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RESEARCH PAPER

Homology modeling and structure prediction of thioredoxin (TRX) protein in wheat (*Triticum aestivum* L.)

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Abstract

Wheat is an important dietary cereal often associated with beneficial health effects. A study was carried out to investigate the *in silico* analysis of homology modeling and 3D structure prediction of Thioredoxin (TRX) protein in *Triticum aestivum*. Primary structure prediction and physicochemical characterization were performed by computing theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY). In this study homology modeling, a high quality of protein 3D structure has been predicted for the hypothetical amino acid sequence. Thioredoxin of *Triticum aestivum* was compared to the 1XFL solution structure of *Thioredoxin h1* from *Arabidopsis thaliana* predicted structure through ROSETTA. However, the quality of the homology model performed through SWISS-MODEL depended on the quality of the sequence alignment by BLAST and template structure. Comparative assessment of secondary structure modeled using GOR IV, HNN and SOPMA revealed greater percentage of residues as alpha helix and random coils against the beta sheets. Structure comparison by VAST for the ROSETTA modeled structure indicated no hits for the entire sequence unlike that of SWISS modeled

structure, which indicated 60 structure neighbours for the entire residues. Tertiary structure was predicted using homology modeling by taking template PDB-1fxl and the modeled protein energy were minimized. The models were validated using protein structure checking tools PROCHECK. These structures will provide a good foundation for functional analysis of experimentally derived crystal structures.

Key words: Antioxidant, function prediction, homology modeling, procheck validation, structure comparison, thioredoxin and *Triticum aestivum.*

Introduction

Thioredoxins (TRXs) proteins are ubiquitous disulfide reductases (14kDa average) and it act as antioxidants by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange. TRX was discovered in *E. coli* and identified as the hydrogen donor for Ribonucleotide reductase (Laurent *et al*., 1964). Since this discovery, many functions have been attributed to thioredoxins. They are considered to be the major factor responsible for maintaining proteins in their reduced state inside cells and are important agents in transcription factor activation, signaling, apoptosis and reduction of peroxiredoxins, among other processes (Arner and Holmgren, 2000). In wheat, thioredoxin h acts as an early wake-up call in seed germination, facilitating the mobilisation of nitrogen and carbon through the activation of a protease (thiocalsin) (Besse *et al*., 1996), inactivation of proteinaceous inhibitors such as a-amylase and trypsin inhibitors and reduction of disulfide bonds of storage proteins (gliadins and glutenins) thereby increasing hydrolysis (Kobrehel *et al*., 1992). The abundance of thioredoxin *h* in the endosperm is controlled by the embryo via hormones. Gibberelins enhance the disappearance of thioredoxin *h*, whereas abscisic acid shows the opposite effect (Lozano *et al*., 1996). Wheat seeds contain at least four thioredoxin isoforms and analysis of different cultivars revealed important variations in the relative abundance of these isoforms (Runquist *et al*., 1999). Wheat seed quality depends on a set of parameters including the structural organization of the storage compounds, the protection of tissues against oxidative stress, (during seed desiccation and germination, or grain filling), and the activation of the metabolism of seed cells upon imbibition. For these processes, redox control is an important determinant. The redox potential of a cellular compartment describes the tendency of the system to gain or lose electrons when new species are introduced. In this respect, thiol/disulfide exchanges are of great importance because they act as regulatory switches for numerous proteins in response to modifications in the cellular compartment redox state. This is the case for the activity of many seed enzymes, storage proteins (Wong *et al*., 2004), and the antioxidative system. Among the proteins concerned in the redox control, the redoxin family is characterized by

the typical motif -CXXC-, in which two cysteines are separated by two other residues (Martin 1995). The most important representatives of this redox motif are the thioredoxin-fold proteins, which include thioredoxin (Trx), glutaredoxin (Grx), protein disulfide isomerase, and nucleoredoxin. This motif presents some degree of flexibility because one of the Cys residues can be replaced by threonine or serine (Fomenko and Gladyshev 2003), as suggested by the subgroups of momothiol Grx and Trx. The motif is employed by redox protein for the active site that permits the oxidation, reduction, or isomerization of disulfide bonds of target proteins.

The Trx system is a major antioxidant system that responds to the cellular redox state. Plants possess two Trx systems, each one characterized by a distinct enzyme that catalyzes the reduction of the Trx and of the electron donor. The first system has been traditionally located in the chloroplast involved in carbon metabolism and oxidative regulation. With the advance of proteomics techniques, chromatography coupled with gel-based or mass spectrometry can experimentally identify thioredoxin/glutaredoxin target proteins or proteins that undergo thiol-disulfide transitions (Hisabori *et al*., 2005; Rouhier *et al*., 2005; Yano *et al*., 2001). By the reason of technical limitation, further experiments are needed to eradicate the false positive proteins. Current situation there is not much information available on characterization of TrxR protein sequence of *Triticum aestivum* involved in redox mechanism. Unavailability of 3-dimensional structure of enzymes is one of the major hindrances in elucidatingthe interactions of enzymes with possible inhibitors. Comparative homology modeling is promulgated as the most unswerving computer-based technique for deciphering the 3D structures in the absence of crystal structure of the protein. In this study the antioxidant wheat protein thioredoxin (TRX) have been selected for which three dimensional structures were not available at the protein data bank (PDB). This article describes the *In silico* analysis of homology modeling and 3D structure prediction of antioxidant Thioredoxin (TRX) protein in *Triticum aestivum* which will provide insight into its structure and aid in drug designing.

Materials and methods

The primary sequence of the thioredoxin (Accession No.Q8H6X0) of *Triticum aestivum* (wheat) was retrieved from the EXPASY public domain protein database (http://www.expasy.org/uniprot/Q8H6X0#seq) (Figure1A). The thioredoxin proteins sequences were retrieved (http://www.uniprot.org/uniprot/Q8H6X0.fasta) in FASTA format and used for further analysis.

Primary structure prediction

For TRX protein primary structure prediction, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient (Gill and Von Hippel, 1989), instability index (Guruprasad *et al*., 1990), aliphatic index (Ikai, 1980) and grand average hydropathicity (GRAVY) (Kyte and Doolottle, 1982) were computed using the Expasy's ProtParam server (http://expasy.org/cgi-bin/protparam).

Secondary structure prediction

Secondary structure of this protein was predicted using The FASTA sequences of TRX protein was used to prediction of secondary structure using GOR IV, SOPMA and Hierarchical neural network (HNN) program (http://npsa-pbil.ibcp.fr/cgi-bin/secpred_hnn.pl) (Guermeur *et al*., 1999).

Model building and evaluation

The modeling of the three dimensional structure of the protein was performed by Swissmodel homology modeling programs, (Arnold *et al*., 2006). The SWISS-MODEL depended on the quality of the sequence alignment by BLAST and template structure. Structural analysis was performed and figures representations were generated with Swiss PDB Viewer (Guex and Manuel, 1997) (Fig. 1B). Assessment of secondary structure was modelled using HNN server. Tertiary structure was predicted using homology modeling by taking template PDB-1fxl and modelled protein energy were minimized. 3-D structure of TRX protein in *Triticum aestivum* was compared by NCBI- VAST to the 1XFL solution structure of *Thioredoxin* h1 from *Arabidopsis thaliana* predicted structure through ROSETTA. Validation of the tertiary structure of TRX protein was done by PROCHECK.

Results and discussion

Primary structure prediction

Thioredoxin (TRX) protein sequences were retrieved from the EXPASY, a public domain protein database. The FASTA format of thioredoxin (TRX) proteins sequences were retrieved (http://www.uniprot.org/uniprot/Q8H6X0.fasta) (Fig. 2). The wheat seed storage proteins are a major source of protein in the human diet, and are responsible for the properties of wheat dough's that allow a wide range of food products. They are also implicated in wheat allergies and Coeliac disease, an

autoimmune condition triggered by some cereal proteins. Gliadins and glutenins are the major storage proteins that accumulate in wheat endosperm cells during seed development (Mowat, 2003). These polymers are among the largest protein molecules known in nature and are the most important determinants of the viscoelastic properties of gluten. Glutenins consist of very large disulfide-linked polymers made up of high molecular weight and low molecular weight (LMW) subunits. LMW glutenins consist of 250-300 residues forming two domains. These proteins have a cysteine residue within the N-terminal domain, which is unlikely to form intramolecular disulfide bonds with cysteine residues within the C-terminal domain, because of the rigidity imposed by the repetitive sequence (Thomson et al. 1992). In this study primary structure of wheat Thioredoxin (TRX) proteins were predicted using Expasy's ProtParam server (http://expasy.org/cgi-bin/protparam). Results showed that TRX protein has 131 amino acid residues and the estimated molecular weight is 14544.5. The calculated isoelectric point (pI) will be useful because at pI, solubility is least and mobility in an electrofocusing system is zero. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of protein is zero. At pI proteins are stable and compact. The computed pI value of TRX protein was 5.00. Computed pI value of protein were less than 7 (pI<7) indicates that these TRX proteins were considered as acidic. The computed isoelctric point (pI) will be useful for developing buffer system for purification by isoelectric focusing method. The total number of negatively charged residues (Asp + Glu) was 20 and Total number of positively charged residues (Arg + Lys) was16. The LMW glutenins have seven cysteine residues in their C-terminal domain, at least one of which is unpaired, thus available for intermolecular bonding. On the other hand, gliadins, in accordance with their mobility in acid PAGE, are divided in four groups: α (fastest mobility), β, γ, and ω gliadins (slowest mobility). All gliadins are low in ionic aminoacids (histidine, arginine, lysine, and free carboxylic groups of aspartic and glutamic acid) (Thomson *et al*. 1992).

The aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains is regarded as a positive factor for the increase of thermal stability of globular proteins. Aliphatic index for the TRX protein sequences was 81.07. Whereas, Expasy's ProtParam computes the extinction coefficient for 280 nm wavelengths and 280 nm is favored because proteins absorb light strongly there while other substances commonly in protein solutions do not. Extinction coefficient of TRX protein at 280 nm is ranging from 19605 to 19480 M^{-1} cm^{-1} with respect to the concentration of Cys residues. The computed extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution.

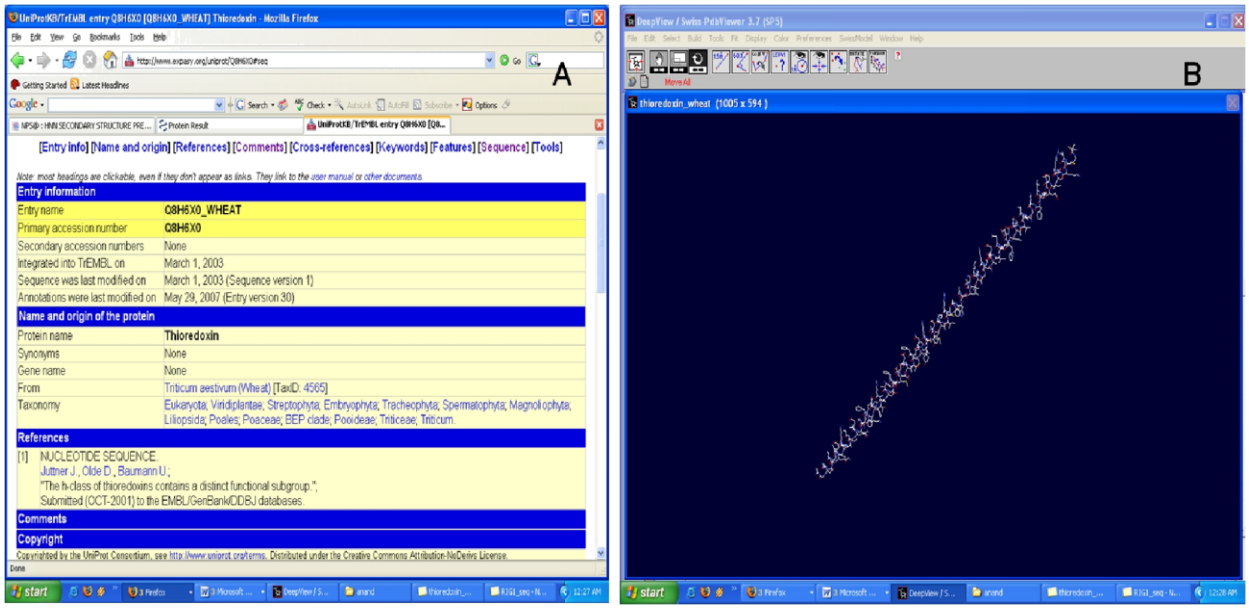

Fig. 1. Source of wheat Thioredoxin (TXR) protein sequence and structure. A) Retrieving of Thioredoxin protein sequence of Triticum aestivum, B) Structure of TRX protein obtained from TRX sequence uploaded SWISS PDB viewer.

>Q8H6X0|Q8H6X0_WHEAT Thioredoxin

MGGCVGKDRSIVEEKLDFKGGNVHVITTKEDWDQKIEEANKDGKIVVANLSASWCGPCRV
IAPVYAEMSKTYPQLMFLTIDVDDLMDFSSTWDIRATPTFFFLKNGQQIEKLVGANKPEL
EKKVQALGDGS

Fig. 2. FASTA format of thioredoxin (TRX) protein sequences.

The very high aliphatic index of TRX protein sequences indicates that TRX proteins may be stable for a wide temperature range. The instability index provides an estimate of the stability of protein in a test tube. There are certain dipeptides, the occurrence of which is significantly different in the unstable proteins compared with those in the stable ones. This method assigns a weight value of instability. Using these weight values it is possible to compute an instability index (II). A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable (Guruprasad *et al*., 1990). The instability index value for the wheat TRX proteins were found to be ranging from 29.32 indicates TRX protein as stable protein. The Grand Average hydropathicity (GRAVY) value for a peptide or protein is calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence. GRAVY indices of TRX are ranging -0.289. This low range of value indicates the possibility of better interaction with water.

View HNN in: [MPSA (Mac, UNIX) , About...] [AnTheProt (PC) , Download...] [HELP]

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         10        20        30        40        50        60        70
          |         |         |         |         |         |         |
 MGGCVGKDRSIVEEKLDFKGGNVHVITTKEDWDQKIEEANKDGKIVVANLSASWCGPCRVIAPVYAEMSK
ccccccccchheehhhhcccccceeeeeeccccchhhhhhhccceeeeeeeecccccchhhhhhhhhhhc
TYPQLMFLTIDVDDLMDFSSTWDIRATPTFFFLKNGQQIEKLVGANKPELEKKVQALGDGS
cccceeeeeeeccchhccccccccccccceeeeccccceeeeecccccchhhhhhhccccc
Sequence  length  :  131
HNN  :
   Alpha  helix     (Hh)  :    32 is  24.43%
   3₁₀  helix       (Gg)  :     0 is   0.00%
   Pi  helix        (Ii)  :     0 is   0.00%
   Beta  bridge     (Bb)  :     0 is   0.00%
   Extended  strand (Ee)  :    32 is  24.43%
   Beta  turn       (Tt)  :     0 is   0.00%
   Bend  region     (Ss)  :     0 is   0.00%
   Random  coil     (Cc)  :    67 is  51.15%
   Ambigous  states (?)   :     0 is   0.00%
   Other  states          :     0 is   0.00%
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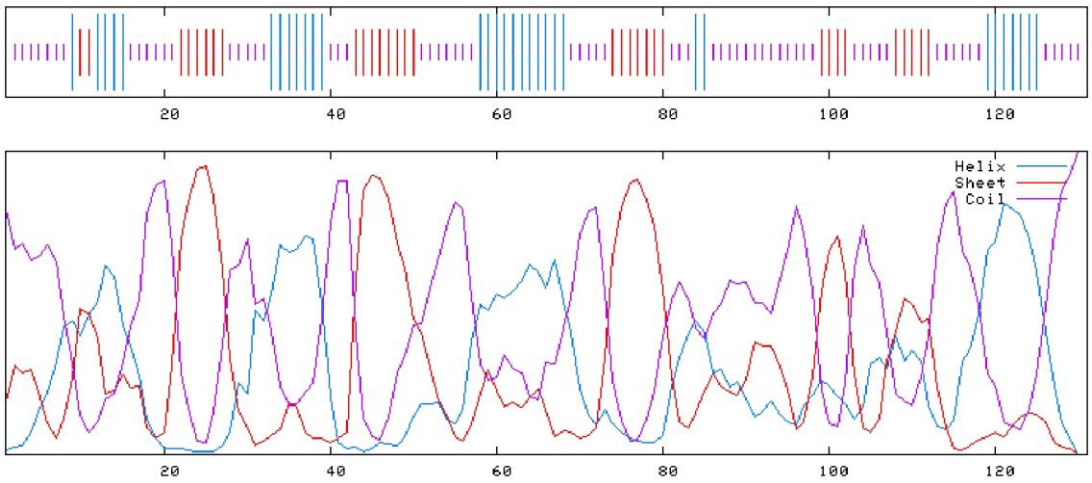

Fig. 3. Hierarchical neural network (HNN) result of secondary structure of TRX protein.

Secondary structure prediction

Formation of alpha helix and betasheet are called as secondary structure of protein. It is made up of two networks: a sequence-to-structure network and a structure-to-structure network. The prediction is thus only based on local information. Secondary structure were predicted by using The FASTA sequences of TRX protein was used to prediction of secondary structure using GOR IV, SOPMA and Hierarchical neural network (HNN) program (http://npsa-pbil.ibcp.fr/cgi-bin/secpred_hnn.pl). The Hierarchical neural network result: UNK_158930 was presented (Figure.3). The results revealed that random coil (51.15 %) dominated among secondary structure elements and alpha helices (24.43 %) and extended strand (24.43 %) are presented at equal percentage.

Tertiary structure prediction

Trx proteins are located in the chloroplast, mitochondria and cytosol of higher plants. In *Arabidopsis*, members of the Trx family are divided into six major groups (*f, m, h, x, y* and *o* type) based on their primary structure. The *m, x* and *y* types are related to prokaryotic Trx, whereas *f, h* and *o* types are specified to eukaryotic organisms (Gelhaye et al. 2004). The chloroplastic Trx was associated with light regulation of carbon metabolism through regulation of the reducing pentose phosphate as well as the C4 pathway (Gelhaye *et al*., 2005). In this study three dimensional structures are predicted for proteins where such data is unavailable. The modeling of the three dimensional structure of the TRX protein was performed by SWISS-MODEL homology modeling programs, (Arnold *et al*., 2006). The SWISS- MODEL depended on the quality of the sequence alignment by BLAST and template structure. Structural analysis was performed and figures representations were generated with Swiss PDB Viewer (Guex and Manuel, 1997). The TRX protein structural analysis results were shown in (Fig. 4). In protein structure prediction, homology modeling, also known as comparative modeling, is a class of methods for constructing an atomic-resolution model of a protein from its amino acid sequence (the "query sequence" or "target"). In this study approximately all homology modeling techniques rely on the identification of one or more known protein structures (known as "templates" or "parent structures") likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. The sequence alignment and template structure are then used to produce a structural model of the target. Since protein structures are more conserved than protein sequences, detectable levels of sequence similarity usually involve significant structural similarity. The quality of the homology model is dependent on the quality of the sequence alignment and template structure. Here, the template taken here was PDB – 1xfl.

TRX protein tertiary structure was predicted using homology modeling by taking template PDB-1fxl. The modeled TRX protein was uploaded in chemsite pro for energy was minimization. Modeled TRX protein energy minimization was showed in Figure 5. The energy landscape of a biomolecule possesses an enormous number of minima, or conformational substrates. Nonetheless, the goal of energy minimization is simply to find the local energy minimum, i.e., the bottom of the energy well occupied by the initial conformation. The energy at this local minimum may be much higher than the energy of the global minimum. Physically, energy minimization corresponds to an instantaneous freezing of the system; a static structure in which no atom feels a net force corresponds to a temperature of 0 K.

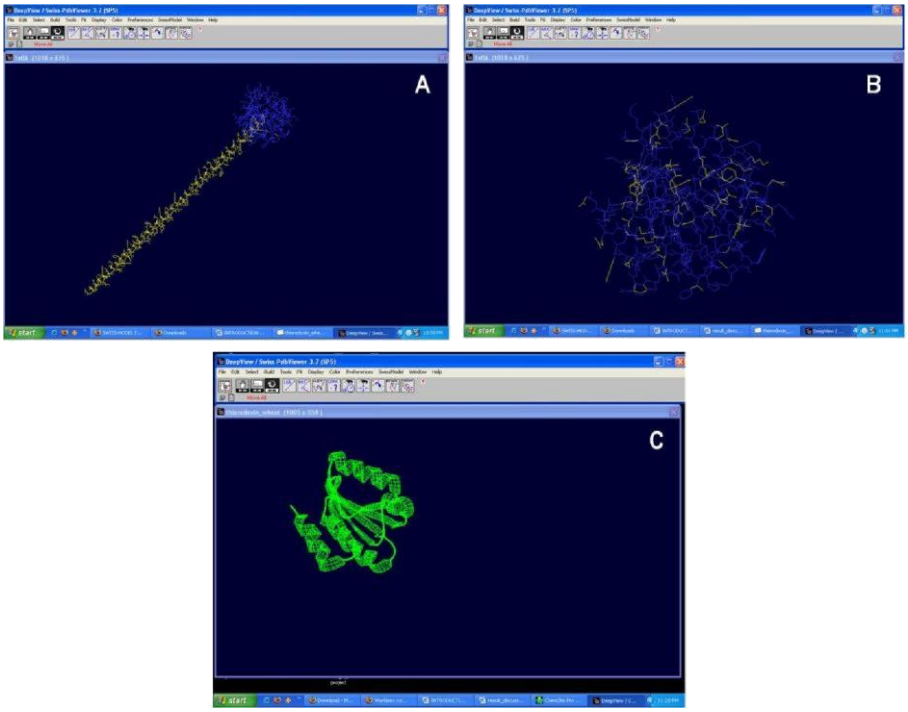

Fig. 4. 3-D structure of TRX protein. A) Thioredoxin protein sequence and template protein structure were uploaded in SWISS-PDB viewer, B) Thioredoxin protein sequence was fitted on the template structure and submitted to SWISS MODEL server, C) Modeled TRX protein structure.

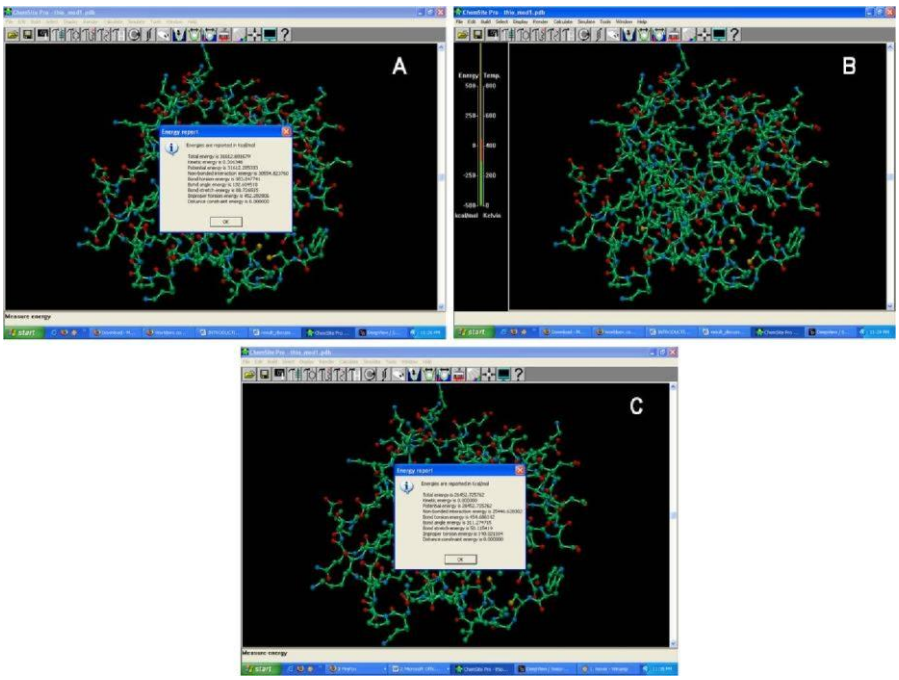

Fig. 5. 3-D Structure of energy minimized TRX protein. A) Modeled thioredoxin protein were uploaded in chemsite pro for energy minimization, B) Thioredoxin protein energy minimization, C) Structure of energy minimized thioredoxin protein.

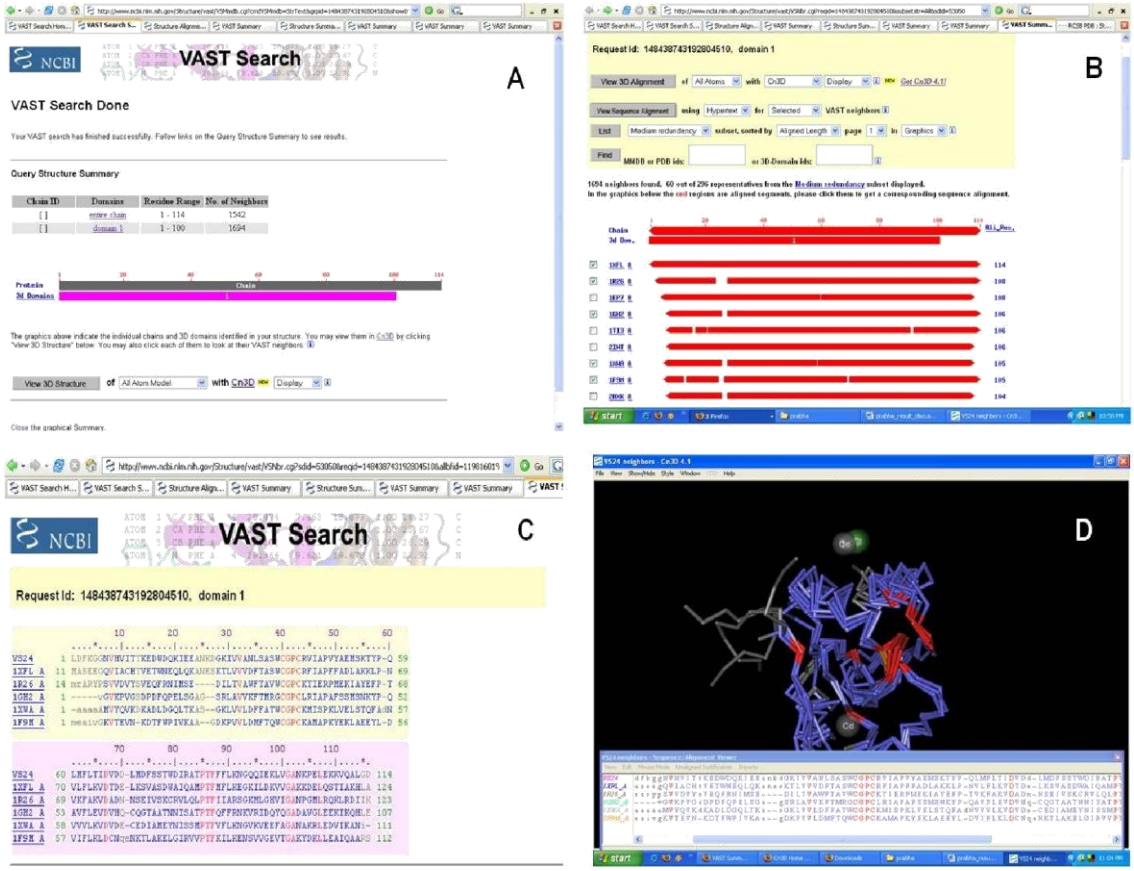

Fig. 6. Tertiary structure of Wheat TRX protein compared by NCBI- VAST to the 1XFL solution structure of *Thioredoxin* h1 from *Arabidopsis thaliana* predicted structure through ROSETTA. A) VAST output view of protein structure neighbours in entrez are determined by direct comparison of 3-dimensional protein structures with the VAST algorithm, B) Thioredoxin whole chain aligned with neighbour, C) Domain1 sequence alignment with neighbours sequence, D) Domain1 superimposed with neighbours. Note: 1F9 -Crystal structure of thioredoxin F from spinach chloroplast, 1XFL -Solution Structure of Thioredoxin h1 from Arabidopsis thaliana, 1GH2 -Crystal structure of the catalytic domain of a new human thioredoxin-like protein and 1R26 -Crystal structure of thioredoxin from *Trypanosoma brucei brucei*.

Validation of tertiary structure

Tertiary structure of TRX protein in *Triticum aestivum* was compared by NCBI- VAST to the 1XFL solution structure of *Thioredoxin h1* from *Arabidopsis thaliana* predicted structure through ROSETTA was showed in Fig. 6 and 7. The default colouring for structure alignments in VS24 neighbours Cn3D 4.1 used red and blue for the regions aligned by the VAST algorithm, where identical aligned residues are red and different but aligned residues are blue; unaligned regions are colored gray. Structure comparison by VAST for the ROSETTA modeled structure indicated no hits for the

entire sequence unlike that of SWISS modeled structure, which indicated 60 structure neighbours for the entire residues. Validation of the tertiary structure by PROCHECK revealed that the structure modeled through SWISS-MODEL to be of high quality with 90.8% of residues in the most favoured region. The predicted structures conformed well to the stereochemistry indicating reasonably good quality.

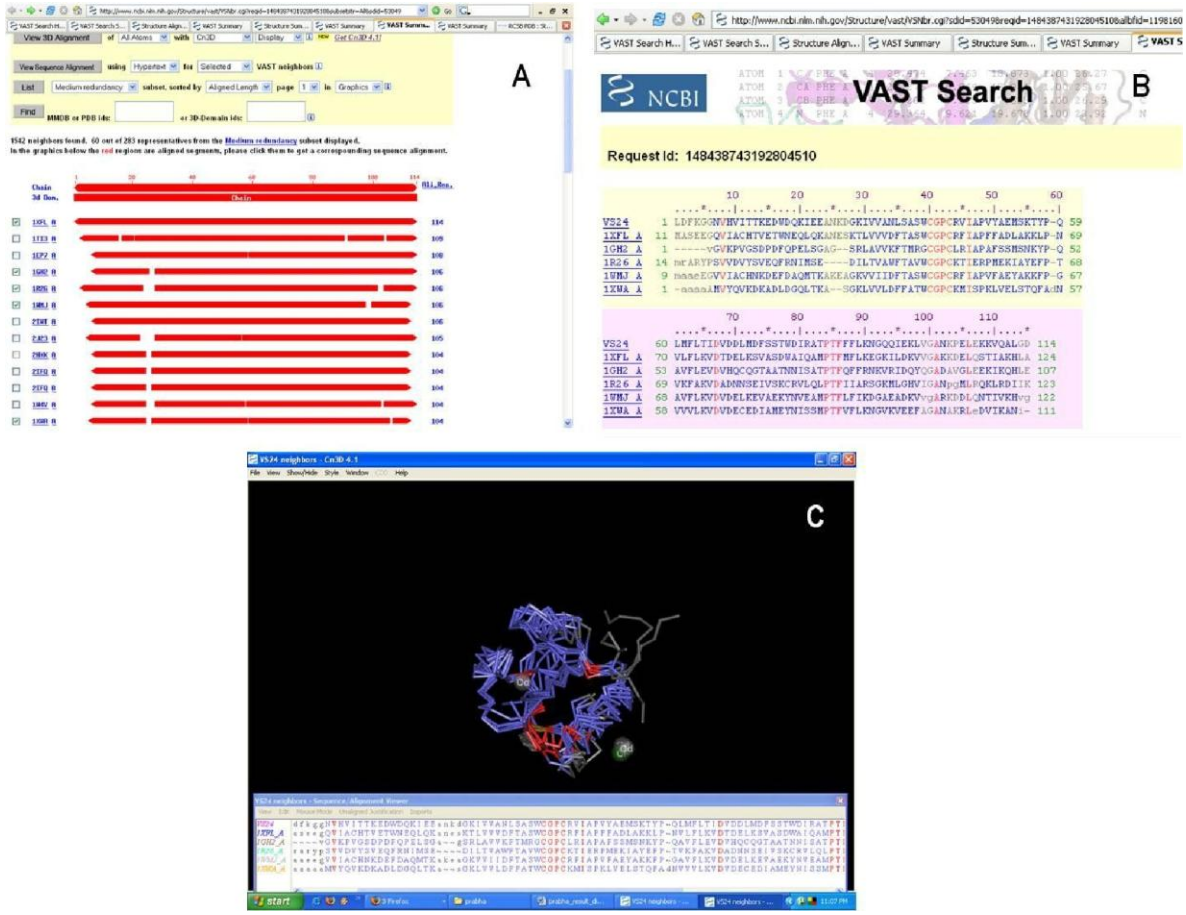

Fig. 7. Tertiary structure of wheat TRX protein sequence aligned with neighbours. A) Thioredoxin whole chain aligned with neighbour, B) Thioredoxin whole chain sequence aligned with neighbour, C) Thioredoxin whole chain superimposed with neighbours. Note: 1XFL - Solution structure of thioredoxin h1 from *Arabidopsis Thaliana*, 1GH2 -Crystal structure of the catalytic domain of a new human thioredoxin-like protein, 1R26 -Crystal structure of thioredoxin from *Trypanosoma brucei brucei,* 1WMJ - Solution structure of Thioredoxin type h from *Oryza sativa* and,1XWA -Drospohila thioredoxin, oxidized, P41212.

Conclusion

In this study Thioredoxin (TRX) protein of *Triticum aestivim* (Wheat) were selected. Primary structure prediction and physicochemical characterization were performed by computing theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index

and grand average hydropathy (GRAVY). Structural analysis was performed and figures representations were generated with Swiss PDB Viewer. The modeling of the three dimensional structure of the TRX protein was performed by SWISS-MODEL homology modeling program. The models were validated using protein structure checking tools PROCHECK. These structures will provide a good foundation for functional analysis of experimentally derived crystal structures.

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