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3D-Structure Prediction of Winged Bean Trypsin Inhibitor and Their Mutant Forms

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Abstract: *Cloning, functional expression and site directed mutagenesis study was carried out previously on winged bean trypsin inhibitor (WbTI2) by our group. Here we report simulated 3-dimentional structure of WbTI2 and two of their mutant forms (R64A and R62Q) which has a direct functional correlation with their 3-D structure.*

Keywords: *WbTI-2, 3-D structure, Mutant protein, Native protein*

1. INTRODUCTION

Winged bean trypsin inhibitor is a very well studied protein of 182 residues having versatile implication with respect to its great therapeutic potential (ref. 1, 2, 3, 4). It is a Kunitz type protease inhibitor primarily reported by kortt et al (ref. 5). Later on cloning, functional expression and mutagenesis study of WbTI-2 gene was done to determine the reactive site loop (RSL) and the key element of the protein (ref. 6). In this study the 3-D structure of the native and mutated WbTI2 was predicted from the amino acid sequence. Position of the RSL with respect to the three dimensional structure of the proteins were also simulated. A comparative study of the 3-D structure native protein and its mutated forms was done which further emphasizes the structure and function correlation of the protein.

The RSL of the protein was determined previously (ref. 7), based on which multiple alignment of the RSL region was done and two amino acids were targeted for mutation namely 64^{th} arginine which was supposedly to be the P1 residue of the protein and the other one is 62^{nd} arginine which was found to be glutamine in all related chymotrypsin inhibitors. Thus both the arginine was changed to alanine and glutamine respectively by site directed mutagenesis technique and expressed protein was assayed for its activity with respect to the native one. The R64A mutant form was found to be totally nonfunctional and the second one i.e. R62Q was found to retain only 40-50% of its normal level of activity (ref.6). On the basis of this previous finding and the sequence of the native and mutated protein three dimensional structures of the proteins were predicted which further emphasize the structure-function correlation of the protein.

2. METHODS AND MATERIAL

2.1. Simulating 3D Structure of the Proteins

Three dimensional structure of the protein were predicted from the amino-acid sequence of WbTI2 (ref: 5) and also the mutated protein sequences using GENO3D (Release 2) [http://geno3D-prabi.ibcp.fr] program. The 'pdb' files generated from the program were viewed by using VMD (visual molecular dynamics) program, available from the site www.ks.uiuc.edu/research/vmd

3. RESULT AND DISCUSSION

The simulated three dimensional structure of native WbTI2 reveals a compact shape and a hydrophobic core like most of other serine protease inhibitor (ref: 8).The native protease has twelve β-pleated sheets (Table: 1), where one of such pleated sheet is exclusively contributed by the predicted RSL region (between 60^{th} -70^{th} amino acids) of the protein which is exposed outwards with a

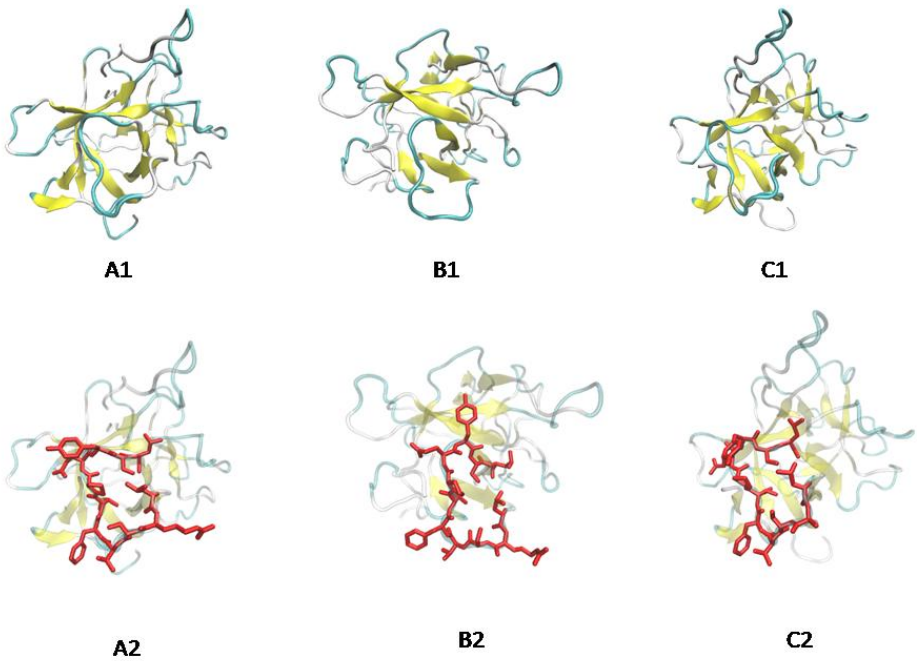
characteristic conformation. The structure shows resemblance with high resolution crystal structure of elastase inhibitor reported by Bode et al (ref: 9), where the serine protease inhibitor has an exposed binding loop of canonical conformation and the binding loop segment (P_3 to P_3') shows anti-parallel β-strand conformation. Similarly the RSL of a pancreatic trypsin inhibitor also found to has a flat shape in its X-ray crystal structure which fits into the active site cleft of the cognate serine protease (ref 10). The predicted 3D structure of the protein (fig: 1) has been found to be very relevant with respect to the function of the protein where a significant structural difference is seen between the native protein and R64A mutant form, especially in the RSL region. Functionally the protein showed complete loss of activity with respect to the native one. On the other hand the R62A mutant showed a bit lesser degree of structural disorientation in its RSL region though certain amount of change is also seen there with respect to the native one. The mutant protein was found to retain 40-50% of its inhibitory activity. The overall 3D structure of both mutated proteins showed a significant structural change (Table: 1). Replacement of conserved sequence of spacer residues particularly those which are involved in non-covalent interactions between the loop and the scaffold has been reported to result in considerable enhancement in loop mobility and reduced rigidity of the scaffold (ref. 11) but this part of work shows significant change of the scaffold resulting from a single amino acid change in the RSL, and the novelty lies in this unique finding where it shows that the structural integrity of the scaffold is also dependent on the conserved sequence of the RSL of the protein.

Table1. *Comparative analysis of the 3D structure of the native and mutated proteins*

	Native WbTI2	R62Q	R64A
Extended conformation (β-plated sheet)	12	14	10
Turns	16	16	14
Isolated Bridge	08	06	06
RSL region Between 60-70 aminoacids	Single exclusive β-plated sheet	Single exclusive β-plated sheet	Single exclusive β-plated sheet

Legends: Different secondary structures found to be present in the native WbTI2 and its two mutants i.e. R62Q and R64A

Figure7. *Predicted 3D structure of the native and mutated WbTI2*



Legends: A1 and A2 showing 3D structure of native WbTI2, B1 and B2 showing 3D structure of R62Q mutant and C1 and C2showing 3D structure of R64A mutant. The RSL region of the proteins are highlighted in red

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