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The tryptophan oxidation pathway in mosquitoes with emphasis on xanthurenic acid biosynthesis

Qian Han[a], **Brenda T. Beerntsen**[b], and **Jianyong Li**[a,*]

a*Deptment of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*

b*Deptment of Veterinary Pathobiology, University of Missouri, 201 Connaway Hall, Columbia, MO 65211, USA*

Abstract

Oxidation of tryptophan to kynurenine and 3-hydroxykynurenine (3-HK) is the major catabolic pathway in mosquitoes. However, 3-HK is oxidized easily under physiological conditions, resulting in the production of reactive radical species. To overcome this problem, mosquitoes have developed an efficient mechanism to prevent 3-HK from accumulating by converting this chemically reactive compound to the chemically stable xanthurenic acid. Interestingly, 3-HK is a precursor for the production of compound eye pigments during the pupal and early adult stages; consequently, mosquitoes need to preserve and transport 3-HK for compound eye pigmentation in pupae and adults. This review summarizes the tryptophan oxidation pathway, compares and contrasts the mosquito tryptophan oxidation pathway with other model species, and discusses possible driving forces leading to the functional adaptation and evolution of enzymes involved in the mosquito tryptophan oxidation pathway.

Keywords

Xanthurenic acid; Mosquito; 3-Hydroxykynurenine; Kynurenine 3-monooxygenase; 3-Hydroxykynurenine transaminase

1. Introduction

Kynurenine and 3-hydroxykynurenine (3-HK) are intermediates in the tryptophan oxidation pathway (Fig. 1). In mammals, kynurenine is the immediate precursor in the pathway leading to the formation of kynurenic acid that serves as a broad-spectrum antagonist at ionotropic excitatory amino acid receptors (NMDA receptors) and protects the central nervous system (CNS) from being overstimulated by excitatory cytotoxins (Stone, 2000). Therefore, there has been extensive research investigating the biochemical pathway leading to the formation of kynurenic acid and the consequences caused by kynurenic acid deficiency (Schwarcz, 1993;Moroni, 1999;Stone, 2001a,c,b). Kynurenine can be oxidized by kynurenine monooxygenase (KMO, EC 1.14.13.9) to form 3-HK. Although 3-HK is a natural metabolite, it is oxidized easily under physiological conditions, stimulating the production of reactive oxygen species (Okuda et al., 1996,1998;Wei et al., 2000). For example, 3-HK can induce apoptosis of neuron cells at micromolar concentrations (Wei et al., 2000). Injection of tryptophan metabolites into adult flies caused severe motor dysfunction (Cerstiaens et al., 2003). Earlier studies have also shown that 3-HK content was quite different in insects at different developmental stages (Linzen, 1974). To maintain physiological conditions, it is

*Corresponding author. Tel.: +1540 231 1182; fax: +1540 231 9070. E-mail address: lij@vt.edu (J. Li).

essential to prevent the accumulation of this reactive compound. In mammals, both kynurenine and 3-HK can be hydrolyzed by kynureninase (EC 3.7.1.3) to anthranilic acid and 3-hydroxyanthranilic acid and the latter two compounds can be either completely oxidized to CO_2 and H_2O through a complicated biochemical pathway or used to synthesize $NAD(P)^+$ (Stone, 1993). Although there have been a number of reports discussing the toxicity of 3-HK in mammals (Okuda et al., 1996,1998;Wei et al., 2000), 3-HK, produced under normal physiological conditions, does not seem to cause any problems in mammals as it can be hydrolyzed and further oxidized via the glutaryl-CoA pathway.

In mosquitoes (likely other insects as well), oxidation of tryptophan to kynurenine and then to 3-HK is a major branch pathway of tryptophan metabolism. However, because mosquitoes do not have kynureninase, the hydro-lysis pathway of 3-HK is not available. Consequently, mosquitoes must deal with 3-HK in a different manner. Our study investigating the tryptophan oxidation pathway in *Aedes aegypti* indicated that mosquitoes have developed an efficient strategy to prevent the accumulation of 3-HK by converting the chemically reactive 3-HK to the chemically stable xanthurenic acid (XA) via transaminase-mediated reactions. For example, the concentration of XA is many folds higher than that of 3-HK in the supernatant of mosquito larval homogenate (Li and Li, 1997; Li et al., 1999) (Li, J., unpublished data). As there have been no reports indicating any toxic effect of XA to living organisms, we proposed that the transamination of the chemically reactive 3-HK to the chemically stable XA serves as the mechanism by which mosquitoes detoxify 3-HK (Li and Li, 1997, 1998;Han et al., 2002).

Recently, the 3-HK to XA pathway has attracted increased attention because it has been found that XA induces the exflagellation of *Plasmodium* microgametocytes (Billker et al., 1998; Garcia et al., 1998), an essential step during sexual reproduction of malaria parasites in mosquitoes. Mosquitoes transmit malaria parasites, dengue fever and West Nile virus, which are major threats to human health and well-being throughout the world. Among them, malaria is considered to be the most prevalent life-threatening disease, with estimates of new cases ranging from 300 million to 660 million cases per year (Snow et al., 2005). The development of insecticide-resistant mosquitoes and drug-resistant parasites demand that new innovative control strategies have to be developed. Therefore, the ability of XA to stimulate *Plasmodium* development provides a potential malaria control strategy by targeting proteins involved in the 3-HK to XA pathway in mosquitoes (Rossi et al., 2005, 2006).

Interestingly, 3-HK also is the initial precursor for the production of ommochromes that are major eye pigments in mosquitoes. Compound eye development and eye pigmentation occur mainly during the pupal and early adult stages (Beard et al., 1995; Li et al., 1999; Rasgon and Scott, 2004; Sethuraman and O'Brochta, 2005). Consequently, the 3-HK to XA pathway must be partially down-regulated in pupae and adults to allow for the accumulation of some 3-HK that then can be transported to the compound eyes for pigmentation (Li and Li, 1997; Li et al., 1999). In this review, we summarize the tryptophan to XA pathway in living organisms, compare and contrast the same pathway in mosquitoes with that of other organisms, and discuss the functional adaptation and evolution of the proteins involved in the 3-HK to XA pathway in mosquitoes.

2. The tryptophan to XA pathway

XA is a metabolite in the tryptophan oxidation pathway and its formation from tryptophan is a complicated process with a number of enzymes involved in the biochemical pathway. The overall process leading to the formation of XA includes oxidation of tryptophan to formylkynurenine, hydrolysis of formylkynurenine to kynurenine, hydroxylation of kynurenine to 3-HK, transamination of 3-HK to a side chain keto acid intermediate, and intramolecular cyclization of the intermediate to XA (Fig. 1). The chemical process and

enzymes involved in this branch pathway of tryptophan metabolism have been studied extensively in mammals. Because metabolites in the tryptophan oxidation pathway influence the color of the compound eyes in insects, there have been a number of earlier reports discussing tryptophan metabolites in relation to insect compound eye development (Howells et al., 1977;Paton and Sullivan, 1978;Summers and Howells, 1978;Akaboshi, 1979;Howells, 1979). However, there have been few studies concerning the enzymes involved in the tryptophan oxidation pathway in insects.

2.1. Tryptophan 2,3-dioxygenase (TDO)

TDO is a heme-containing dioxygenase involved in catalyzing the addition of molecular oxygen (O_2) across the 2,3-double bond of the indole ring of tryptophan, leading to the cleavage of the indole ring to form *N*-formylkynurenine (Tanaka and Knox, 1959; Hayaishi and Nozaki, 1969; Leeds et al., 1993; Dick et al., 2001). It has generally been accepted that the heme prosthetic group of TDO is present as a heme-ferric form (heme-Fe^{3+}) that must be reduced to the heme-ferrous form (heme-Fe^{2+}) prior to mediating tryptophan oxidation (Tanaka and Knox, 1959;Hayaishi and Nozaki, 1969; Ishimura and Hayaishi, 1973;Hitchcock and Katz, 1988). The mechanism leading to TDO activation has attracted a considerable amount of attention. It has been reported that superoxide ($\bullet O_2^-$) or hydrogen peroxide (H_2O_2) or reducing agents (e.g., ascorbate and sodium hydrosulfite) stimulate TDO activity through a reductive activation process (Tanaka and Knox, 1959; Hayaishi and Nozaki, 1969; Brady et al., 1971;Schutz et al., 1972; Ishimura and Hayaishi, 1973; Hitchcock and Katz, 1988). Earlier studies also concluded that tryptophan 2,3-dioxygenase was subject to allosteric regulation and that the enzyme had at least two distinct sites for tryptophan binding, with one as a catalytic site and the other as an allosteric site (Koike et al., 1969; Brady et al., 1971; Schutz et al., 1972; Kobayashi et al., 1989).

The early studies of insect tryptophan oxidation focused on *Drosophila* TDO which also was called tryptophan pyrrolase (Kaufman, 1962; Marzluf, 1965; Phillips et al., 1967; Burnet and Sang, 1968; Tartof, 1969; Baillie and Chovnick, 1971; Jacobson, 1971; Tobler et al., 1971;Sullivan et al., 1974; Mischke et al., 1975; Moore and Sullivan, 1975; Tobler, 1975). Subsequently, it was verified that the *vermilion* gene that affects the color of *Drosophila* compound eyes is *Drosophila* TDO (Searles and Voelker, 1986; Walker et al., 1986; Searles et al., 1990). After the molecular characterization of the *Drosophila* TDO (Gen-Bank accession no. AAC24239), the TDO gene from *Anopheles gambiae* (GenBank accession no. AAC27659) and *Tribolium* (GenBank accession no. AAL15466) also has been molecularly cloned (Mukabayire et al., 1996; Lorenzen et al., 2002). Presently, a number of insect TDO sequences are available in the genomic databases. However, there have been no in-depth studies dealing with any insect TDO at the protein level, likely due to difficulties in isolating sufficient TDO from insects for critical characterization. Recently, we expressed an *Ae. aegypti* TDO (GenBank accession no. AF325458) in a baculovirus/insect cell system, and this recombinant TDO was functionally active as shown by its ability to oxidize tryptophan to formylkynurenine (Fig. 2). The ability to purify milligram quantities of functionally active TDO from transfected insect cells should provide the necessary material required for understanding the structure/function relationship of this important enzyme.

2.2. Kynurenine formamidase

Formylkynurenine, formed by TDO-catalyzed tryptophan oxidation, is hydrolyzed to kynurenine (see Fig. 1). Hydrolysis of formylkynurenine proceeds under physiological conditions, but the rate is low. A specific enzyme, which catalyzes the hydrolysis of formylkynurenine, has been shown to be present in a number of mammalian species and is responsible for the rapid conversion of formylkynurenine to kynurenine in vivo (Mehler and Knox, 1950). This enzyme has been termed kynurenine formamidase (EC 3.5.1.9) and the

enzyme from adult chicken liver (Bailey and Wagner, 1974), rat liver cytoplasm (Arndt et al., 1973), *Drosophila melanogaster* (Moore and Sullivan, 1975,1978), and *Streptomyces parvulus* (Brown et al., 1986) has been partially purified. A mouse kynurenine formamidase recently has been cloned (GenBank accession no. NP_082103) and characterized (Pabarcus and Casida, 2002). A gene coding for kynurenine formamidase also has been identified from a bacterium (Kurnasov et al., 2003). However, no sequence in the *Drosophila* and *An. gambiae* protein databases has been annotated as the kynurenine formamidase. A BLAST search and sequence alignment revealed that the coding sequences with GenBank accession numbers of AAF52391 and EAA03628, respectively, sharing 25% and 35% sequence identity with the mouse formamidase, are likely to be the kynurenine formamidase in *Drosophila* and *An. gambiae*, respectively. The true identity of these two proteins, however, must be functionally verified through critical biochemical characterization.

2.3. Kynurenine 3-monooxygenase (KMO)

KMO (EC 1.14.13.9), a flavin-containing enzyme, catalyzes the hydroxylation of kynurenine to 3-HK in the tryptophan oxidation pathway. KMO has a key role in tryptophan catabolism and the synthesis of ommochrome pigments in mosquitoes. Early studies of KMO were concerned primarily with its role in eye pigmentation in some insects, including *D. melanogaster* (Howells et al., 1977; Paton and Sullivan, 1978; Howells, 1979), *Lucilia cuprina* (Summers and Howells, 1978) and *Musca domestica* (Akaboshi, 1979). In *D. melanogaster*, the *cinnabar (cn)* gene (GenBank accession no. NP_523651) was shown to encode KMO (Warren et al., 1996) and in a mutant strain of *Bombyx mori*, the genetic lesion leading to the white-eye phenotype has been identified (Quan et al., 2002). The gene encoding KMO (GenBank accession no. AAO27576) in the yellow fever mosquito, *Ae. aegypti*, has been named *kynurenine hydroxylase (kh)* (Cornel et al., 1997), and its mutation results in a white-eye phenotype designated as the kh^w strain (Bhalla, 1968; Cornel et al., 1997). Recent efforts to genetically manipulate mosquitoes to control mosquito-transmitted diseases have raised considerable interest in the KMO gene, because it can serve as an excellent marker that indicates the successful production of transgenic *Ae. aegypti* (Coates et al., 1998; Jasinskiene et al., 1998;Sethuraman and O'Brochta, 2005). Sequence analysis of the wild-type and mutant kh^w cDNAs revealed a deletion of 162 nucleotides in the mutant *Ae. aegypti* KMO gene near the 3′-end of the deduced coding region and RT-PCR analysis confirmed the transcription of a truncated mRNA in the mutant strain (Han et al., 2003). The in-frame deletion of the KMO gene results in a loss of 54 amino acids and disrupts a major alpha-helix, which probably accounts for the loss of its activity. Further evidence has shown that the *Ae. aegypti* white-eye (kh^w) mutant strain (Bhalla, 1968) can be complemented by a wild-type copy of the *D. melanogaster cinnabar* gene (Cornel et al., 1997), thereby providing genetic evidence that the product encoded by the white-eye gene was KMO. Although the *D. melanogaster cinnabar* gene complements various white-eye and other mutations in Diptera, it does not function well outside this order (Atkinson et al., 2001). However, the general application of the eye-color genes as broad-spectrum transformation markers in species of Coleoptera has also been discussed (Lorenzen et al., 2002).

The catalytic mechanism of KMO has been suggested as being similar to that of a bacterial *p*-hydroxybenzoate hydroxylase (Entsch et al., 1976a,b;Breton et al., 2000). Sequence comparison between the *Ae. aegypti* KMO and *Pseudomonas putida* hydroxybenzoate hydroxylase (PHBH), an extensively characterized flavin monooxygenase, revealed similarity between the two enzymes. Although *Ae. aegypti* KMO and *P. putida* PHBH share only limited overall sequence identity (16%), the *Ae. aegypti* KMO, like the *P. putida* PHBH, contains a similar sequence motif $xhxhGxxGxxxhxxh(x)_8hxhE(D)$, which is a well-known fingerprint for a dinucleotide binding domain, where x is any residue and h is a hydrophobic residue (Eppink et al., 1997). Moreover, the amino acid sequence, $_{153}$DYIAGCDGFHGISR$_{166}$, which has been

reported to play a dual function in both FAD and NAD(P)H binding in hydroxybenzoate hydroxylase, also is highly conserved in the *Ae. aegypti* KMO (residues $_{172}$DLIVGCDGAYSAVR$_{185}$). Based on these observations, the catalytic mechanism of KMO likely is similar to that of hydroxybenzoate hydroxylase, a prototype for a FAD-dependent monooxygenase. However, some sequence fragments that are highly conserved in KMOs are not present in hydroxybenzoate hydroxylase. For example, the residues 60–68, 225–230, 233–240 and 326–332 in the *Ae. aegypti* KMO likely play relevant roles in either folding stability or catalysis. KMO has been cloned from several insect species and the biochemical and kinetic parameters of the enzyme have been analyzed for a few of them (Hirai et al., 2002;Quan et al., 2002). For example, recombinant *Ae. aegypti* KMO showed a high substrate specificity for kynurenine with optimum activity at 40 °C and pH = 7.5. Its K_m for kynurenine, NADPH and NADH was 0.89, 0.82 and 5.17, respectively, suggesting that it favors NADPH as a reducing agent. KMO is considered to be an important pharmaceutical target for the development of drugs for neurodegenerative diseases (Pellicciari et al., 2003;Moroni et al., 2005;Samadi et al., 2005); therefore, there is an increasing need to determine its 3D structure.

2.4. Kynurenine aminotransferase

In humans and other mammals, kynurenine aminotransferase (KAT) I and II are the primary enzymes involved in the transamination of 3-HK (Okuno et al., 1991; Guidetti et al., 1997; Han et al., 2004). However, the 3-HK transamination function of KAT was rarely highlighted in the published literature. KAT, as implied by its name, catalyzes the transamination of kynurenine to kynurenic acid, a broad-spectrum antagonist at ionotropic excitatory amino acid receptors (NMDA receptors) that protects the central nervous system from being over-stimulated by excitatory cytotoxins (Stone et al., 1996). Although mammalian KAT efficiently catalyzes both the kynurenine to kynurenic acid pathway and the 3-HK to XA pathway, the emphasis on KAT function has been exclusively on its role in the biosynthesis of kynurenic acid (Moroni, 1999; Stone and Darlington, 2002; Schwarcz, 2004). Initially, it was assumed that a similar KAT was responsible for the 3-HK to XA pathway in *Ae. aegypti*. The *Ae. aegypti* KAT (GenBank accession no. AAK97625) was successfully cloned and its deduced sequence shares high sequence identity with mammalian KATs (Fang et al., 2002). Subsequently, an *Ae. aegypti* recombinant KAT was expressed in insect cells, and biochemical characterization revealed that the mosquito KAT had high activity towards kynurenine, but it showed no activity for 3-HK (Fang et al., 2002; Han and Li, 2004). By following the 3-HK transamination active fractions during chromatographic separation, we eventually purified the 3-HK transaminase (HKT) from *Ae. aegypti* and cloned its gene (GenBank accession no. AAL29468) based on partial protein sequences (Han et al., 2002; Han and Li, 2002). Surprisingly, however, its primary sequence shares the highest sequence identity (45–46%) with the mammalian alanine glyoxalate aminotransferases (AGTs) in the GenBank database (GenBank accession nos. CAA53527, AAA31158, NP_057911, BAA02632 for cat, rabbit, mouse, and human, respectively).

3. Functional adaptation and evolution of mosquito AGTs

In mammals, KAT is primarily responsible for the 3-HK to XA pathway. The mosquito KAT (Fang et al., 2002;Han and Li, 2004; Han et al., 2005), sharing high sequence identity (>45%) with mammalian KATs, would logically be considered the probable enzyme responsible for the 3-HK to XA pathway in mosquitoes. In contrast, the protein (GenBank accession no. AAL29468) with high 3-HK transamination activity, shares limited similarity (<10%) with KAT sequences, but has high sequence identity (>45%) with mammalian AGTs. Consequently, the sequence most likely would have been annotated as a mosquito AGT if it had been randomly cloned or determined through a genome project, and its functional assignment as an AGT likely would have been assumed. Apparently, the primary function of this particular mosquito enzyme

has shifted or expanded to include the transamination of 3-HK to XA (Fig. 1); accordingly, we named this enzyme *Ae. aegypti* HKT (Han et al., 2002). The completion of the *An. gambiae* genome project made it possible to search its database for similar sequences, which led to the discovery of two coding sequences (GenBank accession nos. CAJ14970, and XM_309676) that showed high sequence identity with the *Ae. aegypti* HKT. BLAST analysis of the *Ae. aegypti* EST databases also identified a second AGT-like fragment, which subsequently led to the isolation of its full-length cDNA (GenBank accession no.ABA26661). While both *An. gambiae* and *Ae. aegypti* possess a second AGT-like sequence, only one AGT is present in other species, including humans. These data raise two interesting questions: why have two AGT-like sequences evolved in mosquitoes and what is the driving force behind the shift in primary function from an AGT to HKT for one of the coding sequences.

3.1. Primary function of AGT

AGT plays an important physiological role in living organisms and has been studied in a variety of animals, plants and fungi. It converts glyoxylate to glycine using pyruvate as the amino group acceptor. The enzyme is present in peroxisomes or mitochondria in different mammalian species. In general, the peroxisomal AGT is responsible for detoxifying glycolate-derived glyoxylate, while the mitochondrial AGT is involved in converting hydroxyproline-derived glyoxylate into glycine (Watts, 1992; Danpure, 1997; Holbrook and Danpure, 2002;Takayama et al., 2003; Birdsey et al., 2004; Birdsey et al., 2005). In plants, it has been shown to be involved in the photorespiratory glyoxylate cycle within peroxisomes (Rehfeld and Tolbert, 1972; Liepman and Olsen, 2001). In yeast, disruption of AGT can lead to glycine auxo-trophy. For example, a yeast AGT deletion strain was unable to grow when glucose was the only carbon source, unless glycine was supplemented (Schlosser et al., 2004). The importance of hepatic AGT in minimizing endogenous oxalate production in some mammals is clearly shown by the autosomal recessive disorder of glyoxylate metabolism known as primary hyperoxaluria type 1. This disorder is a potentially lethal condition in which AGT deficiency leads to excessive oxalate synthesis and excretion and the deposition of insoluble calcium oxalate in the kidney (Danpure et al., 1996; Danpure, 2001). AGT also substantially contributes to the metabolism of serine in humans, dogs, cats, and rabbits, regardless of its localization in mitochondria or in peroxisomes (Rowsell et al., 1979; Beliveau and Freedland, 1982; Xue et al., 1999). Therefore, it is clear that AGT is essential in living organisms from yeast to humans.

3.2. AGT sequences in mosquitoes

By searching an early release of the *Ae. aegypti* EST sequences (http://www.tigr.org/tdb/e2k1/aabe/) with the *Ae. aegypti* HKT, we found that *Ae. aegypti* has an additional AGT-like fragment. This discovery subsequently led to the successful cloning of a new *Ae. aegypti* AGT (GenBank accession no. ABA26661) that shares 48% identity with the previously reported *Ae. aegypti* HKT (Han et al., 2002) and ~45% identity with mammalian AGTs. BLAST analysis of the *An. gambiae* genomic database revealed that two putative AGT-like sequences with accession numbers of EAA07245 and EAA05410, respectively, also are present in that mosquito species. Recently, the EAA07245 sequence has been identified as the *An. gambiae* HKT (Rossi et al., 2005). Sequence alignment revealed 73% identity between the two functionally verified proteins, *Ae. aegypti* HKT (GenBank accession no. AAL29468) and *An. gambiae* HKT (Gen-Bank accession no. EAA07245), and 80% identity between the second *Ae. aegypti* (GenBank accession no. ABA26661) and putative *An. gambiae* AGT (GenBank accession no. EAA05410). Our recent data showed that *Ae. aegypti* HKT was present only in the larval stages, while its second putative AGT was present during the pupal and adult stages (Han, et al., 2006). BLAST analysis of the genomes of other available model species (including cyanobacteria, archaea, yeast, plants, fruit fly, honeybee, frog, fish, rat, mouse, and human) revealed only one AGT or putative AGT in their databases, which raises essential questions about the physiological roles, functional differentiation, and evolution of mosquito AGTs.

3.3. Functional differences of mosquito AGTs

Based on phylogenetic analysis (Fig. 3), it seems that the functionally verified mosquito HKTs are slightly closer to the mammalian AGTs than the putative *Ae. aegypti* and *An. gambiae* AGT sequences (Fig. 3). While our previous study determined that the mosquito HKT is most efficient in catalyzing the transamination of 3-HK to XA, it does have AGT activity (Han et al., 2002;Han and Li, 2002), suggesting that the HKT function might have evolved after the AGT function of the protein or vice versa. Recently, we have expressed the human AGT (GenBank accession no. BAA02632) and initial biochemical characterization of the human enzyme showed that it was highly efficient in mediating the glyoxylate to glycine pathway while also displaying detectable HKT activity (Han et al., 2006). Nonetheless, the ability of human AGT to use 3-HK as a minor substrate suggested the possibility that the mosquito AGT became well-adapted to the 3-HK substrate at its active site during the HKT functional evolution process. In contrast, when the second putative *Ae. aegypti* AGT sequence (GenBank accession no. ABA26661) was expressed and characterized, the protein displayed high AGT activity, but showed no HKT activity (Han et al., 2006). Interestingly, although the rather extensively characterized mammalian AGTs have been assumed to represent a typical AGT, based on substrate specificity, it is the *Ae. aegypti* sequence (GenBank accession no. ABA26661) that behaves like a true AGT.

3.4. Possible driving force for mosquito AGT functional adaptation and evolution

Compared with mammalian AGTs, it is clear that one mosquito AGT-like sequence has evolved as HKT and the other has evolved as a true AGT. In mammals, 3-HK can be hydrolyzed by kynureninase (EC 3.7.1.3) to 3-hydroxyanthranilic acid that then can be either completely oxidized to CO_2 and H_2O through a complicated biochemical pathway or used to synthesize $NAD(P)^+$ (Stone, 1993). Therefore, oxidative stress due to 3-HK accumulation does not seem to be a major problem in mammals. In contrast, mosquitoes do not have kynureninase; therefore, there is no 3-HK hydrolysis pathway available. Consequently, the high conversion efficiency of 3-HK to XA in mosquito larvae is considered to be a mechanism by which mosquitoes detoxify the chemically reactive 3-HK (Han et al., 2002). During mosquito development, larvae have high HKT activity, but this enzyme activity becomes essentially undetectable in pupae and adults. In mosquitoes, as well as other insects, larvae are the active feeding stages. Conceivably, some of the ingested tryptophan will be oxidized via the kynurenine pathway, leading to the production of 3-HK. During the pupal stage, mosquitoes undergo an extensive tissue/body transformation, but do not ingest any food. Accordingly, the tryptophan oxidation pathway likely becomes a minor event, which may explain, in part, the down-regulation of HKT in pupae.

Although 3-HK is toxic in general, it also serves as a precursor for eye pigments in the compound eyes. In mosquitoes, compound eye development occurs primarily during the pupal stages, so some accumulation of 3-HK actually becomes necessary for compound eye pigmentation in the pupal and early adult stages. Indeed, 3-HK becomes a major electrochemically active compound in mosquito pupae. Further analysis indicated that most of the 3-HK was transported to the compound eyes rather than remaining in circulation in the body (Li et al., 1999). These data suggest that the complete down-regulation of mosquito HKT in pupae and adults could be due, in part, to the need for 3-HK for eye pigmentation. The requirements for 3-HK detoxification during the larval stages and the preservation of 3-HK for eye pigmentation during the pupal and early adult stages provide a reasonable explanation for the molecular regulation of the HKT during mosquito development.

Other than mosquitoes, only one AGT or putative AGT sequence is present in other model species (including *Drosophila*), which logically leads to a question as to why two AGT-like sequences have evolved in mosquitoes. Moreover, based on the protein profile or activity

profile in *Ae. aegypti*, the functionally verified HKT is present in larvae and the second AGT is present in pupae and adults, which is an interesting phenomenon that requires further explanation and justification. Our recent biochemical characterization of the second *Ae. aegypti* AGT showed that this AGT, unlike mammalian AGTs, functions virtually exclusively on the glyoxylate to glycine pathway (*i.e.*, functions as a true AGT) (Han et al., 2006). It has been shown that AGT is essential in human and yeast (Danpure et al., 1996; Danpure, 2001; Schlosser et al., 2004) and it likely is crucial in insects. In our previous study, we demonstrated that *Ae. aegypti* HKT, though highly efficient in mediating the 3-HK to XA pathway, also has AGT activity. It is quite possible that the same enzyme is involved in the detoxification of 3-HK and metabolism of glyoxylate during larval development. During the pupal and adult stages, 3-HK needs to be preserved and transported to the compound eyes for eye pigmentation, but the presence of high HKT activity in pupae likely would prevent 3-HK from accumulating. The requirement of 3-HK for eye pigmentation may explain why HKT has to be down-regulated in pupae and adults. On the other hand, if mosquito HKT also plays a role in the glyoxylate to glycine pathway, down-regulating HKT in pupae or adults would affect the glyoxylate metabolism. Western analysis using anti-mosquito AGT antibodies showed that the second *Ae. aegypti* AGT was present in pupae and adults (Han et al., 2006), but was not detected in larvae. Because the second AGT (GenBank accession no. ABA26661) has no activity for 3-HK, its presence satisfies the need for glyoxylate metabolism without affecting compound eye pigmentation (Han et al., 2006).

The above discussion provides a rather logical explanation as to why mosquitoes have two individual AGTs. Compound eye pigmentation occurs in a number of insect species, including *Drosophila*. Consequently, one could also ask why *Drosophila* does not need two AGTs. *Drosophila* is similar to mosquitoes in that it does not have kynureninase; therefore, accumulation of 3-HK should be a potential problem for *Drosophila* as well. Analysis of 3-HK and XA in the larval stages revealed that the concentration of 3-HK was about 2–3-fold lower in *D. melanogaster* larvae than in *Ae. aegypti* larvae, but the relative concentration of XA was about 20-fold higher in mosquito larvae than in *Drosophila* larvae (Li and Li, 1997;Li et al., 1999) (Li J., unpublished data). In mosquitoes, larvae are aquatic and microorganisms serve as their primary food resources, so their diets are protein-rich. In contrast, *Drosophila* larvae get their food from fruit resources, so their diets are rich in carbohydrates and glycolate, the precursor of glyoxylate. It seems clear that mosquito larvae have a significant amount of tryptophan that needs to be dealt with via the kynurenine oxidation pathway. This difference in physiological requirements likely explains why one of the AGT-like sequences in mosquitoes evolved to detoxify 3-HK via transamination.

4. Conclusion

In summary, the study of tryptophan metabolism has revealed that the fates of some tryptophan metabolites are quite different in mosquitoes than those of other species. To prevent 3-HK from accumulating during the larval stages, an AGT-type of enzyme in mosquitoes has expanded its function such that transamination of the chemically reactive 3-HK to the chemically stable XA becomes its primary function or, at least, one of its primary functions. To preserve 3-HK for eye pigmentation and to keep the operation of glyoxylate to glycine during pupal and adult stages, a second AGT, which has no detectable HKT activity and which is highly specific for the transamination of glyoxylate to glycine, has evolved in mosquitoes. These data provide an interesting example of protein functional adaptation and evolution in meeting the physiological requirements of mosquitoes.

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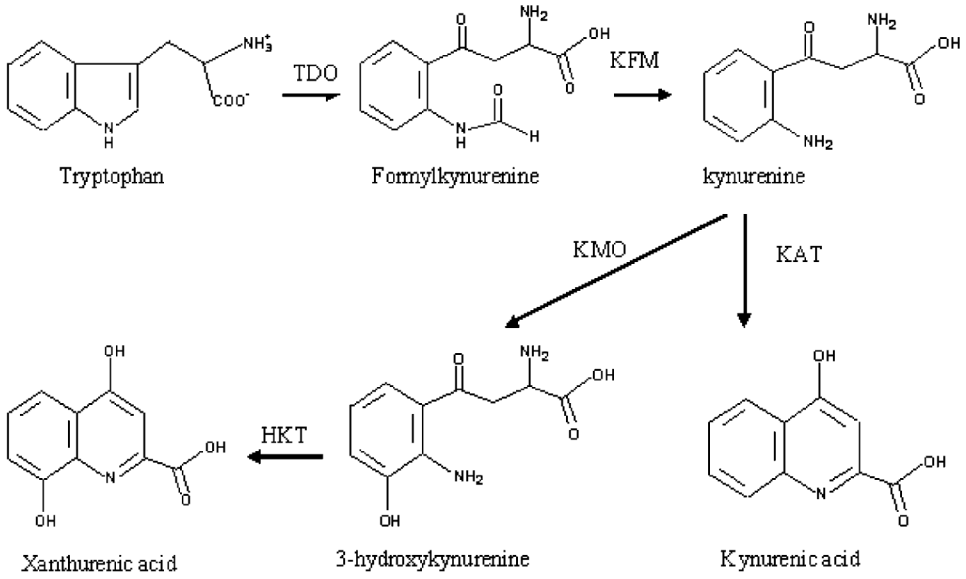

Fig. 1.

Biochemical pathway of tryptophan to xanthurenic acid in mosquitoes. TDO, tryptophan dioxygenase; KFM, kynurenine formamidase; KMO, kynurenine monooxygenase; KAT, kynurenine aminotransferase; HKT, 3-hydroxykynurenine transaminase.

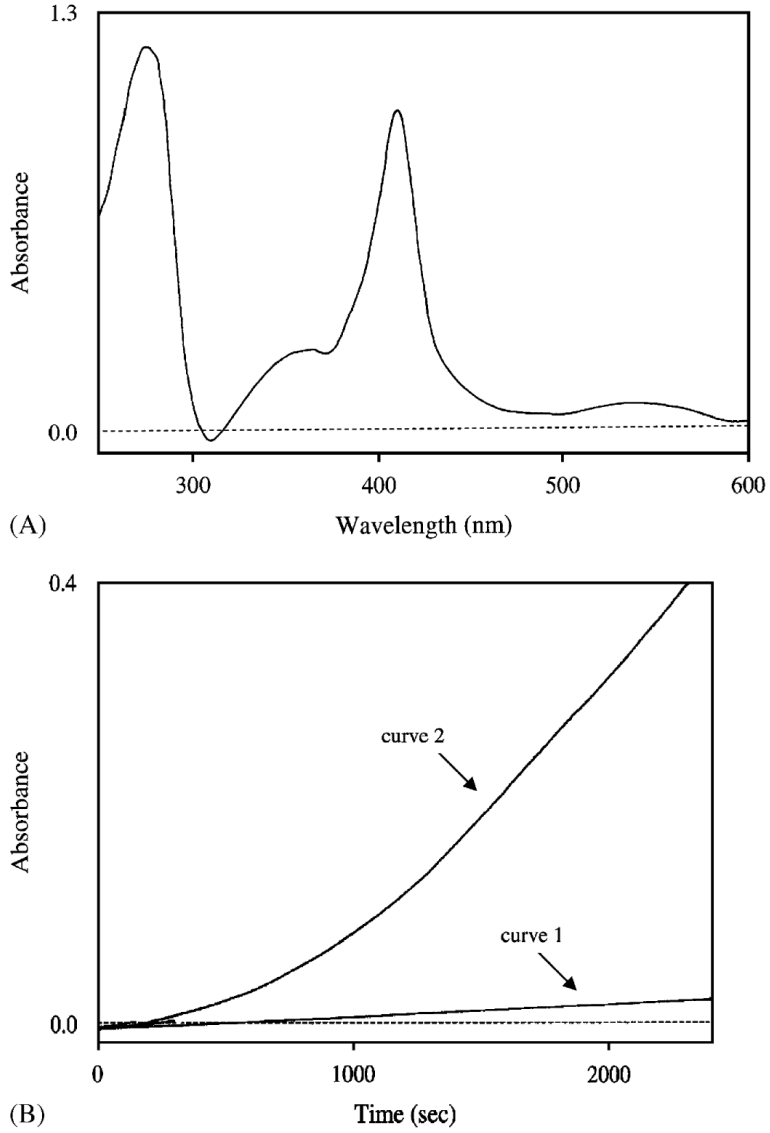

Fig. 2.

Spectral characteristics and biochemical activity of the recombinant *Ae. aegypti* tryptophan 2,3-dioxygenase (TDO). (A) Spectral characteristics of the purified recombinant *Ae. aegypti* TDO (about 1 mg in 1.0 ml of phosphate buffer, pH 7.5). TDO is a heme-containing enzyme. The presence of an absorbance peak with a λ_{max} at 405 nm indicates that the heme prosthetic group is present in the enzyme. (B) Production of formylkynurenine in a TDO and tryptophan reaction mixture. Presence of an initial lag phase in formylkynurenine production in a TDO and tryptophan reaction mixture suggests that *Ae. aegypti* TDO is present as an inactive form, and addition of tryptophan results in its progressive activation. Curve 1 and curve 2 illustrate absorbance change at 321 nm in tryptophan solutions in the absence and presence of TDO (20 μg/ml reaction mixture), respectively.

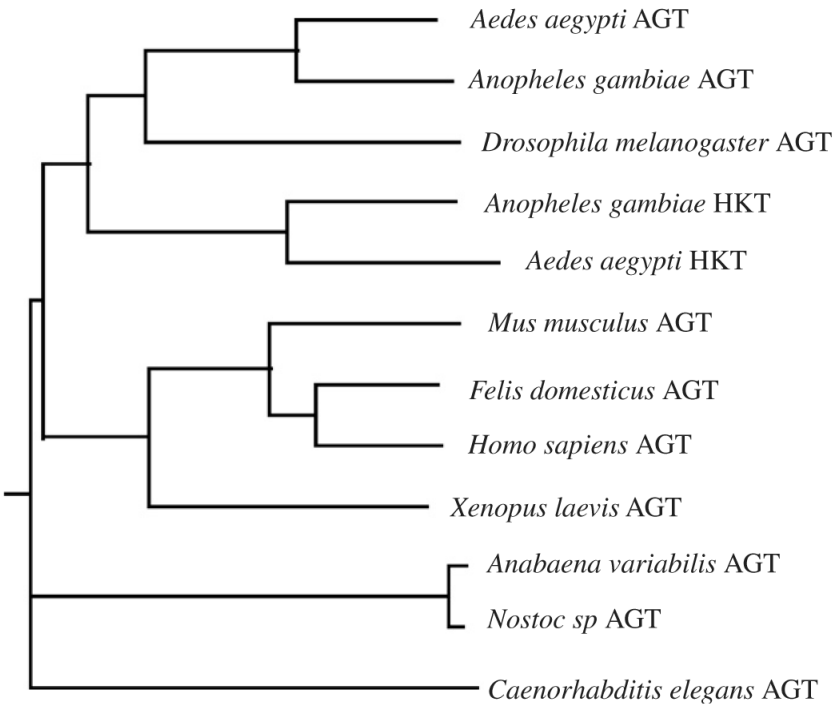

Fig. 3.

A phylogenetic tree of alanine glyoxalate aminotransferases (AGT) from several representative model species. The phylogenetic tree was generated using CLUSTALW program in biology workbench.