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Insect Arylalkylamine *N*-Acetyltransferases as Potential Targets for Novel Insecticide Design

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Abstract

Crop protection against destructive pests has been at the forefront of recent agricultural advancements. Rapid adaptive evolution has led to insects becoming immune to the chemicals employed to quell their damage. Insecticide resistance is a serious problem that negatively impacts food production, food storage, human health, and the environment. To make matters more complicated are the strict regulations in place on insecticide development, driven by rising public concern relating to the harmful effects these chemicals have on the environment and on society. A key component to solving the problem of insecticide resistance, while keeping public welfare in mind, is the identification of novel insect-specific protein targets. One unexplored target for the development of new targeted insecticides are the insect arylalkylamine *N*-acetyltransferases (iAANATs). This group of enzymes, shown to be intrinsic in the development of the insect cuticle, is an untapped well of potential for target-specific inhibition, while offering enough variety to ensure protection for non-target enzymes. In this review, we highlight kinetic, genetic and bioinformatic data showing that the iAANATs are intriguing insecticide targets that should be specific only for particular insect pests. Such a pest-specific insecticide would minimize environmental harm by eliminating such non-discriminate attacks which have made insecticides such a highly regulated industry, and would have negligible toxicity to humans and other mammals.

Keywords: Insecticide; *Bombus terrestris*; Neonicotinoid; Arylalkylamine *N*-acetyltransferase

Introduction

Ever-increasing insecticide resistance illustrates the uphill battle faced by the food and agricultural industries. Newly developed insecticides are quickly becoming classified as "non-renewable resources", with a measure of effectiveness based on how long they will be commercially viable as a primary concern [1]. Adding to this concern is the link between current insecticide usage and declining bee populations [2]. Thus, the identification and exploitation of novel targets for insecticide design is critical to debase the detrimental effects of insecticide resistance and environmental damage. One such avenue, which holds potential, are the arylalkylamine *N*-acetyltransferases. Arylalkylamine *N*-acetyltransferases (AANAT), often called dopamine- or serotonin *N*-acetyltransferases, are members of the Gcn5-related *N*-acetyltransferase (GNAT) superfamily of enzymes. These enzymes catalyze the acetyl-CoA-dependent acetylation of an amine or arylalkylamine (**Figure 1**) [2].

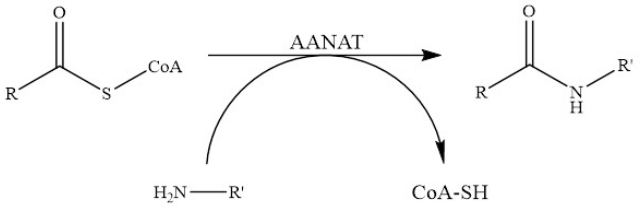

Figure 1 Mechanism for the AANAT-catalyzed *N*-acetylation of amines and arylalkylamines.

Since the initial characterization of *Drosophila melanogaster* AANAT by Maranda et al. [3], a large number of insect genes encoding AANAT and AANAT-like proteins have been described, highlighting the biological importance of these enzymes [4-10]. In vertebrates, AANAT catalyzes the rate-limiting step in the biosynthesis of melatonin [8] and has a key role in regulation of the sleep-wake cycle [9]. AANATs found in insects (iAANAT) are similarly involved in important biological processes, including insect-specific roles. It is a testament to the versatility of this group of enzymes that each insect family has developed family-unique AANATs to facilitate their own needs. iAANATs are vital to cuticle morphology by catalyzing the acetylation of dopamine to *N*-acetyldopamine, a precursor to the sclerotization, and the hardening of the exoskeleton [6,10]. In addition to dopamine, a variety of biogenic amine neurotransmitters also serve as acetyl group acceptors, meaning that the iAANATs are involved in neurotransmitter inactivation and, thus, may contribute to the regulation of neural signaling networks [11].

An iAANAT-targeted insecticide would not only disrupt neural signaling, but would also inhibit cuticle development, decrease the structural stability, result in a non-conforming appearance (harming the insects' ability to mate), and diminish the cuticle-mediated protection against injury and infection. The aforementioned AANAT-regulated production of melatonin (by catalyzing the acetylation of serotonin) manages the life span of *Drosophila melanogaster*, illustrating the toxicity potential of these biogenic amines if their inactivation is interrupted. Insects are particularly susceptible to iAANAT inhibition because insects lack the enzyme monoamine oxidase, an enzyme which functions in mammals to inactivate arylalkylamines [12]. It has long been an endeavor of ours to examine the variety of functions of the iAANATs, and to ascertain the fatty acid amides they produce. Thus far, we have successfully identified, cloned and characterized iAANATs from *Drosophila melanogaster* and *Bombyx mori*. Other groups have characterized iAANATs from other insects, including *Aedes aegypti*, *Tribolium castaneum* and *Antheraea pernyi* [11,13-18]. A picture of diversity and specificity for iAANATs emerges from the kinetic analyses showing a broad range of amine and acyl-CoA thioester substrates for the iAANATs, knockdowns demonstrating the detrimental effects of targeting iAANATs, and sequence comparisons between the iAANATs revealing insect-specific targeting potential. These data point towards the iAANATs as excellent targets for the design of novel insecticides.

Results and Discussion

Aside from the biogenic amines which have shown cross-species functionality, acyl-CoA chain length specificity differs between specific iAANATs, ranging from acetyl-CoA to oleoyl-CoA. Based on the specificity of the acyl-CoA thioester substrates, some iAANATs function in the formation of both short- and long-chain *N*-acylamides, whereas others function only to catalyze the formation of short-chain or long-chain *N*-acylamides. For example, iAANATs found in *D. melanogaster* show wildly differing results, especially with regards to acyl-chain length for their respective acyl-CoA substrates. Acetyl-CoA is a substrate for *D. melanogaster Dm*-AANATA, K_m = 39 ± 12 µM, but apparently had no appreciable affinity for the long-chain acyl-CoA, arachidonoyl-CoA [13]. Yet in the same organism, *Dm*-AANATL2 accepted both acetyl-CoA and arachidonoyl-CoA as substrates, with Km values of 6.1 ± 0.3 µM and 1.9 ± 0.25 µM, respectively, illustrating the differences in between the iAANATs found in the same organism [18]. This trend is similarly seen in comparing two iAANATs expressed by *B. mori*. Acyl-chain length had negligible effect on the Km values for the acyl-CoA substrates for *Bm*-iAANAT while *Bm*-iAANATL3 preferred acyl-CoA substrates with an acyl chain length of 2-10 carbon atoms, with longer chain acyl-CoA thioesters not acceptable as substrates, at all (**Table 1**).

A review of the data for the amine substrates for the iAANATs again point to specific metabolic functions for the specific members of the iAANAT family. Differences of several orders of magnitude are found for amine K_m values when comparing iAANATs (**Table 2**). For example, the K_m for dopamine with *Dm*-AANATA is 25 ± 2 µM and that for *Bm*-iAANATL3 is 330 ± 23 µM. Differences like this are congruent for numerous amines, signifying a divergence in binding specificity in the active site between iAANATs.

Table 1 K_m values for the iAANATs found in *D. melanogaster* and *B. mori* with respect to different acyl chain lengths.

Acyl-CoA	(K_m) (µM)			
	***Dm*-AANATA**	***Dm*-AANATL2**	***Bm*-iAANATL3**	***Bm*-iAANAT**
Acetyl-CoA	39 ± 12	6.1 ± 0.27	90 ± 3.6	0.31
Butyryl-CoA	36 ± 2	1.8 ± 0.17	14 ± 0.75	N/A
Hexanoyl-CoA	23 ± 3	N/A	13 ± 0.54	N/A
Octanoyl-CoA	18 ± 3	N/A	10 ± 1.0	N/A
Decanoyl-CoA	220 ± 60	N/A	12 ± 5.5	N/A
Palmitoyl-CoA	N/A	9.9 ± 1.6	N/A	1.1 ± 0.3
Oleoyl-CoA	N/A	3.6 ± 0.58	N/A	1.7 ± 0.51
Arachidonyl-CoA	N/A	1.9 ± 0.25	N/A	1.2 ± 0.67

Table 2 Binding constants for iAANATs found in *D. melanogaster* and *B. mori* with respect to different amine acceptors.

Amines	(K_m) (µM)			
	***Dm*-AANATA**	***Dm*-AANATL7**	***Dm*-AgmNAT**	***Bm*-iAANATL3**
Tyramine	12 ± 1	42 ± 2	N/A	63 ± 6.7
Octopamine	10 ± 2	120 ± 10	N/A	18 ± 0.77
Dopamine	25 ± 2	170 ± 20	N/A	330 ± 23
Tryptamine	33 ± 3	26 ± 2	N/A	97 ± 8.8
Norepinephrine	32 ± 6	230 ± 50	N/A	140 ± 8.6
Phenethylamine	56 ± 13	320 ± 40	N/A	N/A
Serotonin	110 ± 8	160 ± 20	N/A	1100 ± 63
4-methoxyphenethylamine	780 ± 60	190 ± 20	N/A	N/A
4-phenylbutylamine	270 ± 20	610 ± 30	N/A	N/A
3,4-dimethoxyphenethylamine	3200 ± 300	320 ± 30	N/A	N/A
Histamine	N/A	520 ± 50	N/A	1700 ± 98
Putrescine	N/A	81 ± 11	51 ± 3	N/A
Agmatine	N/A	790 ± 100	0.3 ± 0.02	N/A

Spermine	N/A	N/A	18 ± 3	N/A
N8-acetylspermine	N/A	N/A	9.1 ± 1	N/A
Spermidine	N/A	N/A	17 ± 0.5	N/A
Cadaverine	N/A	N/A	32 ± 6	N/A

Kinetic analyses provide a limited evaluation of the active site differences between the iAANATs. The K_m values are not $K_{dissociation}$ values and a comparison (or ratio) of K_m values may only provide an estimate ratio of $K_{dissociation}$ values for an acyl-CoA or amine substrate. However, the highlighted differences in the K_m values imply a large enough distinction in substrate affinity to suggest that insecticides can be developed that will only target iAANATs in insect pests. Another aspect in considering the iAANATs as insecticide targets is to evaluate expression knockdown data. If the expression knockdown of a potential insecticide target is not deleterious to the insect, an inhibitor targeting it will probably not be a useful insecticide. One such knockdown was performed by Long et al. on *Bombyx mori* iAANAT2 [18].

The inhibition of *Bm*-iAANAT2 expression led to an increase in melanin deposition in larvae and adults, resulting from increase cellular concentrations of dopamine. As mentioned, any deviation in the appearance of the insect would have a naturally detrimental effect on its chances of mating [19]. Another iAANAT knockdown was performed in *T. castaneum*, the red flour beetle, an insect regarded as a serious pest to the food industry and hence a perfect model for insecticide target discovery. Noh et al. [6] demonstrated that along with darkening of the cuticle, the knockdown resulted in a separated, misshapen elytron (the wing casing of the beetle), and misfolding of the hindwings of the beetle. The evidence demonstrates that targeting iAANAT compromised the structural integrity of the *T. castaneum* exoskeleton. This naturally leaves the insect vulnerable to a host of environmental threats, including disease, predation, and the aforementioned inability to mate.

While the experimental data point toward the iAANATs as excellent targets for insecticide development, insecticides have come under scrutiny for their toxicity against the honey bee, *Apis melifera*, and the bumblebee, *Bombus terrestris*. Neonicotinoid insecticides are particular offenders, claimed to be involved in colony collapse disorder (CCD) [20]. It is here the AANATs stand out as insecticide targets. An examination of the sequences for numerous iAANATs reveals several unique iAANAT families. The "traditional" iAANAT gene is easily identifiable through a CoA binding pocket motif (synonymous with the NAT superfamily) and an insect specific motif, FxDEPLN. This motif contains functionally and structurally important residues. Due to the link of the enzyme to dopamine acetylation, this "traditional" iAANAT is often referred to as dopamine *N*-acetyltransferase (DAT). DAT is conserved among all insects. However, genome mining suggests that most insects contain several other types of iAANATs, also with characteristic motifs. These other families of iAANAT generally exhibit very low sequence homology outside of their phylogenic grouping. Examples of this can be found in the mosquito family (namely *Aedes* and *Culex*) with greater than 80% homology among other family members. Compared against insect genomes outside of this however, there is generally less than 30% homology. To add to this, *A. melifera* and *B. terrestris* both contain an iAANAT specific for *Apidae* (bees). *Coleoptera* (beetles) contain several more. This is further illustrated in **Figure 2.**

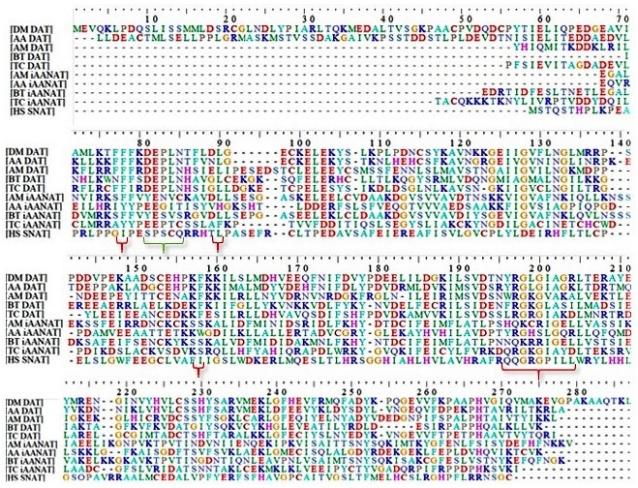

Figure 2 Primary sequence alignment of various insect AANATs, as well as human serotonin *N*-acetyltransferase (SNAT). DAT specifies the conserved dopamine *N*-acetyltransferases found in all insects. iAANATs are the genus-specific AANATs. Bracketed in green is the amine binding pocket and highlighted in red are residues that make up the CoA binding pocket. Both are noticeably generally conserved among DATs, but varied among genus-specific iAANATs. The nomenclature is as follows: AA – *Aedes aegypti*; DM – *Drosophila melanogaster*; TC – *Tribolium castaneum*; BT – *Bombus terrestris*; AM – *Apis mellifera*; HS – *Homo sapiens*. Accession numbers: 'AA DAT' – XP_001661400.1; 'DM DAT' – NP_995934.1; 'TC DAT' – XP_972873.2; 'BT DAT' – XP_012167469.1; 'AM DAT' – XP_016770090.1; 'BT iAANAT' – XP_020723819.1; 'AM iAANAT' – XP_001122379.2; 'AA iAANAT' – XP_001649422.1; 'TC iAANAT' – XP_973841.1; 'HS SNAT' - NP_001079.1.

Conclusion

Importantly, the regions responsible for binding of both the acyl-CoA and the amine substrate vary considerably between these iAANAT families, despite very high structural homology. In all iAANATs, the overall secondary and tertiary structure of the amine binding pocket is very similar. However, differences in specific residues are responsible for changes in binding affinities. In *Diptera*- (fly) specific iAANATs, for example, many hydrophobic residues are replaced with residues containing nucleophilic groups and hydrogen bond acceptors that are not found in other iAANATs [14]. This suggests that by exploiting these catalytically relevant, yet insect-specific residues, it is possible to design compounds that bind to a specific iAANAT

family, such as *Diptera* iAANAT or *Coleoptera* iAANAT, which would not bind (or would bind with low affinity) to others, leaving non-targeted species unaffected.

These comparisons of iAANATs reveal the potential for high insecticide specificity, by solely targeting pests or disease vectors, and keeping agriculturally or ecologically important species unharmed. Additionally, iAANATs share little homology to AANATs expressed in vertebrates. This is in stark contrast to other types of insecticides, such as the aforementioned neonicotinoids, as well as organophosphates and organochlorines, which are highly non-specific, and often cause severe environmental side-effects [20]. Further work must be done to design and develop iAANAT-specific inhibitors to solidify these as legitimate targets. However, the evidence discussed herein indicates the iAANATs are excellent insecticide targets and the development of iAANAT-targeted inhibitors has the potential to solve many of the problems faced by current insecticide development.

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Competing Interests

The authors declare no competing interests.

Authors' Contributions

B. G. O., A. J. H., and D. J. M. contributed equally to the creation of this article.

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