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Molecular Docking Studies of Kunitz Type of Soya Bean Trypsin Inhibitor from Erythrina Caffra with Curcumin and its Natural Analogues

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Abstract:
Proteinase inhibitors (PIs) are the natural, defense-related proteins present in the seeds of leguminous plants. PIs are the major cause of poor protein digestibility and low nutritive value of soyabean and other related leguminous plant proteins. They combine to protein digesting enzymes mainly trypsin and chymotrypsin thus disturbing protein digestion in the intestinal tract. In the present study crystal structure of Kunitz type of trypsin inhibitor from Erythrina caffra which belongs to soyabean trypsin inhibitor family (PDB ID-1TIE) is retrieved from RCSB Protein Data Bank and molecular docking studies were performed by taking Curcumin (CID 969516), which is a major constituent of turmeric, 6 Gingerol (CID 442793),which is an active constituent of ginger and Isoeugenol (CID 853433 ,) which is a phenylpropanoid occurs in the essential oils of plants respectively as ligands. Docking is performed by using autodock suite. Binding energies are calculated and interactions are studied. Our findings suggest that these ligands show stable binding with the macromolecule with much lesser binding energies suggesting that curcumin and its structural analogues can be used for clinical trials in preventing pancreatic hypertrophy of rats fed on soyabean diet.

Key words*: Protease Inhibitors (PIs), Binding Energies, Docking, Curcumin*

1. Introduction

Proteolytic enzymes are digestive enzymes necessary for protein digestion, degradation and turnover. Damaged and misfolded proteins are broken down by these enzymes to provide free amino acids necessary for growth. As a defense strategy against pests and pathogenic microorganisms, plants produce protease inhibitors (PIs) which inactivate proteolytic enzymes of pests as well as those secreted by pathogenic microorganisms thus cause a drop in the availability of necessary amino acids and their growth (De Leo et al., 2002). Information on the distribution and functional properties of protease inhibitors (PIs) can be retrieved from a database called PLANT-PIs which currently contains 495 inhibitors identified in 129 different plants (De Leo et al., 2002). Molecular weight of PIs ranges from 4 to 85 kDa and they are often resistance to heat, extremes in pH, and proteolysis because of high content of cysteine residues (Richardson, 1991) PIs are synthesized as pre-proteins and further processed to produce the native PIs which form stable complexes with target proteases thus block or alter their active sites (Laing and McManus, 2002}. PIs have currently been classified into 48 families (Rawlings et al., 2004) on the basis of sequence relationship, protein folding and sequence homologies of their inhibitor domains, Soybean trypsin inhibitor which belongs to Serine protease inhibitor category (Mello et al., 2002; Haq and Khan, 2003) is the first PI isolated. PIs, which contribute 1 to 10 % of total proteins found in storage organs, such as seeds and tubers and whenever taken in, PIs interact with proteases found in gastrointestinal tract influencing the digestibility and nutritional quality of leguminous proteins.

2. Methods and Resources

The NCBI Entrez protein database (protein data bank) was accessed at internet. Crystal structure of Kunitz type of trypsin inhibitor from Erythrina caffra which belongs to soyabean trypsin inhibitor family (PDB ID-1TIE) and homologous to Kunitz type soyabean trypsin inhibitor from soyabean is retrieved from RCSB Protein Data Bank. RCSB Protein Data Bank is a repository for the 3-D protein structural data. These structures are obtained by X-ray crystallography or NMR spectroscopy, submitted by scientists around the world. In the present study 1TIE is docked with curcumin (CID 969516) which is a major constituent of turmeric, 6 Gingerol which is an active constituent of ginger (CID 442793) and Isoeugenol (CID 853433) which is a phenylpropanoid occurs in the essential oils of plants respectively Structures of these ligands were retrieved from pubchem which is a database for chemical molecules and prepared for docking by using Openbabel., which allows us to search, convert, analyze data from molecular modeling. Macromolecule is prepared by using Discovery studio 3.5 from Accelrys which is a well-known suite of softwares for macromolecule simulation systems. Docking is performed by using Autodock 4.2, which is simulation software for molecular modeling and Protein-ligand docking, available under General Public License. Autodock is frequently used for calculating and displaying feasible docking modes of pairs of protein and corresponding ligands (protein-ligand docking) and for calculating binding energies of their interactions .It has two main programs:- AutoDock for docking of the ligand to a set of

grids describing the target protein and AutoGrid for pre-calculating these grids, maintained by The Scripps Research Institute and Olson Laboratory (Morris et al, 2009). In the present study entire surface of the macromolecule is searched for docking and very large grid maps are created. Docked structures are analyzed by using Discovery studio 3.5 and Pymol 1-1 (Accelrys Software Inc**,** PyMOL Molecular Graphics System).

3. Results

In the present study Kunitz type of trypsin inhibitor from Erythrina caffra which belongs to soyabean trypsin inhibitor family (PDB ID-1TIE) is docked with curcumin (CID 969516), 6 Gingerol (CID 442793) and Isoeugenol (CID 853433) respectively. Binding energies of 1TIE (trypsin inhibitor from Erythrina caffra) with curcumin, 6 Gingerol and isoeugenol are calculated, its values -2.13 kcal, 1.2 kcal and -2.89 kcal respectively. Following Table shows values of binding energy and intermolecular energy of 1TIE with Curcumin, 6-Gingerol and isoeugenol and figures show interactions of residues of 1TIE with Curcumin, 6-Gingerol and isoeugenol respectively. Out of 10 docked conformations the best one with minimum binding energy is shown in the following table and figures. Every conformation is a combination of translation, quaternion and torsion angles and is characterized by intermolecular energy, internal energy and torsional energy. The combination of first and third gives binding energy. Our results indicate that these ligands show effective binding with 1TIE .We propose that curcumin, 6 Gingerol and Isoeugenol can be used for clinical trials for the prevention of pancreatic hypertrophy caused by high soya protein intake in rats and for adding more nutritive value to soya beans and related leguminous proteins.

Macromolecule	Binding energy /Intermolecular energy with Curcumin.	Binding energy /Intermolecular energy with 6 Gingerol	Binding energy/Intermolecular energy with Isoeugenol
1TIE	-2.13 kcal /3.15 kcal	-1.2 kcak /-4.78 kcal	-2.89 kcal /-3.79
Interacting Residues	ILU 144, TYR 160	LEU 50. GLU 49, TRP 23	PRO144,LYS72,CYS132, TRY113, GLU133

Table 1: Results of molecular docking of 1TIE with CURCUMIN, 6-GINGEROL AND ISOEUGENOL

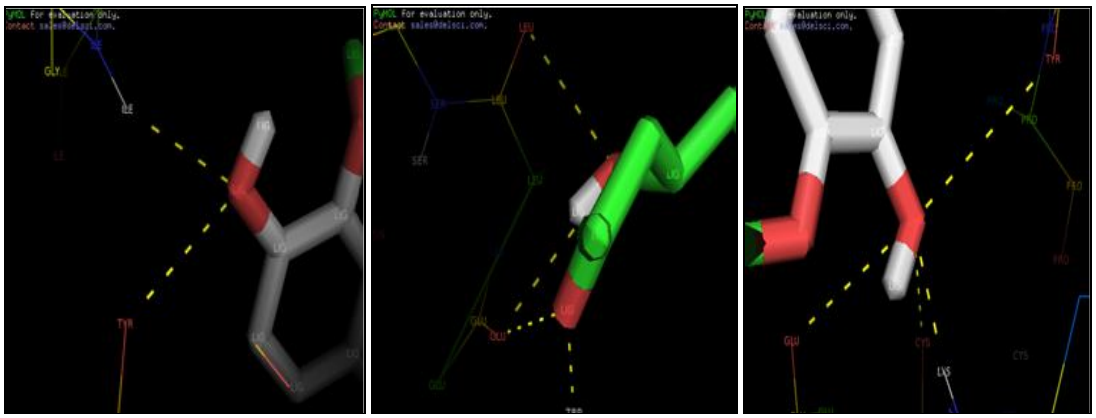

Figure 1: Interaction between 1TIE and Curcumin
Figure 2: Interaction between 1TIE and 6 Gingerol
Figure 3: Interaction between 1TIE and Isoeugenol

4. Discussion

Some pests and pathogenic microorganisms of plants secrete extracellular enzymes causing proteolytic digestion of plant parts and play an important role in the pathogenesis. As a line of defense against these pathogens and pests plants also secret protease inhibitors (PIs) which are active against enzymes secreted by microorganisms as well as insect gut proteases. Soya-bean trypsin inhibitor (Ham & Sandstedt, 1944; Kunitz 1945) when added to a diet of rats led to growth inhibition. Kunitz-type of PIs inhibits trypsin, chymotrypsin and subtilisin activities (Park et al., 2005). Pancreatic hypertrophy is seen is rats and chicks fed on raw soya beans. In pancreatic hypertrophy, sulphur containing amino acids which are essential constituents pancreatic enzymes are diverted to the synthesis of these enzymes which are irretrievably lost by excretion causing growth reduction. As the release of cholecystokinin (CCK) which is a stimulating hormone secreted from intestinal mucosa is inhibited by free trypsin (Wilson *et* al. 1978). trypsin inhibitor can indeed be the major cause responsible for the poor growth of animals fed on raw soya beans and it may have deleterious effects on human beings too. In the present study we have docked trypsin inhibitors with Curcumin which is a major curcuminoid of Indian spice turmeric and its structural analogues 6 Gingerol and Isoeugenol.. Our results showed much stable binding interactions between trypsin inhibitor and ligands.So we predict that by reducing the amount of trypsin inhibitor or inactivating it by natural means surely we can enhance nutritional quality of soya beans and other leguminous crops

5. References

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