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ENZYMATIC ACTIVITY IN THE INTESTINAL WALL CELLS DURING
METAMORPHOSIS OF THE HAWK MoTH (CELERIO EUPHORBIAE)

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The breakdown of disaccharides (maltose, lactose, saccharose, trehalose) and
the activity of \( \gamma \)-glutamyltransferase (EC 2.3.2.2), cobalt-activated acylase, leucyl-
aminopeptidase (EC 3.4.1.1) and alanylaminopeptidase (EC 3.4.1.2) are higher in the
intestines of feeding caterpillars and "running" caterpillars than in hibernating
pupae and adult moth; the ability to degrade lactose and trehalose is null in adult
moth and the activity of the peptidases is the lowest.

Non-phosphorylating transglycosylation was studied using p-nitrophenyl-N-malto-
side and p-nitrophenyl-N-lactoside as donors and acceptors of glucosyl residue;
the reaction with p-nitroaniline malsoside was the highest in caterpillars, threefold
lower in pupae and 15-fold lower in adult moth. Maltose was the most efficient
donor with p-nitroaniline-N-glycoside, and saccharose with p-nitrophenyl-N-ribose.

Biochemical changes associated with metamorphosis of the hawk moth have
been studied by Heller & Piechowska (1971). They also reported on the activity of
\( \alpha \)-amylase in the hawk moth tissues, including the intestine. We have extended these
studies and examined hydrolysis and transglycosylation of disaccharides and the
peptidase activity in the extracts from the hawk moth intestine.

The developmental cycle of the hawk moth involves three main stages: feeding
caterpillar, pupa and moth, and some intermediate metamorphic stages, such as
"running" caterpillars and "spindle" stage. All these stages are characterized by
specific digestive function of the intestine, and also changes in the kind of food and
manner of feeding. Caterpillars of \textit{C. euphorbiae} belong to monophagous animals,
as they feed on one plant only (\textit{Euphorbia ciperissias}). This precludes the effect of
changes in the food on the enzymatic activities of the intestine. In the period preceding
pupation, caterpillars cease feeding and "run" for a proper place for pupation;
at that time they empty their digestive tract of food remnants, and the digestive
function of the intestine declines. In pupae the intestine serves mainly as the organ
collecting final products of metabolism, which are eliminated in the form of the

[173]
so-called meconium at hatching of the adult moth. During their short life the adult moth feed only occasionally, sucking nectar through a coiled syphonet which they insert into the interior of the flower still keeping in flight (this gave rise to the taxonomic name of the family: the hawk moth, Sphingidae).

In the present work we have attempted to relate the digestive function of particular enzymes with the kind of food and the metamorphic stage of the hawk moth. Data on the transglycosylation ability of the digestive enzymes provide additional information on characteristics of intestinal hydrolases of C. euphorbiae during metamorphosis.

**Materials and Methods**

**Material.** The intestines from caterpillars at the last feeding period (after the last moulting), "running" caterpillars, hibernating pupae and adult moth were dissected, immediately washed with cold distilled water to remove their contents, blotted dry on filter paper and weighed. The extracts were prepared in a Potter homogenizer at 0 - 2°C in 0.05 M-phosphate buffer, pH 7.0 (4:1, v/w). The extract was centrifuged at 3000 g for 15 min was used for the experiments.

**Assay methods.** The activity of γ-glutamyltransferase and cobalt-activated acylase were assayed with γ-L-glutamyl-α-naphthylamide and L-butyryl-γ-L-glutamyl-β-naphthylamide, respectively, using the reagent kits prepared by the Sera and Vaccines Plant (Kraków, Poland). The activity of leucylaminopeptidase was measured with L-leucyl-β-naphthylamide, and that of alaninaminopeptidase with L-alanyl-β-naphthylamide as described by Sobiech & Szewczuk (1974a,b).

The breakdown of maltose, lactose, saccharose and trehalose was assayed under conditions described by Dahlqvist (1961), and glucose liberated was determined with Glucostat (Worthington, Freehold, N.J.; U.S.A.).

The transglycosylation activity was assayed in two systems: I, homologous, i.e. the same oligosaccharide, pN(Glc)₂ or pN(GalGlc) served both as donor and acceptor of glucosyl residue; II, heterologous, i.e. a disaccharide served as a donor, and p-nitroaniline monosaccharide as an acceptor of the transferred sugar residue. In the latter system, p-nitroaniline N-glycosides of glucose, mannose, lyxose and ribose served as acceptors, whereas maltose, isomaltose, cellobiose, trehalose, saccharose and lactose as donors. Donors and acceptors of sugar residues were used at final concentration of 40 mM. Incubations were carried out in 100 mM-acetate buffer, pH 5.0, at 30°C. The reaction products were separated by chromatography and determined quantitatively (Hutny & Kossobudzki, 1968). The enzyme activity was expressed in nmol/min per mg protein.

Protein was determined colorimetrically (Bradford, 1976) using bovine serum albumin as standard.

**Reagents.** p-Nitrophenyl-N-glucoside¹ and p-nitrophenyl-N-maltoside were synthesized as described by Kossobudzki (1967). p-Nitroaniline N-glycosides of ribose,

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¹ Abbreviations used: Glc, glucose; pNGlc, p-nitrophenyl-N-glucoside; pN(GalGlc), p-nitrophenyl-N-galactoside; pN(Glc)₂, p-nitrophenyl-N-maltoside; pN(Glc)₃, p-nitrophenyl-N-maltotrioside; pNGal, p-nitrophenyl-N-galactoside; pNRib, p-nitrophenyl-N-riboside.
lyxose, mannose and lactose were prepared by the method of Kossobudzki & Wróbel-Twarowska (1977). L-Alanyl-β-naphthylamide was from Ferak (West Berlin), glucose from Loba Chemie (Vienna, Austria), maltose from Reachim (U.S.S.R.), trehalose from Chemapol (Prague, Czechoslovakia), and cellobiose from the United Pharmaceutical Works (Czechoslovakia). Other reagents were supplied by POCh (Gliwice, Poland).

RESULTS AND DISCUSSION

Hydrolysis of disaccharides

Maltose, saccharose, lactose and trehalose were hydrolysed by various disaccharidas- ses (Fig. 1). Maltose and saccharose were substrates for several α- and β-glycosidases, and the breakdown of these disaccharides was the greatest. The rate of hydrolysis of lactose and trehalose was lower, and each sugar was hydrolysed by a single specific enzyme: β-galactosidase and trehalase, respectively.

![Activity graphs for saccharose, maltose, lactose, and trehalose](image)

Fig. 1. Glycosidase activity with the disaccharides indicated, in the extracts from the intestine of feeding caterpillars (1), “running” caterpillars (2), pupae (3) and moth (4) of Celerio euphorbiae. The reaction was carried out in 50 mM-phosphate buffer, pH 7.0, containing 5 mM-NaCl at 37°C.

The highest hydrolytic activity with all the disaccharides studied was in the intestinal extracts from feeding caterpillars. At the later developmental stages this activity became lower, the decrease starting at the phase of "running” caterpillars. In hibernating pupae and adult moth, trehalose and lactose were practically not hydrolysed. The breakdown of saccharose underwent relatively the smallest changes; it became somewhat lower in "running” caterpillars and in pupae, and again was
slightly higher in the intestine of adult moth. The maintenance of the saccharose-
hydrolysing activity, and also almost unchanged degradation of maltose during
metamorphosis were probably due to the occurrence of saccharose, melicitose and
maltose both in the leaves and flower nectar of *Euphorbia ciperissias* (Bailey, 1975).
The early results of Stober (1927) suggested the presence of invertase in the hawk
moth intestine.

Of special interest are changes in trehalose breakdown; this disaccharide serves
in several insects as a glucose carrier. Distribution of trehalose in *C. euphorbiae*
tissues was studied by Mochnacka & Petryszyn (1959). They have demonstrated that
trehalose appears in haemolymph of "running" caterpillars and pupae, whereas
in other tissues it was synthesized only in pupae but vanished prior to the emergence
of the imago. Besides, trehalose was not found by those authors in surge leaves.
Our results indicate that trehalase activity is the highest in the intestines of feeding
caterpillars, and is practically absent in intestines of pupae and adult moth. It seems
possible that the lack of trehalase in the tissues of feeding caterpillars, observed
by Mochnacka & Petryszyn (1959) could be due to its hydrolysis by trehalase, whereas
accumulation of the disaccharide could result from low activity of the enzyme or
its absence.

On comparing the rate of disaccharide hydrolysis by extracts from intestines
of feeding caterpillars and from mammalian small intestines it is evident that hydro-
lysis of maltose in caterpillars is higher by a factor of five than in piglets (Hall *et al.*,
1983), whereas breakdown of saccharose, trehalose and lactose is similar to the
values reported for porcine (Dahlqvist, 1961; Stevens & Kidder, 1972) and bovine
intestines (Siddons, 1968).

*Transglycosylation*

At all developmental stages of the hawk moth, non-phosphorylating transglyco-
sylation with pN(Glc)$_2$ both as a donor and acceptor of glucosyl residues showed
a pH optimum of 5.0 - 5.2, but a high activity was still observed over the pH range
from 4.4 to 6.0.

Also extracts from the hawk moth intestine catalysed, although to a smaller
extent, transglycosylation in which pN(GalGlc) was both an acceptor and donor
of the glucosyl residue.

Transglycosylation with pN(Glc)$_2$ in the intestine was 3 - 5 times higher in cater-
pillars than in pupae, and 13 - 19 times higher than in the adult moth (Table 1).
The differences in transglycosylation with p-nitrophenyl-$\beta$-lactoside at the successive
developmental stages were much less pronounced; the highest values were observed
in caterpillars, the lowest in adult moth. However, it should be noted that liberation
of pNGlc and pNGal was about three times as high as that of p-nitrophenyloligo-
saccharides. This results from the hydrolysis of the substrate which occurs independ-
ently of transglycosylation.

It was found that maltose was a more efficient donor of glucosyl residues than
saccharose, lactose, trehalose and cellobiose in the transglycosylation reaction with
p-nitroaniline $N$-glycosides of glucose, mannose, ribose and lyxose as acceptors. The results obtained (Fig. 2) indicate that pNGlc and pNRib are the best acceptors. The remaining p-nitroaniline $N$-glycosides showed but traces of acceptor properties.

![Graph](image)

Fig. 2. Transglycosylation with pNGlc (A) or pNRib (B), and maltose (1), isomaltose (2), cellobiose (3), saccharose (4), lactose (5) in the intestine of feeding and “running” caterpillars, pupae and moth of _Celerio euphorbiae_ during metamorphosis.

Formation of pN(Glc)$_2$ was several-fold higher with maltose than with other disaccharides in pupae and adult moth. In the extracts from caterpillars, formation of pN(Glc)$_2$ was also detected. With trehalose, no transglycosylation products were found. This should be ascribed either to low trehalase activity in _C. euphorbiae_ intestine, or to a lack of transferase activity of this hydrolase. This last possibility has been confirmed in the case of the enzyme from pig intestine (Dahlqvist, 1960). It is noteworthy that also saccharose can be a good donor of the sugar residue, since p-nitrophenoxyoligosaccharides were among the reaction products. Similarly as with pN(Glc)$_2$, also the highest activity towards disaccharides and pNGlc or pNRib was found in caterpillars. In general, transglycosylation was twice or three times higher in the homologous than in the heterologous transferase systems.

In the life cycle of _C. euphorbiae_, caterpillars feed much more greedily than adult moth, and their intestinal glycosidases show much higher activity as compared with pupae or adult moth. This concerns both the hydrolytic and transferase activities. In pupae, the differences between the homo- and hetero-transglycosylation systems
Table 1

Non-phosphorylating transglycosylation in the intestine of the hawk moth (Celerio euphorbiae) during metamorphosis

The results are mean values ± S.D. from 6 determinations and are expressed in nmol/min per mg protein. Each determination was made in samples from 5 individuals (caterpillars) and 8-10 individuals (pupae and moth).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Substrate:</th>
<th>p-nitrophenyl-N-maltoside</th>
<th>p-nitrophenyl-N-lactoside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Product:</td>
<td>pNGlc</td>
<td>pN (Glc)₃</td>
</tr>
<tr>
<td>Feeding caterpillars</td>
<td>1781.6±190.1</td>
<td>575.9±61.8</td>
<td>347.5±37.2</td>
</tr>
<tr>
<td>&quot;Running&quot; caterpillars</td>
<td>1155.0±114.1**</td>
<td>402.1±50.1**</td>
<td>370.6±46.3</td>
</tr>
<tr>
<td>Pupae</td>
<td>379.5±29.8**</td>
<td>156.7±11.2**</td>
<td>142.9±10.1**</td>
</tr>
<tr>
<td>Adult moth</td>
<td>52.6±7.6**</td>
<td>31.6±3.1**</td>
<td>98.7±7.5**</td>
</tr>
</tbody>
</table>

* p≤0.1; ** p≤0.01.
were insignificant. In the adult moth, transglycosylation was very low and could
be detected only with maltose as a donor.

The heterotransglycosylation activities with pNRib as an acceptor of sugar
residue (Fig. 3) and various disaccharides as donors were similar to those with
pNGlc. The extract from the intestine of adult moth showed no transglycosylation
activity with pentose as an acceptor. At variance with the results with pNGlc, sac-
charose proved to be a better donor than maltose for the pNRib acceptor.

![Graph showing enzyme activities](image)

Fig. 3. The intestinal peptidase activity in feeding (1) and “running” (2) caterpillars, pupae (3) and
moth (4) of Celerio euphorbiae. Conditions of the reaction are given under Materials and
Methods.

Transglycosylation of intestinal enzymes of animals and man, studied under
similar conditions, was lower than the activity found in the caterpillar intestine.
Differences were observed also in the optimum pH value, which for vertebrates
is 6.0 (Sobiech & Kotoński, 1980; Kotoński et al., 1983) and for the hawk moth
about 5.0.

\[ \gamma \text{-Glutamyltranspeptidase, cobalt-activated acylase, leucylaminopeptidase and alanyl-
aminopeptidase} \]

The activity of the peptidases studied declined gradually during metamorphosis
of *C. euphorbiae*. The activity of \( \gamma \)-glutamyltranspeptidase in the intestines of cater-
pillars and pupae (Fig. 3) was lower than in the small intestine of man and other
animal species (Sobiech, 1981). On the other hand, its activity in the feeding caterpillar was higher than in the intestine of bee, pigeon, guinea pig and mouse (Sobiech & Szewczuk, 1974b; Sobiech, 1981), and lower than in rabbit, hen, pig, hamster and man (Sobiech & Szewczuk, 1977; Sobiech, 1981).

The activity of cobalt-activated acylase in caterpillar intestine was half that observed in rat, threefold lower than in rabbit, five times lower than in hamster and mouse, and 12 times lower than in guinea pig (Słowińska et al., 1978).

The activity of leucylaminopeptidase in feeding caterpillars was three times higher than in guinea pig (Sobiech & Szewczuk, 1974a). The alanylaminopeptidase/leucylaminopeptidase ratio in caterpillar intestine was 1.6, whereas in the serum and tissues of man it exceeds 2.0 (Wieczorek & Sobiech, 1979).

The results presented show the decline of the enzymatic activities during metamorphosis, associated with changes in the digestive function of the intestine. Changes in the activity of the enzymes studied do not follow the same pattern. It seems that this is due to the changes in concentration of the enzymatic substrates in food. These enzymes, the substrates for which are present both in leaves and nectar, retain their activity, whereas the activity of those enzymes declines, for which the substrates are no more available in nectar.

REFERENCES


AKTYWNOŚĆ ENZYMATYCZNA W JELICIE MOTYŁA WILCZOMLECZKA (CELERIO EUPHORBIAE) W CZASIE METAMORFOZY

Streszczenie

Rozkład disacharydów i aktywność γ-glutamylotransferazy, acylazy aktywowanej przez kobalt, leucyloaminopeptydazy i alanyloaminopeptydazy była wyższa w jelitach gąsienic żerujących i „biegających” w porównaniu z poczwarkami i motylami. U dorosłych motyli zanika zdolność rozkładu laktozy i trehalozy oraz najniższa jest aktywność peptydaz.


Received 16 September, 1983;
Revised 15 November, 1983