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Plasmodium and host carbonic anhydrase: molecular function and biological process

Research Article

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Summary

Carbonic anhydrase (CA) is an enzyme that catalyzes an interconversion of CO_2 and HCO^{3-}. CA is present at high levels in humans and *Plasmodium spp*. However, the function of CA in malarial infection is not well characterized. Here, the author used a new gene ontology technology to predict molecular function and biological process of CA. Using GoFigure server, the molecular function and biological process of human and *P. falciparum* CA are predicted. Comparing to human CA, the *P. falciparum* CA has similar molecular functions as carbonate dehydratase activity and zinc ion binding. Although the basic sequences for human and *P. falciparum* CA are totally different, the molecular functions are similar. This finding implies that any treatment aiming at blocking the functions of *P. falciparum* CA can affect human CA. Thus any drug targeting at *P. falciparum* CA might not be a magic bullet. The more specific structural antagonist that can directly block amino acid of *P. falciparum* CA is more favorable.

I. Introduction

Carbonic anhydrase (CA) is an enzyme that catalyzes an interconversion of CO_2 and HCO^{3-}. CA is present at high levels in humans and *Plasmodium spp* (Reungprapavut et al, 2004). Existence of at least three isozymes was demonstrated in *P. falciparum* and a rodent malarial parasite *P. berghei* (Reungprapavut et al, 2004). Krungkrai et al found that the parasite enzyme activity was sensitive to well-known sulfonamide-based inhibitors of both bacterial and mammalian enzymes. They noted that the enzyme inhibitors had antimalarial effect against in vitro growth of *P. falciparum* (Krungkrai et al, 2002). Reungprapavut et al noted that *P. falciparum* carbonic anhydrase was a possible target for chemotherapy (Reungprapavut et al, 2004).

In malarial infection, CO_2 is essential for the growth of intraerythrocytic malarial parasite to synthesize pyrimidine through CO_2 fixation and regulate intracellular pH and CO_2 transport across the plasma membrane of erythrocytes, which are facilitated by CA (Sein and Aikawa, 1998). However, the function of the CA in malarial infection is not well characterized. Krungkrai et al noted that a full understanding host and parasite CA promised advances in malarial treatment (Sein and Aikawa, 1998). Here, the author used a new gene ontology technology to predict the molecular function and biological process of this enzyme.

II. Materials and methods

A. Getting the sequence

The database Unitprot (Bairoch et al, 2005) was used for data mining of the amino acid sequence for human host and *P. falciparum* CA.

B. Prediction of molecular function and biological process

The author performed prediction of molecular function and biological process of human and *P. falciparum* CA using a novel gene ontology prediction tool, GoFigure (Bairoch et al, 2005). GoFigure is a computational algorithm tool which was recently developed in gene ontology (Bairoch et al, 2005). The tool accepts an input DNA or protein sequence, and uses BLAST to identify homologous sequences in gene ontology annotated databases (Bairoch et al, 2005). The approach uses a BLAST search to identify homologs in public databases that have been annotated with gene ontology terms (Bairoch et al, 2005). These include: SwissProt, Flybase (Drosophila), the Saccharomyces Genome Database (SGD), Mouse Genome Informatics (MGI) and Wormbase (nematode) (Bairoch et al, 2005). The contents of

results will show molecular function as well as biological process of the studied protein (Bairoch et al, 2005). The prediction of molecular function and biological process were presented and compared.

III. Results

A. Sequence

From searching of the database Uniprot, sequence of human and *P. falciparum* CA was derived as shown in **Figure 1**.

B. Prediction of molecular function and biological process

Using GoFigure server, the molecular function and biological process in human and *P. falciparum* CA is predicted. The molecular function and biological process of human and *P. falciparum* CA are presented in **Figure 2** and **Figure 3**, respectively. The molecular function of human CA is "Carbonase dehydratase activity", "Zinc ion binding" and "Lyase activity" and the molecular function of *P. falciparum* CA is "Carbonase dehydratase activity", and "Zinc ion binding". The biological processes of human and *P. falciparum* CA are "One carbon compound metabolism" and "One carbon compound metabolism", respectively.

IV. Discussion

CA is an enzyme that is believed to have a significant role in malarial infection. The malarial parasite *P. falciparum* encodes for an alpha-carbonic CA possessing catalytic properties distinct of that of the human host, which was only recently purified (Krungkrai et al, 2002). CA inhibitors are possible effective antimalarial drug (Krungkrai et al, 2005). Recently, Krungkrai et al said that the vital contribution of CA to parasite survival made the enzyme an attractive target for therapeutic evaluation (Krungkrai et al, 2001). In addition, there are some current researches on the possible use of CA inhibitors to kill cancer kills. The possible mechanisms are inhibition of CA isozymes which predominate in tumor cell membranes, perhaps causing acidification of the intercellular milieu, or inhibition of intracellular isozymes which provide bicarbonate for the synthesis of nucleotides and other essential cell components (Supuran et al, 2001).

Roles of both host and parasite CA in cellular level metabolism during a malarial infection have been proposed (Sein and Aikawa, 1998; Sein and Aikawa, 1998). Until present, the function of *P. falciparum* CA, correlating to human CA, is not well explored and there is a need for better understanding function of these proteins. In this work, the author explores and compares the potential functions of malarial and human carbonic anhydrase by gene ontology.

Based on recent advance in the genomics technology, current microarray technology permits examination of gene expression patterns of ten thousands of genes (Bairoch et al, 2005). A challenge facing the biologist interpreting such data is recognizing the function of many of the hits identified in a single experiment (Khan et al, 2003). While one can check the literature, a rapid means to get some idea of potential function of a gene product is to obtain the ontology terms that describe the gene (Khan et al, 2003). The gene ontology is developed for this specific purpose. Many gene ontology tools have been constructed and launched. Here, the author used a gene ontology tool to perform a comparative study on the predicted function of human and *P. falciparum* CA. This bioinformatics approach may be of interest to predict gene
functions as an enormous inflow of information is derived from current genome projects on malarial organisms.

A) human CA

ASPDWGYDDK NGPEQWSKLY PIANGNNQSP VDIKTSETKH DTSLKPISVS

YNPATAKEII NVGHSFHVNF EDNDNRSVLK GGPFSDSYRL FQFHFHWGST

NEHGSEHTVDGVKYSAELHVAHWNSAKYSSLAEAASKADGLAVIGVLMKV

GEANPKLQKV LDALQAIKTK GKRAPFTNFD PSTLLPSSLD FWTYPGSLTH

PPLYESVTWI ICKESISVSS EQLAQFRSLL SNVEGDNAVP

MQHNNRPTQPLKGRTVRASF

B) *P. falciparum* CA

MKDLKERELKNISDVYLNLFDD-DNYAWNNNYNKPWMKG------DFFYY

YEYFIKKIVINRQNNIFQIKAARDGIIPFGVLFTTEQPAMFYADQIFHFA------

PSEHTFQGSGNRREIEMQIFHSTNYFYDIQDDKSKYKKKYGLHIIYNNLK

KNSKETSKKDSSRYHSYLMSFLMNSLSNEQLQNYKNKKKR----------

IKKMKNQYEVISITFTSAEINASTINAEKKLPSEKFLRTIINVSSAVHVGSGNK---

Figure 1. Sequence of human and *P. falciparum* CA.

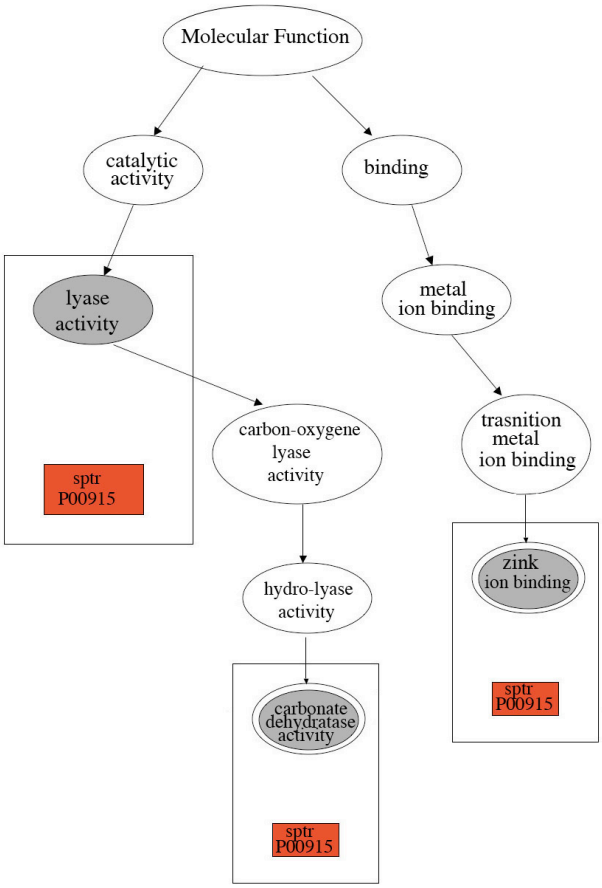

Figure 2. Expected molecular function of human CA.

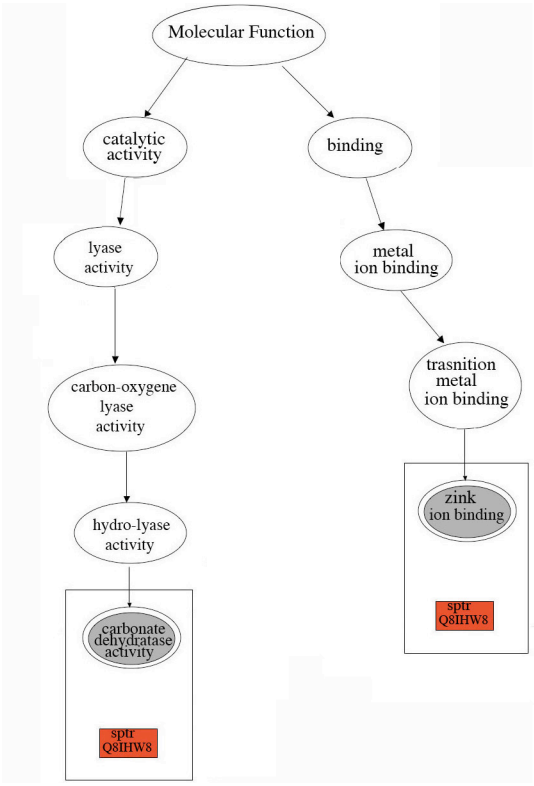

Figure 3. Expected biological process of *Plasmodium falciparum* CA.

Compared to human CA, the *P. falciparum* CA has similar molecular functions as carbonase dehydratase activity and zinc ion binding. However, human CA has additional significant activity as lyase activity. It is a well known fact that crucial enzymes such as lactate dehydrogenase (LDH) are highly conserved among the species and throughout evolution and thus it is not surprising that this applies also to CA. Although the basic sequences for human and *P. falciparum* CA are totally different, the molecular functions are similar. This implies that any treatment aiming at blocking the functions of *P. falciparum* CA can affect human CA. Thus any drug targeting at *P. falciparum* CA might not be a magic bullet. More specific structural antagonist that can directly block amino acid of *P. falciparum* CA is more favorable.

However, some concerns on this conclusion should be addressed. While the enzymes may have similar or identical functions among the species, there can be substrates that are preferred by the mammalian or the protozoan enzyme. For example, the quantification of growth inhibition of anti-malarial drugs is often done measuring LDH activity in the parasitized red blood cells. There is ample quantity of LDH in human red blood cell, but the substrates used by the parasitic LDH are highly selective for the parasitic enzyme. Thus, one can envision that screening for an anti-malarial drug would compare the various candidate drugs in regards to their ability to inhibit at lower concentrations the protozoan CA than compared to the mammalian CA. Overall, in order to give significance to the conclusion, it has to evaluate whether the enzymes have same substrate specificity and whether all anti-malarial drugs have the same dose range of toxicity when tested on parasite cultures and on mammalian cell cultures.

Indeed, three of fourteen CA isozymes detected in mammalians have been identified in *P. falciparum* (Reungprapavut et al, 2004). This can confirm that human CA and *P. falciparum* CA share common substrates. This can be the reason for the fact that there are issues with currently marketed sulfonamide drugs on undesirable side effects (Lee et al, 2004; Sheth, 2004). Based on the basic principles of chemical reaction in organic chemistry, the dose ranges of the same antimalarial drugs for the same enzymatic blocking reaction of enzymes using the same substrate depend directly on those enzymes. Basically, the molecular weight of human CA is significantly higher than *P. falciparum* CA. This implies that the amount of CA inhibitors for human CA is more than that of *P. falciparum* CA. Therefore, it can imply that CA inhibitors inhibit at lower concentrations the protozoan CA than compared to the mammalian CA. However, the ideal CA inhibitors should be selective for the reactions without identical substrate between host and parasite. An ultimate proof of the biological functions would still require biochemical experiments. Further experimental studies are needed before making a conclusion on this topic. Nevertheless, the findings in this study not only support the previous knowledge on malarial CA but also give the new view on the function of malarial CA.

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