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TOXOCARA CANIS ARGININE KINASE: GENE STRUCTURE, FUNCTIONAL ANALYSIS OF SITE-DIRECTED MUTANS AND SCREENING OF SUBSTRATE ANALOGS AS POTENTIAL INHIBITORS

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Arginine kinase (AK) is an enzyme and is a member of phosphagen kinase family widely distributed among the invertebrates. Generally AK is found as monomers. However, true dimeric and contiguous dimeric AKs have been reported. Furthermore, AK plays an important role in intracellular energy metabolism. In our study we determined the exon/intron organization of the *Toxocara canis* AK (TCAK) gene. *T. canis* AK has the seven-exon/six-intron gene structure. Only a few splice junctions were conserved in nematodes. Moreover, the gene structures of nematode AKs are highly divergent and variable suggesting a frequent loss and gain of introns during the course of arginine kinase evolution. In contrast, the intron positions in molluscan AKs are highly conserved. We noted that Guanidine specific (GS) region which is variable in length is a possible candidate for the guanidine-recognition site. Therefore, we introduced site-directed mutants to the GS region of TCAK and measured the enzyme activity of muted and wild type TCAK. The K_m value of mutant (Alanine to Serine) was decreased indicating a high affinity for substrate arginine than wild-type. In addition, the K_m value of mutant (Serine to Glycine) was increased to 0.19 mM. Meanwhile, K_m value (0.19 mM) of the double mutant (Alanine-Serine to Serine-Glycine) was slightly increased compared to 0.12 mM in the wild-type. These results suggest that these substitutions of the amino acids on the GS region of TCAK have no significant influence on activity for the substrate arginine. We evaluated the effects of green and black tea on TCAK in-vitro and several other chemicals such as anthelmintics (pyrantel pamoate, flubendazole, thiabendazole and milbemycin), plant extract (*Azadiracta indica*), an aminoglycoside antibiotic (aminosidine), a citrus flavonoid glycoside (rutin) and commercially available catechin mixture (containing epicatechin, epicatechingallate, epigallocatechin and epigallocatechingallate) against the TCAK. Green and black tea (1:10 dilution) produced about 15% and 25% inhibition on TCAK, respectively. Extract of *Azadiracta indica* produced 5% inhibition on TCAK. However, other chemicals used in this trial did not elicit significant inhibition on TCAK. Based on our results, we suggest that, green and black tea and extract of *Azadiracta indica* inhibit recombinant TCAK in-vitro.