

Study on Folate Binding Domain of Dihydrofolate Reductase in Different Plant species and Human beings

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Abstract:

Data base (NCBI and TIGR) searches are made to retrieve protein sequences of different plant species namely *Medicago truncatula*, *Pisum sativum*, *Ricinus communis*, *Arabidopsis thaliana*, *Vitis vinifera*, *Glycine max*, *Daucus carota*, *Oryza sativa* Japonica Group, *Arabidopsis lyrata* subsp. *lyrata*, *Brachypodium distachyon*, *Oryza sativa* Indica Group, *Zea mays* and careful alignment of derived sequences shows 95% or higher identity. Similarly, DHFR sequence of human being is also retrieved from NCBI. A phylogenetic tree is constructed from different plant and human DHFR domain using the Neighbour - Joining method in MEGA 5.05. Conservation score is performed by using PARALINE. Result suggests that folate binding domain of dihydrofolate reductase is conserved (score 8.06) and excepting some minor variations the basic structure of the domain in both plant species and human being is rather similar. Human DHFR domain contains PEKN sequence near active site, though proline is common for all the selected organisms but the other sequences are different in plants. The plant domain is always associated with TS (Thymidylate synthase). Plant based system is predicted to be an effective model for assessment of MTX (Methotrexate) and other antifolate drugs.

Keywords: DHFR, Methotrexate, phylogenetic tree.

Background:

Methotrexate (MTX) is a chemical [MF: C₂₀H₂₂N₈O₅; MW: 454.44; chemical name N-(4-(((2,4-diamino-6-pteridiny)methyl)methylamino) benzoyl) [1] sensitive to hydrolysis, oxidation and light and clinically used for the treatment of cancer, severe psoriasis and rheumatoid arthritis [2]. MTX acts as a competitive inhibitor of dihydrofolate reductase (DHFR, EC 1.5.1.3) blocks DNA replication and RNA synthesis and a target of chemotherapeutic agents in malignant disease [3, 4]. In plants and protozoa, DHFR and Thymidylate synthase (TS) functionally correlate to each other and the activities of the both enzymes are restricted in a single polypeptide [5, 6]. TS is a ubiquitous enzyme present in both prokaryotic and eukaryotic

cell and plays a significant role in nucleotide biosynthesis [7]. X ray crystallography reveals similarity in tertiary structure of DHFR in bacteria and vertebrate [8]; while, in human there exists 7 parallel and 1 antiparallel strand of β sheet leading to carboxy terminus end. Inhibitor like MTX is reported to bind with folate binding domain of DHFR the an extended cavity located on one side of central β -sheet [9].

DHFR-TS enzyme is identified for the first time in *Arabidopsis thaliana* by cDNA sequence analysis and DHFR specific sequence is found to be located upstream of TS coding region. DHFR-TS cDNA sequence are also identified in other higher plants namely, *Daucus carota* [10], *Glycine max* [11] and *Zea mays*

[12] among others. Present study describes the core DHFR and its folate binding domain from the available data sources of plant and human beings. Phylogenetic analysis based on protein sequences conservenss, secondary structure and hydrophobicity, is conducted to ascertain relatedness among plant species as well as between plant domains and human. The objective of the work is to foresee whether MTX can target plant DHFR as it is a potent inhibitor of human DHFR.

Methodology:

Data retrieval

Using key words namely 'Plant DHFR', 'HumanDHFR', 'Plant DHFR-TS', a search is performed from database NCBI (<http://www.ncbi.nlm.nih.gov/>) and TIGR (<http://www.tigr.org>) for retrieving data on plant DHFR. A careful alignment is then carried out with the derived sequences from database. Sequences that shared 95% or higher identity are considered as likely alleles [13, 14].

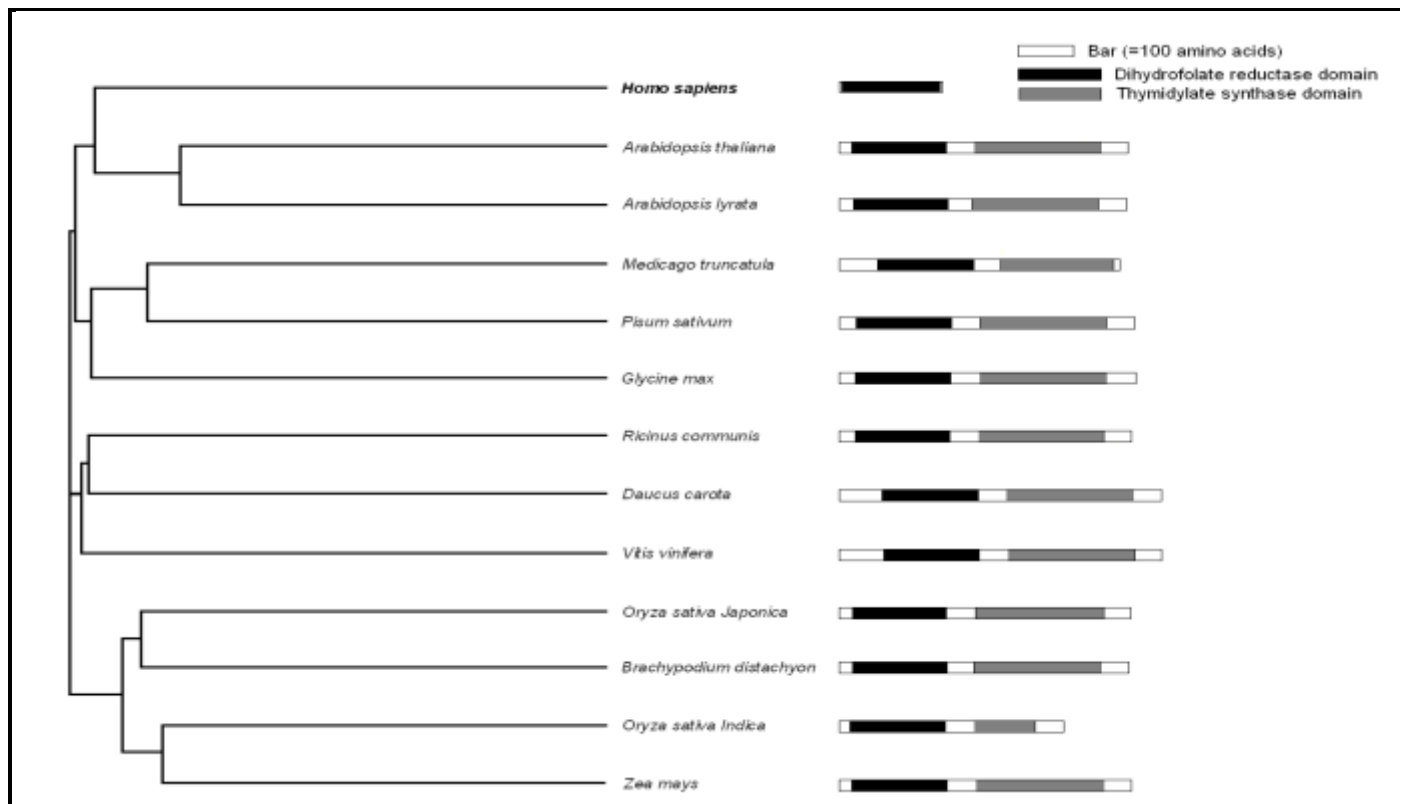


Figure 1: Dendrogram of DHFR protein domains of plants and human beings.

BLAST searches for folate binding domain

Similarly using key words namely, 'Human DHFR' and 'Homo sapiens DHFR' human folate binding domain is retrieved from NCBI data base. Moreover all DHFR domain sequences are used to retrieve other available DHFR sequences with high percentage of identity using BLAST against NCBI. A sequence alignment is created using CLUSTAL Xv.1.8 [15] and a phylogenetic tree is constructed from different plant and human folate binding domain using the neighbor joining (NJ) method in MEGA 5.05 [16]. Conservation scoring is performed by PRALINE (<http://www.ibi.vu.nl/programs/pralinewww/>) [17].

Results & Discussion:

Identification and characterization of predicted DHFR genes

A total of 12 predicted plant DHFR-TS homologues with protein sequences are identified. The plant DHFR regions are with 173 amino acids long stretch as compared to 187 for human [5]. In case of folate binding domain it is 131 amino acids long for selected plants species whereas it is 130 for human. The relationship of plant and human DHFR domains is presented in **Figure 1**. As all DHFR domains are highly conserved, a phylogenetic tree is constructed after multiple

sequence alignment using predicted protein sequences to possess better precision on the evolutionary relationship.

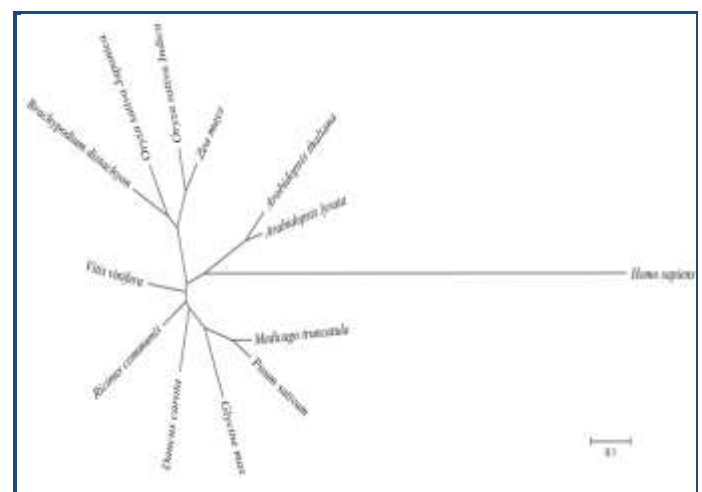


Figure 2: Unrooted phylogenetic tree of folate binding domain of 12 different plant species along with human after multiple sequence alignment using predicted protein sequence.

Phylogenetic analysis of predicted folate binding domains

Dendrogram and unrooted phylogenetic tree (Figure 1 & 2) reveals close relationship among plant species of related taxonomic members. Both analyses showed the identical results. Cluster comprising 8 of *Zea mays*, *Oryza sativa* Indica Group, *Brachypodium distachyon* and *Oryza sativa* Japonica Group of family Poaceae; *Glycine max*, *Pisum sativum* and

Medicago truncatula of Fabaceae; *Arabidopsis thaliana* and *Arabidopsis lyrata* of Brassicaceae; *Vitis vinifera*, *Daucus carota* and *Ricinus communis* are dicotyledons and belong to the family Vitaceae, Apiaceae and Euphorbiaceae respectively. Human beings are mammalian belonging to the family Hominidae form separate cluster and distantly apart from the plant species.

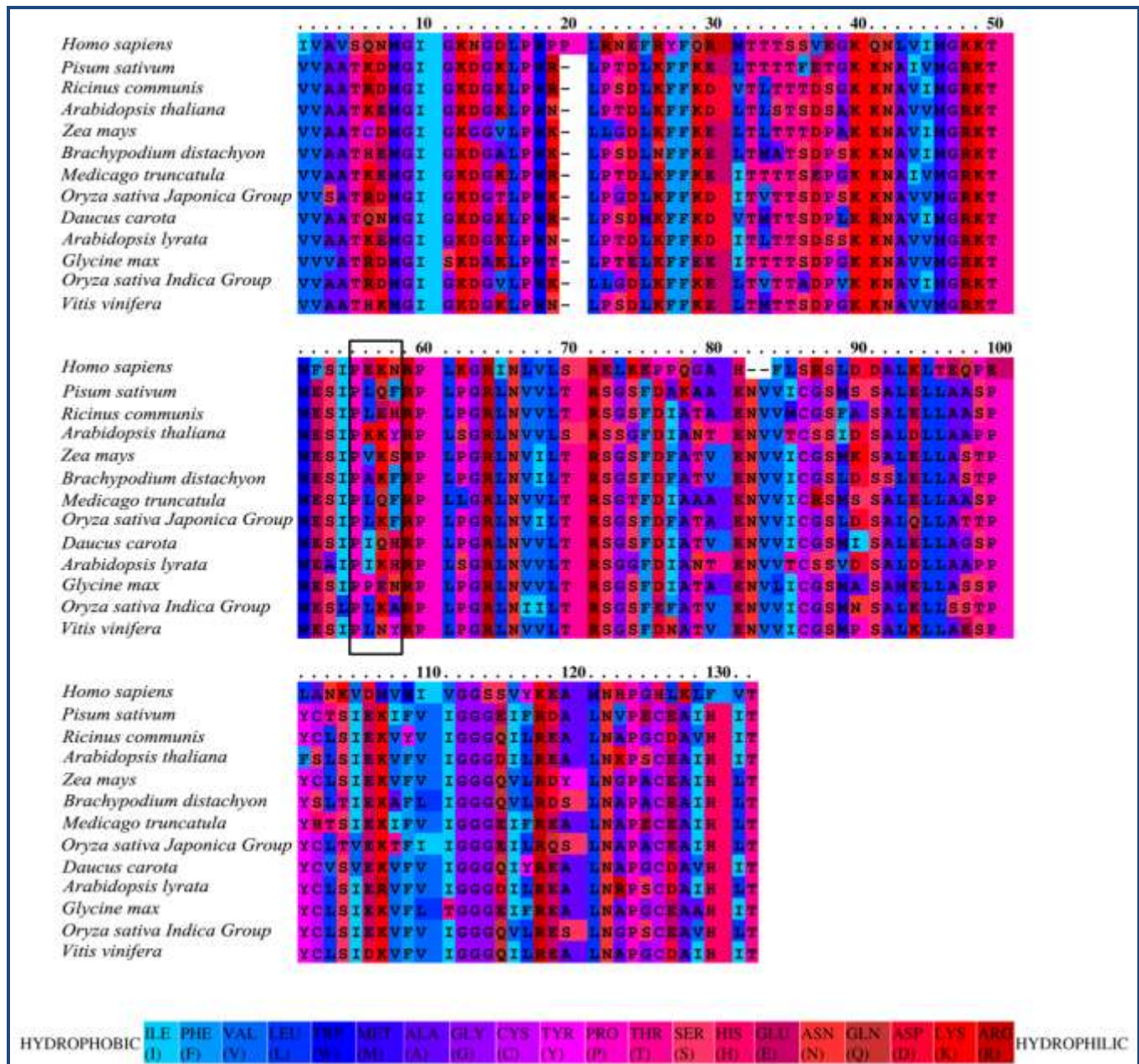


Figure 3: Comparison of folate binding sites of amino acid sequence from plants and human; hydrophobicity of amino acids are represented in color.

Conservation score performed using PARALINE software reveals that the alignment score of DHFR is 8.06 which indicates that the domain is well conserved. Few dissimilarities exist between human and plant DHFR domains. In the 20th position of the human DHFR domain, there is an additional proline residue moderately polar uncharged in association with an imino group providing structural rigidity to the β sheet [18]. The human DHFR domain possesses proline-Glutamic acid-Lysine-

Aspartic acid (PEKN) sequence near the active site (Figure 3); although in other selected organisms the sequence does not exist apart from the presence of proline [19]. In plant DHFR domain 2 extra amino acids are noted to be present at the 82nd and 83rd positions. The present investigation reveals that the basic structure of the folate binding domain is more or less similar in both plant species as well as in human beings, though MTX binds with human as well as plant folate binding domains with

different affinity. As MTX is a potent inhibitor to human folate binding domain therefore it is expected that MTX as well as other antifolate agents may be effectively used for plant system as well, which will be simple and cost effective.

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