Secondary and tertiary structure prediction of fenugreek (Trigonella foenum-graecum) protein

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ABSTRACT

In-silico development of protein models in fenugreek (Trigonella foenum-graecum) has opened up new vistas using the modern computational tools. The identification of protein in fenugreek having homology with the protein domain of humans and E-coli shows that the database available on Apiaceae family are very low. The estimated molecular weight of identified for fenugreek AM-1 protein was 11372.8 and was predicted as basic. A channel in fenugreek transmembrane protein has been identified through which a ligand (an ion or a small molecule) might pass. The present finding may be a valuable addition to the proteomic information available on fenugreek. Further validation can be performed using wet lab experiments.

Key words: Hydropathicity, PMDB, Protein, Script.

INTRODUCTION

Fenugreek (Trigonella foenum-graecum) is a member of Fabaceae. The name fenugreek comes from foenum-graecum, meaning Greek hay, as the plant was traditionally used to scent inferior hay (Helambe and Dande, 2012). The genus name, Trigonella, is also derived from the old Greek name, referring to the triangular shape of the flowers (Petropoulos, 2002). Fenugreek contains protein, fibre, vitamin C, niacin, potassium, iron and alkaloids. It also contains a compound diosgenin which has oestrogen-like properties, as well as steroidal saponins. These compounds impart many benefits to fenugreek.

Fenugreek seeds are rich in protein (25-5%), fat (7-9%), unavailable carbohydrate (48%), mucilaginous matter (20%) and saponins (4-8%). Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration (Bukhari et al., 2008).

Fenugreek is being cultivated abundantly in India claiming 70-80% of world’s export share (Harish et al., 2011). The state of Rajasthan supplies 83-90% of this and ranks first in fenugreek production in India (Mathur et al., 2009). Inspite of large potential and high content of protein in fenugreek seeds, however, no reports on molecular structure predictions are available on Trigonella spp. native to this region. The present study was therefore aimed to focus on understanding the molecular structure and its characterization based on its stability and different physiochemical properties. This helped in further determining the molecular nature and function of the fenugreek protein (Jethra et al., 2015). The present study will additionally assist in developing and determining other protein structures for crop improvement.

MATERIALS AND METHODS

Translation: National Centre for Biotechnology Information (NCBI) a public domain database (http://www.ncbi.nlm.nih.gov/) was used to retrieve sequenced data available and to prepare a protein structure using different translation tools (Transeq and Sixpack) (Choudhary and Jethra, 2014) and perl script was developed to get six reading frames.

Primary structure prediction: Primary structure prediction were computed using the Expasy’s Prot Param server (http://expasy.org/cgi-bin/protparam), which included different parameters like theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient (Gill, et al., 1989), instability index (Guruprasad et al., 1990), aliphatic index (Ikai, 1980) and grand average hydropathicity (GRAVY) (Kyte, et al., 1982).

Secondary structure prediction: Secondary structure of this protein was predicted by LOMETS and PSIPRED software’s using the translated amino acid sequences in FASTA format (Guermeur, et al., 1999)

Model building and evaluation : Ab-initio and threading Meta servers (Zhang, 2008) were used for modeling the three dimensional structure of the protein (Jethra et al., 2014).

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RESULTS AND DISCUSSION

Primary structure prediction: In the present study, Expasy’s ProtParam server (http://expasy.org/cgi-bin/protparam) was used to determine the physiochemical properties or the primary structure of fenugreek AM-1 protein (Identity given by PDBSum). The translated primary sequence, with 102 amino acid residues of AM-1 protein was used for primary structure prediction. The estimated molecular weight was 11372.8. The isoelectric point (pI) was also calculated which is useful because at pI, solubility is least and mobility in an electro focusing system is zero. The computed pI value of fenugreek AM-1 protein was 10.38 which was greater than 7 (pI>7) indicating that the predicted protein was basic in nature. The Aliphatic index determined for the protein sequence was 68.82. The Aliphatic Index (AI) is defined as the relative volume of a protein occupied by aliphatic side chains and is regarded as a positive factor for the increase of thermal stability of globular proteins.

Total number of negatively charged residues (Asp + Glu) and total number of positively charged residues (Arg + Lys) were estimated to be 8 and 15 respectively. The protein structure comprised of 1583 atoms in total. Expasy’s ProtParam also computed the extinction coefficient at 280 nm wavelengths as 280 nm is favored because proteins absorb light strongly there while other substances do not. Extinction coefficient of the protein at 280 nm was 3105 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 (Jethra, et al., 2012).

The instability index provides an estimate of the stability of protein in a test tube. A protein whose instability index was smaller than 40 was predicted as stable, a value above 40 predicts that the protein may be unstable (Guruprasad, et al., 1990). The instability index value for fenugreek protein was found to be 34.26, indicating that the identified protein was a stable protein. A GRAVY index for the protein was -0.653. This low range of value indicates the possibility of better interaction with water. The Grand Average hydropathicity (GRAVY) value for a peptide or protein was calculated as the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.

Secondary structure prediction: The model preparation of protein’s secondary structure was performed using ProteinPredict, SOPMA PSIPRED and JPRED3 (Figure 1). The protein sequences in FASTA format were used to derive secondary structure of protein. Secondary structure of protein was predicted by the formation of alpha helix and β-sheets. The results revealed that random coil (69.61%) dominated among secondary structure elements and alpha helices (4.90%) and extended strand (25.49%) were also present (Figure 2). It was developed using two networks: a sequence-
to-structure network and a structure-to-structure network (Mihasan, 2010).

**Tertiary structure prediction:** SAVES server was used for further verification and stability check for 3D structure (Figure 3) of fenugreek AM-1 protein which showed 78.4% residues or empirically distributed data-points present in the structure lie in most favored region and 14.2% residues in additional allowed region of Ramachandran plot showing the stability and good quality of protein 3D structure. PDBSum gave the types of residues and their density in the predicted structure (Jethra et al., 2014). Although we also found the conformation values of $\phi$ and $\psi$ angles possible for all amino acid residues present in the protein. The protein predicted was with an unidentified structure and function in *Trigonella foenum-graecum*. MEMSAT-SVM was used to identify a channel in fenugreek transmembrane protein, through which a ligand (an ion or a small molecule) might pass. The channel can be defined by the pore-lining residues, (Figure 4) which in most cases are accessible to solvent, and approximated by an axis, which ideally connects the two entrances N-Terminal (Cytoplasmic) and C-Terminal (Extracellular) at position 61-76 Pore-Lining of the pore and passes through the centre of the channel. Disorderedness of the fenugreek AM-1 protein structure was predicted and was removed for further analysis (Figure 5).

The prepared model was submitted to PMDB (Id No. PM0079210_A) for identification and functional annotation of the protein. The results were showing only 83.5% match with *CATH domain superfamily* Single helix bin (2k9y, transferase) with pDomTHREADER. FFPred predicted its functions in 2 categories: 1) Biological Process: phosphate-containing compound metabolic process with a probability of 95.9% and 2) Molecular function: ATP binding with 98.1% probability.
The present finding is first report of *in-silico* protein modeling for cumin. The identified fenugreek AM-1 protein is unique and having homology only with the reported bacterial and human protein domains. This had happened due to low strength of cellular database available on fenugreek or other related crops. The identified protein is having a match with the CATH domain, showing its function in protein synthesis.

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**REFERENCES**


