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Comparative Genome Analysis of *Plasmodium falciparum* Triosephosphate Isomerase and Cytochrome Oxidase; Effect of Antimalarial Drugs on the Enzyme Stability

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***Abstract*—**
Background: *Malaria is a major global public health challenge. Plasmodium falciparum happens to be the most virulent among the causative parasites of malaria. The development of drug resistance in Plasmodium falciparum strains has built up a great interest in the search for new antimalarial drugs and drug targets. As part of a program to develop metabolic enzymes as potential drug targets, the 3-dimensional structure of Plasmodium falciparum triosephosphate isomerase was determined. The focus on glycolytic and electron transport chain enzymes in the malaria parasite results from the observation that in the asexual stage of the parasite in the human red blood cells, the energy requirements of the organism are almost exclusively met by glycolysis and the electron transport chain enzyme of the parasite remains highly stable and resistant.*
Materials and Methods: *The amino acid sequences of the experimental enzymes were mined from the NCBI database and sequence aligment between the triosephosphate isomerase and cytochrome oxidase of P. falciparum and their respective human orthologs were performed using the ClustalW sequence alignment software. The alignments were visualized using the Bioedit software which is a biological sequence alignment editor. The MEGA7 software was used to view and highlight the protein conserved domains and variable sites while the prediction of the protein domain was done using the PSIPRED. The amino acid composition graph of the P. falciparum enzymes was also plotted using specific functions on the MEGA7 software.*
Results: *Here, we present a computational analysis of the amino acid composition of Plasmodium falciparum triosephosphate isomerase and cytochrome oxidase which are cytosolic and mitochondrial enzymes respectively. An alignment was also carried out with the human orthologs of each of the respective analysed parasite enzyme sequence. This comparison with the human enzymes was used to predict their functional similarity in respect to therapeutic drug design and the predicted potency of the drug for prophylaxis and disease treatment.*
Conclusions: *Antimalarial drugs targeted at the Plasmodium falciparum triosephosphate isomerase tends to act faster compared to the mitochondrial cytochrome oxidase drug target counterparts. The initial takes advantage on the cytosolic instability of the disulfide bonds which has been analysed also to be of a very minute quantity in the enzymes.*

***Keywords*—** *Plasmodium falciparum; Resistance; Alignment; Orthologs; Prophylaxis.*

I. BACKGROUND

Parasitic organisms acquire organic material from their environments and convert this material into energy or their own substance (i.e., biomolecules) [8]. Cells are made up of distinct classes of biomolecules with specific functions. These macromolecules are synthesized from small molecular weight precursors. These precursors are components of interconnected metabolic pathways [25]. The malaria parasite exhibits a fast growth and multiplication rate during many stages of its life cycle. This makes it necessary for the parasite, like all other organisms to acquire nutrients and metabolize these biological molecules in order to survive and reproduce [17]. It is a clear concept that the parasite's metabolism will be intertwined with that of the host's because of the intimate relationship between the host and parasite [13].

These interactions between the host and the parasite are further complicated by the complex life cycle of the parasite involving vertebrate and invertebrate hosts as well as different locations within each of these hosts. A better understanding of the parasite's metabolism may lead to the development of novel therapeutic procedures which exploits the uniqueness of the parasite [13, 27]. The blood-stage of the parasite actively ferments glucose as a primary source of energy. The *Plasmodium falciparum* metabolic steps involved in the conversion of glucose to lactate are essentially the same as that found in other organisms [25].

All of the enzyme activities in *Plasmodium falciparum* have been identified including some of the genes which have already been cloned. The parasite exhibits a high rate of glycolysis and utilizes up to 75 times more glucose than uninfected Red blood cells [16, 17]. Most of the glucose is converted to lactate and the high lactate dehydrogenase activity is believed to function in the regeneration of NAD^+ from NADH which is produced earlier in the glycolytic pathway by glyceraldehyde-3-phophate dehydrogenase. Most of the glucose utilized by the parasite is converted to lactate i.e. an approximate value of 85% of the utilized glucose by the parasite is converted into lactate. However, some of the glycolytic intermediates may be diverted for synthetic purposes. For example, enzymes of the pentose phosphate pathway have been identified. This pathway provides some of the ribose sugars needed for nucleotide metabolism and provides for the regeneration of reduced NADPH to be used in biosynthesis or defense against reactive oxygen species. Similarly, the further metabolism of pyruvate may provide intermediates in several biosynthetic pathways [8].

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The TCA cycle and oxidative phosphorylation are generally carried out in the mitochondria of eukaryotes. These processes are generally assumed to be non-functional in the blood-stage parasite as depicted by the acristae mitochondria [21]. However, recently a functional electron transport chain and oxidative phosphorylation have been demonstrated in the blood-stage parasite [27]. In addition, the parasite mitochondrion does have a membrane potential but cytochrome oxidase is present. The antimalarial drug atovaquone has been shown to inhibit electron transport and to collapse the mitochondrial membrane potential in the malaria parasite [5, 23]. One of the most important functions of the mitochondrion during the blood stage is for the synthesis of pyrimidine.

This study was conducted to explore the capabilities of computational methods to predict the most stable and efficient enzyme of the malaria pathogen *Plasmodium falciparim* based on their sub cellular localization *and activity*, and furthermore to identify drug targets, given currently available large scale data. For this purpose, the amino acid sequences were compiled from available resources, which is able to fulfill metabolic functions known from the literature to occur in *Plasmodium falciparum*. A common approach to get an insight into the stability of an enzyme is to analyze the amino acid composition.

II. Materials and Methods

Gene Mining

The human and *Plasmodium falciparum* cytochrome oxidase were obtained from the GenBank database [19] while triosephosphate isomerase amino acid sequences were obtained from the UniProtKB/Swiss-Prot database [18]. The GenBank sequence database is an open access, annotated collection of all publicly available nucleotide sequences and theirprotein translations. This database is produced and maintained by the National Center for Biotechnology Information (NCBI) [12]. The UniProt consortium comprises the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource (PIR) which hosts a large resource of bioinformatics databases and services [26].

Sequence Alignment

The alignment of the *Plasmodium falciparum* cytochrome oxidase and Ttriosephosphate isomerase with their respective human orthologs was carried out using the ClustalW alignment tool [9]. ClustalW was used because it gives a better accuracy within the shortest time frame and is computationally stronger compared to most other alignment tools. The aligned amino acid sequences were viewed with the biological sequence alignment editor “Bioedit” [11].

Protein Variable Sites

The aligned amino acid sequences variable sites were viewed and highlighted using one of the functions implemented in the Molecular Evolutionary Genetic Analysis software (MEGA 7.0) [24].

Protein Domain

The PSIPRED (Psi-blast based secondary structure prediction) protein domain prediction method obtained from the expasy server was used in predicting the conserved domain in each of the analysed amino acid sequences [3]. The result of this analysis was presented in a graphical format.

Amino Acid Composition

The composition of the amino acid constituent of each analyzed protein was calculated using a function implemented for such an action in the MEGA 7.0 software [24].

III. Results and Discussion

Sequence Alignment

Fig. 1. Amino acid sequence alignment of Triose phosphate isomerase. The alignment in this figure is targeted at indicating the Triose phosphate isomerase protein conserved domains and variable sites in both human and the plasmodium parasite. The human and plasmodium falciparum Triose phosphate isomerase amino acid sequences were obtained from the UniProtKB/Swiss-Prot datbase with accession number P60174.3 and Q07412.1 respectively.

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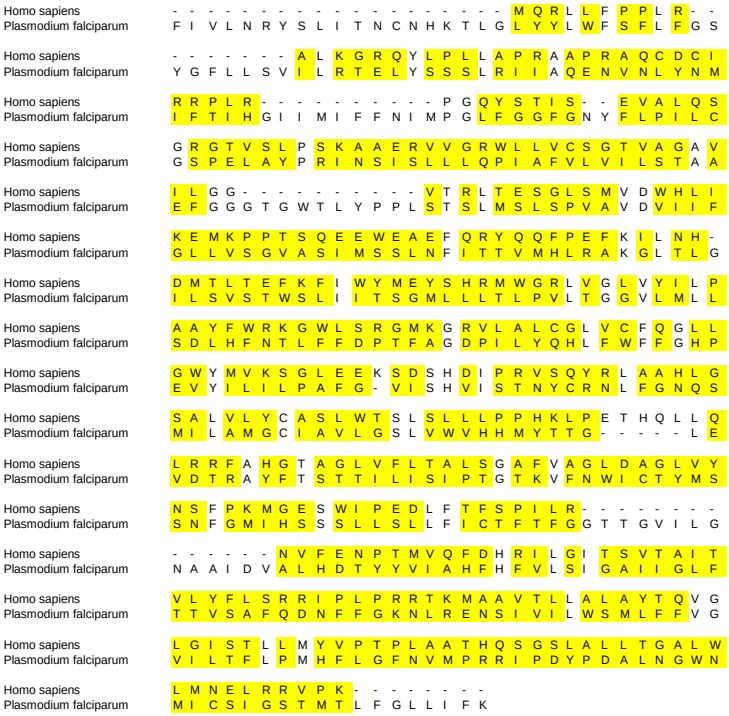

Fig. 2. Amino acid sequence alignment of Cytochrome oxidase. The alignment in this figure is targeted at indicating the Cytochrome oxidase protein conserved domains and variable sites in both human and the plasmodium parasite. The human and plasmodium falciparum Cytochrome oxidase amino acid sequences were obtained from the GenBank database with accession number BAF84343.1 and BAO27318.1 respectively.

Amino Acid Composition

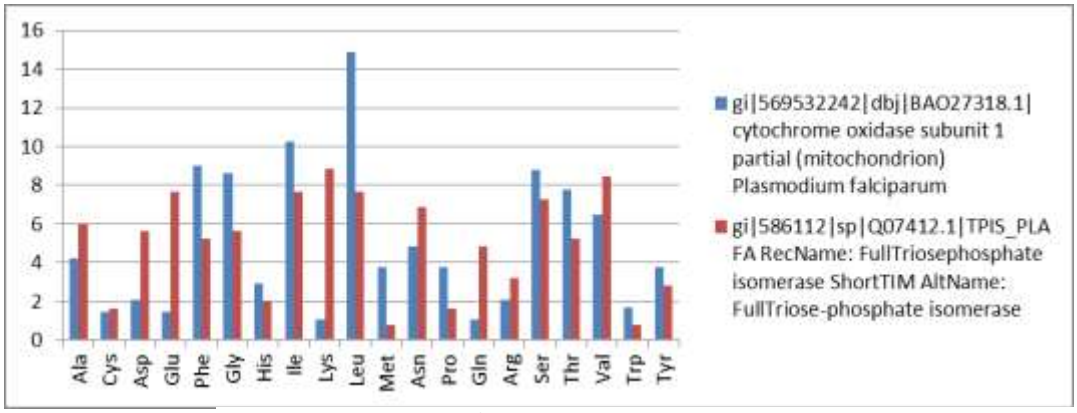

Fig. 3. Amino acid composition graph. Based on the low propensity of the amino acid side chains to be in contact with polar solvent, some amino acids are classified as hydrophobic. They include alanine, valine, cysteine etc which are in higher quantity in triose phosphate isomerase which is a cytosolic enzyme.

3D Structure Models

Fig. 4. Human Cytochrome oxidase 3D structure.

Fig. 5. *Plasmodium* Cytochrome oxidase 3D structure.

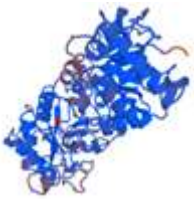

Fig. 6. Human Triosephosphate isomerase 3D structure.

Fig. 7. *Plasmodium* Triosephosphate isomerase.

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Aligned Terminal Profile

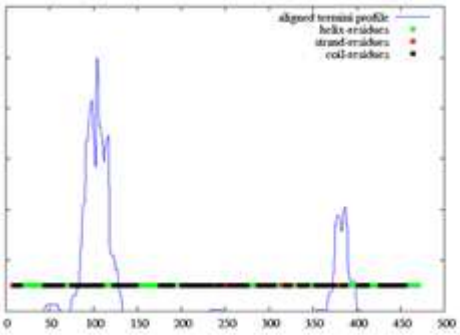


Fig. 8. Cytochrome oxidase.

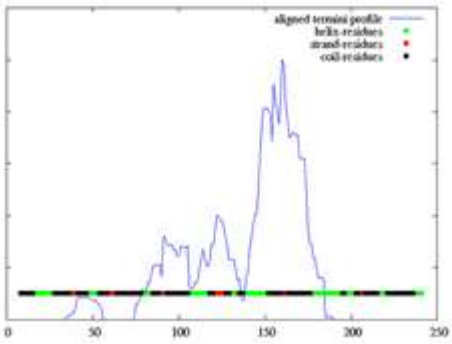
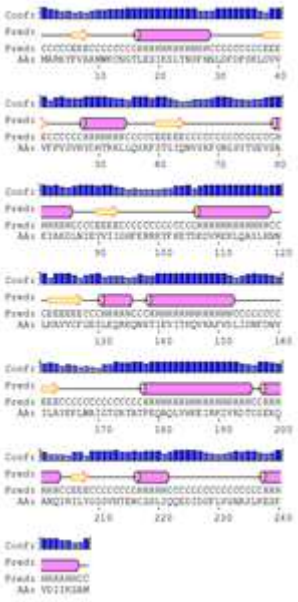


Fig. 9. Triosephosphate isomerase.

Plasmodium falciparum Cytochrome oxidase and Triosephosphate isomerase aligned termini profile: Indicates the density of the end points of PSI-BLAST alignments which were generated between the query sequence and all the PSI-BLAST hits which were found, given a PSI-BLAST run against a database with all sequence fragments removed.

Protein Domain



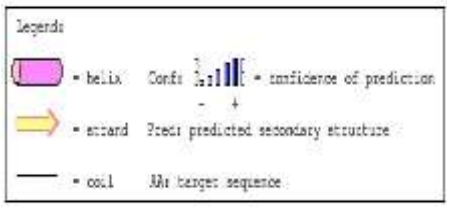
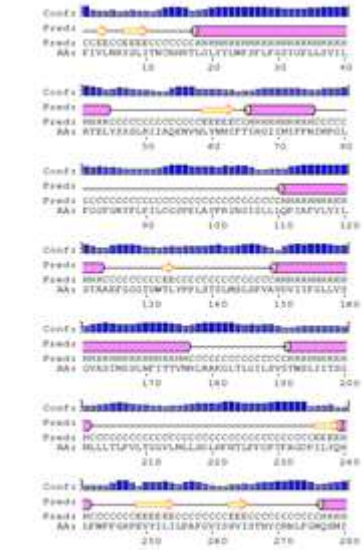


Fig 10. Triosephosphate isomerase.



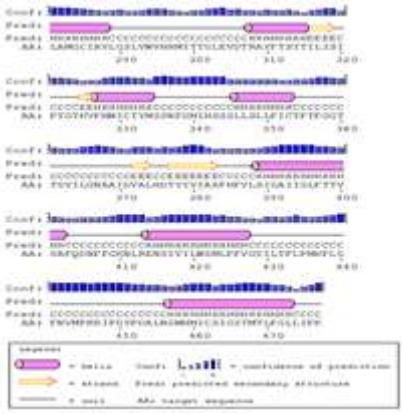


Fig. 11. Cytochrome oxidase.

For the development of an enzyme as a drug target, it is important to determine the key structural differences between the host and pathogen enzymes [15]. This can then in principle be used to develop selective inhibitors [22]. The alignment of the *Plasmodium* Cytochrome oxidase and Triose phosphate isomerase amino acid sequence with their respective human ortholog indicates the protein variable sites and conserved domain. This is also necessary for specificity in drug target during the drug design process [30].

The Protein folding knowledge is one of the basic requirements in any effort aimed at understanding protein structure and function [1]. Folding analysis will reveal evolutionary relationships, which are quite very difficult to detect at the sequence level. This may also help in a better

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understanding of a mechanism of function of a protein, its biological activity and role in living organisms [2]. From the study of the relationships between the amino acid sequence and the fold, we may also get deeper knowledge of the basic principles of protein structures, learn how to design new proteins with a defined activity, and how to modify the existing ones.

A domain is the basic building block of a protein structure, although many proteins only contain a single domain. Certain protein domains have some clearly defined functions associated with them and they are characterized by a spatially separated unit of the protein structure which may have sequence or structural resemblance to another protein structure or domain. For a hydrogen bond to be formed, two electronegative atoms have to interact with the same hydrogen which is the case in an alpha helix [20]. The hydrogen is covalently attached to the hydrogen-bond donor, which happens to be one of the atoms, but interacts electrostatically with the other which is the hydrogen bond acceptor, O. In proteins essentially all groups capable of forming H-bonds both the main chain and side chain, independently of whether the residues are within a secondary structure or some other type of structure are usually H-bonded to each other or to polar solvents which in most cases are water molecules [7]. Due to their electronic structure, water molecules may accept two hydrogen bonds, and donate another two, thus being simultaneously engaged in a total of four hydrogen bonds.

The amino acids with non-polar side chains are of great importance in the analysis because they provide rigidity to the protein structure [29]. By imposing certain torsion angles on the segment of the polypeptide chain, they preserve the protein three-dimensional fold [6]. Cysteine is encoded by the codons UGU and UGC. The thiol side chain in cysteine often participates in enzymatic reactions, as a nucleophile. The thiol is susceptible to oxidization to give the disulfide derivative "cystine", which serves an important structural role in many proteins [14].

The disulfide bonds are known to be generally unstable in the cytosol [4]. Disulfide bonds in proteins are formed by oxidation of the thiol groups of cysteine residues. The other sulfur-containing amino acid, methionine, cannot form disulfide bonds. Cysteine residues play a valuable role by cross linking proteins, which increases the rigidity of proteins and also functions to confer proteolytic resistance and since protein export is a costly process, minimizing its necessity is advantageous [28]. Inside the cell, disulfide bridges between cysteine residues within a polypeptide support the protein's tertiary structure [10].

IV. Conclusion

The activities of the cytochrome oxidase, which is located in the plasma, the nuclear membrane as well as in the mitochondria of the *Plasmodium falciparum* can be inhibited completely by specific antimalarial drugs. Some other antimalarial drugs has also been shown to inhibit electron transport and to collapse the mitochondrial membrane potential in the malaria parasite but on the other hand, triosephosphate isomerase has been described as an attractive and most important target for drug design against parasites because of its important role in glycolysis. Thus, because of the instability of their disulfide bonds, the glycolytic enzymes have been shown to provide a better drug target.

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