Glucose Utilization in the Regulation of Chitin Synthesis in Brown Planthopper

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Abstract

Glucose-6-phosphatase (G6Pase) and hexokinase (HK) are two key enzymes in the glycolysis and gluconeogenesis pathways, which catalyze the synthesis and degradation of glucose in insects, respectively. G6Pase and HK play an important role in insect growth by regulating the metabolism of glucose, leading to the efficient metabolism of other macromolecules. However, it is unclear whether these genes could be investigated for pest control through their actions on chitin metabolism. We studied the potential functions of G6Pase and HK genes in the regulation of chitin metabolism pathways by RNAi technology. Interference with G6Pase expression did not affect trehalase and chitin metabolism pathways in brown planthopper, Nilaparvata lugens (Stål). However, knockdown of the HK gene resulted in a significant decrease of expression of genes associated with the trehalase metabolic pathway but had no significant effect on trehalase activity, trehalase content, or glucogen content. Additionally, HK knockdown resulting in downregulation of the genes involved in chitin metabolism in the brown planthopper. These insects also showed wing deformities and difficulty in molting to varying degrees. We suggest that the silencing of HK expression directly inhibited the decomposition of glucose, leading to impaired chitin synthesis.

Key words: brown planthopper, glucose-6-phosphatase, hexokinase, trehalose metabolism, chitin metabolism

Rice (Oryza sativa L.) is the most important cereal crop in China and is the staple food for more than two-thirds of the world’s population. Surprisingly, its annual yield loss due to pests and disease reaches levels of 24–41% (Boddupally et al. 2018). Among the most destructive of these pests is Nilaparvata lugens (Stål) (Hemiptera: Delphacidae). It is a single-feeding pest with strong migratory ability and can damage rice directly by sucking phloem sap through its mouthparts (Cheng et al. 2013). In addition, brown planthopper can also carry rice viruses, representing an additional threat to rice productivity (Lu et al. 2016). Although traditional insecticides have proven to be effective in controlling the population of brown planthopper (Sun et al. 2013, Yang et al. 2017), the excessive use of chemical pesticides has led to many problems, including increased production costs, toxicity to natural enemies of brown planthopper and their ‘resurgence’, and harm to agro-ecosystems and human health (Rola and Pingali 1993, Becker et al. 1996, Nauen and Denholm 2005, Wang et al. 2008, Bottrell and Schoenly 2012). Therefore, the development and utilization of biological pesticides have become an important avenue of research.

Manipulating the regulation of insect chitin synthesis has become a favorable avenue of investigation in the development of new pest control methods (Arakane et al. 2005, Wang et al. 2012, Mansur et al. 2014, Xu et al. 2017, Wu et al. 2019, Wang et al. 2019). The exoskeleton of insects is composed of the stratum corneum and an important role in the growth and development of insects, including protection, support, movement, and as a barrier against environmental stress (Merzendorfer and Zimoch 2003, Zhu et al. 2016). However, an exoskeleton with a rigid structure has limitations on the growth and development of insects. Insects regularly form a new stratum corneum to replace the old stratum corneum, and chitin plays a key role in this process (Nakabachi et al. 2010). Chitin is considered the second most abundant biomolecule. It is a polymer of N-acetylglucosamine linked by β-1,4 glycosidic bonds, synthesized by chitin synthase, a transmembrane protein (Merzendorfer and Zimoch 2003). Chitin is found in many organisms, including microorganisms such as fungi, protists and algae, arthropods such as insects, crustaceans and arachnids, and other invertebrates such as sponges, coelenterates, mollusks and nematodes (Sobala et al. 2015). In insects, chitin is the central component of the epidermis, the trachea and the peritrophic matrix (PM) (Zhao et al. 2014).

The sugar present in insect’s blood (hemolymph)—trehalose—is a nonreducing sugar consisting of two glucose molecules linked by alpha, alpha-1,1-glycosidic linkages, widely found in bacteria, yeast, fungi, plants and invertebrates, but not present in mammals (Becker et al. 1996, Elbein 1974, Wingler 2002, Elbein al. 2003, Frison et al. 2007). Trehalose is not only an energy source but also an...
important stress protection agent that shields the body from damage in various adverse conditions (Becker et al. 1996, Elbein et al. 2003, Tang et al. 2010, Shukla et al. 2015, Liu et al. 2016). Therefore, it plays a key role in insect development and physiological activities. It has been reported that the concentration of trehalose in the hemolymph of insects affects the rate of energy consumption during flight (Clegg and Evans 1961), the synthesis of chitin during molting (Candy and Kilby 1962), cold resistance (Wyatt 1967), food selection and feeding behavior (Thompson and Dahlan 1999), and the oogenesis of insects (Santos et al. 2012).

Glucose is the most widely used carbohydrate in animals for energy production, the provision of macromolecular precursors, and signaling molecules in liver and adipose tissue (Vaulont et al. 2000). Hexokinase (HK) is the first enzyme in the glucose metabolism pathway, as well as a multifunctional protein with roles in transcriptional regulation (Niederacher and Entian 1991, Herrera et al. 1995) and apoptosis (Gottlob et al. 2001, Bryson et al. 2002), as well as acting as a cytokine neurotransmitter interleukin (neurotrophic factor) (Gurney et al. 1986, Chaput et al. 1988, Faik et al. 1988). In addition, HK is also involved in pathogen-host interactions; for example, HK was first reported in Nematocida parisii (Cuomo et al. 2012) and has also been shown in Paranosema (Antonospora) locustae by biochemical experiments (Reinke et al. 2017), and in P. locustae (Senderskiy et al. 2014, Timofeev et al. 2017). Locustae HK is predicted to contain a signal peptide, while showing the presence of the host’s indirect immunofluorescence assay (IFA) (Senderskiy et al. 2014, Timofeev et al. 2017). The special function of secreted hexokinase has been studied in Trachipleistophora hominis, where it acts as a regulator to increase glycolysis or ATP production on the surface of parasites (Ferguson and Lucocq 2019). Hexokinase irreversibly catalyzes ATP-dependent glucose phosphorylation, producing ADP and glucose-6-phosphate (G-6-P), which is the initial step in the glycogen synthesis, glycolysis, or pentose phosphate pathways (Miyamoto and Amrein 2017). Glucose-6-phosphatase (G6Pase), a gluconeogenesis-specific enzyme, glucose-6-phosphatase (G6Pase), first appeared in echinoderms but also found in echinoderms, molluscs, and vertebrates (Miyamoto and Amrein 2017). G6Pase hydrolyzes glucose-6-phosphate (G-6-P) to free glucose in the terminal step of gluconeogenesis and glycogenolysis. It is one of the rate-limiting enzymes in the gluconeogenesis pathway and its expression can be regulated by the IRS/PI3K/Akt signaling pathway (Barthel and Schmoll 2003).

Most of the current research in the field focuses on the regulation of chitin synthesis by trehalose. However, the question of whether the use of glucose has an effect on the synthesis of chitin remains unanswered. To address this question, we interfered with HK and G6Pase—the enzymes that directly regulate glucose synthesis and degradation—to explore the potential function of glucose utilization in chitin synthesis. If glucose metabolism is used to regulate the synthesis of chitin in brown plant hopper, a novel mechanism for the prevention and control of brown planthopper or other rice pests will be exposed.

### Materials and Methods

#### Test Insect

*N. lugens* used in this study were from the laboratory breeding population, and the test insect source comes from the Hangzhou population of the China Rice Research Institute. Feeding conditions of brown planthopper: *O. sativa* is a susceptible strain TN1 (Taichung Native 1), temperature 25 ± 1°C, light–dark cycle 16:8 (L:D) h, relative humidity 70 ± 5%.

#### RNA Extraction and cDNA Preparation

The total RNA from brown planthopper was obtained by Trizol extraction under RNase free conditions. RNA integrity was assessed using gel electrophoresis, concentration and purity was checked on the NanoDropTM 2000 micro-assay spectrophotometer. RNA was stored at −80°C before use. cDNA was synthesized using the PrimeScript RT reagent kit (TaKaRa) according to the manufacturer’s instructions. The synthesized cDNA was stored at −20°C.

#### Synthesis of dsRNA

The dsRNA primers for the synthesis of dsHK, dsG6Pase, and dsGFP were designed using Primer 5 software (specific primer sequences are shown in Table 1). cDNA was first polymerase chain reaction (PCR) amplified with the primers of interest. The PCR products were subject to T cloning, followed by a subsequent amplification with primers containing the T7 promoter sequence. Cross-PCR reactions were performed using a T7 RiboMAXTM Express RNAi System kit (Promega) to synthesize dsRNA. The integrity of dsRNA was determined by gel electrophoresis, and the concentration and purity of synthesized dsRNA were measured using NanoDropTM 2000 microassay spectrophotometer.

### Table 1. Primers used in the dsRNA synthesis and qRT-PCR detection

| Gene Type | Gene Type | Primer set | Forward Primer (5′-3′) | Reverse Primer (5′-3′) | Length of target fragment (bp) |
|-----------|-----------|------------|------------------------|------------------------|-----------------------------|
| dsRNA     | dsHK      | dsHK       | TGGTGGCGAAGAAGTGAA     | TCCAGATACGAGCCGTG      | 409                         |
|           |           | qRT-dsHK   | T7- GTGTTGCGAAGAAGTGAA | T7-TCCAGATACGAGCCGTG   | 459                         |
| dsRNA     | dsG6Pase  | dsG6Pase   | GGGTGCTCTATCGTGTTG     | AGGCCATGCTGGTG         | 414                         |
|           |           | qRT-dsG6Pase | T7- GGGTGCTCTATCGTGTTG | T7- AGGCCATGCTGGTG      | 464                         |
| dsRNA     | dsGFP     | dsGFP      | AAGGCCCCAGACGCTGCTG    | GCAGAATTACCATAGCCACT   | 132                         |
|           |           | qRT-dsGFP  | T7- AAGGCCCCAGACGCTGCT | T7- CAGCAGACCAGGTGCTG   | 409                         |

T7 : GGATCCTAATACGACTACATAGG.
Microinjection of Brown Planthopper
The volume of dsRNA per injection was determined by a standard capillary under a microscope. The brown planthoppers were anesthetized with CO₂ and placed in the groove of a pre-prepared agarose gel. The dsGFP, dsHK, and dsG6Pase were injected into the lateral epidermis of the two pairs of hind paws in the chest of the brown planthopper. 50 ng of dsRNA was injected into each brown planthopper. Finally, the injected brown planthoppers were transferred to a glass tube containing fresh rice, and the surviving brown planthoppers were taken for subsequent experiments with 48 h after injection.

Tissue Collection
The material for exploring the developmental expression of brown planthopper was from 1st of 4th instar nymph to 3rd days after reaching adulthood. Sampling was carried out at 24 h intervals, and three samples were collected in parallel to assess developmental expression patterns. Material from the head, foot, wing, epidermis, ovary, and fat body were obtained through anatomical mirror dissection of LEICA EZ4. The samples were stored at −80°C.

Gene Expression by qRT-PCR
Total RNA was extracted from the brown planthopper material, and synthesized cDNA by reverse transcription as previously described. The relative expression levels of G6Pase and HK genes at different developmental stages and in different tissues were detected by qRT-PCR. The brown planthopper 18S gene was selected as an internal reference gene. qRT-PCR primers are shown in Table 1. Gradient PCR was performed to verify the optimal annealing temperature of the primers, and agarose gel electrophoresis was performed to ensure the specificity of the PCR product.

Table 2. Primers used for qRT-PCR
| Gene name       | Genebank number | Forward primer (5′-3′)                  | Reverse Primer (5′-3′)                  | Length of target fragment (bp) |
|-----------------|-----------------|----------------------------------------|----------------------------------------|-------------------------------|
| QNI18S          | KU556839.1      | CGCTACTACCGGATTTGAA                   | GGAACACCTTGTAGACTTT                 | 165                           |
| QNIPGM1         | KU556840.1      | TCTCTGGTGTGTTGTTG                    | CCTTCAGCTTGGGAGA                    | 127                           |
| QNIGP           | KU556838.1      | GCTGCTATGGCTATGGTTGTTTC               | GACCCCAAGACCTGAAA                    | 141                           |
| QNIGS           | KU556837.1      | GCTCAGAGCTATGCTTTCTAG                | TCTGAGTGGTGGCCACTTTCCTT             | 202                           |
| QNLUGPase       | KU556842.1      | ATACAAGATGGCCGCTAA                   | TTGTGGCAAGGTGATAGGCA                | 136                           |
| QNITP51         | GQ397450        | AAGACTGAGGCCGAATGGT                   | AAGTGGAATGGAATGCA                    | 154                           |
| QNITP2          | KU556826        | AGATGGCCGACCAACA                      | TCAACGGCCGAATGACT                    | 161                           |
| QNITP3-3        | KU556827        | GTGATGGCCTGGTGGCTAT                  | CCGGTTACATGGGAGATAGGCA              | 224                           |
| QNITRE1-1       | FJ790319        | GCCATTTTGGAAGAATGG                    | CCGGTTACATGGGAGATAGGCA              | 132                           |
| QNITRE1-2       | KU556829        | GATGCGAGGTATTAA                      | AATGGGCTTCAAGATCAA                   | 178                           |
| QNITRE2         | GQ397451        | TCACGGTGGTCCAGGCT                    | GTGAAACCACCTGTGAGTAC                | 197                           |
| QNIIHK          | KU556830        | GTGCCGGAAGAAGAATGG                     | CCAAGTGTCACCAAA                    | 105                           |
| QNIFAT          | KU556833        | CCCCCAGCCAGTCCCTCCG                    | CCGGTTACATGGGAGATAGGCA              | 132                           |
| QNIG6Pase       | KU556841.1      | AGACCTGGCAGCTAATAG                   | GAGGATGGCCAGCCAA                     | 132                           |
| QNIG6P1         | KU556832.1      | GTTGACCGGTGCTGGAGGAAG                 | TACAGTGCTGGTGGCC                     | 147                           |
| QNIG6P2         | KU556831.1      | ACAAAGGGACGATCGAGAAG                   | ACCTGTGCTGGTGCC                     | 85                            |
| QNIG6P3         | XM_022345379.1  | ATGTCAGGTGGTGAAGACCT                  | CCTGAAATGGGGATGTA                    | 179                           |
| QNIGNPNA        | KU556834        | TGAGCTGCTGGACGT                     | TACCTGTGCTGGTGCC                     | 120                           |
| QNIUP           | JF330415        | AAGCCAGATTTAACAGCGGATC                | ACACCATCCGAGATGAC                    | 222                           |
| QNI1CHS1        | AEL88648        | CCGGAGAGCCAGATTCACAGA                 | AGCTCTGGTGGTCAGTGGCC                 | 141                           |
| QNI1CHS1a       | JQ400014        | TGTTCTGCTGCAAATCAATCAAAA              | ACACCAATCCGAGATGAC                    | 141                           |
| QNI1CHS1b       | JQ400013        | GCTGCTTGGTCTTCCCAT                   | ACACCAATCCGAGATGAC                    | 187                           |
disruption, 800 μl of PBS was added and centrifuged at 1,000 g for 20 min at 4°C. 350 μl of the supernatant was taken at 4°C, 20,800 x g for 1 h, and the remaining supernatant was used for the determination of total protein and trehalose concentrations. The supernatant after ultracentrifugation was used to measure glucose, protein concentration, and soluble trehalase activity assay, and the pellet was suspended in PBS for determination of glucose, protein concentration, and membrane-bound trehalase activity, the specific steps refer to the method of Zhang et al. (2017).

Statistical Analysis
The relative copy number of the gene was determined by qRT-PCR, using the $2^{-\frac{\Delta\Delta CT}{\Delta}}$ method. The reactions were performed in triplicate and with three biological replicates. The control group was the results of the brown planthopper injected with dsGFP. The converted values were analyzed as follows (Chen et al. 2018):

\[
2^{-\Delta\Delta CT} = 2^{-[(CT \text{ control group} - CT \text{ control 18s}) - (CT \text{ test group} - CT \text{ test group 18s})]}
\]

Difference analysis was performed by using SPSS software, and one-way analysis of variance (ANOVA) data analysis method was employed to analyze potential differences. Differences were considered significant if the $P$ value was between 0.01 and 0.05, and extremely significantly different the $P$ value was less than 0.01. The analyzed data was plotted using SigmaPlot 10.0 software.

Results
Developmental and Tissue Expression Patterns of G6Pase and HK
In order to explore the specific expression profiles of HK and G6Pase genes in different developmental stages of brown planthopper, we...
examined the expression levels of HK and G6Pase genes from the fourth instar to the third day of adulthood. The results showed that the expression of HK and G6Pase was relatively stable in the nymphal stage. However, HK increased significantly in the early stage of the fourth instar nymph and then decreased significantly in the adult stage. The expression of G6Pase gradually increased across developmental time, reaching its peak at the final time point, while the expression of HK showed a significant downward trend (Fig. 1A and B).

We also examined the expression levels of HK and G6Pase genes in different tissues of the adult brown planthopper, namely, foot, wing, midgut, ovary, and fat. The tissue expression profiles showed that the G6Pase gene was highly expressed in the ovary of the brown planthopper, followed by the head and fat body, and its expression in the wing was relatively low. The expression level of HK gene was highest in the head, followed by foot, and the expression level in wings and ovaries was relatively low (Fig. 1C and D).

Expression of G6Pase and HK in Brown Planthopper After RNAi

The expression of G6Pase and HK genes were knocked down using RNAi technology. The results showed that compared to the control group, the expression levels of these genes were significantly decreased after 48 h of interference with the G6Pase or HK gene in brown planthopper. Which indicate that RNAi effectively inhibited the expression of G6Pase or HK genes. In addition, the results showed that the expression of G6Pase was significantly decreased after interference with HK, and there was no significant change in the expression of HK gene after interference with G6Pase (Fig. 2).

Expression of Trehalose Metabolism-Related Genes in Brown Planthopper Following G6Pase and HK Knockdown

To assess the effect of G6Pase and HK inhibition on trehalose metabolism in the brown planthopper, we analyzed the expression of key genes by qRT-PCR. After 48 h of G6Pase knockdown, the expression levels of trehalose metabolism-related genes in brown planthopper were mostly unchanged, except for the downregulation of TPS1 and TPS3 genes, and the upregulation of TPS2. After HK knockdown, the expression levels of trehalose metabolism-related genes in brown planthopper were all significantly or extremely significantly decreased, with the exception of TPS2 and UGPase (Fig. 3).

Effects of G6Pase and HK Gene Inhibition on Trehalase Activity in Brown Planthopper

We next tested the enzymatic activities of soluble and membrane-bound trehalase. The results showed that there was no significant change in soluble trehalase activity and membrane-bound trehalase activity in the brown planthopper after inhibition of G6Pase or HK expression (Fig. 4).

Effects of G6Pase and HK Gene Knockdown on the Trehalose and Glucose in Content in Brown Planthopper

Interference with G6Pase or HK gene expression has little effect on the content of trehalose in the brown planthopper. Compared with the control group, there was no significant difference in the content of trehalose in the brown planthopper after G6Pase or HK gene inhibition (Fig. 5B). However, the glucose content in the brown planthopper was significantly increased after G6Pase knockdown, whereas there was no significant difference in the glucose content following HK knockdown (Fig. 5A).

Evaluation of Chitin Level in Brown Planthopper Following G6Pase and HK Inhibition

We next sought to investigate the effects of G6Pase and HK knockdown on chitin metabolism, and employed qRT-PCR to test the expression of related genes. Our results demonstrate that perturbation of G6Pase activity does not significantly impact the expression of genes involved in chitin metabolism in brown planthopper, except in the case of G6P12 and UAP (Fig. 6A). In contrast, inhibition of HK expression resulted in significant or extremely significant downregulation of the genes involved in chitin metabolism. In addition, in the brown planthopper also showed wing deformities (Fig. 6A) and difficulty in molting to varying degrees (Fig. 6B).

Fig. 2. Relative expressions of G6Pase and HK after injection for 48 h in brown planthopper. qRT-PCR data showing mRNA levels of NlG6Pase and NlHK relative to NL-18S mRNA level following RNAi targeting. First day of instar nymph were chosen as the targets for dsRNA injection. * indicates significant difference and ** indicates extremely significant difference, the same below.

Fig. 3. Expression level of trehalose metabolic pathway genes in brown planthopper after RNAi. Expression of three trehalose-6-phosphate synthases (TPS), three trehalases (TRE), two phosphoglucomutase (PGM), UDP-glucose pyrophosphorylase (UGPase), glycogen synthase (GS), and glycogen phosphorylase (GP) at 48 h relative to the NL-18S mRNA level were measured using qRT-PCR.
Discussion

G6Pase is a member of the nonspecific esterase family with polymorphism. The function of G6Pase is mainly to catalyze the hydrolysis of acetyl groups (Alterio et al. 2010). In the study of insects’ G6Pase, G6Pase was expressed only in the brain in Drosophila melanogaster and Anopheles gambiae, suggesting that it has a neuronal function, and implicating its involvement in neuronal conduction (Miyamoto and Amrein 2017). Notably, there is a difference between the expression patterns of N. lugens to Drosophila melanogaster or Anopheles gambiae. In our tissue expression pattern of G6Pase, G6Pase was expressed in various tissues of the brown planthopper, and was highly expressed in the ovary, followed by brain and fat bodies (Fig. 1C).

The expression of G6Pase in brown planthopper was maintained at a low level in the nymphal stage and increased in the adult stage (Fig. 1A). Interestingly, G6Pase has a sharp rise and fall at the end of the fifth and adulthood (Fig. 1A), suggesting that G6Pase may be involved in the emergence of brown planthopper.

Hexokinase is a rate-limiting enzyme that plays a key role in glucose homeostasis and energy metabolism through glucose (Glc) phosphorylation and Glc signaling (Ge et al. 2019). In the study of insect hexokinase, Bombyx mori muscle hexokinase is almost type I and is present in almost all tissues: hexokinase in testis and malphigian tube consisted of type I and type II, midgut contained type I, II, and IV hexokinases, while fat body tissues had types I, III and IV (Yanagawa 1978). Tadano (1987) detected HK-1 in the head, chest, and abdomen of the adult Aedes togoi; in contrast, HK was concentrated in the chest in Anopheles stephensi (Gakhar and Nagpal 1996). Our tissue expression pattern shows that HK is ubiquitous in various tissues of brown planthopper, and has the highest expression level in the brain (Fig. 1D), which is consistent with the findings of mammals (Lawrence et al. 1984) and the findings of Ge et al. (Ge et al. 2019). Consistently, HK has shown to be evolutionarily conserved in the organic central nervous system (Lawrence et al. 1984, Ge et al. 2019). In relation to its expression throughout development, HK-2 and HK-3 existed at all developmental stages in the study of An. stephensi (Gakhar and Nagpal 1996). In addition, HK-1 did not exist in all adult stages, its activity increased during larval growth, and was the highest at the last age observed (Gakhar and Nagpal 1996). Studies have also shown that the expression levels of DM1 and DM2 (two hexokinase isoenzyme sequences) are uniform in the 2nd and 3rd instar larvae, pupa and adult stages of Drosophila (Jayakumar et al. 2001). Our results demonstrate that the expression of HK is maintained at a relatively high level.
Background and Objectives

Chitin is a crucial component of the exoskeleton of insects, playing an essential role in their growth and development. The biosynthesis of chitin is a complex metabolic process that involves the coordinated regulation of various genes. In this study, we aimed to investigate the potential function of glucose metabolism in the regulation of chitin synthesis in the brown planthopper (Nilaparvata lugens). We selected glucose-6-phosphate isomerase (G6Pase) and hexokinase (HK) as candidates for RNA interference (RNAi) experiments to evaluate their roles in chitin metabolism.

Methods

RNAi was employed to knock down the expression of G6Pase and HK in N. lugens. The expression levels of key genes involved in the chitin metabolic pathway were examined using qRT-PCR. The artificial juvenile hormone (20E) was used to induce nymphal molting to examine the effects on chitin synthesis.

Results

After RNAi of G6Pase or HK, we observed a significant decrease in the expression of G6Pase and HK genes, respectively. The expression of genes involved in the chitin metabolic pathway was significantly reduced in the RNAi-treated individuals compared to the controls. Additionally, the chitin content in the nymphs was significantly reduced after RNAi of HK.

Conclusion

Our findings suggest that glucose metabolism plays a critical role in the regulation of chitin synthesis in N. lugens. The interference with G6Pase and HK results in a significant decrease in chitin content, indicating the importance of these enzymes in the glucose metabolism pathway for chitin synthesis.

Future Directions

Further studies are needed to elucidate the molecular mechanisms underlying the regulation of chitin synthesis by glucose metabolism. The integration of genomic and transcriptomic data with metabolic studies will provide a comprehensive understanding of the complex interplay between glucose metabolism and chitin biosynthesis in insects.
HK were expressed at all stages and were relatively stable in the nymphal stage. However, G6Pase was significantly decreased in the larva-adult stage, indicating that it may relate to the molting process. In terms of tissue-specific expression, we found that G6Pase is highly expressed in the ovary, followed by the brain; while HK is highly expressed in the brain.

We evaluated the possibility of the brown planthopper HK and G6Pase genes as new targets in pest control. The results showed that interference with G6Pase had little effect on chitin synthesis, while knockdown of HK showed strong interference. Our results indicate that the knockdown of HK inhibits chitin synthase CHS1a, CHS1b, and CHS1b in the brown planthopper, which causes the chitin not to be synthesized normally.

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