Eur. J. Entomol. **94**: 167–176, 1997
ISSN 1210–5759

Distribution, stability and binding specificity of a hemagglutinin in the corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae)

OLUSOLA A. ADEYEYE and JING-QIU CHENG

Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282, USA

Noctuidae, *Helicoverpa zea*, lectin, hemagglutinin, binding, erythrocytes

Abstract. A hemagglutinin (HA) was detected during all developmental stages of the corn earworm *Helicoverpa zea*. The HA titer was relatively low at the onset of each developmental stage; it increased very rapidly during each stage and declined considerably just before the onset of the next. The highest HA titer of 4,096 was attained during days 3–5 of fifth-instar larvae. In contrast, the lowest titer of 8 was obtained in adult insects. Female pupal and adult *H. zea* had higher HA titers than their male cohorts. The hemagglutinin agglutinated red blood cells (RBCs) from a broad range of animals. Its binding specificity was unusual in that the hemagglutination was inhibited by sucrose, N-acetylneuraminic acid, lactose, galactose, maltose, galactosamine and N-acetyl-galactosamine in that order of decreasing effect. The midgut fluid of *H. zea* larvae contained agglutinins with a specificity that suggests that they were exogenously derived from dietary ingredients. Unlike *H. zea* hemagglutinin, which failed to agglutinate fresh mouse erythrocytes, hemagglutinins found in the diet and midgut fluid caused strong agglutination of mouse erythrocytes. HA titer fluctuated between and within the various life stages. Unlike many insect lectins, *H. zea* hemagglutinin is relatively stable after being subjected to repeated cycles of freezing and thawing. Although it is rapidly inactivated at ≥ 40°C, this lectin is relatively stable at 20°C. Since it is inhibited by 5 mM EDTA, it is probable that the binding action of this lectin is dependent on the presence of divalent cations.

INTRODUCTION

Lectins are ubiquitous proteins; they have been found in plants, animals and microorganisms. They agglutinate cells and bind sugars specifically and reversibly (Nakamura et al., 1992). Indeed, as Sharon & Lis (1990) noted, the carbohydrate specificity of lectins is exploited conventionally by the hapten inhibition technique, in which different monosaccharides, oligosaccharides, or glycopeptides are tested for their ability to inhibit either hemagglutination or polysaccharide precipitation by the lectin. Agglutination by lectins is also inhibited differentially by some oxidizing agents and chelating agents. Although there are more than 20,000 records of lectins from diverse groups of organisms (Gauss, 1993), much remains to be elucidated about their precise physiological and ecological roles (Sharon & Lis, 1990; Zelck & Becker, 1992). Even so, it is known that soluble, as well as membrane-bound lectins, participate in the processes of cell adhesion, opsonization, phagocytosis, and cytolysis (Vasta & Marchalonis, 1984). Thus, they are important for the recognition of non-self and cellular defense (Sharon & Lis, 1989) and are involved in cell differentiation (Grubhoffer & Maťha 1991). In particular, lectins occurring as integral proteins on hemocyte surfaces enable hemocytes to bind directly to carbohydrate determinants on target cells (Renwrantz & Stahmer, 1983; Richards & Renwrantz, 1991).

Conversely, cell surface lectins of target cells may bind to carbohydrates on hemocytes (Zelck & Becker, 1992).

Because of the involvement of lectins in the recognition of non-self, a number of studies have concentrated on the role of lectins in parasite-host relationships (Lehane & Msangi, 1991; Grubhoffer & Dusbábek, 1991; Grubhoffer & Maťha, 1991; Grubhoffer et al., 1991; Mohamed et al., 1992; Kawauchi et al., 1993; Kopáček et al., 1993; Volf et al., 1993). Thus, the occurrence, tissue distribution and immunochemical characteristics of the lectins of a few ectoparasitic arthropods are well described. However, few studies have focused on the role of lectins in the dynamic interactions between plants and insect herbivores. Pusztai et al. (1993) reported that plant lectins act as anti-nutritive growth factors in rats. Plant lectins may also function as protective allelochemicals against insect herbivores. Huesing et al. (1991) and Habibi et al. (1993) found, respectively, that the cowpea weevil *Callosobruchus maculatus* and the leafhopper *Empoasca fabae* were adversely affected by plant lectins. Can insect lectins conversely function as a defense against dietary toxins? If so, lectin activity should be higher during the actively feeding stage of a holometabolous insect because it is at this stage that they must detoxify dietary toxins. The polyphagous herbivore, *Helicoverpa zea* Boddie, is a major crop pest that feeds on corn, cotton, tobacco, and legumes. Feeding as it does on diverse plants, *H. zea* must cope with the ingestion of many types of protective allelochemicals including host plant lectins. *H. zea* is, therefore, an ideal insect model for studying the role of lectins in plant-insect-herbivore interaction. The present report focuses on the distribution, stability, and specificity of an agglutinin found in the tissues of the corn earworm, *H. zea*.

MATERIAL AND METHODS

Erythrocytes, reagents and insects

Twelve types of red blood cells (RBCs) were used to determine the hemagglutinating specificity of corn earworm lectin. Fresh rabbit and mouse blood cells were collected from laboratory animals and washed in buffered physiological saline (BPS). A 2% (V/V) PBS suspension of these cells was used for hemagglutination assays. All chemicals and the remaining 10 erythrocyte types were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). The erythrocytes were glutaraldehyde-stabilized and lyophilized; they include three human erythrocyte types (groups A, B and O), and erythrocyte types from six other mammals (horse, sheep, porcine, rat, guinea pig, rabbit) and chicken. Fertilized eggs of corn earworms were obtained from the USDA Insect Biology and Population Dynamics lab in Tifton, Georgia. The insects were reared at 25°C with a 14L : 10D photoperiod. The insects were allowed to feed ad libitum on a pinto bean and wheat germ based artificial diet as described by Perkins et al. (1973). Eclosing adults were fed on a 10% sucrose solution. Under these conditions, the larval development lasted approximately 13 days. The first, second, third, fourth, and fifth larval instars lasted approximately 1.5, 1.5, 2.0, 2.5 and 5.5 days, respectively; pupal stage lasted 10 days.

Hemagglutinin distribution among corn earworm tissues and diet

Hemolymph, the gut, and fat body were collected from larval, pupal and adult insects. In addition, the wing pad and reproductive organs were collected from pupal and adult insects, respectively. Because of the difficulty of collecting sufficient volume of hemolymph from earlier instars, hemolymph was collected from the third larval instar onwards. To collect larval hemolymph, the first and second pairs of abdominal legs of the larvae were ablated, and the hemolymph collected in a pre-cooled phosphate buffered physiological saline (PBS, 0.02 mM PBS in 0.87% NaCl, 0.03% phenylthiocarbamide and 0.01% phenylmethylsulfonyl fluoride, pH 7.2) in a ratio of 1 : 3, hemolymph to PBS. Pupal hemolymph was collected by puncturing the wing pad region of the pupal integument. Because adult *H. zea* insects have considerably less hemolymph, samples were obtained by dissecting the abdomen and rinsing the internal organs

and tissues in PBS (5 adults to 1 ml PBS). The mixture was centrifuged at 13,000 rpm for 10 min at 5°C, and the supernatant collected and stored at −10°C until it was tested. Infranatant hemocytes were collected from 20 ml of hemolymph, and washed five times in PBS to ensure total removal of plasma. They were subsequently suspended in PBS; 1 volume of hemocytes was suspended in 9 volumes of PBS. The fat body, gut minus content, reproductive system and wing pads were collected from the respective specimens which were dissected in an ice-cold bath; these organs and tissues were rinsed thoroughly in cold PBS. Organs and tissues were homogenized separately with a tissue homogenizer (model 985–370, Biospec Products Inc., USA). The homogenates were centrifuged at 13,000 rpm for 10 min at 5°C; the supernatants were collected and stored as above. Hemolymph or tissue extract samples were pooled from a group of ten insects; they were microtitrated against fresh rabbit RBCs to detect hemagglutination. Each analysis was replicated twice.

PBS extracts of pinto beans, wheat germ, yeast and the combined meridic diet of *H. zea* were assayed against rabbit erythrocytes. Their binding specificity was compared with those of the hemolymph, midgut and midgut fluid.

Hemagglutination activity (HA) titer

Two-fold serial dilutions (12.5 µl of sample plus 12.5 µl PBS) of hemolymph samples were placed in 96 well V-shaped microtitration plates. Subsequently, 25 µl of 2% rabbit erythrocyte suspension in PBS was added into each well. The plate was vortexed for 30–60 s. In our preliminary microtitration assays, duplicate samples were kept either at room temperature for 1 h or at 5°C over-night, before observing the hemagglutination reaction. Because both conditions yielded identical results, most assays were carried out at room temperature for 1 h. The reciprocal value of the highest dilution of a sample with a positive reaction was designated as the titer of the sample. The protein concentrations of the hemolymph samples were determined spectrophotometrically using the Bradford Coomassie Assay (BCA) kits by Pierce (Pierce Chemical Co., Rockford, Illinois, USA).

Binding specificity of corn earworm hemagglutinin

We elected not to determine the inhibitory effects of a barrage of oxidizing and chelating agents against *H. zea* lectin activity. Rather, because ethylenediamine tetraacetic acid (EDTA) and dl-dithiothreitol (DTT) are ingredients of the hemolymph bleeding solution routinely used in our laboratory, we determined the effects of EDTA and DTT on the agglutinating potency of *H. zea* lectin. Hemagglutinin binding specificity was determined by microtitrating *H. zea* hemolymph against the different types of erythrocytes. Further, five monosaccharides, five disaccharides and six sugar derivatives (see Table 2) were tested to determine their inhibitory effect on corn earworm lectin. Sugars were serially diluted two-fold in PBS to a final volume of 12.5 µl in the wells of the microtitration plates. Then, 12.5 µl of hemolymph sample having a HA titer of 4 was added into each well. The plate was kept at room temperature for 30 min to ensure binding. Subsequently, 25 µl of fresh rabbit RBCs were added to the wells, mixed and the results obtained after 1 h at room temperature.

Stability of corn earworm hemagglutinin

Hemagglutinins differ considerably in their stability during storage. Two independent sets of experiments were conducted to ascertain the stability of *H. zea* hemagglutinin. First, samples were subjected to repeated cycles of freezing and thawing. Hemolymph samples were placed into Eppendorf tubes and frozen at −10°C for 30 min. Afterwards, the tubes were transferred into water bath for 15 min at 20°C to thaw the samples. The HA titer was subsequently determined using fresh rabbit RBCs. Independently, fresh hemolymph samples from same group of insects were subjected to short-term (2 to 6 h) and long-term (24 to 96 h) storage at different temperatures (5, 20, 40 and 60°C). At scheduled intervals, the lectin titers were determined by hemagglutinating microtitration against fresh rabbit erythrocytes.

To tests the effects of EDTA and DTT on hemagglutination activity, hemolymph (12.5 µl) was added into Eppendorf tubes containing 12.5 µl PBS. PBS buffered solutions of ethylenediamine tetraacetic acid (EDTA) or dl-dithiothreitol (DTT) were introduced into each tube to obtain the hemolymph samples containing 1 mM or 5 mM EDTA or DTT. The samples were incubated at 20°C, and their HA titers were determined as outlined above.

RESULTS

Hemagglutinin distribution among corn earworm tissues

Plasma (hemocyte free) showed high HA titers regardless of the insect's developmental stage or sex (Table 1). Larval and pupal hemocytes also had high HA titers. HA was not detected in the fat body and reproductive system of *H. zea*. The midgut of larval, pupal and adult male insects had no HA. By contrast, the midgut of adult female insects consistently had a weak hemagglutination reaction. Likewise, very low HA titers were detected in extracts from the pupal wing pads. The HA titer in the washing solutions (supernatants) was the same as that of the hemolymph and decreased very slowly following washing. After five washings, the decrease in the HA titer was less than 50%. This result suggested that the lectin of the corn earworm was located on the hemocytes.

A very high HA titer also was obtained for the midgut fluid of the corn earworm larvae. However, we subsequently found that the erythrocyte binding specificity of the midgut fluid differed from those of all other *H. zea* tissues (Table 1) but was identical with those of the diet (Table 3). It was easy to distinguish corn earworm HA from diet HA by checking them with fresh mouse erythrocytes; *H. zea* lectin failed to react with mouse erythrocytes while lectin in the diet reacted quite strongly with mouse erythrocytes.

TABLE 1. The distribution of hemagglutination activity (HA) among various corn earworm tissues*.

Tissue extracts	5th instar larva (3-day-old)	Pupa (2-day-old)		Adult (4-day-old)	
		Male	Female	Male	Female
Hemolymph	+++	+++	+++	++	++
Hemocytes	+++	++	++		
Fat body	–	–	–	–	–
Midgut	–	–	–	–	+
Midgut fluid	+++	–	–		+
Hindgut	–	–	–		
Reproductive system	–	–			–
Wing pads of pupa		+	+		

* Hemagglutination activity was determined using fresh rabbit RBCs. HA titer: low (+), middle (++), high (+++) or negative (–).

Hemagglutination activity (HA) titer

Hemagglutinins were detected at all life stages of the corn earworm. However, HA titers fluctuated from one stage to another, and within each stage. The fluctuation in HA titers approximated changes in hemolymph concentration during the various developmental changes. The highest HA titer from egg extract, larval, pupal and adult hemolymph were 64, 4096, 2048 and 32, respectively. HA titer of pupal and adult insects was dependent on their sex; HA titers were higher in the female than in the male pupae and adults (Table 4). In 3rd and 4th-instar larvae, the HA titer was low at the beginning of each instar, then increased with the growth of the insect and reached the maximum titer by the end of the instar. In 5th-instar larvae, HA titer peaked in fully grown larvae and was maintained for 2 days while the insect developed into the pre-pupal stage. Prior to pupation, the HA titer decreased sharply (Fig. 1). HA titers peaked within 2 days after pupation; subsequently it declined progressively until adults eclose (Table 4).

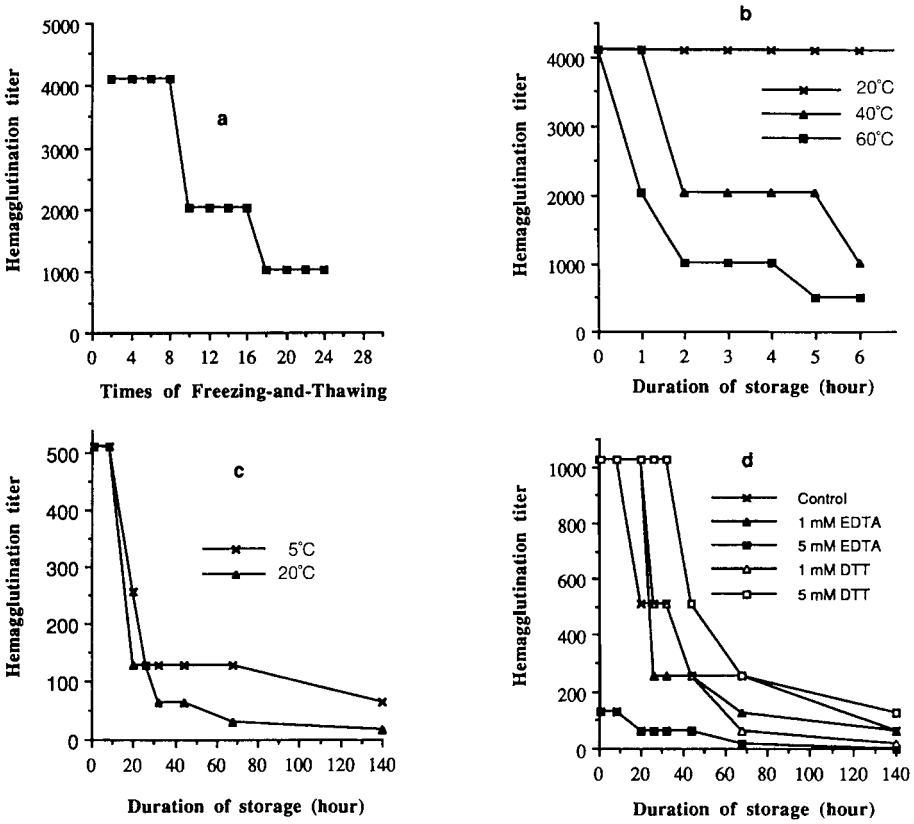

Fig. 1. Fluctuations in hemagglutinin titer and protein concentration in the hemolymph of fourth- and fifth-instar *Helicoverpa zea* larvae. HA titers were determined using fresh rabbit RBCs.

TABLE 2. The specificity of hemagglutination activity (HA) of corn earworm lectin against red blood cells (RBCs)*.

RBCs	HA specificity	RBCs	HA specificity
Human group A	N	Chicken	P
Human group B	N	Guinea Pig	P
Human group O	P	Rat	P
Horse	P	Rabbit	P
Sheep	P	Rabbit (fresh)	P
Porcine	P	Mouse (fresh)	N

* RBCs were glutaraldehyde-stabilized except fresh rabbit and mouse cells. P – positive; N – negative.

Binding specificity of corn earworm hemagglutinin

All erythrocyte types, except human group A, B and freshly-bled mouse erythrocytes, agglutinated with the hemolymph samples (Table 2). High HA titers were obtained with rabbit, sheep, porcine and guinea pig erythrocytes; human group O, horse and chicken erythrocytes showed low HA titers. The reactivity of the erythrocytes was dependent on

whether the cells were fresh and fixed. For example, the HA titer obtained using freshly-bled rabbit RBCs was eight times higher than that of the glutaraldehyde-fixed rabbit RBCs.

Of the 16 sugars and their derivatives tested in the experiment, seven (N-acetylneuraminic acid, D-galactose, D-galactosamine, N-acetyl-galactosamine, sucrose, maltose, and lactose) inhibited agglutination of fresh rabbit erythrocytes at final concentrations ranging from 16 to 125 mM (Table 5). The most effective inhibitors were sucrose and N-acetylneuraminic acid.

TABLE 3. Hemagglutination activity* (HA) resulting from corn earworm larval diet and its ingredients.

RBCs	Extracts of diet and its main ingredients			
	Diet	Pinto beans	Wheat germ	Yeast
Human Group A	++	+++	+++	–
Human Group B	++	+++	+++	–
Horse	+	++	–	–
Sheep	+	++	+++	–
Porcine	++	+++	+++	–
Chicken	+++	+++	+++	–
Rabbit	++	++	++	–
Rabbit (freshly-bled)	++	+++	+++	–
Mouse (freshly-bled)	++	+++	+++	–

HA titer: low (+), middle (++), high (+++) or negative (–).
* Hemagglutination activity was determined using fresh rabbit RBCs.

TABLE 4. The HA titer* and protein concentration in the hemolymph of corn earworm pupae and adults.

Development stages		Male		Female	
		HA titer	Protein (mg/ml)	HA titer	Protein (mg/ml)
Pupae	0-day-old	512	17.4	2,048	20.4
	2-day-old	1,024	27.6	2,048	26.7
	4-day-old	512	20.4	1,024	33.7
	6-day-old	512	25.7	512	25.5
	8-day-old	4	20.4	4	14.0
Adult	1-day-old	4		8	
	5-day-old, unfed	8		16	
	5-day-old, fed	4		32	

* HA titers were determined using fresh rabbit RBCs.

Stability of corn earworm hemagglutinin

Repeated cycles of freezing and thawing the hemolymph caused a decrease in HA titers. However, this process failed to induce a total loss of hemagglutination (Fig. 2a). Eight cycles of freezing and thawing reduced HA by 50%. The corn earworm hemagglutinin appeared to be relatively stable at room temperature. At 20°C, HA titer of the sample remained unchanged for eight hours (Fig. 2b, c), while at 5°C the HA titer decreased even more slowly; and the sample maintained 50% HA for 20 h. At 60 and 40°C, the sample

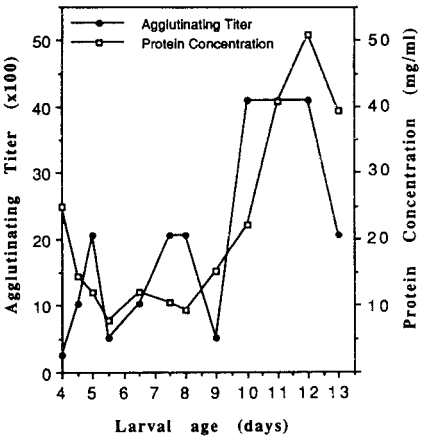

Fig. 2. Effects of various physical and chemical treatments on the hemagglutinin titer of *Helicoverpa zea* hemolymph. HA titers were determined using fresh rabbit RBCs.

lost its 50% of HA within one and two hours, respectively. At these elevated temperatures, a four to eight-fold reduction in HA titer occurred within 6 h (Fig. 2b).

Adding 1 mM EDTA to the sample appeared to make it more stable than the control; the HA titer remained unchanged for 20 h (Fig. 2d). However, increasing EDTA concentration to 5mM resulted in 88% reduction of HA within one hour. This result indicates that the divalent cations probably play an important role in the function of corn earworm HA. DTT was a good stabilizing agent for the corn earworm HA. The sample containing 5 mM DTT maintained its HA titer for 32 h.

TABLE 5. Binding specificity of corn earworm lectin using various sugars[a].

Mono- and di-saccharides	Inhibitory concentration[b] (mM)	Saccharide derivatives	Inhibitory concentration (mM)
D(+)-Glucose	x[c]	D(+)-Glucosamine	x
D(+)-Mannose	x	D(+)-Mannosamine	x
D(+)-Galactose	62	D(+)-Galactosamine	125
D(–)-Fructose	x	N-Acetyl-D-Glucosamine	x
L(–)-Fucose	x	N-Acetyl-D-Galactosamine	125
Sucrose	16	N-Acetyneuraminic acid	16
Maltose	62		
Lactose	31		
Trehalose	x		
Cellobiose	y[c]		

[a] Binding specificity was determined using fresh rabbit RBCs.
[b] Inhibitory concentration: The minimun final concentration of saccharide with the inhibitory effect.
[c] x and y: Without inhibitory effect at the final concentration of 125 mM (x) or 62 mM (y).

DISCUSSION

We report the occurrence, tissue distribution, binding specificity and stability of a putative new hemagglutinin that occurs in all developmental stages of the corn earworm, *H. zea*. This hemagglutinin agglutinated with erythrocytes from different animals; however, it did not agglutinate with mouse erythrocytes. Its sugar binding specificity was atypical of that reported for many arthropods. Like many arthropod lectins, it was inhibited by galactose and galactose derivatives (Chen et al., 1993; Grubhoffer & Maťha, 1991; Mckenzie & Preston, 1992). However, it also bound to sucrose and maltose even though glucose, of which these two sugars are partially comprised, failed to bind with the agglutinin. Moreover, the inhibitory concentration of sucrose was one-fourth that of galactose. This result

indicates the possibility that the *H. zea* hemagglutinin has two binding sites, one for monosaccharides and the other for di-saccharides. Otherwise, the binding activity may be attributed to the presence of more than one tissue specific hemagglutinin.

Many insect lectins are thermo-labile; their HA titers greatly decline after storage at warm temperatures, or after repeated freezing-and-thawing. Hence, insect lectins are typically handled cautiously at cold temperatures. In contrast, *H. zea* agglutinin is fairly stable even after repeated cycles of freezing-and-thawing.

EDTA is often incorporated into insect hemolymph buffers. It is a chelating agent which binds divalent cations such as calcium and magnesium. We found that 1 mM EDTA had a stabilizing effect on *H. zea* agglutinin. However, 5 mM EDTA appears to inhibit or denature corn earworm hemagglutinin. This result suggests that the activity of the corn earworm hemagglutinin is dependent on divalent cations. Such cation-protein linkage has been previously reported for lectins of other arthropods (Grubhoffer & Matha, 1991). Five millimolar DTT stabilized corn earworm HA. However, the actual role of DTT in this process is not clear. DTT inhibits the melanization of hemolymph. However, samples which were allowed to melanize did not show a decrease in HA titer.

Pinto beans and wheat germ are the major ingredients in the diet of corn earworm larvae as formulated by Perkins et al. (1973). Each has an agglutinin which contributed to the pool of agglutinins present in the diet, and in the midgut fluid. The specificity of reactions against rabbit and mouse erythrocytes revealed that agglutinin activity in the midgut fluid was exogenous in origin. Further, plant-derived agglutinins were restricted to the gut lumen whereas *H. zea* agglutinin occurred in the hemocoel.

Corn earworm agglutinin titer was related to the development of the insect; HA titers peak during key periods of metamorphosis. In 3rd and 4th-instar larvae, highest HA titers were attained at the end of each instar as the insects prepared to molt to the next instar. Similarly, the highest HA titer in the 5th-instar larvae occurred just before the initiation of pupation. Amanai et al. (1991) reported that in the silkworm, *Bombyx mori*, hemocytes appeared to be the major source of lectin synthesis. Our results indicate that this may also be true of *H. zea*. Of all the tissues we tested only the hemocytes exhibited hemagglutinating activity. We observed (unpublished data) that during pre-pupation, most hemocytes transform into adipocytes. This transformation is coincidental with the peak of hemagglutinating titer just before the onset of pupation. Further studies are required to elucidate the relationship, if any, between the transformation of the hemocytes and hemolymph lectin titers.

Lepidopterans purge their gut prior to molting to successive stages. Such purges eliminate potential dietary toxins, and the need to invest a huge defensive arsenal against them. Reprocessing these lectins just before molting ensures that the same materials can be recycled into those that are needed during metamorphosis. Assuming that these agglutinins are indeed used as opsonizing agents against dietary toxins, their titer should decline sharply during the non-feeding pupal stage. This, indeed, is the case. Although the pupae do not feed, they undergo other cellular processes such as differentiation and cell adhesion. Because these other processes involve lectin activity, the decline in hemagglutinin titer during the pupal stage may be due to the lack of challenge by dietary non-nutrients. Adult *H. zea* feed on nectars that probably present little or no toxicological challenge. The present report has established that agglutination titers are elevated during the feeding periods of *H.*

zea. Even so, some questions have emerged. Is the agglutinin a lectin? Does the agglutinin detoxify plant allelochemicals? Which group of allelochemicals can be detoxified by the agglutinin? Efforts are underway to answer some of these questions.

ACKNOWLEDGEMENT. This study was supported by National Science Foundation grant # DEB-9207642 to O.A. Adeyeye.

REFERENCES

AMANAI K., SAKURAI S. & OHTAKI T. 1991: Site of hemolymph lectin production and its activation in vitro by 20-hydroxyecdysone. *Arch. Insect Biochem. Physiol.* **17**: 39–52.

CHEN C., RATCLIFFE N.A. & ROWLEY A.F. 1993: Detection, isolation and characterization of multiple lectins from the hemolymph of the cockroach Blaberus discoidalis. *Biochem. J.* **294**: 181–190.

GAUSS D.H. 1993: Lectins: Insights into the state of knowledge by literature searches. In Gabius H.-J. & Gabius S. (eds): *Lectins and Glycobiology*. Springer-Verlag, Berlin, Heidelberg, pp. 3–5.

GRUBHOFFER L. & DUSBÁBEK F. 1991: Lectin binding analysis of Argas polonicus tissue glycoproteins. *Vet. Parasitol.* **38**: 235–247.

GRUBHOFFER L. & MAŤHA V. 1991: New lectins of invertebrates. *Zool. Sci.* **8**: 1001–1003.

GRUBHOFFER L., VEREŠ J. & DUSBÁBEK F. 1991: Lectin as the molecular factors of recognition and defence reaction of ticks. In Dusbábek F. & Bukva V. (eds): *Modern Acarology*. Academia, Prague and SPB Academic Publishing, The Hague, pp. 381–388.

HABIBI J., BACKUS E.A. & CZAPLA T.H. 1993: Plant lectins affect survival of the potato leafhopper (Homoptera: Cicadellidae). *J. Econ. Entomol.* **86**: 945–951.

HUESING J.E., MURDOCK L.L. & SHADE R.E. 1991: Rice and stinging nettle lectins: insecticidal activity similar to wheat germ agglutinin. *Phytochemistry* **30**: 3565–3568.

KAWAUCHI H., HOSONO M., TAKAYANAGI Y. & NITTA K. 1993: Agglutinins from aquatic insects: Tumor cell agglutination activity. *Experientia* **49**: 358–361.

KOPÁČEK P., GRUBHOFFER L. & SODERHALL K. 1993: Isolation and characterization of a hemagglutinin with affinity for lipopolysaccharides from plasma of the crayfish Pacifastacus leniusculus. *Dev. Comp. Immunol.* **17**: 407–418.

LEHANE M.J. & MSANGI A.R. 1991: Lectin and peritrophic membrane development in the gut of Glossina morsitans morsitans and a discussion of their role in protecting the fly against trypanosome infection. *Med. Vet. Entomol.* **5**: 495–502.

MCKENZIE A.N.J. & PRESTON T.M. 1992: Biological characteristics of the Calliphora vomitoria agglutinin. *Dev. Comp. Immunol.* **16**: 85–93.

MOHAMED H.A., INGRAM G.A. & MOLYNEUX D.H. 1992: Carbohydrate-binding specificities of anti-erythrocyte lectins (hemagglutinins) in Anopheles gambiae gut extracts and hemolymph. *Med. Vet. Entomol.* **6**: 217–224.

NAKAMURA C.V., ESTEVES M.G.J, ANDRADE A.F.B, ALVIANO C.S., SOUZA W.D. & GLUSTER J. 1992: Cell surface saccharides in three Phytomonas species differing in host spesificity. *J. Protozool.* **39**: 303–309.

PERKINS W.D., JONES R.C., SPARKS A.N., WISEMAN B.R., SNOW J.W. & MACMILLIAN W.W. 1973: Artificial diets for mass rearing of the corn earworm (Heliothis zea). *USDA ARS Prod. Res. Rep. No. 154*. 7 pp.

PUSTAI A.S., EWEN W.B., GRANT G., BROWN D.S., STEWART J.C., PEUMANS W.J., VAN DAMME E.J.M. & BARDOCZ S. 1993: Antinutritive effects of wheat-germ agglutinin and other N-acetylglucosamine-specific lectins. *Br. J. Nutr.* **70**: 313–321.

RENWRANTZ L. & STAHMER A. 1983: Opsonization properties of an isolated haemolymph agglutinin and demostration of lectin-like recognition molecule at the surface of haemocytes of Mytilus edulis. *J. Comp. Physiol. (B)* **149**: 535–546.

RICHARDS E.H. & RENWRANTZ L. 1991: Two lectins on the surface of Helix pomatia haemocytes. *J. Comp. Physiol. (B)* **161**: 43–54.

SHARON N. & LIS H. 1989: Lectins as cell recognition molecules. *Science* **246**: 227–234.

SHARON N. & LIS H. 1990: Legume lectins – a large family of homologous proteins. *FASEB J.* **4**: 3198–3208.

VASTA G.R. & MARCHALONIS J.J. 1984: Summation: Immunological significance of invertebrate lectins. *Progr. Clin. Biol. Res.* **154**: 177–191.

VOLF P., GRUBHOFFER L. & MUŠKA M. 1993: Detecting and identification of tissue specific lectins of the tsetse fly, Glossina tachinoides. In: *Management of Insect Pest Nuclear and Related Molecular and Genetic Techniques.* International Atomic Energy Agency, Vienna, pp. 557–565.

ZELCK U. & BECKER W. 1992: Biomphalaria glabrata: Influence of calcium, lectins, and plasma factors on in vitro phagocytic behavior of hemocytes of noninfected or Schistosoma mansoni-infected snails. *Exp. Parasitol.* **75**: 126–136.

Received February 14, 1996; accepted April 16, 1996