INTERNATIONAL JOURNAL OF ENHANCED RESEARCH IN SCIENCE TECHNOLOGY & ENGINEERING
VOL. 1 ISSUE 3, DEC.-2012 ISSN NO: 2319-7463

Structural characterisation and homology modeling of Arginine kinase of *Trypanasomabrucei* - An *In Silico* Approach

V. Kabila

Associate Professor, Department of Zoology, Sri Meenakshi Government Arts College for Women (Autonomous) Madurai 625002

ABSTRACT

***Trypanasomabrucei*, the protozoan that causes trypanasomiasis, one of the Neglected Tropical Diseases (NTD), is found in the order kinetoplastida, which is significant from a medical and veterinary standpoint. In developing nations, this is one of the most important public health issues. Research interest in the field of drug discovery has been made possible by the elucidation of pathogen genomes and the capacity to map targets between parasite and human enzymes. Since the structural and functional characteristics of arginine kinase of *T.brucei* has not yet been documented, the present study has been taken up. The 3D structure was modeled and putative binding sites were identified. The enzyme arginine kinase of *T.brucei*was found to be cytoplasmic, stable, hydrophilic and with ATP guanido phosphotransferase domain. Ramachandran plot has 88.6% amino acids lying in the favorable region, making the predicted model a good one. The modeled 3D structure was found to have a good QMEAN, PROSA and ProtSAVscores. This showed that the predicted 3D structure has an overall good quality. The presence of ligand binding sites in the selected protein makes the present study a favourable starting point towards rational drug designing for human trypanasomasis.**

Key Words: Arginine kinase, homology modelling, *Trypanasomabrucei*

INTRODUCTION

Protozoans including *Trypanasomabrucei, T. cruzi*, and *Leishmania major* are among the critically important medicinal and veterinary protozoans found in the order Kinetoplastida. Tritryps are the parasites that cause trypanasomiasis, chagas sickness, and leishmaniasis, among other non-communicable diseases. These infections rank among the most important public health issues in underdeveloped nations [1].

African Trypanasomiasis is caused by an extracellular parasite called *T. brucei*. Its life cycle alternates between the procyclic form seen in the midgut of Tsetse flies and the bloodstream form found in the blood and tissue fluids of mammals. Significant changes in the energy metabolism occur during differentiation and the shift from one host to another, allowing the parasite to adapt to the various host settings.[1] .

Research interest in the field of drug discovery has increased significantly during the past ten years with respect to neglected tropical illnesses. This has been made possible by the elucidation of pathogen genomes and the capacity to map targets between parasite and human enzymes for which numerous target-based drug development experiments have been carried out[2].

Phosphagen kinases are important phospho transferases in the metabolism of energy. The reversible transfer of a phosphate between ATP and guanidine molecules is catalyzed by these highly conserved enzymes in cells that exhibit rapid and fluctuating rates of energy turnover [2].

One important phosphotransferase enzyme, arginine kinase, contributes to the parasite's metabolic plasticity and maintains the homeostasis of the cell's energy levels in response to environmental changes. [3]. It is crucial in supplying the energy required for cellular activity up until metabolic processes like glycolysis are activated because it catalyzes the reversible phosphorylation between phospho arginine and ADP.It also plays a crucial role in the resistance mechanism against several stresses, including reactive oxygen species, trypanocidal medicines, and pH. This pathway is found to be an ideal point to start the process of target identification as arginine kinase is completely lacking in mammal [4].

The structural database scan revealed that the 3D structure of the arginine kinase produced by *T. brucei*is not yet available. Additionally, Trypanosomatid Kinases constitute a large family of possible biological targets to investigate while developing antikinetoplastid drugs. As a result, the aim of the current study is to develop a 3D structure using comparative modeling for the arginine kinase of *T. brucei*..Moreover, finding binding sites would make this protein acceptable for docking research, which in turn aids in the development of anti-Trypanasomiasis treatments.

II. MATERIALS AND METHODS

To perform the structural analysis of arginine kinase C9ZXW0 *of Trypanasomabrucei*of various computational tools and servers were used. The aminoacid sequence of Arginine kinase T .brucei from Uniprotkb of NCBI database (http: //ncbi.nlm.nih. gov.in). The FASTA format of selected sequence was downloaded and used for further structural analysis.

(A)Primary Structure Prediction
The primary sequence of the selected protein was submitted to ProtParamtool to analyse the primary structure.

(B)Secondary structure prediction
The analysis of secondary elements of C9ZXW0 was performed by SOPMA server. CDD BLAST was performed to find out the presence of conserved domain .

(C)Prediction of tertiary structure
For the selected Arginine kinase of*T.brucei*, only the primary sequence was available. The data on 3D structural information was not available in any of the structural databases such as Swiss model Repository and Protein Model Portal . Hence homology modeling was done to deduce the three-dimensional structure the protein.

Swissmodel server was used to select the suitable template in automated mode. The 3D structure of the query protein was predicted and this was subjected to a series of tools to check its functional annotation quality. The predicted 3D structure was subjected to energy minimization using Modrefiner.. The generated model was evaluated by PROCHECK . The Z score was also predicted by Swissmodel server. The refined model was submitted to PROSA and PROTSAV to know about the overall quality factor of the protein. The generated model was visualized by Swiss PDB viewer.

Binding site analysis was also performed by 3DLigandsite and GHECOM tools

RESULTS

(A) Physicochemical parameters
Results from ProtParam exhibited various physicochemical parameters of Ferrous transport protein of *T.brucei* (Table.1). The sequence length of selected protein was found to be with 356 aminoacids and the composition of amino acids was also recorded(Table1) The instability index was recorded as 34.42, which makes the protein stable. The positive value indicated that the protein was hydrophilic .(Table1).

The grand average of hydropathicity (GRAVY) was calculated to be -0.499.

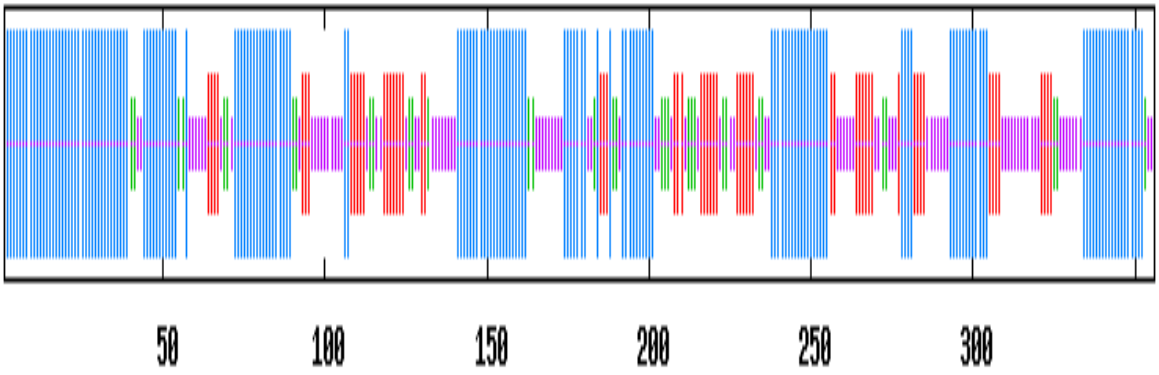
Table1: Biophysical parameters of Arginine kinase C9ZXW0 of *T. brucei*

Aminoacids	Residue	%
Ala (A)	24	6.7%
Arg (R)	18	5.1%
Asn (D)	12	3.4%
Asp (D)	29	8.1%
Cys (C)	5	1.4%
Gln (Q)	14	3.9%
Glu (E)	26	7.3%
Gly (G)	25	7.0%
His (H)	8	2.2%
IIe (I)	15	4.2%
Leu (L)	38	10.7%
Lys (K)	33	9.3%
Met (M)	6	1.7%
Phe (F)	15	4.2%
Pro (P)	12	3.4%
Ser (S)	18	5.1%
Thr (T)	20	5.6%
Trp (W)	2	0.6%
Tyr (Y)	13	3.7%
Val (V)	23	6.5%
Pyl (O)	0	0.0%
Sec (V)	0	0.0%

The instability index (II) is computed to be 34.42
Aliphatic index: 83.54
Grand average of hydropathicity (GRAVY): -0.499

(B) Secondary structure

SOPMA server predicted α helix, extended strand, β turn, and random coils . It was found that Arginine kinase of C9ZXW0 has more α helix (46.63%) (Fig.1).
Sequence length : 356
Alpha helix (Hh) :46.63%
Random coil (Cc) :27.25%
Extended strand(Ee) **:**16.85%



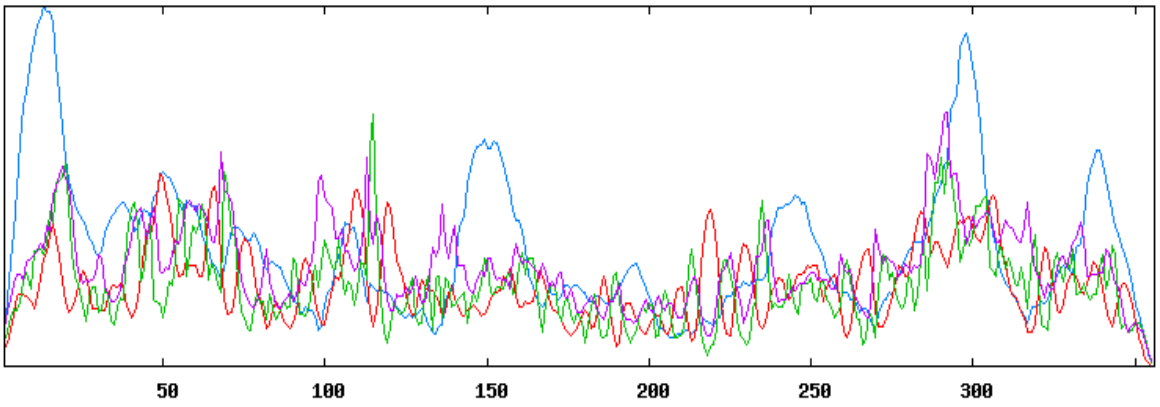


Fig.1. Secondary structural elements predicted by SOPMA Server

The CDD Blast hits recorded that the query protein has Arginine kinase belongs to Phosphagen kinase Superfamily (Fig2).

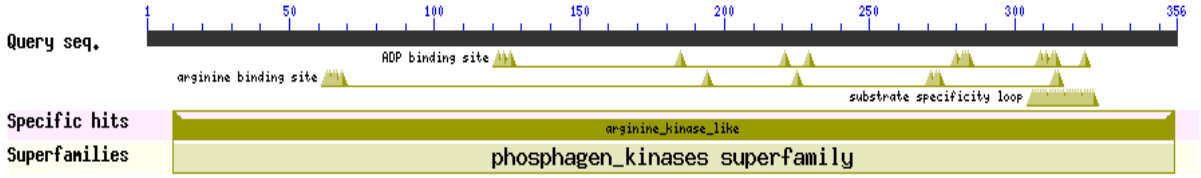
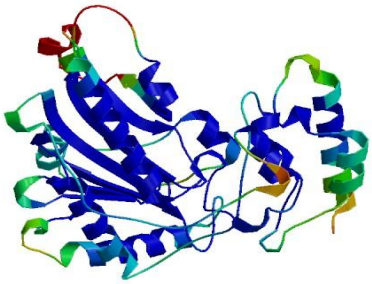


Fig 2 : CDD BLAST results of C9ZXW0 of *T.brucei*

(C)Tertiary Structure:

The tertiary structure was predicted by homology modeling. The Swiss model server modelled 3D structure for the Arginine kinase (Fig. 3). The query has more than 90 % of residues in most favourable region (Fig. 4.), showing that the model obtained was fairly a good one.



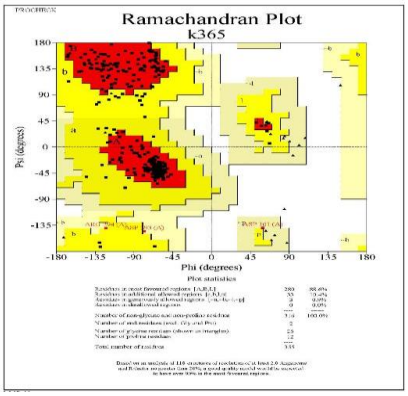


Fig.3: Homology model of Arginine kinase of*T. Brucei*

Fig. 4: Ramachandran plot

The normalised Qmean score of swissmodel graph revealed the predicted model (red star) lies within the black area (Fig 5), showing that the model shows close similarity with the available PDB structures

This was found to be in consistent with the results predicted by PROSA server, showed that the query protein was found to be similar to the available Xray and NMR structures .(Fig .6)

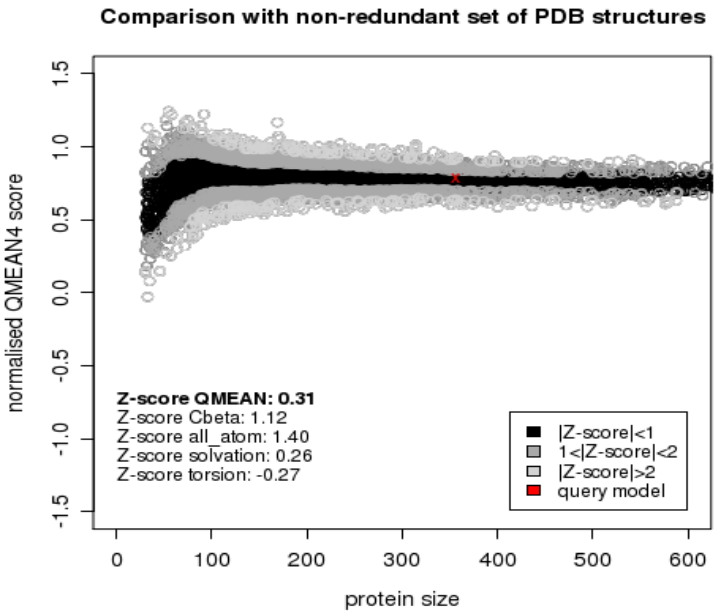
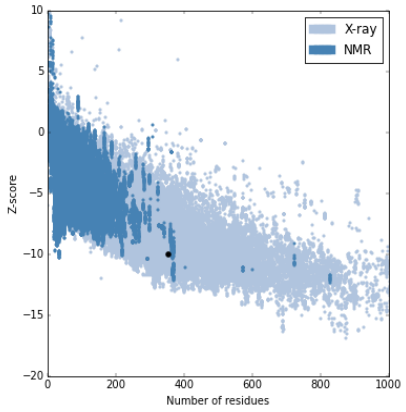


Fig 5: Showing the protein of the modeled query (in red colour) with its Z score. The modeled query lies well within the good scoring areas of other non-redundant PDB structures



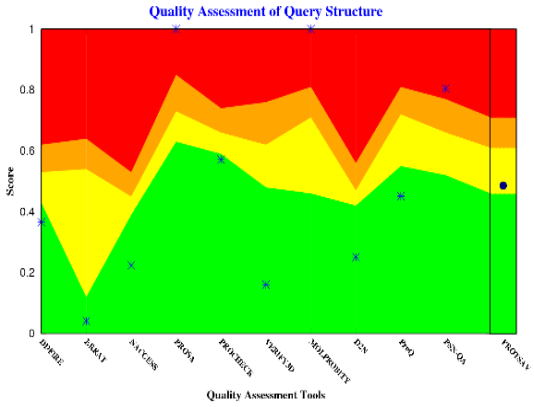


Fig.6 **Fig.7**

Results of PROSA (Fig6) and PROTSAV (Fig7)of Arginine kinase(C9ZXW0) showing the position of query protein well within the good score area of X-Ray and NMR structuresThe black dot shows that the model lies in the area of 2-5A°rmsd.

Thus the results computed by various *insilico* tools showed that the structure of the predicted protein was stable and with good quality.

Binding site analysis was also performed by 3DLigandsite and GHECOM .The modeled protein arginine kinase was found to be with 18 binding sites (Fig.8).

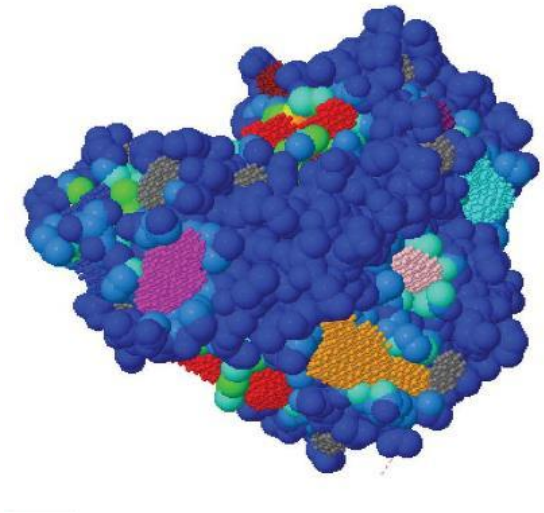


Fig-8

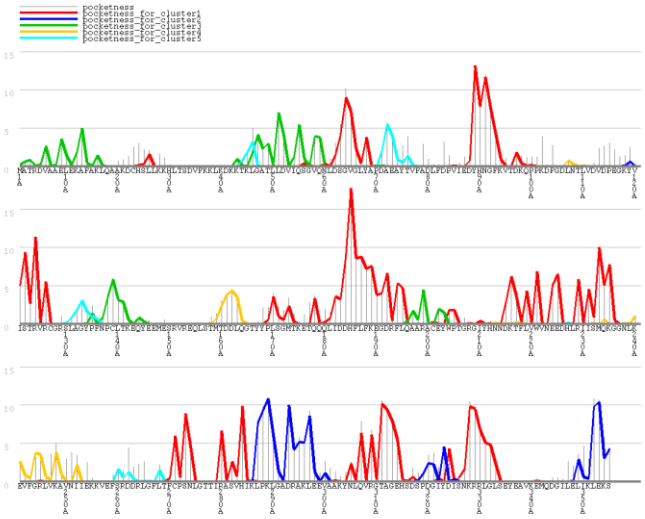


Fig.9

The predicted 3D model(Fig 8) of arginine kinase with putative binding pockets (Fig.9) for C9ZXWO of *T.brucei*. The graph predicted by GHECOM provide the details of various pockets available in the selected protein (Fig 9). It also reveals the interacting amino acids in these sites.

DISCUSSION

The parasitic protozoan *T.bruceigambiense* has the ability to adapt its metabolism to a wide range of environmental condition and selection pressure which include the availability of quality of carbon sources in different mammalian and insect host[1]. Arginine kinase of *T.brucei*is an important component in resistance mechanism to different stress factors such as trypanocidal drugs pH, starvation etc. Therefore the enzyme such as phosphokinases associated in energy metabolism becomes an important candidate for trypanocidal therapeutic drugs [3].

The construction of 3D models and docking studies have become the most essential part of identification and validation of therapeutic targets. Ideal targets are genes or proteins of parasites that are absent or quite different from mammalian host. And they must also play a crucial role for the parasite so that interference with their function will have a damaging effect on the parasite [3]. The target chosen for the present study fulfils these criteria. Moreover, there is no 3D structure model available in the protein structural databases. This makes the present study , the elucidation of structure and identification of binding sites of arginine kinase of *T.brucei* a favourable starting point for rational drug designing for human trypanasomiasis.

The relationship between the arginine transport rate, arginine kinase activity ,the parasite's stage and replication capacity was established which implies the crucial role played by this enzyme as regulator of energy reserve and growth[3].

Moreover, Arginine kinase serve as a ready source not only of ATP but also of inorganic phorphate which in essential for active metabolism.In addition, it is highly critical for the survival of the *Trypanasoma* under stressful condition ; particularly for the bloodstream form which are constantly being exposed to prooxidants in the mammalian host blood environment. [5], [6].

Furthermore, the protein structural databases do not contain any 3D structure models of the selected enzyme, This makes the current study which aims to elucidate the structure and identifies the binding sites of T. brucei's arginine kinase a good starting point to design targets for human trypanasomiasis.

In the present study , Protparam results showed that the enzyme arginine kinase was found to be stable (Table 2). The presence of ATP-guanidophospho transferase domain in the present study confirms the role of phospho transferase nature of arginine kinase [3], [4].

In silico structural charaterisation and determination of 3D structure by comparative modeling revealed good scores in PROSA and PROTSAV computations (Fig .6 and 7) ; this make the enzyme arginine kinase , a good model to be utilized in the development of new diagnostic tools for detection of infection .

For the investigation of ligand or small molecule binding through docking studies, the prediction of eighteen probable ligand binding sites (Fig. 8 and 9) will offer some insight. Further studies are required to elucidate arginine kinase to be used as a validated drug.

REFERENCES

[1]. G.D.Alonoso., C.A.Pereira, S.Remedi C. Paveto. L.Cochella.S.Ivaladi, B.Gerezde, M.Burgos.H.N.Torres., and M.M.Flwia .Arginine kinase of the flagellated protozoa *Trypanosomacruzi* . Regulation of its expression and catalytic activity.*FEBS*Letters , 2001, 498:22-25.

[2]. B.R.Jarilla T.,Agatsuma. Phosphagen Kinases of parasites: Unexplored chemotherapeutic Targets. *Kar.J.Para*., 2010, 48:281 -284.

[3]. C.A.Pereira.A.M. Silber, E.G.Rey. New Enzymes as potential therapeutic targets for *Trypanosomiases* and *Leishmaniasis. SANE Huidaur publication*.,2011PP.126-137.

[4]. C.A.Pereira,G.D.Alonso,M.C.Paveto,A.Iribarren,M.L.Caban,H.N.Torres,M.M.Flawia. *Trypanosoma cruzi*arginine kinase characterization and cloning.A novel energetic pathway in protozoan parasites. *J.Bio.Chem., 2000, 275:1495-1501.*

[5]. M.A.Ferreira., A.C.R.Guimarase, P.V.d.S.ZCapriles.., L.E.Dardenne, W.M.Degrave. A new approach for potential drug target discovery through *in silico* metabolic pathway analysis using *Trypanosoma cruzi* genome information. *Mem .Inst .Oswaldo .Cruz ,Rio de Janeiro* ., 2009,104(8): 1100-1110.

[6]. K.Uda, N.Fujimoto, Y.Akiyama, K.Mizuta ,K.TanKa, W.R.Ellington, T. Suzuki. Evolution of the arginine kinase family .*Comp BiochemPhysiol D: Geno and Prot*.2006, 1:209-218.