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Studies on the Salivary Glands of Rice Plant Leafhoppers

IV. Carbohydrase Activities

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α-Glucosidase which hydrolyzed sucrose and trehalose and β-glucosidase which acted on phenolic glucosides such as arbutin and salicin were found to occur in the salivary glands of *Nephotettix cincticeps* UHLER, *Inazuma dorsalis* MOTSCHULSKY, *Laodelphax striatellus* FALLÉN and *Nilaparvata lugens* STÅL. Cellulase (using carboxymethyl cellulose as a substrate), pectinase (pectic acid), amylase (soluble starch), α-galactosidase (melibiose), β-galactosidase (lactose) and α-fructofuranase (raffinose) were not detected. In the salivary glands of *N. cincticeps* and *I. dorsalis* both the α- and β- glucosidase activities were localized in the III-cells of the principal gland by histological methods. In the salivary glands of *L. striatellus* and *N. lugens* the α-glucosidase was demonstrated to originate in the G-follicle in the principal gland, but β-glocosidase in the B- and D-follicles.

INTRODUCTION

The earlier papers (SŌGAWA, 1967a, b) have dealt with the nature and origin of the sheath material which is an obvious salivary product that lines the path made by the stylets through the tissues of the plant to form the stylet sheath. The secretion of the sheath material appears to be an essential part of the feeding process of phytophagous hemipterous insects. This is, however, a specially modified function of the salivary glands in these insects. Undoubtedly the elaboration of digestive enzymes is their original and more general function, as in the insects belonging to the other orders. In fact, the occurrence of digestive enzymes in the salivary glands of hemipterous insects has long been known (e.g. BAPTIST, 1941; BRAMSTEDT, 1948; GOODCHILD, 1952; NUORTEVA, 1954). However such knowledge in the glands of leafhoppers seems to be still limited, only the investigations by HERFORD (1935), SAXENA (1954) and NUORTEVA (1956) are available. Also, the localization of the digestive enzymes in the salivary glands of leafhoppers has been yet unknown. The present studies were, therefore, undertaken in order to detect and localize the digestive enzymes, glucosidases, in the salivary glands of rice plant leafhoppers.

MATERIALS AND METHODS

Species examined. The following species were used at adult stage: *Nephotettix*

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cincticeps UHLER (Deltocephalidae), *Inazuma dorsalis* MOTSCHULSKY (Deltocephalidae), *Laodelphax striatellus* FALLÉN (Delphacidae) and *Nilaparvata lugens* STÅL (Delphacidae).

Method for paper chromatographic study. The salivary glands were taken out in the distilled water from the live insects and placed on a microscopic hollow glass. When 10 pairs of the glands were collected, 30 μl of substrate solution was deposited on them, and the glands were torn with fine needles within the drop of the substrate solution. The hollow glass was then confined in a moist vessel and maintained at 38°C for 24 hr. The substrate solutions were made up with each 10 mg of soluble starch, maltose, sucrose, trehalose, cellobiose, melibiose, lactose and raffinose per 1 ml of 1/30 M Sörensen's phosphate buffer, pH 6.0. In the cases of carboxymethyl cellulose and pectic acid, McIlvaine's phospho-citrate buffer, pH 4.5, was used. Also the solutions prepared by mixing 4 parts of 1% aqueous solutions of arbutin (hydroquinone-β-D-glucopyranoside), salicin (saligenin-β-D-glucopyranoside) and cellobiose and 1 part of McIlvaine's phospho-citrate buffer, pH 4.5, were used. After incubation, the solutions were directly subjected to paper chromatography in many cases. If necessary, protein was precipitated and removed from the incubated solutions by addition of ethanol followed by centrifugation. Each substrate solution alone was kept under the same condition and used as the control.

For the paper chromatographic assay, Toyo-filter paper No. 50 was used. The samples were developed ascendingly with *n*-butanol-acetic acid-water (4 : 1 : 5 or 4 : 1 : 2 v/v) solvents. Sugars and phenolic compounds on the filter paper were detected by benzidine-acetic acid (benzidine 0.5 g, glacial acetic acid 20 ml, water 80 ml) and phenol reagent (Ishizu Pharmaceutical CO., LTD.) followed by exposing to ammonium vapor respectively.

Method for histochemical study. The salivary glands were fixed in 10% formalin in 0.8% sodium chloride solution for 30 minutes, washed with distilled water for 10 min and incubated with substrate solution at 38°C for 6 hr. The composition of the substrate solution for α-glucosidase is as follows:—

1. 1 mg of 6-bromo-2-naphthyl-α-D-glucopyranoside (Sigma Chemical Company) dissolved in 0.5 ml of ethanol.
2. 1 ml of 1/15 M Sörensen's phosphate buffer, pH 6.0.
3. 3.5 ml of distilled water.

The composition of the substrate solution for β-glucosidase is as follows:—

1. 1 mg of 6-bromo-2-naphthyl-β-D-glucopyranoside (The Borden Chemical Company) dissolved in 0.5 ml of ethanol.
2. 1 ml of McIlvaine's phospho-citrate buffer, pH 4.5.
3. 3.5 ml of distilled water.

After the incubation period, the salivary glands were rinsed in distilled water and treated briefly with 0.1% aqueous solution of naphthanil diazo blue B containing 0.1% $NaHCO_3$. As a control, the salivary glands treated with boiling water were submitted to the same procedures.

RESULTS

1. *Carbohydrase activities in the salivary glands*

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Sucrose and trehalose were commonly hydrolyzed by the salivary glands of the four species of the rice plant leafhopper. A trace activity of maltase was exceptionally found in the glands of *N. cincticeps*, but not sufficiently to permit the

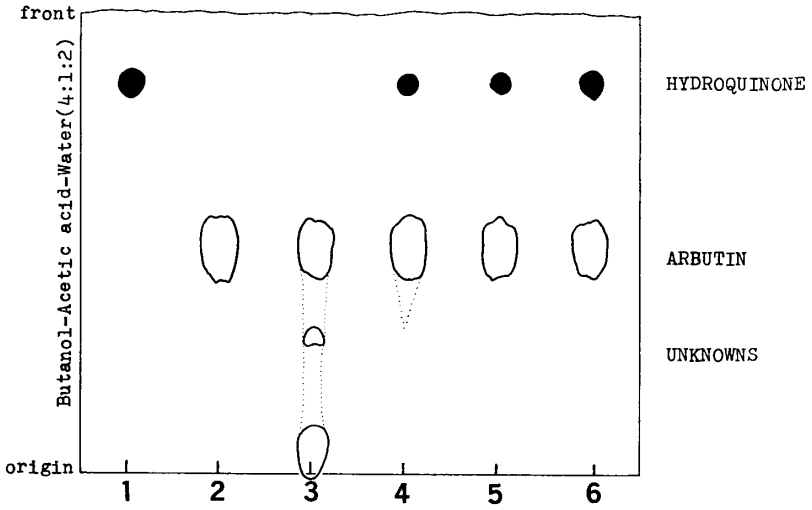

Fig. 1. The paper chromatograms showing β-glucosidase activities of the salivary glands of rice plant leafhoppers on arbutin as substrate. 1. Authentic hydroquinone, 2. Arbutin (check), 3. *N. cincticeps*, 4. *I. dorsalis*, 5. *L. striatellus*, 6. *N. lugens*. The solid spots, hydroquinone, were detected by the phenol reagent only. The outline spots, abrutin and unknowns, were colored by exposing to ammonium vapor after spraying the phenol reagent.

Table 1. Carbohydrate Activities in the Salivary Glands of Rice Plant Leafhoppers

Substrate	Enzyme	*N. cincticeps*	*I. dorsalis*	*L. striatellus*	*N. lugens*
Cellulose	Cellulase	−	−	−	−
Pectic acid	Pectinase	−	−	−	−
Starch	Amylase	−	−	−	−
Maltose	α-Glucosidase	±	−	−	−
Sucrose	α-Glucosidase α-Fructofuranase	+	+	+	+
Trehalose	α-Glucosidase	+	+	+	+
Cellobiose	β-Glucosidase	±	±	±	±
Arbutin	β-Glucosidase	+	+	+	+
Salicin	β-Glucosidase	+	+	+	+
Melibiose	α-Galactosidase	−	−	−	−
Lactose	β-Galactosidase	−	−	−	−
Raffinose	β-Galactosidase α-Fructofuranase	−	−	−	−

+ Presence, ± Trace, − Absence

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conclusion that the results is positive. Although cellobiose was not attacked at pH 6.0, it seemed to be slightly hydrolyzed at pH 4.5 by the salivary glands of the four species. The phenolic glucosides, such as arbutin and salicin, were also splitted into glucose and aglycons, hydroquinone and saligenin. However, in the case of hydrolysis of arbutin by the glands of *N. cincticeps*, unknown phenolic products were recognized instead of hydroquinone by the paper chromatography (Fig. 1). Cellulose, pectic acid, starch, melibiose, lactose and raffinose were not hydrolyzed. These results were summarized in Table 1.

2. Localization of glucosidases in the salivary glands

The sites of α- and β-glucosidase activities were examined histochemically. The principle of the method is as follows: the enzymic hydrolysis of the artificial glucosides, 6-bromo-2-naphthyl-α-D-glucopyranoside and 6-bromo-2-naphthyl-β-D-glucopyranoside, yields glucose and naphthol. The liberated naphthol is visualized by coupling with diazonium salt, naphthanil diazo blue B, to form colored, water-insoluble azo dyes.

The anatomical terms used here are the same as those used in the earlier paper (SŌGAWA, 1965).

α-Glucosidase. In the salivary glands of *N. cincticeps* and *I. dorsalis,* the III-cells gave positive coloration, purplish blue to deep blue (Fig. 2-A), while in the glands of *L. striatellus* and *N. lugens* the G-follicle was positive (Fig. 2-B). No coloration appeared in the glands treated with boiling water.

β-Glucosidase. The III-cells of *N. cincticeps* and *I. dorsalis* were positively colored in deep blue, as in the result of test for α-glucosidase (Fig. 2-A). On the other hand, in the salivary glands of *L. striatellus* and *N. lugens,* the B-follicle was intensely colored in deep blue, and the D-follicle was also colored to a reduced extent (Fig. 2-B). In this test, the IV-cells of *N. cincticeps* and *I. dorsalis* and the A-follicle of *L. striatellus* and *N. lugens* took up purplish shades. This seemed to be due to adsorption of the naphthol liberated from the site of enzyme activity to the incubation solution in these tissues. Completely negative results were shown by the glands treated with boiling water.

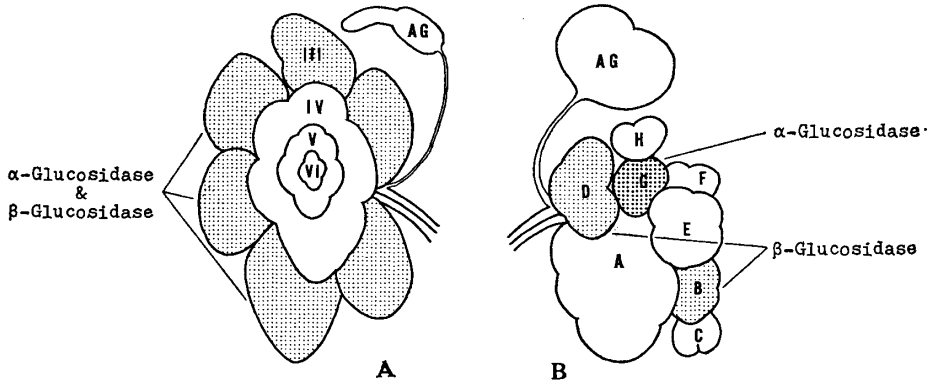

Fig. 2. The localization of α-glucosidase activities in the salivary glands of *N. cincticeps* and *I. dorsalis*(A) and in those of *L. striatellus* and *N. lugens*(B).

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DISCUSSION

Invertase and trehalase activities were found to occur commonly in the salivary glands of the four species of the rice plant leafhopper. Concerning to the invertase activity in the salivarys glands, it is of interest to note that a main sugar in the rice plant is sucrose (e. g. MURAYAMA et al., 1955). It is apparent that the salivary invertase plays an important role in the digestion of sucrose ingested; and the assumption, which is generally applicable, that a correlation can be drawn between the presence of a high concentration of a specific substance in the food and the presence of corresponding digestive enzymes is true in this case. The invertase in the salivary glands was not α-fructofuranase but α-glucosidase, because raffinose with free fructosyl unit was not hydrolysed. This salivary invertase is assumed to act largely within the alimentary canal after it has been swallowed with the plant juice. On the other hand, trehalase activity in the salivary glands is somewhat problematical since trehalose is not apparently a normal constituent of the rice plant. In view of the evidence that trehalose is important as a blood sugar in insects, the trehalase may be an intracellular enzyme and take no part in digestion. There is also another possibility that both the invertase and trehalase activities are due to the same α-glucosidase. In spite of the occurrence of strach in the plant tissues, no amylase activity was detected in the salivary glands of the rice plant leafhoppers. This seems to be connected with their feeding habits as indicated by SAXENA (1954), who has shown that amylase is present in the salivary glands of mesophyll feeders but not in those of phloem feeders. Of four species examined here, *N. cincticeps* and *L. striatellus* have been found to suck from the vascular bundles (NAITO and MASAKI, 1967; SONKU and SAKURAI, 1965). In addition to the α-glucosidase activities, a β-glucosidase activity which was in effect preferably on phenolic glucosides was detected in the salivary glands of the rice plant leafhoppers. Using the histochemical techniques it was demonstrated that the α- and β-glucosidase were elaborated in the same secretory cells, the III-cells, of the principal salivary glands of *N. cincticeps* and *I. dorsalis*. This indicates that the both types of glucosidase are simultaneously discharged. It is, however, uncertain whether or not the β-glucosidase serves as a digestive enzyme since its natural substrate is obscure. On the other hand, in the salivary glands of *L. striatellus* and *N. lugens*, both the glucosidases come from different secretory cells. The α-glucosidase activity was found to originate in the G-follicle, while the β-glucosidase in the B- and D-follicle. The localization of the α-glucosidase gives rise to some discussion, because the G- and H-follicle have been demonstrated to contain an unsaturated lipid and regarded as a source of the stractural precursor of the sheath material (SŌGAWA, 1967b). The sheath materials discharged by the rice plant leafhoppers are evidently sudanophilic, indicating the presence of lipid component (SŌGAWA, 1967a). It is difficult to account that the digestive enzyme is secreted along with the sheath material. Similar conflicting evidence can be quoted from the studies on the salivary glands of a plant bug, *Oncopeltus fasciatus* (DALL.). MILES (1960) has shown that the contents of the lateral lobe of the principal salivary glands are sudanophilic in the histochemical property and enter into the composition of the sheath material. But BRONSKILL et al. (1958) have found strong proteinase and lipase and weak amylase and invertase in this lateral lobe (the terms used for

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the lateral and posterior lobes are the reverse of those used by MILES.). These enzymes have been proved to be discharged into the feeding medium by FEIR and BECK (1961). In the cases of *N. cincticeps* and *I. dorsalis*, it is very possible that the lipid moiety of the sheath materials is secreted from the V-cells which produced no digestive enzyme (SŌGAWA, 1967b). However, it is of interest to point out that an another type of secretory cells, the III-cells, contains an unsaturated lipid (SŌGAWA, 1967b) as well as α-glucosidase. The histochemical reactions for lipids in the II-cells are much resembling to those in the G-follicle of *L. striatellus*. This fact indicates that some fraction of the lipid secretion of the salivary glands of the rice plant leafhoppers has no connection with the formation of the stylet sheath, and functions, for example, as lubricant or vehicle of the digestive enzymes. Through the above discussion, it is possible that only the H-follicle is a true source of the lipid moiety of the sheath material, and the G-follicle is originally the secretory tissue for digestive enzyme. There the possibility will remain that the lipid secretion of the G-follicle enter adventitiously into the sheath material. MILES (1959 a, b) has ascertained that a certain plant bug and aphid secrete a watery saliva other than the sheath material and sucked back with nutrient fluid. DAY et al. (1952) has also suggested the secretion of such watery saliva by a leafhopper, *Orosius argentatus* (EVANS). This type of saliva seems to be a vehicle for the digestive enzymes in the salivary glands.

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