

Homology Modeling and Structural Analysis of NHX Antiporter of *Leptochloa fusca* (L.)

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Abstract

Eukaryotic NHX transporters are trans-membrane proteins which are vital for cellular homeostasis. Plants vacuolar NHX antiporters have been suggested to be involved in sequestering Na⁺ into vacuoles, thus preventing toxic effects of Na⁺ in the cytoplasm. Primary and secondary structure analysis of NHX antiporter of *Leptochloa fusca* suggested that this antiporter is a hydrophobic protein containing a significant proportion of alpha helices. In this study, a three-dimensional structures of NHX antiporter predicted by *in silico* 3D homology modeling studies. The resulting model was refined by energy minimization, subjected to the quality assessment from both geometric and energetic aspects and was found to be of reasonable quality. Validation of 3D structure was done by plotting Ramachandran plot and calculation of QMEAN score. This predicted information will help in better understanding of mechanisms underlying to salt tolerance in monocot plants and use of this information in protein engineering to improve plants to high salinity conditions.

Keywords: *Leptochloa fusca*; NHX; Homology modeling

Abbreviations: LfNHX: *Leptochloa fusca* NHX; 3D: Three dimensional; HM: Homology modeling

Introduction

Intracellular pH regulation is important for growth and cellular functions. In prokaryotic and eukaryotic cells, NHX exchangers play a key role in regulation of cytosolic pH. NHX transporter is a ubiquitous membrane protein localized in cytoplasmic and organelle membranes and is present virtually in all cell types, including bacteria, plants and mammals. This membrane protein transports Na⁺ and H⁺ in opposite directions across cell membranes. In higher eukaryotic cells, NHX exchangers function to remove excess protons from the cytosol by taking up Na⁺ from the external environment, a process that is driven by the sodium gradient generated by Na⁺/K⁺-ATPase [1,2].

In higher plants, physiological and biochemical data suggest that NHX antiporter and/or alterations in channel selectivity play a more prominent role in adaptation to elevated cytosolic Na⁺ than primary ion pump activity. Moreover, the dominance of a large central vacuole within all mature plant cells and its capacity to sequester toxic cations, bestows on this organelle a prominent role in salinity adaptations [3,4].

Despite all of the available sequence information we have, 3D structure and structure-function studies of these transporters, especially in halophyte plants which are the best materials for studying the molecular basis of salt tolerance, no study has not yet been initiated.

Structure prediction by homology modeling (HM) can help in understanding the 3D structure of a given protein. This subject will help in elucidating the mechanisms of protein function, since function is determined by 3D structure [5,6]. *Leptochloa fusca* is an attractive model plant to study the mechanism of salt tolerance [7]. But the 3D structure of LfNHX has not been determined until now and studying of its 3D structure may provide additional information about its molecular properties. In the current study an attempt has been made to generate model of the LfNHX using homology modeling techniques.

Material and Methods

The seeds of *L. fusca* (L.) were obtained from the Agricultural Biotechnology Research Institute of Iran (ABRII). *L. fusca* (L. (seeds were grown in sandy soil and 6 weeks-old plants were used for RNA isolation. The full length sequence of LfNHX gene was determined by RT-PCR, and rapid amplification of cDNA ends (RACE) method. The sequences were submitted to NCBI Gene Bank (Accession No: JN085959). The same sequence was used for the further studies such as homology modeling and other secondary and functional characterization.

Primary and Secondary Structure Prediction

In this study, secondary structure of LfNHX was predicted using ExPasy's ProtParam server (<http://expasy.org/cgi-bin/protparam>) based on the gene sequence and, secondary structure of this protein was predicted using GOR IV and SOPMA [8].

Prediction of 3D Structure of NHX Antiporter

In order to predict the 3D structure of LfNHX, two web servers were used. In the first approach, we used automated mode of phyre2 server [9]. In all 540 identical amino acids between query and templates showed positive matches. This is followed by deducing up of model on the basis of based on identical folds. The model constructed by this method showed 67 % coverage and template alignment confidence

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(99.9 %) values. Similar studies were carried out by using a Hidden Markov Model based server SAM-T08 server [10]. The server generates clusters from query and then aligns the clusters against a HMM database to generate a model.

Model Optimization

Modeler 9v8 [11] was used for optimization of SAM-T08 predicted model. Optimization was performed in 10 steps. Finally, the all-atom models were subjected to a short run of energy minimization by using Q-mean algorithm, to relieve unfavorable steric interactions and to optimize the stereochemistry. Q-mean score [12] and Ramachandran plot statistics were used for evaluation of predicted models.

Functional Characterization

Interpro scan and Fingerprint scan were used to predict the signatures and the motif regions in the sequence. InterPro Scan is a tool that combines different protein signature recognition methods native to the InterPro member databases into one resource, corresponding to InterPro and GO annotation. CYS_REC (<http://sunl.softberry.com/berry.phtml?topic>) was used to locate "SS bond" between the pair of cysteine residues, if present.

Analysis of Ligand Binding Sites and Pockets

Ligand binding sites were predicted by QSITE FINDER at <http://www.modelling.leeds.ac.uk/qsitefinder/>

Submission of the Modeled Proteins in Protein Model Database (PMDB)

The models generated for NHX protein were successfully submitted in Protein model database, PMDB [13] without any stereochemical errors. The submitted model can be accessed via their PM0078210.

Results and Discussion

Protein structure validation

Q-mean score [12] was used for evaluation of predicted models. Q-mean is a parameter between 0-1. Scores equivalent to 1 are similar to crystallographic structure. The model Predicted by Swiss model generated score of 0.03, score of phyre2 was 0.09 and SAM-T08 predicted model reached 0.16. Predicted models by Phyre2 and Swiss model did not cover all of query residues while in the SAM-T08 output, all of 540 residues were modeled (Figure 1). SAM-T08 predicted better model based on Q- mean scores which were later optimized by Modeler software. After optimization predicted model Q-mean score reach to 0.25.

Ramachandran plot for LfNHX has been illustrated in Figure 2. The validation was performed with PROCHECK (Figure 2). A plot has been drawn between the Phi (Φ) and Psi (Ψ) torsion angles of all residues of the predicted proteins, except the amino acids present at the chain termini. Glycine residues are shown in black triangles. The residues in the core (red colored) region represent the most favored combinations of the torsion values indicating low-energy regions and the additional/generously allowed regions are in yellow. Altogether more than 90% of the residues were found to be in favored and allowed regions, which validate the quality of homology models. The overall G-factor for LfNHX was -0.3.

Primary and secondary structure prediction

The findings suggest that all the NHX protein under study were

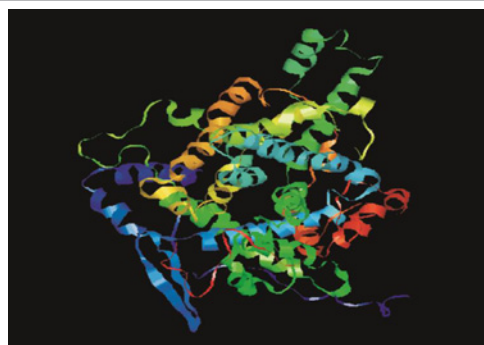


Figure 1: SAM-T08 predicted model of NHX.

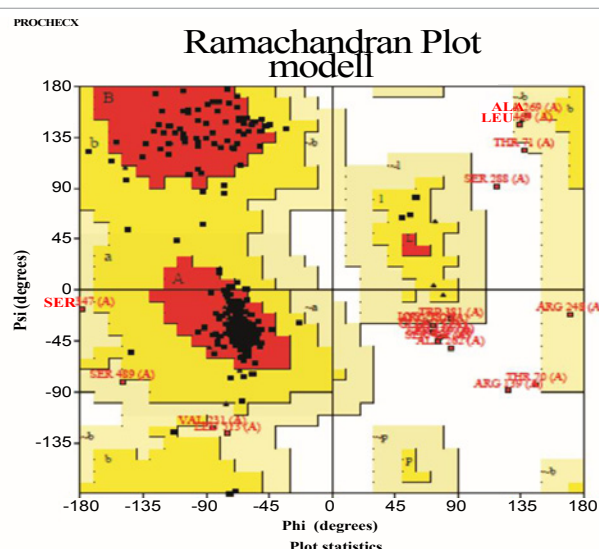


Figure 2: Validation of the model through Procheck.

hydrophobic in nature due to presence of high non-polar residues content. LFNHX protein has high percentage of alanine, leucine and serine. Results also showed that the maximum number of amino acid present in the sequence was found to be leucine (12 %) and the least was for cysteine (1.5 %). The total number of positively charged residues (Arg + Lys) was 44 and the total number of negatively charged residues (Asp + Glu) was 31.

Secondary structure analysis revealed that Lf NHX modeled protein had mixed secondary structures i.e. alpha helices, extended strand, random coil and beta turn. The analysis revealed that alpha helices were dominated among secondary structure elements, followed by random coils, extended strand and beta turns (Table 1). The high percentage of helices in the structure makes the protein more flexible for folding, which might increase protein interactions [14].

Functional characterization of LfNHX

The fingerprint scan of the sequence showed ten fingerprints, which share two common motifs for most of the sequences (Table 2). Di-sulphide bridges plays an important role in determining the thermo-stability of the enzymes, CYS_REC was used to determine the Cysteine residues and disulphide bonds. CYS_REC predicted 8 Cysteine residues in Lf NHX but no disulphide bonds (not shown) detected for this. The extensive hydrogen bonds may provide stability to these

Secondary structure	SOPMA	GOR4
Alpha helix	32.41%	26.30%
310 helix	0.00%	0.00%
Pi helix	0.00%	0.00%
Beta bridge	0.00%	0.00%
Extended strand	29.81%	25.93%
Beta turn	10.19%	0.00%
Bend region	0.00%	0.00%
Random coil	27.59%	47.78%
Ambiguous states	0.00%	0.00%
Other states	0.00%	0.00%

Table 1: Secondary structure of Lf NHX by SOPMA & GOR4.

Finger Print	No. of Motifs
NAHEXCHNGR	4
KV9CHANNEL	2
EDTRANSPORT	2
MG045FAMILY	2
MINTRINSICP	2
TCRTETB	2
ANGIOTENSINR	2
LIPOCALINIMR	2
YEAST73DUF	2
SUGRTRANSPORT	2

Table 2: Finger PRINT scan result of NHX protein.

proteins in absence of disulphide bonds. Binding pockets prediction of Lf NHX revealed many binding sites indicating that there is more than one binding site, giving insights to competitive inhibition with the active site or the presence of multiple substrate binding sites.

Submission of modeled protein in PMDB

The modeled structures of NHX proteins were successfully deposited in Protein Model Database (PMDb). The PMDB ID for the submitted modeled protein is PM0078210.

Conclusion

The LfNHX theoretical 3D model was predicted using homology modeling, refined with energy minimization and assessed for stereochemical and energetic aspects. The predicted structure can be used for further studies to understand the role of each fold of this protein in the function. Also the 3D predicted structure can be used as a basal structure for performing point mutation to improve the transformation efficiency of this protein for developing new recombinant bacterial and plant species.

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