ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol. Vol. 48, No. 4: 281-284, December 2010
DOI: 10.3347/kjp.2010.48.4.281

▣ MINI-REVIEW

Phosphagen Kinases of Parasites: Unexplored Chemotherapeutic Targets

Blanca R. Jarilla[1,2] and Takeshi Agatsuma[1,*]

[1]Department of Environmental Health Sciences, Kochi Medical School, Oko, Nankoku City, Kochi 783-8505, Japan; [2]Department of Immunology, Research Institute for Tropical Medicine, Alabang, Muntinlupa City 1781, Philippines

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 catalizing 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), glycocyamine kinase (GK), hypotaurocyamine kinase (HTK), lombricine kinase (LK), opheline kinase (OK), taurocyamine kinase (TK), and thalessemine 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 between 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

• Received 28 July 2010, accepted 28 August 2010.

* Corresponding author (agatsuma@kochi-u.ac.jp)

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 *Steinernma carpocapsae*. Platzer 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 endosplasmic 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 taurocyamine 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 trypanosomiases [37]. Paveto et al. [38] demonstrated that the polyphenols catechin gallate or gallocatechin 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 toxocariasis. Results of IgG-ELISA using recombinant *T. canis* AK showed high sensitivity for detection of toxocariasis 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.

ACKNOWLEDGEMENTS

We thank our colleagues in Department of Environmental Health Sciences, Kochi Medical School for their support and efforts. We also wish to thank Prof. Suzuki and Associate Prof. Uda for their kind invaluable encouragements throughout the experiments.

REFERENCES

1. Wyss M, Smeitink J, Wevers RA, Wallimann T. Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism. Biochim Biophys Acta 1992; 1102: 119-166.
2. Ellington WR. Evolution and physiological roles of phosphagen systems. Annu Rev Physiol 2001; 63: 289-325.
3. Morrison JF. Arginine kinase and other invertebrate guanidine kinases. In Boyer PC, ed, The Enzymes. New York, USA. Academic Press. 1973, p 457-486.
4. Robin Y. Phosphagens and molecular evolution in worms. BioSystems 1974; 6: 49-56.
5. Thoai VN. Homologous phosphagen phosphokinases. In Thoai VN, Roche J, eds, Homologous Enzymes and Biochemical Evolution. New York, USA. Gordon and Breach. 1968, p 199-229.
6. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J 1992; 281: 21-40.
7. Sauer U, Schlattner U. Inverse metabolic engineering with phosphagen kinase systems improves the cellular energy state. Metab Eng 2004; 6: 220-228.
8. Goil MM. Study of phosphagen in two trematodes. Z Parasitenkd 1980; 61: 271-275.
9. Pereira CA, Alonso GD, Paveto MC, Iribarren A, Cabanas ML, Torres HN, Flawiá MM. *Trypanosoma cruzi* arginine kinase characterization and cloning. A novel energetic pathway in protozoan parasites. J Biol Chem 2000; 275: 1495-1501.
10. Pereira CA, Alonso GD, Ivaldi MS, Silber AM, Alves MJM, Bouvier LA, Flawia MM, Torres HN. Arginine metabolism in *Trypanosoma cruzi* is coupled to parasite stage and replication. FEBS Lett 2002; 526: 111-114.
11. Uda K, Fujimoto N, Akiyama Y, Mizuta K, Tanaka K, Ellington WR, Suzuki T. Evolution of the arginine kinase family. Comp Biochem Physiol Part D Genomics Proteomics 2006; 1: 209-218.
12. Alonso GD, Pereira CA, Remedi MS, Paveto MC, Cochella L, Ivaldi MS, Gerez de Burgos NM, Torres HN, Flawia MM. Arginine kinase of the flagellate protozoa *Trypanosoma cruzi*: regulation of its expression and catalytic activity. FEBS Lett 2001; 498: 22-25.
13. Miranda MR, Canepa GE, Bouvier LA, Pereira CA. *Trypanosoma cruzi*: oxidative stress induces arginine kinase expression. Exp Parasitol 2006; 114: 341-344.
14. Pereira CA, Alonso GD, Ivaldi S, Bouvier LA, Torres HN, Flawia MM. Screening of substrate analogs as potential enzyme inhibitors for the arginine kinase of *Trypanosoma cruzi*. J Eukaryot Microbiol 2003; 50: 132-134.
15. Canonaco F, Schlattner U, Pruett PS, Wallimann T, Sauer U. Functional expression of phosphagen kinase systems confers resistance to transient stresses in *Saccharomyces cerevisiae* by buffering the ATP pool. J Biol Chem 2002; 277: 31303-31309.
16. Canonaco F, Schlattner U, Wallimann T, Sauer U. Functional expression of arginine kinase improves recovery from pH stress of *Escherichia coli*. Biotechnol Lett 2003; 25: 1013-1017.
17. Livingstone DR, Dezwaan A, Leopold M, Marteijn E. Studies on the phylogenetic distribution of pyruvate oxidoreductases. Bio-

chem Syst Ecol 1983; 11: 415-425.
18. Thompson SN, Platzer EG, Lee RW. Phosphoarginine-adenosine triphosphate exchange detected in vivo in a microscopic nematode parasite by flow 31P FT-NMR spectroscopy. Magn Reson Med 1992; 28: 311-317.
19. Platzer EG, Wang W, Thompson SN, Borchardt DB. Arginine kinase and phosphoarginine, a functional phosphagen, in the rhabditoid nematode *Steinernema carpocapsae*. J Parasitol 1999; 85: 603-607.
20. Wickramasinghe S, Uda K, Nagataki M, Yatawara L, Rajapakse RPVJ, Watanabe Y, Suzuki T, Agatsuma T. *Toxocara canis*: Molecular cloning, characterization, expression and comparison of the kinetics of cDNA-derived arginine kinase. Exp Parasitol 2007; 117: 124-132.
21. Nagataki M, Wickramasinghe S, Uda K, Suzuki T, Yano H, Watanabe Y, Agatsuma T. Cloning and enzyme activity of a recombinant phosphagen kinase from nematodes (in Japanese). Jpn J Med Technol 2008; 57: 41-45.
22. Matthews BF, MacDonald MH, Thai VK, Tucker ML. Molecular characterization of arginine kinases in the soybean cyst nematode (*Heterodera glycines*). J Nematol 2003; 35: 252-258.
23. Stein LD, Harn DA, David JR. A cloned ATP: guanidine kinase in the trematode *Schistosma mansoni* has a novel duplicated structure. J Biol Chem 1990; 265: 6582-6588.
24. Shoemaker CB. The *Schistosoma mansoni* phosphagen kinase gene contains two closely apposed transcription initiation sites and arose from a fused gene duplication. Mol Biochem Parasitol 1994; 68: 319-322.
25. Awama AM, Paracuellos P, Laurent S, Dissous C, Marcillat O, Gouet P. Crystallization and X-ray analysis of the *Schistosoma mansoni* guanidine kinase. Acta Crystallogr Sect F Struct Biol Cryst Commun 2008; 64: 854-857.
26. Jarilla BR, Tokuhiro S, Nagataki M, Hong SJ, Uda K, Suzuki T, Agatsuma T. Molecular cloning and characterization of a novel two-domain taurocyamine kinase from the lung fluke *Paragonimus westermani*. FEBS Lett 2009; 583: 2218-2224.
27. Stein LD, Harn DA, David JR. A cloned ATP: guanidine kinase in the trematode *Schistosma mansoni* has a novel duplicated structure. J Biol Chem 1990; 265: 6582-6588.
28. Uda K, Saishoji N, Ichinari S, Ellington WR, Suzuki T. Origin and properties of cytoplasmic and mitochondrial isoforms of taurocyamine kinase. FEBS J 2005; 272: 3521-3530.
29. Compaan DM, Ellington WR. Functional consequences of a gene duplication and fusion event in an arginine kinase. J Exp Biol 2003; 206: 1545-1556.
30. Suzuki T, Tomoyuki T, Uda K. Kinetic properties and structural characteristics of an unusual two-domain arginine kinase from the clam *Corbicula japonica*. FEBS Lett 2003; 533: 95-98.
31. Suzuki T, Kawasaki Y, Furukohri T, Ellington WR. Evolution of phosphagen kinase. VI. Isolation, characterization and cDNA-derived amino acid sequence of lombricine kinase from the earthworm *Eisenia foetida*, and identification of a possible candidate for the guanidine substrate recognition site. Biochim Biophys Acta 1997; 1343: 152-159.
32. Keiser J, Utzinger J. Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. Trends Parasitol 2007; 23: 555-562
33. Nwaka S, Ramirez B, Brun R, Maes L, Douglas F, Ridley R. Advancing drug innovation for neglected diseases-criteria for lead progression. PLoS Negl Trop Dis 2009; 3: e440.
34. Cavalli A, Bolognesi ML. Neglected tropical diseases: Multi-Target-Directed ligands in the search for novel lead candidates against *Trypanosoma* and *Leishmania*. J Med Chem 2009; 52: 7339-7359.
35. Krasky A, Rohwer A, Schroeder J, Selzer PM. A combined bioinformatics and chemoinformatics approach for the development of new antiparasitic drugs. Genomics 2007; 89: 36-43.
36. Fernandez P, Haouz A, Pereira CA, Aguilar C, Alzari PM. The crystal structure of *Trypanosoma cruzi* arginine kinase. Proteins 2007; 69: 209-212.
37. Silber AM, Colli W, Ulrich H, Alves MJM, Pereira CA. Amino acid metabolic routes in *Trypanosoma cruzi*: Possible therapeutic targets against Chagas' disease. Curr Drug Targets Infect Disord 2005; 5: 53-64.
38. Paveto C, Guida MC, Esteva MI, Martino V, Coussio J, Flawia MM, Torres HN. Anti-*Trypanosoma cruzi* activity of green tea (*Camellia sinensis*) catechins. Antimicrob Agents Chemother 2004; 48: 69-74.
39. Wu XA, Zhu WJ, Lu ZR, Xia Y, Yang JM, Zou F, Wang XY. The effect of rutin on arginine kinase: inhibition kinetics and thermodynamics merging with docking simulation. Int J Biol Macromol 2009; 44: 149-155.
40. Wickramasinghe S, Yatawara L, Nagataki M, Takamoto M, Watanabe Y, Rajapakse RPVJ, Uda K, Suzuki T, Agatsuma T. Development of a highly sensitive IgG-ELISA based on recombinant arginine kinase of *Toxocara canis* for serodiagnosis of visceral larva migrans in the murine model. Parasitol Res 2008; 103: 853-858.