Journal of Insect Physiology 58 (2012) 83–88

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Xanthurenic acid is an endogenous substrate for the silkworm cytosolic sulfotransferase, bmST1

Akira Kushida [a], Ryo Horie [b], Kenji Hattori [a], Hiroshi Hamamoto [b], Kazuhisa Sekimizu [b], Hiroomi Tamura [a,*]

[a] Graduate School of Pharmaceutical Science, Keio University, Minatoku, Tokyo, Japan
[b] Graduate School of Pharmaceutical Science, The University of Tokyo, Bunkyoku, Tokyo, Japan

ARTICLE INFO

Article history:
Received 14 July 2011
Received in revised form 5 October 2011
Accepted 10 October 2011
Available online 20 October 2011

Keywords:
Bombyx mori
Cuticle
Malpighian tubule
Sulfotransferase
Xanthurenic acid

ABSTRACT

Sulfotransferase enzymes are known to regulate physiologically active substances such as steroids and catecholamines in mammals. Although invertebrates also express sulfotransferases, their biological function is mostly unclear. In a previous study, we reported that 4-nitrocatechol and the gallete ester are substrates for the silkworm sulfotransferase bmST1. The K_m of bmST1 for these substrates is high. However, endogenous substrates of bmST1 have not yet been determined. We therefore investigated endogenous bmST1 substrates and carried out a detailed expression profile analysis of bmST1. We found that xanthurenic acid, a tryptophan metabolite, is a possible endogenous substrate of bmST1. The K_m of bmST1 for xanthurenic acid is low, in the μM range, which is lower than that for previously reported substrates. Additionally, xanthurenic acid is a tryptophan metabolite that characteristically shows toxicity *in vivo*. High dose administration of xanthurenic acid resulted in inhibition of cuticular biosynthesis. The expression of the bmST1 gene reached a maximal level in the Malpighian tubule at the 4th molting stage, when amino acid metabolism might be activated. Our results suggest that bmST1 plays a role in detoxification of xanthurenic acid in the silkworm.

1. Introduction

Sulfotransferases (SULTs) are phase II drug-metabolizing enzymes that catalyze the transfer of a sulfonate group from 3′-phosphoadenosine 5′-phospho sulfate (PAPS) to phenols, enols, alcohols, or amines (Falany, 1997). In mammals, SULTs are cytosolic enzymes found in most body tissues, including liver, intestine, brain, adrenal gland and platelets. In most cases, sulfoconjugation increases the polarity and water solubility of molecules, thereby facilitating biliary or urinary excretion and detoxification of these compounds. The SULTs comprise a multigene family and a number of subfamily genes have been identified based on molecular cloning studies (Yamazoe et al., 1994). Recent reports have identified and characterized SULTs of non-mammalian vertebrates and invertebrates. For example, in invertebrates, the armyworm sulfotransferase SULT101A1 catalyzes retinol dehydration (elimination of a sulfate-group) (Grün et al., 1996) and the tick sulfotransferases Sult1 and Sult2 catalyze dopamine conjugation (Yalcin et al., 2010). We also reported characterization of SULTs from *Caenorhabditis elegans* (ceST1), *Drosophila melanogaster* (dmST1-4) and *Bombyx mori* (bmST1) (Hattori et al., 2006, 2007, 2008).

B. mori is the most commonly used species of insect in commercial silk production and one of the most widely used insects in biophysical and biochemical studies. Although Huang et al. reported the presence of 42 putative UDP-glucosyl transferase (UGT) genes in the genome of *B. mori* (Huang et al., 2008), only two cytosolic SULT genes have been assigned in the genome database. Furthermore, no significant SULT activity has been observed towards common phenolic substrates, even though relatively high expression of SULT mRNA was detected in the gut or Malpighian tubule (Hattori et al., unpublished data). Bacterially expressed SULT proteins showed no activity towards common phenolic compounds such as simple phenols, catecholamines, hydroxysteroids or catechins; weak activities were observed only for 4-nitrocatechol and gallates (Hattori et al., 2007). To determine the physiological role of bmST1, we initiated an investigation of endogenous substrates of bmST1 and examined the expression profile of this gene during insect development.

2. Materials and methods

2.1. Materials

Xanthurenic acid, 3-hydroxy-DL-kynurenine and 3-hydroxyanthranilic acid were purchased from Tokyo Chemical Industry

* Corresponding author. Address: 1-5-30 Shibakoen, Minatoku, Tokyo 105-8512, Japan. Tel.: +81 354002634; fax: +81 354002689.
E-mail address: tamura-hr@pha.keio.ac.jp (H. Tamura).

0022-1910/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.10.003

(Tokyo, Japan). Kynurenine and kynurenic acid were purchased from SIGMA Aldrich (St. Louis, MO). L-Tryptophan, 8-hydroxyquinoline-2-carboxylic acid, 20-hydroxyecdysone and silica TLC plates were purchased from Nacalai Tesque (Kyoto, Japan), Wako Pure Chemical Industries (Osaka, Japan), ENZO Life Sciences (Farmingdale, NY) and Merck (Whitehouse Station, NJ), respectively.

2.2. Insect breeding and treatment with tryptophan metabolites

B. mori (Hu·Yo×Tsukuba·Ne) eggs were purchased from Ehime Sansyu (Ehime, Japan) and were raised on artificial diet Silk Mate 2S (Nosan corporation, Kanagawa, Japan) at 27 °C. Reagents were kneaded into the artificial diet and administered orally from day 1 of the 5th instar larval stage, or 50 μL of the reagents were injected into the hemolymph between semilunar markers using a 1 mL syringe, starting from day 2 of the 5th instar larval stage.

Cuticular thickness was measured according to the method of Tanaka (1970). In brief, the epidermis was collected and tanned to isolate the cuticle. The thickness of the cuticle around semilunar markers was determined using a micrometer.

2.3. Protein preparation

Recombinant bmST1 was expressed and purified according to a previous study (Hattori et al., 2007). Cytosolic fractions were prepared from tissues obtained from 5th instar larvae by homogenization of the tissue under liquid N_2, followed by centrifugation (105 k × g, 60 min). Protein concentrations were determined using the Bradford method (Bio-Rad, Hercules, CA).

2.4. Enzymatic activity

Sulfotransferase activity was measured by using PAP[^{35}S] according to previously described procedures (Hattori et al., 2007). [^{35}S]-labeled sulfated compounds were separated using silica TLC (developing solvent; 2-propanol/chloroform/methanol/water = 10:10:5:2) (Shampengtong and Wong, 1989) or were extracted with 1-butanol that was acidified with sodium phosphate (pH 2) (extraction efficiency was more than 90%). Radioactivity was visualized using the image analyzer FLA-7000 (GE Healthcare, Buckinghamshire, UK) or was counted using the liquid scintillation spectrometer LSC-6101 (Aloka, Tokyo, Japan).

2.5. Quantitative PCR

Total RNA was isolated using ISOGEN (Nippon Gene, Tokyo, Japan), and their quantity and purity were checked by OD_{260} and OD_{260}/OD_{280} respectively. First strand cDNA was synthesized from 2.5 μg of total RNA using ReverTra Ace (TOYOBO, Osaka, Japan) with random primers, according to the manufacturer's protocol. Real-time PCR was performed using FastStart Universal SYBR Green Master (Roche Applied Science, Upper Bavaria, Germany) and the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Samples were denatured at 95 °C for 10 min, and cDNA products were amplified with 40 cycles of denaturation at 95 °C for 15 s, then annealing and extension at 60 °C for 60 s. Calculations of the initial relative amounts of mRNA were performed according to the cycle threshold method (Higuchi et al., 1993) using the dilution series of highest expressing sample as standards and analyzed by a software, BioRad CFX manager ver. 2.0. The mRNA levels were normalized using the 18S rRNA levels. The primers used were 5′-TTT CTC GGA CGA GGT CAC TTT C-3′ (forward) and 5′-GTC TCG CCA GTT CTG CCT TT-3′ (reverse) for bmST1 (233 bp), 5′-GCT GTC CCT TTT GCC GTT T-3′ (forward) and 5′-GTC GCT GAC GCA TCA TTT GT-3′ (reverse) for Ftz-f1 (150 bp), and 5′-TGG TTG CAA AGC TGA AAC TTA AAG-3′ (forward) and 5′-AGT CAA ATT AAG CCG CAG GC-3′ (reverse) for 18S rRNA (75 bp) (Hukkanen et al., 2003). Primer specificities were checked using Melt Curve Analysis and agarose gel electrophoresis.

2.6. Data analysis

Kinetic parameters were calculated with Mathematica 8 (Wolfram, Champaign, IL) using the NonlinearModelFit function. Previous data were also recalculated using this function. STATISTICA 8 (StatSoft, Tulsa, OK) was utilized for statistical analysis. The results were analyzed by one-way ANOVA followed by Dunnett's or Tukey's test if significant differences ($p < 0.05$) were observed.

3. Results

3.1. Identification of an endogenous substrate of bmST1

In order to identify endogenous substrates of bmST1, we screened a large number of sulfate acceptors, as listed in Table 1, and found that xanthurenic acid, a tryptophan metabolite (Fig. 1), is a substrate of bmST1. Unlike xanthurenic acid, other tryptophan metabolites were not conjugated by bmST1 at a concentration of 1 μM (Fig. 2A). The kinetic parameters of the reaction of bmST1 and xanthurenic acid were then determined. The K_m of bmST1 for xanthurenic acid (0.388 ± 0.046 μM) is lower than that for the previously described bmST1 substrates 4-nitrocatechol and propyl gallate (1717 ± 260 and 273 ± 75 μM, respectively) and the V_{max} (6.70 ± 0.19 nmol/min/mg protein) is higher (5.12 ± 0.38 and 0.33 ± 0.04 nmol/min/mg protein, respectively) (Fig. 2B). Thus, xanthurenic acid appeared to be a specific and a good substrate for bmST1.

Xanthurenic acid has two hydroxy groups. In mammals, xanthurenic acid sulfate has been reported to act as a natriuretic hormone and the 8-hydroxy group is sulfated (Cain et al., 2007). We therefore tested the effect of bmST1 on compounds that lack one of the two hydroxy groups of xanthurenic acid; kynurenic acid and 8-hydroxyquinoline-2-carboxylic acid, that lack the 8- and the 4-hydroxy group, respectively. Only the 8-hydroxyquinoline-2-carboxylic acid was sulfated at a high concentration (100 μM), but neither compound was sulfated at a low concentration (1 μM) (Fig. 2C). These results suggested that the 8-hydroxy group is sulfated by bmST1.

To verify that sulfotransferase proteins in silk worm tissues can sulfonate xanthurenic acid, cytosols from the Malpighian tubule and the midgut of 5th instar larvae, in which bmST1 mRNA is abundantly expressed, were assayed for sulfotransferase activity toward xanthurenic acid. Xanthurenic acid sulfate was detected in a manner that depended on addition of 1 μM xanthurenic acid to both tissues (Fig. 2D).

3.2. Expression analysis of the bmST1 gene

To elucidate the physiological function of bmST1, the expression of the bmST1 gene during development was examined using real-time PCR analysis (Fig. 3). The highest expression was observed in the egg, followed by the 3rd to the 5th instar larvae, whereas significantly low level expression was observed in the 2nd (Dunnett's, $p = 0.045$), pupa (Dunnett's, $p = 0.0082$) and adult (Dunnett's, $p = 0.0026$) stages, compared to that of the egg (Fig. 3A). The bmST1 mRNA expression level was quantified in several tissues around 4th molting stage. The highest expression was observed in the Malpighian tubule at the 4th molting stage (Tukey's, $p = 0.005$). The level of bmST1 expression in Malpighian tubule dramatically increased more than 10 times during 4th molting stage (Tukey's, $p = 0.037$) and gradually decreased at the beginning of 5th stage (Tukey's,

Table 1
Substrates screened as bmST1 substrates.

1-Naphthol	1-Naphthylamine	2,6-Dichloro-4-nitrophenol	2-Aminonaphthol
2-Naphthol	3,4-Dihydroxymandelic acid	3,4-Dihydroxyphenylacetic acid	3,4-Dihydroxyphenylalanine
4-Aminophenol	4-Nitrophenol	Adrenaline	Alpha-tocopherol
Ascorbic acid	Bisphenol A	Caffeic acid	Chlorogenic acid
Diethylstilbestrol	Dopamine	Ecdysone	Noradrenaline
Octopamine	Pentachlorophenol	Prostaglandin D2	Prostaglandin E2
Protocatechuic acid	Pyridoxine	Quercetine	Resveratrol
Retinol	Serotonin	Thyroxine	Tyramine
Tyrosine	Vanillin	Xanthurenic acid	

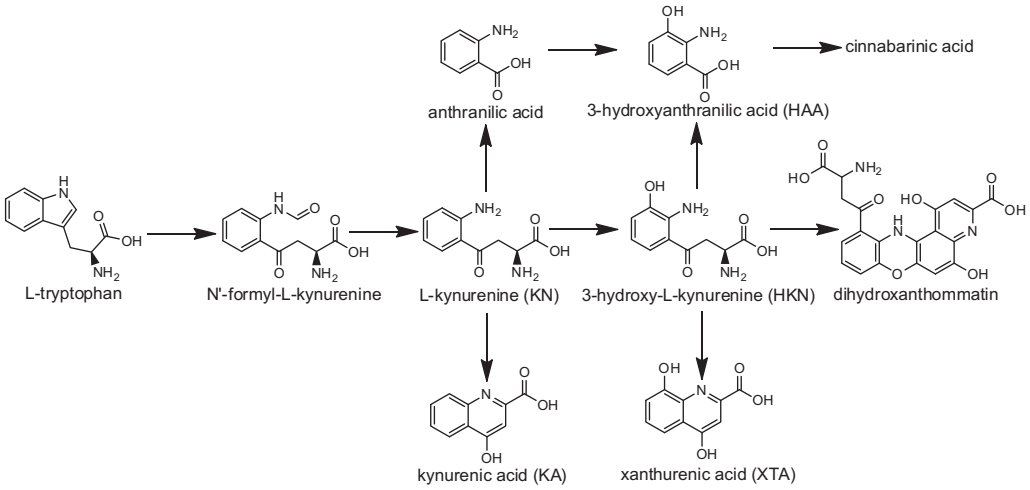

Fig. 1. Kynurenine pathway. This pathway is the main pathway of tryptophan degradation. The pathway branches to form metabolites such as (hydroxy)anthranilic acid, kynurenic acid, dihydroxanthommatin (an ommochrome, i.e. a type of pigment) and xanthurenic acid.

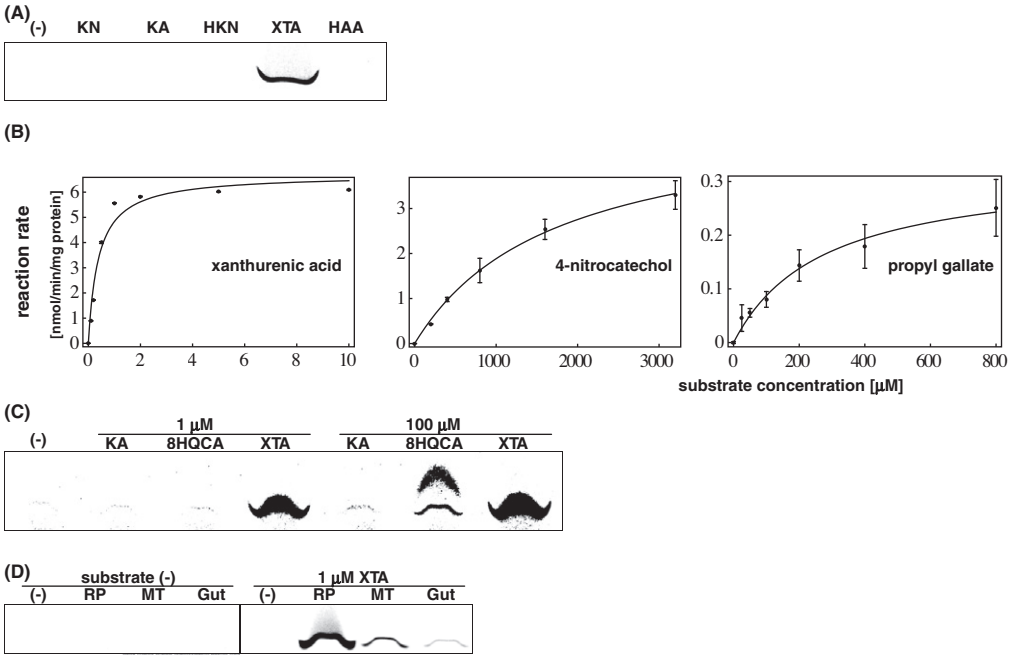

Fig. 2. Sulfation of xanthurenic acid by bmST1. The activity of bmSTI was assayed by silica TLC analysis of [^{35}S]-labeled sulfated substrates. (A) Analysis of bmSTI sulfation of tryptophan metabolites. At a concentration of 1 μM, xanthurenic acid (XTA) was the only compound of typical metabolites of the kynurenine pathway that was sulfated by bmSTI. Kynurenine (KN), kynurenic acid (KA), 3-hydroxykynurenine (HKN) and 3-hydroxyanthranilic acid (HAA) were not sulfated at this concentration. (B) Kinetic parameters of bmST1. The bmSTI enzyme exhibited a lower K_m and higher V_{max} for a xanthurenic acid substrate, than for the previously reported substrates 4-nitrocatechol and propyl gallate (Hattori et al., 2007). Bars, mean ± SD (n = 3) (C) Evaluation of the hydroxy-group substrate preference of bmST1, using 8-hydroxyquinoline-2-carboxylic acid (8HQCA, lacking the 4-hydroxy-group of XTA) and KA (lacking the 8-hydroxy-group of XTA). (D) Measurement of endogenous XTA sulfation activity in cytosols (10 μg) obtained from the Malpighian tubule (MT) and the midgut (Gut) of 5th instar larvae. RP, recombinant bmST1 (0.4 μg).

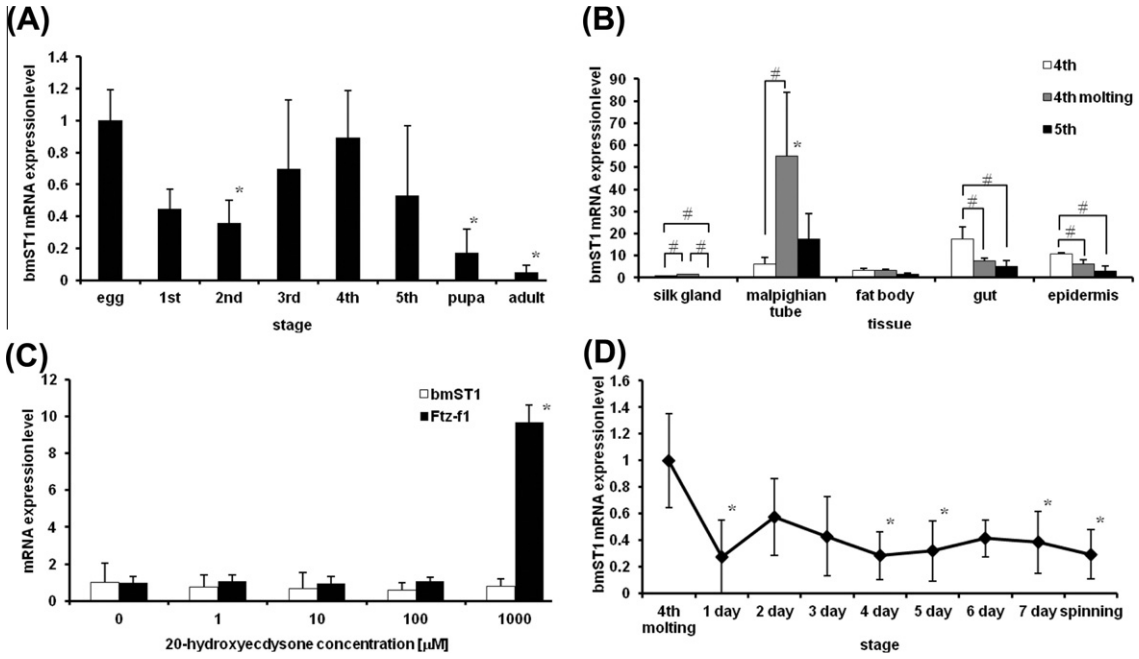

Fig. 3. Expression profile of the bmST1 gene in *B. mori*. Eggs or different developmental stages of *B. mori*, or *B. mori* tissues, were ground in liquid N_2 and total RNA was extracted as described in Materials and Methods. The mRNA levels of bmST1 were quantified using real-time PCR. (A) The mRNA expression of bmSTI at each developmental stage. Relative expression levels were shown by setting the level of the egg as 1.0. Bars, mean ± SD ($n = 3$). Asterisks indicate significant differences from the level of the egg (Dunnett's, $p < 0.05$). (B) Tissue-specific expression of bmST1 mRNA around the 4th molting stage. Relative expression levels were shown by setting the level of the silk grand at 4th stage as 1.0. Bars, mean ± SD ($n = 3$). An asterisk indicates a significant difference between the Malpighian tubule at 4th stage and others (Tukey's, $p < 0.05$). Sharps (#) indicate significant difference between the pairs indicated among the same tissue (Tukey's, $p < 0.05$). (C) Effect of 20-hydroxyecdysone on expression of the bmST1 gene in the Malpighian tubule of 5th instar larvae. The mRNA expression of the established ecdysone responsive gene Ftz-f1 was assayed as a positive control. Bars, mean ± SD ($n = 3$). An asterisk indicates significant difference between the treated group and the control (Dunnett's, $p < 0.05$). (D) Changes in bmST1 gene expression in the Malpighian tubule from after the 4th molting stage to the spinning stage. Bars, mean ± SD ($n = 3$). Asterisks indicate significant differences from the 4th molting stage (Dunnett's, $p < 0.05$).

$p = 0.097$), whereas the expression in other tissues constantly decreased during the stage (Fig. 3B).

To determine if 20-hydroxyecdysone (20-HE) is responsible for induction of the bmST1 gene at this stage, we injected 20-HE into the hemolymph of 5th instar larvae and measured expression of the bmST1 gene in the Malpighian tubule after 24 h. There was no significant induction of bmST1 gene expression even at 1 mM 20-HE, whereas the level of expression of Ftz-f1, an ecdysone responsive gene (Suzuki et al., 2002), significantly increased (Fig. 3C, Dunnett's, $p = 0.00005$). No significant induction of the bmST1 gene was observed at the late 5th instar stage at which 20-hydroxyecdysone is secreted (Fig. 3D).

3.3. Effect of tryptophan metabolites on the growth of B. mori larvae

Tryptophan and its metabolites are known to exhibit toxicity towards insects (Arai and Ito, 1967; Kayser, 1979; Linzen, 1974). We hypothesized that bmST1 might reduce the toxicity of tryptophan metabolites. We therefore analyzed the toxicity of tryptophan metabolites by adding them to the artificial diet and feeding them to 5th instar larvae. After 3 days feeding of tryptophan or its metabolites (4-branched metabolites after kynurenine except for xanthommatin), edema of the intersegmental membrane was observed only following feeding of xanthurenic acid (Fig. 4A). Additionally, growth inhibition was observed at high doses of tryptophan (Dunnett's, $p = 0.0069$) or xanthurenic acid (Dunnett's, $p = 0.045$) (Fig. 4B).

To elucidate the mechanism of the toxicity of xanthurenic acid towards the intersegmental membrane, the thickness of the cuticle was measured. Cuticle thickness decreased in a xanthurenic acid dose-dependent manner (Fig. 4C, Dunnett's, $p = 0.00041$ at 50 μmol/g diet and $p = 0.000009$ at 100 μmol/g diet, respectively).

4. Discussion

In this report, we demonstrated that xanthurenic acid is a highly selective endogenous substrate for the *B. mori* cytosolic sulfotransferase, bmST1. Kynurenic acid, a tryptophan metabolite with a similar structure, was not sulfated, and 8-hydroxyquinoline-2-carboxylic acid was only slightly sulfated at a high concentration (Fig. 2C). Therefore, the hydroxy-groups at the 4 and the 8 position both seem to be essential for recognition by bmST1. SULT1B1 has been shown to catalyze xanthurenic acid sulfation in mammals (Senggunprai et al., 2008), although there is little structural similarity between SULT1B1 and bmSTI (31% amino acid sequence homology). We found that neither the two universal phenol sulfotransferase inhibitors, 2,6-dichloro-4-nitrophenol and pentachlorophenol (Mulder and Scholtens, 1977), nor kynurenic acid, a SULT1B1 specific inhibitor (Senggunprai et al., 2008, 2009), inhibited bmST1 activity (data not shown). Thus, the structure of the substrate binding pocket of this sulfotransferase might differ from those of other phenol sulfotransferases. Further precise investigation, including mutational analysis, should be performed to clarify the structural diversity of this unique sulfotransferase.

Quantitative real-time PCR analysis revealed abundant expression of bmST1 in the Malpighian tubule at the 4th molting stage. The physiological functions of this organ are thought to correspond to those of the kidney in mammals (Dow and Davies, 2006). Preurine is formed in the tubules when nitrogenous waste and electrolytes are transported through the tubule walls. Waste such as urea and amino acids are thought to diffuse through the walls, while ions such as sodium and potassium are transported by active pump mechanisms. Recent reports indicate that the Drosophila tubule expresses very high levels of specific cytochrome P450s as well as glutathione-S-transferases, which suggest that the tubule

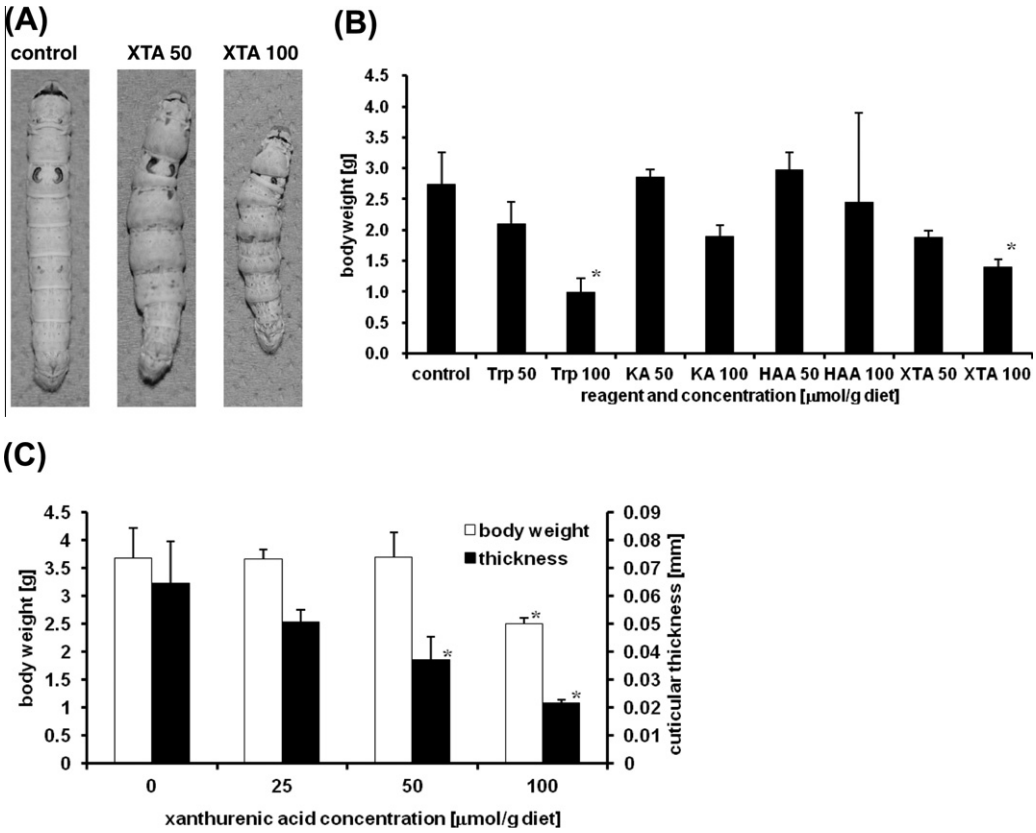

Fig. 4. Toxic effect of xanthurenic acid on 5th instar larvae. (A) Changes in morphology and size due to feeding of high doses (>50 μmol/g) of xanthurenic acid (XTA) for 3 days. (B) Effects of tryptophan and its metabolites (as shown in Fig. 1) on the growth of 5th instar larvae. Feeding of tryptophan or its metabolites was started on day 1 of the 5th instar stage and was continued for 3 days. Body weight was measured on day 4. Bars, mean ± SD ($n = 3$). Asterisks indicate significant differences between treated groups and controls (Dunnett's, $p < 0.05$). (C) Cuticular thickness and body weight was measured after feeding of various concentrations of xanthurenic acid as described in (B). Bars, mean ± SD ($n = 5$). Asterisks indicate significant differences between treated groups and controls (Dunnett's, $p < 0.05$).

plays a major role in metabolism and detoxification of both endogenous solutes, and of xenobiotics such as insecticides (Dow and Davies, 2006). In addition, many kinds of transporters are also expressed in the Drosophila Malpighian tubule (Dow and Davies, 2006). Xanthurenic acid sulfate might be eliminated from the hemolymph to the hindgut via these transporters. These data suggest that bmST1 plays an important role in the detoxification of endogenous solutes and xenobiotics in this organ of the silk worm.

The fact that bmST1 gene expression is induced at the 4th molting stage would be consistent with the fact that this stage corresponds to a possible period of high amino acid metabolic activity. Indeed, the level of 3-hydroxykynurenine, the precursor of xanthurenic acid, increases at the time of molting and spinning (Inagami and Suto, 1954), suggesting the possibility that regulation of bmSTI expression is associated with accumulation of this precursor. However, the result of Fig. 3C indicates that 20-HE might not regulate bmST1 gene expression in the Malpighian tubule. It is possible that tryptophan metabolites or other hormones might function as regulators of the bmST1 gene. Furthermore, the result that the induction of bmST1 gene at 4th molting stage occurred only in the Malpighian tubule among tested tissues suggests that a unique regulation might be employed for bmST1 gene expression in the Malpighian tubule. Further study is needed to clarify the mechanism of regulation of this gene.

In the *in vivo* assay of the toxicity of branched tryptophan metabolites, xanthurenic acid showed characteristic toxicity towards silk worm larvae (Fig. 4). Although the precise mechanism of this toxicity is unclear, a decrease in the thickness of the cuticle might be due to inhibition of cuticular biosynthesis. Growth of the viscera, and an increase in the amount of hemolymph without cuticular outgrowth, might lead to increased internal pressure and edema at the intersegmental membrane. Since this membrane is the weakest part of cuticle, it would then appear as shown in Fig. 4A. The insect cuticle consists mainly of chitin, protein, catechol and lipid that are secreted by epidermal cells (Andersen, 2010; Charles, 2010; Moussian, 2010). Because most cuticular proteins and chitin metabolism are controlled by insect hormones such as ecdysone and juvenile hormones (Charles, 2010; Merzendorfer, 2006; Merzendorfer and Zimoch, 2003; Moussian, 2010), a change in the biosynthesis and concentration of ecdysone, which might be caused by xanthurenic acid (Ohnishi and Naya, 1994), may result in defects of the cuticle. In addition, the silkworm expresses the 14-3-3 gene (Tabunoki et al., 2008), which encodes a target protein of xanthurenic acid-induced apoptosis (Malina and Frueh, 2003). Therefore, increased levels of xanthurenic acid appear to induce apoptosis of epidermal cells in the cuticle.

Xanthurenic acid is a metabolite of the kynurenine pathway that largely involves tryptophan degradation (Fig. 1) (Stone, 1993). It is known that xanthurenic acid is present in mammals, as well as in insects such as flies, mosquitos and the silk worm, which contain xanthurenic acid in their hemolymph and other tissues (Inagami, 1955). In mammals, xanthurenic acid has been reported to have several biological effects such as induction of apoptosis (Malina et al., 2002) and anti-insulin (Kotake and Murakami, 1971), natriuretic (sulfate or glucoside) (Cain et al., 2007) and neurotransmitter (Gobaille et al., 2008) effects. In insects and crustacea, xanthurenic acid depresses ecdysone biosynthesis by inhibition of cytochrome P450 (Ohnishi and Naya, 1994). Additionally, a high dose of tryptophan is known to be toxic to insects, because insects cannot completely degrade tryptophan (Linzen, 1974). Therefore, sulfation

of xanthurenic acid by bmST1 might be crucial for the growth and development of the silkworm.

Acknowledgment

We thank Dr. Natori for helpful discussions.

References

Andersen, S.O., 2010. Insect cuticular sclerotization: a review. Insect Biochemistry and Molecular Biology 40, 166–178.
Arai, N., Ito, T., 1967. Nutrition of the silkworm, *Bombyx mori*. Bulletin of Sericultural Experiment Station 21, 373–383 (in Japanese).
Cain, C.D., Schroeder, F.C., Shankel, S.W., Mitchnick, M., Schmertzler, M., Bricker, N.S., 2007. Identification of xanthurenic acid 8-O-beta-D-glucoside and xanthurenic acid 8-O-sulfate as human natriuretic hormones. Proceedings of the National Academy of Sciences of the United States of America 104, 17873–17878.
Charles, J.P., 2010. The regulation of expression of insect cuticle protein genes. Insect Biochemistry and Molecular Biology 40, 205–213.
Dow, J.A., Davies, S.A., 2006. The Malpighian tubule: rapid insights from post-genomic biology. Journal of Insect Physiology 52, 365–378.
Falany, C.N., 1997. Enzymology of human cytosolic sulfotransferases. The Journal of the Federation of American Societies for Experimental Biology 11, 206–216.
Gobaille, S., Kemmel, V., Brumaru, D., Dugave, C., Aunis, D., Maitre, M., 2008. Xanthurenic acid distribution, transport, accumulation and release in the rat brain. Journal of Neurochemistry 105, 982–993.
Grün, F., Noy, N., Hämmerling, U., Buck, J., 1996. Purification, cloning, and bacterial expression of retinol dehydratase from *Spodoptera frugiperda*. The Journal of Biologycal Chemistry 271, 16135–16138.
Hattori, K., Hirayama, M., Suzuki, H., Hamamoto, H., Sekimizu, K., Tamura, H., 2007. Cloning and expression of a novel sulfotransferase with unique substrate specificity from *Bombyx mori*. Bioscience, Biotechnology, and Biochemistry 71, 1044–1051.
Hattori, K., Inoue, M., Inoue, T., Arai, H., Tamura, H., 2006. A novel sulfotransferase abundantly expressed in the Dauer larvae of *Caenorhabditis elegans*. The Journal of Biochemistry 139, 355–362.
Hattori, K., Motohashi, N., Kobayashi, I., Tohya, T., Oikawa, M., Tamura, H., 2008. Cloning, expression, and characterization of cytosolic sulfotransferase isozymes from *Drosophila melanogaster*. Bioscience, Biotechnology, and Biochemistry 72, 540–547.
Higuchi, R., Fockler, C., Dollinger, G., Watson, R., 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology (NY) 11, 1026–1030.
Huang, F.F., Chai, C.L., Zhang, Z., Liu, Z.H., Dai, F.Y., Lu, C., Xiang, Z.H., 2008. The UDP-glucosyltransferase multigene family in *Bombyx mori*. BMC Genomics 9, 563.
Hukkanen, J., Väisänen, T., Lassila, A., Piipari, R., Anttila, S., Pelkonen, O., Raunio, H., Hakkola, J., 2003. Regulation of CYP3A5 by glucocorticoids and cigarette smoke in human lung-derived cells. The Journal of Pharmacology and Experimental Therapeutics 304, 745–752.
Inagami, K., 1955. Chemical and genetical studies on the formation of the pigment in the silkworm. (VIII) Xanthurenic acid and 4,8-dihydroxyquinoline in the silkworm pupae. The Journal of Sericultural Science of Japan 24, 295–299 (in Japanese).
Inagami, K., Suto, Y., 1954. 3-Hydroxykynurenine metabolism in the larva and the pupa. The Journal of Sericultural Science of Japan 24, 85–91 (in Japanese).
Kayser, H., 1979. Ommochrome formation and kynurenine excretion in *Pieris brassicae*: relation to tryptophan supply on an artificial diet. Journal of Insect Physiology 25, 641–646.
Kotake, Y., Murakami, E., 1971. A possible diabetogenic role for tryptophan metabolites and effects of xanthurenic acid on insulin. The American Journal of Clinical Nutrition 24, 826–829.
Linzen, B., 1974. The tryptophan→ommochrome pathway in insects. Advances in Insect Physiology 10, 117–246.
Malina, H., Richter, C., Frueh, B., Hess, O.M., 2002. Lens epithelial cell apoptosis and intracellular Ca^{2+} increase in the presence of xanthurenic acid. BMC Ophthalmology 2, 1.
Malina, H.Z., Frueh, B.E., 2003. Abnormal signalling of 14-3-3 proteins in cells with accumulated xanthurenic acid. Biochemical and Biophysical Research Communications 310, 646–650.
Merzendorfer, H., 2006. Insect chitin synthases: a review. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 176, 1–15.
Merzendorfer, H., Zimoch, L., 2003. Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. The Journal of Experimental Biology 206, 4393–4412.
Moussian, B., 2010. Recent advances in understanding mechanisms of insect cuticle differentiation. Insect Biochemistry and Molecular Biology 40, 363–375.
Mulder, G.J., Scholtens, E., 1977. Phenol sulphotransferase and uridine diphosphate glucuronyltransferase from rat liver in vivo and vitro. 2,6-Dichloro-4-nitrophenol as selective inhibitor of sulphation. Biochemical Journal 165, 553–559.
Ohnishi, M., Naya, Y., 1994. Effect of xanthurenic acid on P-450-dependent biotransformation by molting glands *in vitro*. Cellular and Molecular Life Sciences 50, 654–657.
Senggunprai, L., Yoshinari, K., Shimada, M., Yamazoe, Y., 2008. Involvement of ST1B subfamily of cytosolic sulfotransferase in kynurenine metabolism to form natriuretic xanthurenic acid sulfate. The Journal of Pharmacology and Experimental Therapeutics 327, 789–798.
Senggunprai, L., Yoshinari, K., Yamazoe, Y., 2009. Inhibitory effects of kynurenic acid, a tryptophan metabolite, and its derivatives on cytosolic sulfotransferases. Biochemical Journal 422, 455–462.
Shampengtong, L., Wong, K.P., 1989. An *in vitro* assay of 20-hydroxyecdysone sulfotrasferase in the mosquito, *Aedes togoi*. Insect Biochemistry 19, 191–196.
Stone, T.W., 1993. Neuropharmacology of quinolinic and kynurenic acids. Pharmacological Reviews September 45, 309–379.
Suzuki, Y., Matsuoka, T., Iimura, Y., Fujiwara, H., 2002. Ecdysteroid-dependent expression of a novel cuticle protein gene BMCPG1 in the silkworm, *Bombyx mori*. Insect Biochemistry and Molecular Biology 32, 599–607.
Tabunoki, H., Shimada, T., Banno, Y., Sato, R., Kajiwara, H., Mita, K., Satoh, J., 2008. Identification of *Bombyx mori* 14-3-3 orthologs and the interactor Hsp60. Neuroscience Research 61, 271–280.
Tanaka, K., 1970. Studies on the structure of the integuments of *Bombyx mori* L. and several other species of Lepidoptera. II. On the histological structure of the cuticle of silkworm larvae. The Journal of Sericultural Science of Japan 39, 231–238 (in Japanese).
Yalcin, E.B., Stangl, H., Pichu, S., Mather, T.N., King, R.S., 2010. Monoamine neurotransmitters as substrates for novel tick sulfotransferases, homology modeling, molecular docking, and enzyme kinetics. ACS Chemical Biology 18, 176–184.
Yamazoe, Y., Nagata, K., Ozawa, S., Kato, R., 1994. Structural similarity and diversity of sulfotransferases. Chemico-Biological Interactions 92, 107–117.