and 64% of the optimum (at pH 6.2) respectively.

The increase in the incubation period (Figure 3), the enzyme concentration (Figure 4) and the substrate concentration (Figure 5) enhanced the rate of hydrolysis of the substrates and the concentration of the hydrolytic end-products. The latter inhibited the activity of the enzymes.

Dialysis reduced the activity of melezitase by 4.8%; K^+ and Li^{++} ions increased its activity upto 0.001 *M* final concentration and thereafter an increase in their concentraion inhibited its activity; while *Tris* increased the activity at 0.025 *M* final concentration by 85.62%.

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Latency of Taste Nerve Signals in Frog (*Rana catesbeiana*)

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Summary. The latency of frog gustatory neural impulses to 1.0 *M* NaCl was a mean of 86 msec. Electrical stimulation of taste cell membranes produced gustatory neural impulses with the mean 5 msec latency. It is concluded that most of the 86 msec latency of taste nerve responses to 1.0 *M* NaCl is due to the latency of taste receptor potential following the onset of gustatory stimulation.

Vertebrate taste cells make functional contact with gustatory nerve fibres. According to present knowledge, transduction of taste stimuli into neural signals can be described by the following scheme: a) adsorption of taste stimuli onto the taste receptor membrane[2]; b) receptor potential of taste cells[3–7]; c) postsynaptic potential at the subsynaptic nerve fibre membrane[8]; and d) generation of gustatory impulses at the nerve terminal[8]. Because of technical difficulty, there is no full physiological understanding of the time course between the successive events mentioned above. However, electrophysiological investigations have revealed that the time required for the whole process, from the onset of strong gustatory stimulation of tongue to the initiation of the first gustatory nerve impulse, is about 35–50 msec[9,10]. When such a latency of gustatory nerve impulses produced by taste stimulation is compared with that produced by electrical stimulation of gustatory cells and nerve terminals, analysis of factors associated with the latency would be feasible. With this approach, I attempted to examine the properties of latency of gustatory impulses and to analyze the factors determining the latency.

Materials and methods. Tongues of bullfrogs (*Rana catesbeiana*) anesthetized with urethane were used in the experiments. A fungiform papilla was fully drawn into a recording suction electrode filled with Ringer saline[11], and the action potentials which were conducted antidromically from other papillae to the gustatory nerve fibres of the suctioned papilla were recorded (Figure 1a). The presentation of taste solutions was done with a taste-stimulus-delivering device, composed of electric interval timers and solenoid valves[12]. A nozzle of the gustatory stimulator was put in the centre of the fungiform papillae population functionally connected with the papilla studied. The solution from the nozzle flowed over the tongue sur-

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