

Phosphagen Kinases of Parasites: Unexplored Chemotherapeutic Targets

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Abstract: Due to the possible emergence of resistance and safety concerns on certain treatments, development of new drugs against parasites is essential for the effective control and subsequent eradication of parasitic infections. Several drug targets have been identified which are either genes or proteins essential for the parasite survival and distinct from the hosts. These include the phosphagen kinases (PKs) which are enzymes that play a key role in maintenance of homeostasis in cells exhibiting high or variable rates of energy turnover by catalyzing the reversible transfer of a phosphate between ATP and naturally occurring guanidine compounds. PKs have been identified in a number of important human and animal parasites and were also shown to be significant in survival and adaptation to stress conditions. The potential of parasite PKs as novel chemotherapeutic targets remains to be explored.

Key words: phosphagen kinase, parasite, chemotherapeutic target

INTRODUCTION

Phosphagen kinases are phosphotransferases that play a key role in cellular energy metabolism. These highly conserved enzymes catalyze the reversible transfer of a phosphate between ATP and guanidine compounds in cells that display high and variable rates of energy turnover [1,2]. Eight PKs have been identified at present including the well-studied creatine kinase (CK) which is the sole PK in vertebrates. In addition to CK, the following PKs are found across a wide variety of invertebrate species: arginine kinase (AK), glycoamine kinase (GK), hypotaurocyamine kinase (HTK), lombricine kinase (LK), opheline kinase (OK), taurocyamine kinase (TK), and thallemine kinase (ThK) [3,4,5].

Phosphagen systems mainly function as temporal energy buffers during periods when demand for energy exceeds ATP production since phosphagens can accumulate in much higher intracellular concentrations and diffuse faster compared with ATP [6]. PKs also function in intracellular energy transport or as spatial energy buffers that shuttle energy between ATP-producing and -consuming sites as exhibited by the interplay be-

tween mitochondrial and cytosolic CK isoforms of the phosphocreatine shuttle [7]. Cellular phosphagens also trap considerable amounts of inorganic phosphate (Pi) which is liberated upon net phosphagen hydrolysis. This results in enhancement of intracellular proton buffering capacity, preventing acidification of the cytosol by protons liberated by cellular ATPase activity. Moreover, the release of Pi exerts an indirect regulatory effect on glycogenolysis and glycolysis since Pi is required for the activation of these metabolic pathways [2,4]. Phosphagen kinases identified in parasites are hypothesized to act as temporal energy buffers during parasite muscle contraction or they may have regulatory effects in the glycolytic pathways when parasites are in an oxygen poor environment [8].

PROTOZOAN PHOSPHAGEN KINASES

Pereira et al. [9] have cloned and characterized a 40-kDa AK from the protozoa *Trypanosoma cruzi*, the causative agent of Chagas disease. Likewise, from *Trypanosoma brucei* which causes human sleeping sickness and Nagana in livestock, AK activity was detected in fractions from procyclic forms. These AKs have comparable specific activities and share 82% amino acid identity with each other [10]. Protozoan AKs appear to be closely related to the AKs from arthropods [11] indicating the possibility that *Trypanosoma* AKs were acquired by horizontal gene transfer [9].

T. cruzi AK has a putative actin-like actin binding domain

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suggesting a relationship with cytoskeletal structures related to cell movement [9]. This AK could function as a modulator of energetic reserves under stress starvation condition since it was observed that AK activity increased continuously during the exponential phase of growth of the parasite [12]. AK has also been proposed to participate in the oxidative stress response systems in *T. cruzi* [13] and overexpression of this enzyme increases the survival capability of *T. cruzi* under pH [14] and nutritional stress conditions [10]. Correspondingly, in *Saccharomyces cerevisiae* and *Escherichia coli* which were engineered to express functional arginine kinase systems, the AK facilitated improvement in the recovery from stress and in stabilizing intracellular ATP levels during the starvation phase [15,16].

NEMATODE PHOSPHAGEN KINASES

The first measurement of AK activity in a nematode was done by Livingstone et al. [17] for the mammalian endoparasite *Ascaris lumbricoides*. Thompson et al. [18] also observed, by NMR spectroscopy, the in vivo exchange of phosphoarginine and adenosine triphosphate in the rhabditoid nematode *Steinernema carpocapsae*. Platzter et al. [19] further studied *S. carpocapsae* AK and their results indicated that this enzyme is a significant component of the energy metabolism both in 3rd stage juvenile (J3) and adult worms, probably playing a key role in aerobic/anaerobic metabolic transitions. AK was also cloned from the zoonotic nematodes *Ascaris suum* and *Toxocara canis* which can both cause visceral larva migrans (VLM) in humans. Both of these AKs have signal peptide on the N-terminal domain presumably targeting this protein to the cytosol or endoplasmic reticulum [20,21]. A similar signal peptide was identified in 1 of the 4 AKs from the free-living nematode *Caenorhabditis elegans* and it was proposed that this particular AK (AK4) is targeted to the mitochondria [11]. Besides in *C. elegans*, the presence of multiple AKs was also reported for the soybean cyst nematode (SCN) *Heterodera glycines*. Matthews et al. [22] have recently cloned 2 AKs from SCN which share 71% amino acid identity and are both expressed constitutively throughout the nematode's life cycle.

TREMATODE PHOSPHAGEN KINASES

In trematode species, contiguous 2-domain phosphagen kinases with a molecular mass of 80 kDa have been identified [23-26]. The PK from *Schistosoma mansoni*, having activity for tauro-

cyamine as well as for other guanidine substrates [25], was shown to be developmentally regulated and highly expressed in the cercariae stage [23]. The 2-domain PKs from *Paragonimus westermani* [26], *Schistosoma japonicum*, and *Eurythrema pancreaticum* (Tokuhiro et al., personal communication) showed specific activity only for the substrate taurocyamine. This implies that TK is not anymore exclusive to annelid as claimed by previous studies [27]. It appears that the presence of 2 catalytic domains on a single polypeptide chain of trematode PKs do not affect the conformational movements during substrate binding since significant activity was observed for the full-length construct of the enzyme. This is in contrast with the contiguous dimeric AKs from the mollusks in which only the second domain showed activity [28,29]. In addition, trematode PKs also showed an uncharacterized 6-amino acid deletion on the guanidine specificity (GS) region. This region has been proposed by Suzuki et al. [30,31] as a potential candidate for the guanidine substrate recognition site. These trematode PKs, though having activity for taurocyamine, interestingly share higher amino acid sequence identity to molluscan AKs rather than annelid TKs and the phylogenetic tree topology showed that it could be possible that trematode PKs have evolved from an AK gene [26].

PARASITE PHOSPHAGEN KINASES AS POTENTIAL CHEMOTHERAPEUTIC TARGETS

At present, drugs are usually available for the treatment of several parasitic infections. However, there is still a need to develop new chemotherapeutic agents due to the possibility of drug resistance especially for infections treatable only by 1 or 2 drugs as in the case of a number of food-borne trematodiasis and water-borne parasitic infections. For instance, praziquantel is the only drug use to treat schistosomiasis and is also the drug of choice for clonorchiasis, opisthorchiasis, and paragonimiasis [32]. Furthermore, there are currently available treatments that can be toxic to humans in high doses, such as those available for Chagas disease and cutaneous leishmaniasis [32].

The advances in molecular biology have accelerated the rate by which drug targets can be identified. Ideal targets are gene and proteins of parasites that are absent or quite different in the mammalian host [33]. These drug targets must also play a crucial role for the parasite so that interference with their functions will have a damaging effect on the parasite [34]. With the recent success of certain kinase inhibitors, identification of kinase targets in parasites and screening these against inhibitors

have become a promising area of research [35]. Because PKs are significant in maintenance of energy homeostasis, PKs that are absent in mammalian tissues could be potential drug target for new chemotherapeutic agents against parasites or they can be utilized in the development of new diagnostic tools for detection of infection.

Since AK has been identified to be important in stress adaptation of *T. cruzi*, and with the recent elucidation of its crystal structure [36] this enzyme can be a potential target for the development of new chemotherapeutic agents against trypanosomiasis [37]. Paveto et al. [38] demonstrated that the polyphenols catechin gallate or galocatechin gallate found in the green tea *Camellia sinensis* can inhibit the activity of recombinant *T. cruzi* AK. Arginine analogs, agmatine, canavanine, nitroarginine, and homoarginine can also inhibit trypanosome AK [14]. In addition, it has been shown that the flavonoid rutin is a non-competitive inhibitor of AK from the muscle of the insect pest locust [39]. The AK from *T. canis* was also suggested as possible novel drug target for VLM in humans [20] and that the recombinant *T. canis* AK could be used as antigen for immunodiagnosis of toxocarasis. Results of IgG-ELISA using recombinant *T. canis* AK showed high sensitivity for detection of toxocarasis in mouse model though the specificity of this antigen still needs further evaluation [40].

To this point, research on PKs from parasite is still on its preliminary stage. Further studies are needed to elucidate the specific physiologic roles of these enzymes in the parasites' survival. It is also a prerequisite to fully understand the substrate binding mechanisms and enzyme kinetics which are vital in designing of drugs targeting these enzymes. The potential of parasite PKs as novel and effective drug targets for the control and possible eradication of important parasites is yet to be fully explored.

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