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Original Article

A Systematic Study on Structure and Function of ATPase of *Wuchereria bancrofti*

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ABSTRACT

Background: Analyzing the structures and functions of different proteins of *Wuchereria bancrofti* is very important because till date no effective drug or vaccine has been discovered to treat lymphatic filariasis (LF). ATPase is one of the most important proteins of *Wuchereria bancrofti*. Adenosine triphosphate (ATP) converts into adenosine diphosphate (ADP) and a free phosphate ion by the action of these ATPase enzymes. Energy releases from these dephosphorylation reactions drive the other chemical reactions in the cell. **Materials and Methods:** In this study we worked on the protein ATPase of Wuchereria bancrofti which has been annotated from National Center for Biotechnology Information (NCBI). Various computational tools and databases have been used to determine the various characteristics of that enzyme such as physiochemical properties, secondary structure, three-dimensional (3D) structure, conserved domain, epitope, and their molecular evolutionary relationship. **Result:** Subcellular localization of ATPase was identified and we have found that 55.5% are localized in the cytoplasm. Secondary and 3D structure of this protein was also predicted. Both structure and function analysis of ATPase of Wuchereria bancrofti showed unique nonhomologous epitope sites and nonhomologous antigenicity sites. Moreover, it resulted in 15 ligand drug-binding sites in its tertiary structure. **Conclusion:** Structure prediction of these proteins and detection of binding sites and antigenicity sites from this study would indicate a potential target aiding docking studies for therapeutic designing against filariasis.

Key words: ATPase, docking studies, epitope, Filariasis, *Wuchereria bancrofti*

INTRODUCTION

Wuchereria bancrofti is one of the most common human parasitic filarial nematode that is found mainly in tropical regions.[1] *Wuchereria bancrofti* is endemic in more than 78 nations and affects 128 million persons worldwide.[2,3] It is mainly found in Central Africa, South and Central America, Nile delta, and the tropical regions of Asia including Southern China and the Pacific. There are three parasites which cause filariasis and it is one of them.[4] It spreads by a mosquito vector and generally there are six genera and 70 species of mosquitoes responsible for spreading. Humans are the only recognized ultimate host of *Wuchereria bancrofti*. Although monkeys have been infected artificially, they are not hosted in the wild. It is responsible for about 90% of lymphatic filariasis (LF).[5] Filaria also causes chronic acute bacterial dermatolymphangioadenitis (ADLA) attacks which can cause renal disease, chyluria, nephritic syndrome, hematuria, proteinuria, and glomerulonephritis. Patients with LF can also have cystitis with urethral block, rheumatic problems, tropical vaginal hydroceles, fibrosing mediastinitis, and bladder pseudotumors. Typically ADLA occurs when a

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mature worm dies and the lymph vessels surrounding it are vexed due to the host's immune response.[6] Several therapeutic drugs are available, some of them are diethylcarbamazine (DEC), ivermectin, or albendazole. No effective drug or vaccine has been discovered to treat LF till date.[7] A mixture of albendazole and ivermectin or DEC and albendazoles can be used for eliminating microfilaria, but they only interrupt the mature females reproductive capability.[8,9] An indication of the efficiency of this combination treatment is still inadequate[10-13] and quantitative efficiency estimates are not yet available. Hence, the new antifilarial drug combinations are estimated to increase compliance, coverage, and decrease costs by familiarizing simple effective drug delivery and distribution methods. For this purpose to design new drugs or vaccines against an organism, its proteins can be used by detecting its domains and predicting ligand binding sites.[14] Among all these proteins, ATPase is one of the most significant proteins. It is a class of enzymes that are crucial for the formation of adenosine diphosphate (ADP) and a free phosphateion from decomposition of adenosine triphosphate (ATP). These dephosphorylation reaction releases energy, which the enzyme hitches to motivate other chemical reactions in the cell. It is also known as transmembrane ATPase, which has different types such as F-ATPases, A-ATPases, V-ATPases, P-ATPases, and E-ATPases. Among them V-ATPases function in the acidification of intracellular sections in eukaryotic cells.[15,16] Moreover, ATPases can be used as drugs or vaccine targets. ATPases are involved in various cellular functions and numerous human diseases. Therefore, gorgeous therapeutic drug targets and plentiful ATPase inhibitors are already on the marketplace. However, maximum of these drugs per form their activity without binding to the nucleotide-binding site. To design competitive ATP inhibitors are a substitute approach to inhibit ATPase's. This method has been successfully used to design protein-kinase inhibitors and it depends on the arrangement of the nucleotide-binding site.[17] In this study, we directed a bioinformatics investigation of structure and function of ATPase of *Wuchereria bancrofti* to predict the potential drug target sites and vaccine target site.

MATERIALS AND METHODS

Sequence retrieval

Amino acid sequences of ATPase protein of *W. bancrofti* was retrieved from National Center for Biotechnological Information (NCBI; http://www.ncbi.nlm.nih.gov/) and the accession number of this protein is EJW84114.1(gi | 402590183). Various computational tools and databases were used to analyze the different properties, like physicochemical, subcellular localization, secondary protein and three-dimensional (3D) structure, epitopes, and antigenicity sites.

Physiological–biochemical characterization

The Expasy Protparam server was used (http://us.expasy.org/tools/protparam) for the physicochemical characterization and to know the molecular weight, theoretical isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient instability index, and grand average hydropathicity (GRAVY) of this ATPase protein.[18]

Subcellular localization and signal peptide

Subcellular localization of the protein was identified by Psort (http://psort.nibb.ac.jp/form2.html). SignalP program (http://www.cbs.dtu.dk/services/SignalP/) was used to predict the signal peptide.[19]

Secondary structure prediction

PSIpred (http://bioinf.cs.ucl.ac.uk/psipred/) was done to analyze the secondary structure of this protein.[20] Secondary structural properties of the protein includes alpha helix, 310 helix, Pi helix, beta bridge, extended strand, beta turns, bend region, random coil, ambiguous states, etc.

Identification and comparison of conserved domain

Conserved Domain Database -Basic Local Alignment Search Tool (CDD-BLAST)(http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/) was done, to find out the conserved domain and to reveal any domain similarity with the *Hs*ATPase.[21]

Molecular evolution

Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) was used for searching evolutionary relationship among different organisms with the same protein ATPase.[22]

3D structure prediction

Phyre2, a web-based software (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id = index), was used to predict the 3D structure of this protein. Predicted structure was then refined by Modrefiner (http://zhanglab.ccmb.med.umich.edu/ModRefiner/). Finally the protein was visualized by Jmolserver and evaluated with PROCHEK server by Ramachandran plot analysis.[23-25]

Active site prediction

3DLigandSite (http://www.sbg.bio.ic.ac.uk/~3dligandsite/) is an automated software that was used to predict the ligand binding sites. Analysis of 3D ligand binding sites is a vital task for the modeled protein to do further work on its docking studies and it gives us an idea to make a grid before docking.[26,27]

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B-cell epitope and antigenicity

The position of the linear B-cell epitopes was done by BepiPred (http://www.cbs.dtu.dk/services/BepiPred/) and Immune Epitope Database (IEDB) Analysis Resource (http://tools.immuneepitope.org/tools/bcell/iedb_input) was used to predict the antigenic sites.[28-30]

RESULT

Physical and chemical characteristics

The amino acid sequence of ATPase *W. bancrofti* (NCBI reference sequence: Gi | 402590183) contained 563 amino acid with a molecular weight of 64,160.1Da and the predicted pI is 9.37. The estimated half-lives of the protein in mammals, yeast, and *E. coli* were 30, >20, and >10 h, respectively, and its instability coefficient was 44.88, which was the characteristic of an unstable protein. The grand average of hydrophobicity (GRAVY of ATPase was − 0.644 and extinction coefficient 46,535. Therefore, the ATPase was an unstable and alkaline enzyme.

Molecular evolution and conserved domain

The molecular evolutionary analysis showed that the ATPase of *W. bancrofti* was more primitive in comparison to other nematodes and also from the host (*Anopheles, Culex*) and human [Figure 1]. It was largely destined from *Homo sapiens*.

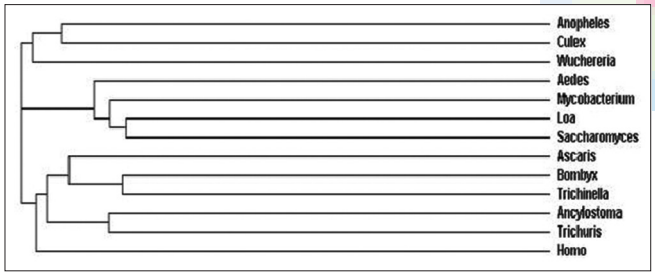


Figure 1: Phylogenetic analysis of adenosine triphosphate (ATP)ase of different species

On the other hand, conserved domain analysis showed that it contains AAA superfamily, a DUF3523 multidomain, and a nonspecific superfamily PPV_E1_C [Figure 2].

Subcellular localization

ATPase of *W. bancrofti* was predicted to be localized 47.8% in the cytoplasm, 21.7% in mitochondria, 17.4% in the nucleus, and 4.3% each in the vacuoles, vesicles of secretory system, and in the endoplasmic reticulum. The prediction was done by k-nearest neighbors (KNN) prediction. Moreover the cytoplasmic location of this ATPase was 55.5% reliable according to Reinhardt's method for cytoplasmic/nuclear discrimination. Moreover, there was not any signal peptide.

Antigenicity sites and linear B-cell epitopes

Bioinformatics tools predicated several antigenicity sites and linear B-cell epitopes. ATPase possesses 22 antigenicity sites, among them 16 sites were not homologous with the amino acid sequence of HsATPase, such as 49–59, 74–80, 132–146, 186–197, 202–218, 224–232, 239–245, 247–259, 361–368, 390–395, 425–433, 440–453, 471–477, 489–502, 504–510, and 551–556.

On the other hand 35 linear B-cell epitopes were found. Among them nine were homologous with HsATPase amino acid sequence, otherwise nonhomologous sequences are 12–19, 41–54, 65–74, 81–101, 170–178, 183–185, 221–224, 293–297, 379–384, 399–401, 451–460, 477–487, 499–508, 546–563, 2, 10, 56, 270, 333, and 544 [Table 1].

Structure of ATPase

The secondary structure analysis reveals that WbATPase consists of 67.5% α-helix, extend strand 7.28, and random coil 22.56. In case of solvent accessibility; 49.20% was buried, 15.10% intermediate accessibility, and 35.70% showed exposed accessibility [Figure 3]. Seventeen protein

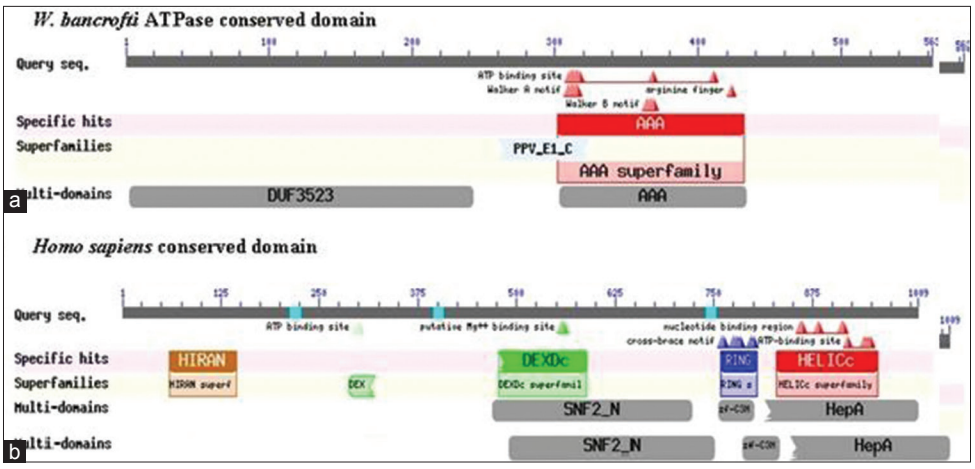


Figure 2: Comparison between (a) WbATPase and (b) HsATPaseconserved domain

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binding site were found. The tertiary structure of WbATPase was predicted by PHYRE2 which showed that the AAA superfamily and DUF3523 multidomain with PPV_E1_C nonspecific domain formed a large indentation [Figure 4]. Predicted model was then refined by Modrefiner and validated with PROCHEK server by Ramachandran plot analysis. PROCHEK analysis showed that 88.8% residues are positioned in the most favored region, 9.0% in additional allowed region, and 0.8% residues in the generously allowed region of the Ramachandran plot.

Ligand Binding Sites

3D ligand showed the 15 active sites of WbATPase with metallic to which drugs can bind. [Figure 5].

DISCUSSION

The physiological–biochemical characterization of *W. bancrofti* ATPase showed that the pI of the protein is 9.87, which indicates that it is an alkaline enzyme. On the other hand the average hydrophobicity of this protein is −0.6, which means that it is an unstable protein in nature.[31]

The molecular phylogenetic analysis reveals that the ATPase of *W. bancrofti* is evolutionarily more ancient than the other nematodes as well as other organisms including the hosts (Ades, Culex, and *Homo sapiens*). But it is highly distant from the HsATPase that permits us to design new drugs or vaccines. The *W. bancrofti* ATPase possess conserved domains (AAA superfamily, a DUF3523 multidomain, and a nonspecific superfamily PPV_E1_C), which are not homologous with HsATPase conserved domains [Figure 2], that can be a potential target site of therapeutic agents.[32] The subcellular localization prediction shows that the ATPase is located in cellular cytoplasm and have segment of amino acids in NORs. This subcellular localization of ATPase may be one of the immune evasion techniques of *W. bancrofti*. The secondary structure annotation showed that 67.5% α-helical with 49.20% solvent accessibility and 17 protein binding sites which can also be used for molecular docking.[33] *W. bancrofti* ATPase is in cytoplasm of the cell with segments in the membrane and the drugs must inhibit and even kill the *W. bancrofti*, but will not be harmful for the normal cells of human. For this reason, drug targets must be some amino acid sequences that do not show the homology with HsATPase. The antigenicity sites—49-59, 74-80, 132-146, 186-197, 202-218, 224-232, 239-245, 247-259, 361-368, 390-395, 425-433, 440-453, 471-477, 489-502, 504-510, 551-556. And the B-cell Epitope sites 12-19, 41-54, 65-74, 81-101, 170-178, 183-185, 221-224, 293-297, 379-384, 399-401, 451-460, 477-487, 499-508, 546-563, 2, 10, 56, 270, 333, 544 without any homology with Human ATPase, can be used as ideal drugs or vaccines

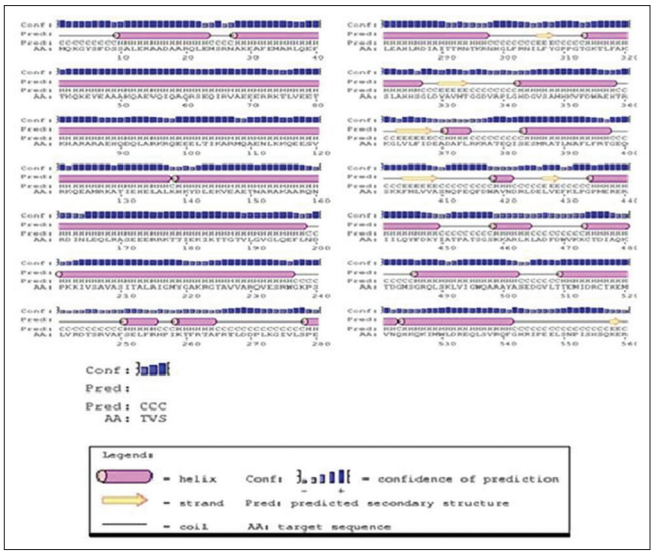


Figure 3: Secondary structure of ATPase



Figure 4: Refined three-dimensional (3D) structure of WbATPase

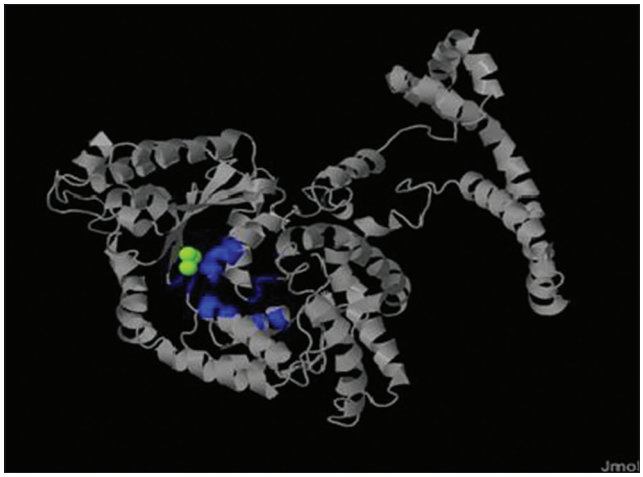


Figure 5: Three-dimensional (3D) ligand binding site. The blue color indicate the ligand binding site, the green color indicate the metallic heterogenes, and the ash color indicate the non-ligand binding site

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Table 1: Linear B-cell epitopes and antigenicity sites of *Wuchereria bancrofti*

Amino acid sequences					
Antigenicity sites		Linear B-cell epitopes			
49-59	316-333	2	65-74	221-224	399-401
74-80	337-355	4-8	81-101	233-243	411-418
132-146	403-411	10	112-126	270	433-437
186-197	425-433	12-19	145-160	293-297	451-460
202-218	440-453	27	170-178	309-316	477-487
224-232	471-477	29	183-185	333	499-508
239-245	489-502	41-54	199	335-346	544
247-259	504-510	56	202	358	546-563
269-288	534-541	58-62	203	379-384	
302-310	551-556				

targets (Pasnik *et al*.,2005 and Dakappagari *et al*., 2000). In tertiary structure of *W. bancrofti* ATPase 3D ligand identified the 10 unique ligand binding site. It is highly notable that the available drugs of filarial DEC, ivermectin, and albendazole[34] are not against the ATPase or its related pathway in *W. bancrofti*. For this reason the ATPase of *W. bancrofti* can be a unique target site of drugs.[35-37] So we strongly believe our research/bioinformatics analysis will be helpful to design new vaccines or drugs against the *W. bancrofti* ATPase.

CONCLUSION

We have taken the ATPase sequence of *W. bancrofti*from the NCBI database and used various bioinformatics tools to determine the various characteristics of that enzyme such as physiochemical properties, secondary structure, 3D structure, conserved domain, epitope, and their molecular evolution. The 3D structure shows superfamily and multidomain, which shows 15 ligand binding sites. The evolutionary relationships of epitopes are highly distant from the HsATPase that permits to design new drugs or vaccines. There are many epitopes and antigenicity sites present, from which the nonhomologous epitopes and antigenicity sites can be used as potential drug or vaccine target against *W. bancrofti* ATPase.

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