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Characterization of glutamine: fructose-6-phosphate aminotransferase from the ixodid tick, *Haemaphysalis longicornis*, and its critical role in host blood feeding ☆

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Abstract

Glutamine: fructose-6-phosphate aminotransferase (GFAT, EC2.6.1.16) is the first, and rate-limiting, enzyme in the hexosamine biosynthetic pathway, and is involved in the regulation of chitin biosynthesis and glycosylation of proteins. We report here the molecular characterization and potential functions of a novel GFAT (HlGFAT) from the ixodid tick *Haemaphysalis longicornis*. HlGFAT consists of 696 amino acids, possesses a class II glutamine aminotransferase domain and two sugar isomerase motifs, and has a close phylogenetic relationship to insect GFAT. *HlGFAT* was expressed at all stages of development and in multiple organs. The transcription levels in the cuticle and midgut were enhanced significantly by blood feeding during the first 3 days and decreased on the fifth day, while those in salivary glands maintained almost the same level during the first 3 days, and decreased to a rather low level at 5 days postinfestation. Endogenous HlGFAT was identified at all developmental stages and in multiple organs, such as epidermis, midgut epithelium, salivary gland, ovary, Malpigian's tubule and trachea. It was identified as a protein of 78.4 kDa using Western blot analysis. Following RNA interference of HlGFAT, engorgement by adult females was reduced significantly. One of the potential mechanisms for this effect may be that the inhibition of HlGFAT limits chitin biosynthesis, so disrupting cuticle growth and possibly peritrophic matrix formation during blood feeding.

Keywords: Glutamine; Fructose-6-phosphate aminotransferase; Tick; RNA interference; Blood feeding; Chitin biosynthesis

1. Introduction

Glutamine: fructose-6-phosphate aminotransferase (GFAT, EC2.6.1.16), known under its trivial name of glucosamine-6-phosphate synthase, is the first, and rate-limiting, enzyme in the hexosamine biosynthetic pathway (Fig. 1). The final product of the pathway, uridine diphosphate-*N*-acetyl-glucosamine (UDP-GlcNAc), is an essential substrate for protein glycosylation and an active precursor of numerous macromolecules containing amino sugars such as chitin, a homopolymer of β-(1-4)-linked *N*-acetyl-D-glucosamine. Chitin is the major component of the cell wall in bacteria and fungi (McMurrough et al., 1971) and the cuticle and peritrophic matrix (PM) in arthropods (Merzendorfer and Zimoch, 2003). Previous studies have revealed that the inhibition of GFAT might have important implications in antibacterial antifungal

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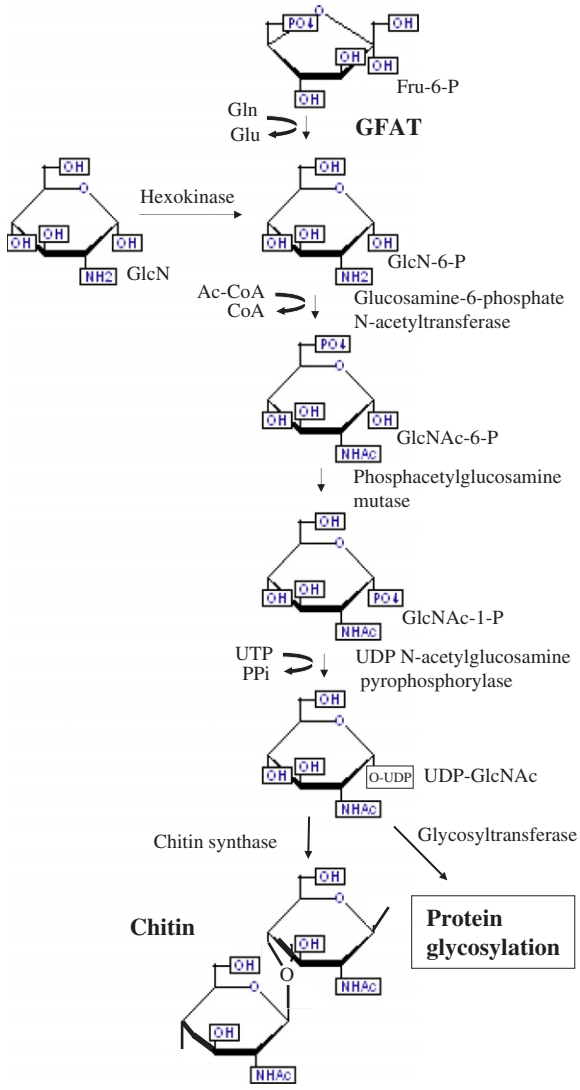

Fig. 1. Glutamine: fructose-6-phosphate aminotransferase (GFAT) is the first, and rate-limiting, enzyme in the hexosamine biosynthetic pathway. The final product, uridine diphosphate (UDP)-*N*-acetylglucosamine, is an active precursor of chitin and an essential substrate for protein glycosylation. GlcN, glucosamine; Fru-6-P, D-fructose 6-phosphate; GlcN-6-P, D-glucosamine 6-phosphate; GlcNAc-6-P, *N*-acetyl- D-glucosamine 6-phosphate; GlcNAc-1-P, *N*-acetyl-D-glucosamine 1-phosphate; UTP, uridine 5′-triphosphate; Ppi, inorganic pyrophosphate; Gln, glutamine; Glu, glutamate.

therapies (Andruszkiewicz et al., 1990). Cuticle is an exoskeleton determining the maximal body size that blood sucking arthropods may reach after feeding (Obenchain, 1982). PM is an extracellular sac derived from secretions of the epithelial cells of the midgut. It functions to protect the midgut epithelium from pathogens, abrasion, toxic compounds, and in certain cases to facilitate digestion (Lehane, 1997; Shao et al., 2001; Matsuo et al., 2003). Hence, GFAT may play critical roles in blood sucking by regulating chitin synthesis, which is supported by the up-regulation of a GFAT gene from the mosquito *Aedes aegypti* during blood feeding (Kato et al., 2002), and the regulation of chitin biosynthesis in the formation of PM lining the mosquito midgut (Kato et al., 2006). On the other hand, since UDP-GlcNAc is also a precursors for glycosylation of proteins, GFAT is most likely involved in regulating the availability of precursors for glycosylation to influence metabolism in humans (McKnight et al., 1992), and to affect synthesis of glue proteins for attachment of fruit fly *Drosophila melanogaster* larva (Graack et al., 2001).

An ixodid tick, *Haemaphysalis longicornis*, being an obligate ectoparasite of a variety of domestic animals, and a dominant tick in Asia, transmits babesiosis and theileriosis between animals. It is also a reservoir of Lyme disease and oriental spotted fever in humans (Uchida et al., 1995; Wang et al., 2000). Like other arthropods, this tick has a cuticle as an exoskeleton and possibly PM lining the gut, both of which contain chitin (Matsuo et al., 2003). Therefore, it would be of interest to elucidate the function of GFAT in tick physiology, as it might facilitate the development of novel tick control strategies. In this study, we isolated and characterized a novel GFAT gene (*HlGFAT*) from the tick *H. longicornis*, determined its transcriptional and translational profiles at different stages and in various organs, and analyzed the in vivo function of this gene by knockdown of gene expression using RNA interference (RNAi), a method that has provided a powerful alternative to traditional genetics and has thus revolutionized the analysis of gene function in normal organisms (Grishok et al., 2001). It is also a useful technique in the knockdown of tick genes (Miyoshi et al., 2004; Narasimhan et al., 2004). Our results showed that HlGFAT may play an important role in tick survival.

2. Materials and methods

2.1. Ticks and animal ethics

The pathogenetic Okayama strain of the hard tick *H. longicornis* (Fujisaki, 1978) was maintained by feeding on rabbits. All animals used in this study were acclimatized to the experimental conditions for 2 weeks prior to commencement of the experiment. Animal experiments were conducted in accordance with the protocols approved by the Animal Care and Use Committee, National Institute of Animal Health (Approval Nos. 441, 508, and 578).

2.2. Isolation of HlGFAT cDNA

A clone with an expression sequence tag of a GFAT homolog was obtained from a *H. longicornis* egg cDNA library constructed previously (You et al., 2001). The cDNA insert was excised from the corresponding phage and cloned into pBluescript SK (+) (Stratagene, La Jolla, CA). A partial fragment of 840 bp was amplified from the recombinant plasmid by PCR using sequence specific primers (5′ACATCGTTGGCTGTGGCG3′ and 5′GTCTGGAAGCCTGTGGAG3′). The PCR product