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Research Article

Prediction of interaction of various apoptotic proteins with Sulforaphane (SFN) isolated from *Brassica oleraceae* using docking studies

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ABSTRACT

Sulforaphane an isothiocyanate formed by hydrolysis of glucosinolates exerts various activities such as antitumor, antioxidant and anti-inflammatory activities. Previous research proved the induction of apoptosis by SFN in HEp-2 cell line. Here we studied the structure activity relationship of sulforaphane with various apoptosis proteins such as p53, bax, bcl-2 and caspases by docking studies using molecular docking server. Molecular models were used to study the interactions of SFN with various binding pockets in the protein. SFN was successfully docked to all the proteins and their binding energies were comparable with positive inducer of apoptosis 5FU. The results were appreciable and in agreement with our earlier research. Hence, SFN from plant source can be developed as a promising lead in adjuvant cancer chemotherapy.

Keywords: Bax; bcl-2; Brassica; docking; p53; Sulforaphane

Background

Various research studies proved that consumption of vegetables and fruits reduce the risk of various types of cancer like breast, lungs, etc. (Gasper AV et al., 2007). Among the various plant groups, Brassica members continue to occupy a greater place in anti-cancer research. The Brassica members include Cabbage, Broccoli, Raddish, cauliflower, Brussels sprouts, etc. The major glucosinolates presents in red cabbage were found to be glucoraphanin, glucobrassicin, sinigrin and progoitrin. Glucosinolates may break down to various hydrolysis products by commensal microflora. These members contain enriched content of glucosinolates which on hydrolysis produces isothiocyanates (ITC), Indoles, nitriles and oxazolindinethiones (Steven JT et al., 2004). Sulforaphane an isothiocyanate also known as 1-Isothiocyanato-4- (methylsulfinyl)-butane isolated from red cabbage (Brassica oleraceae var rubra) is reported to have been antitumor, antioxidant, antimicrobial and anti-inflammatory activities. The isothiocyanate sulforaphane (SFN) has attracted much recent interest, since it was found be the most potent naturally occurring inducer of phase II detoxification enzymes and potent scavenger of reactive oxygen Species (ROS) including superoxide anions and hydroxyl radicals (Davis CD et al., 2002) The mechanism by which these agents exert their anticancer action was reported to be apoptosis. Both pro apoptotic (Bax, Bak, Bid, Noxa, etc.) and anti-apoptotic (Bcl-XL., Mcl-1, Bcl-w, etc.) proteins have been reported to be key regulators of apoptosis. The p53-dependent apoptotic pathway can lead to the cellular protein cleavage (e.g. PARP), DNA damage and cell death. Two major pathways leading to apoptosis exist in cells: the extrinsic pathway, which involves the activation of the death receptor family and the intrinsic pathway, which involves the mitochondria. In both the pathways, an apoptotic death stimulus results in the activation of caspases, the major executioners of this process, either directly or via activation of the mitochondrial death program (Illic N et al., 1996). It is an anti-oxidant and a potent monofunctional inducer, which accounts for its anticarcinogenic properties in animal models. Studies have documented that the consumption of green and yellow vegetables, especially crucifers, is associated with a lower cancer risk and reported to have antimicrobial activity (DTH Verhoeven et al., 1997). The induction of apoptosis by SFN mediated through death receptor has been reported earlier (Jin CY et al., 2007). The p53 pathway of apoptosis has not been clearly understood. In our research sulforaphane was isolated from red cabbage, studied its apoptosis induction through p53 and mediated through bax and bcl-2 (Renuka and Berla, 2012). In this present insilico work the docking interactions of p53 (tumorsuppressor), bax (proapoptotic), bcl-2 (antiapoptotic) proteins, caspase enzymes (cysteine proteases) with SFN (sulforaphane) were studied in reference to 5 fluorouracil.

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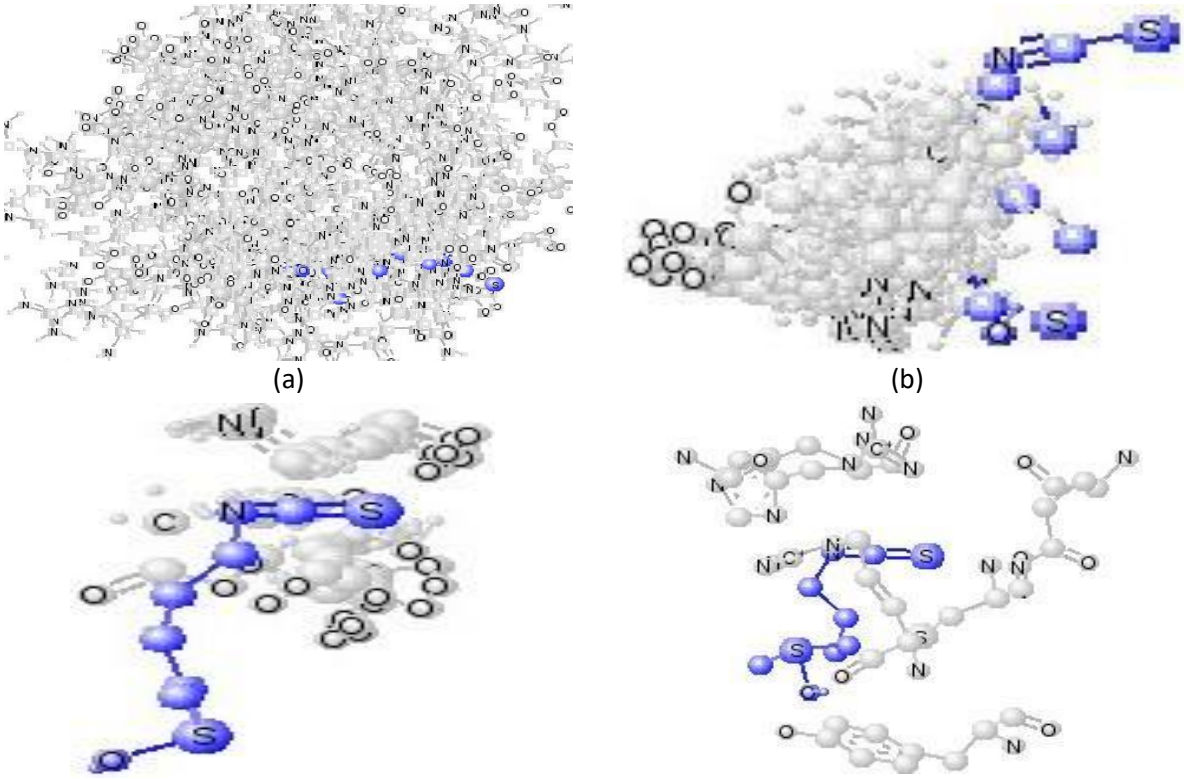
Methodology

Pubchem (pubchem.ncbi.nlm.nih.gov), Smiles online translator (http://cactus.nci.nih.gov/translate) used in

Table 1: comparison of different energies interacting surfaces and frequencies of species

Parameters	p53		bax		bcl-2		casp-3	
	SFN	5FU	SFN	5FU	SFN	5FU	SFN	5FU
Est Free energy of Binding (kcal/mol)	-2.73	-3.80	-4.36	-4.48	--4.85	-4.82	-3.99	-5.48
Est inhibition Constant, Ki	10.06	-1.64	635.99	521.7	277.68	290.91	1.20	96.22
vdW + H bond+dissolve Energy(kcal/mol)	-4.74	-3.64	-6.41	-4.37	-6.75	-4.17	-5.86	-4.61
Electrostatic Energy (kcal/mol)	-0.03	-0.16	-0.05	-0.11	-0.09	0.65	-0.17	-0.87
Total intermol Energy (kcal/mol)	-4.76	-3.80	6.46	4.48	-6.84	-4.82	-6.03	-5.48
Frequency (%)	18	18	33	96	21	65	32	97
Interaction Surface	396.56	250.65	381.6	250.6	393.85	250.64	459.9	319.05



(a) (b) (c) (d)

Figure 1: Illustration of Sulforaphane binding with various apoptosis proteins a) p53 (tumor suppressor) bonding with SFN b)Bax (proapoptotic) protein with SFN c) Bcl-2 (antiapoptotic)protein with SFN d) caspase 3 (Cysteine protease) with SFN as shown in JMol viewer (in built visualisation software of Docking server)

this study are freely available for academic use. Molecular docking server was used on paid subscription. Autodock (http://autodock.scripps.edu) is used for molecular docking calculations. Rasmol is used for visualisation of docking results. MMFF94 semi empirical method can be used to calculate small molecule geometries and electric properties. Detailed methodology can be accessed from server using URL http://www.dockingserver.com/web/gettingstarted.PDB files of various human proteins such as p53, bax, bcl-2 and casp-3 were obtained from Protein data Bank (http://www.pdb.org).

Protein file Preparation

Briefly, PDB files for p53 protein (IHS 5), bax (1F16) bcl-2 (1MK3) casp-3 (1NM3) downloaded from Protein Data Bank (http://www.pdb.org) was uploaded to the server. At protein clean step, charge calculation method was selected as Gasteiger. All the protein chains were selected and hetero atoms are not removed as the binding site is not predicted. All water molecules were selected for cleaning. By completion of this step, protein clean, calculation of protein charges and solvation parameters as well as the protein parameter file created (Bikadi Z et al., 2007). In the next step, a Grid (a three-dimensional box) was created with a dimension of X=20 Angstrom, Y= 20 Angstrom, Z= 20 Angstrom, while a center of mass was kept at a coordinate of X= 103.61, Y= 100.67, Z= 78.536. The protein was made ready for docking experiments.

Ligand file Preparation

The Sulforaphane (SFN) structures were downloaded from Pubchem as .sdf files. The .sdf files were converted to .pdb files using smiles online translator. In the same way .sdf files of 5 fluorouracil were retrieved from pubchem and converted to .pdb files using smile online translator. Molecular docking server was used for the preparation of ligand before docking experiment. Briefly, ligands were uploaded singly to the server. Charge calculation and geometric optimization methods were selected as Gasteiger and MMFF94 respectively; while pH was kept as 7.0 (McDonald IK and J.M. Thornton, 1994). By the end of these process ligand files are ready for the docking.

Docking

Docking was started by selecting two ligands (SFN) and various proteins such as p53, bax, bcl-2 and casp-3 from their respective folders. The number of individuals in the population (ga_pop_size) was kept 150, AutoDock counts for numbers of energy evaluations (ga_num_evals) were kept 25000000 and the number of generations (ga_num_generation) selected as 540000 and rest on other settings kept as default setting. Finally, simulation experiment started with keeping the numbers of run as 100. AutoDock is the most popular molecular docking program used for various molecular docking calculations. Many research reports supported the fidelity and accuracy of Autodock docking tools (Ali Abdullah et al., 2007). Hence, Moleular Docking Server was considered as an appropriate docking tool for this docking experiment using multiple proteins against our ligand SFN (Halgren TA., 1998; Morris GM. et al., 1998; Solis FJ and Wets RJB, 1981) Astex-Viewer and JMOL viewer (default viewer of docking server) and Rasmol viewers were used for the visualization of docking results.

RESULTS AND DISCUSSION

The binding energy for SFN with various human proteins such as p53, bax, bcl-2 and caspase 3 are tabulated in the table. Frequencies of occurrence out of total population for p53 with SFN and 5FU were found

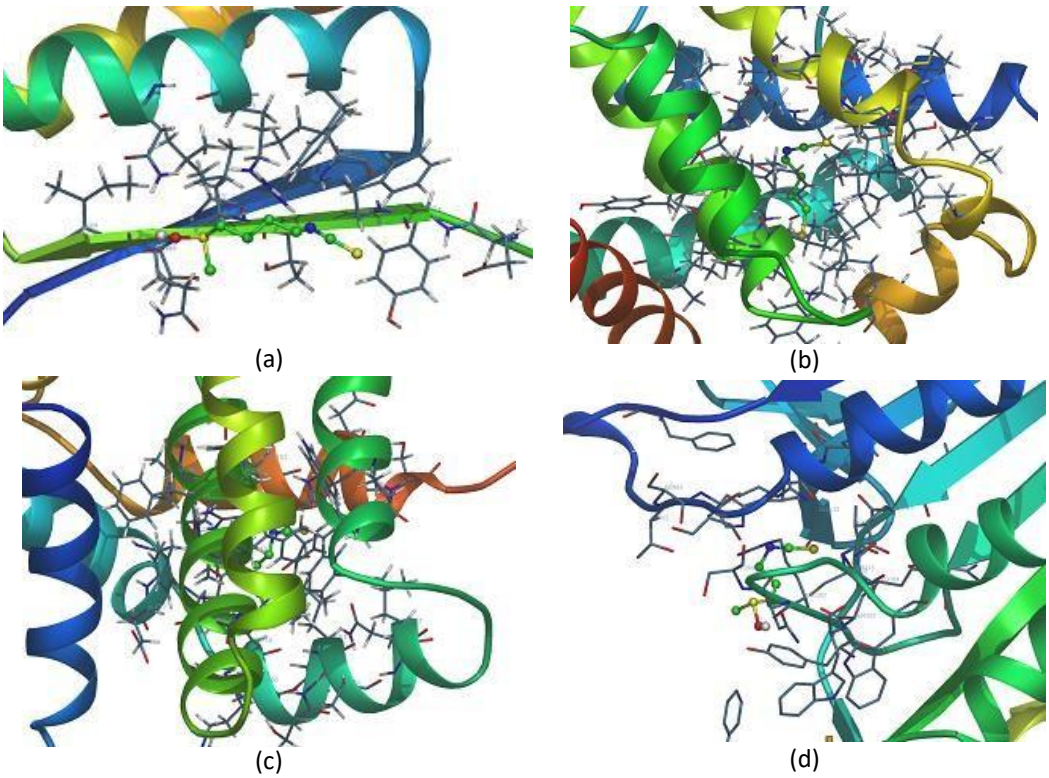


Figure 2: Visualization of lowest energy conformation of binding of SFN with various apoptosis proteins a]p53 with SFN b) Bax with SFN c) bcl-2 with SFN d) Casp-3 with SFN as shown in ASTEX viewer(default viewer of Docking server)

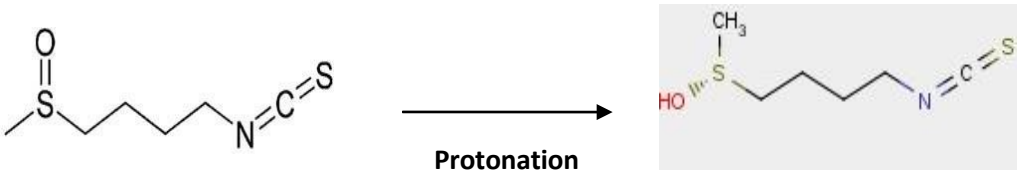


Figure 3: Conversion of Sulforaphane (1-Isothiocyanato-4-(methylsulfinyl)-butane) to protonated form(4 isothiocyanate N –methyl butane1 –SO – thioperoxol) in the ligand preparation step in the server

to be the same as 18 % and for SFN and 5FU with bax, bcl-2 and caspase 3 were tabulated in the table. A comparison of different energies interacting surfaces and frequencies of species are shown in the Table 1. Sulforaphane (1-Isothiocyanato-4- (methylsulfinyl)-butane) is converted to 4 isothiocyanate N –methyl butane1 –SO - thioperoxol by protonation in the ligand preparation process by docking server. In the examination of SFN binding with proteins, SFN contains a hydrophilic sulfinyl group, which forms a hydrogen bond with the amino acids of protein (Ali Abdullah et al., 2007). In general, ITCs (isothiocyanate) including sulforaphane are electrophiles capable of modifying proteins thus initiating a cascade of events like apoptosis (DTH Verhoeven et al., 1997). During docking a series of poses (ligand-protein complexes of particular conformation and mutual orientation) were generated for each molecule. The algorithm for the optimization of the ligand-protein orientation works by the alignment of triplets of ligand atoms on triplets of site points, which are the centers of alpha spheres created in the potential binding sites. From the figure the interaction of various proteins such as p53, bax, bcl-2 and casp 3 protein and SFN depict the various interactions such as hydrogen bond formation hydrophobic and polar interaction between amino acid and the carbon and nitrogen atom of the sulforaphane. While studying the interaction SFN got anchored in p53 protein by forming a hydrogen bond with various amino acids such as THR 6 ILE 9 GLU 16, 20 GLN17 and O1 (3) with GLN 8, 17 LEU 25 and ARG 10. In bax O1 (3) forma a hydrogen bond with LEU 63 whereas N1 (4) with GLU 163. In bcl2 proteins, Caspases are synthesized as relatively inactive zymogens that become activated by scaffold-mediated transactivation or by cleavage via upstream proteases in an intracellular cascade. Caspases (cysteine aspartatic acid proteases) play an essential role at various stages of the apoptotic process. Caspase zymogens possess an N-terminal prodomain, and a linker peptide within the protease domain, which are cleaved to render an active caspase (Mrudula D and Marks G 2002). There exists a polar interaction between ASP 253 with O1 (3) and H1. Members of Brassica genus like Broccoli reported to have been antitumor, antioxidant, antimicrobial and anti-inflammatory activities due to their enriched content of glucosinolates (Fahey JW et al., 2002). These glucosinolates on hydrolysis produce Isothiocyanates, Indoles and other derivatives, which are reported to have anticancer activity in vitro and in vivo. Our previous study has demonstrated to characterize the p53 pathway of apoptosis. by SFN isolated from Brassica oleraceae. Our results displayed SFN from Red Cabbage fraction caused an enhanced expression of bax as well as triggering the down regulation of bcl-2 with a subsequent promotion of the apoptotic activity in HEp-2 cells (Renuka and Berla, 2012). Here in the present study we studied the structure activity relationship of various apoptosis proteins as p53, bax, bcl -2 and casp3 and their interaction with SFN by virtual screening with the help of Molecular docking server. The various hydrogen bonding and hydrophobic interactions reported were responsible for their binding and their energy of binding were found to be closer with 5 Fluorouracil (positive inducer) of apoptosis. Frequency of occurrence for all the proteins seems to fairly appreciable when compared with the 5FU. This parameter can determine the specificity and activity of ligand (SFN) in apoptosis induction. Our finding using this insilico studies also supports our earlier research in signalling pathway involved in apoptosis cascade.

CONCLUSION

We have demonstrated the structure activity relationship of various apoptosis proteins with SFN and proved their stability by studying various interactions involved in the binding. This study may provide an insight to the structural and molecular basis of their interaction in insilico level. Thus, these findings also support the earlier signal transduction research in the same species also in the lead development using SFN against cancer development. Hence, SFN isolated from plant source can be developed as an adjuvant in cancer chemotherapy.

REFERENCES

Ali Abdullah Alshatwi, Tarique Noorul Hasan, Naveed Ahmed Syed, Gowhar Shafi Predicting the possibility of two newly isolated phenetheren ring containing compounds from *Aristolochia manshuriensis* as CDK2 inhibitors Bioinformation 7 (7): 334-338 (2011). (PMID:22355233).

Bikadi, Z Demko L and Hazai E Functional and structural characterization of a protein based on analysis of its hydrogen bonding network by hydrogen bonding plot Arch. Biochem. Biophys. 461, 225-234 (2007) (PMID: 17391641).

Davis, C. D., Zeng, H., Finley, J. E. (2002). Selenium Enriched Broccoli decreases intestinal Tumorigenesis in Multiple intestinal Neoplasia. Journal of nutrition. 132, 302-309. (PMID:11823596).

Dorette T. H. Verhoeven, Hans Verhagen, R. Alexandra Goldbohm, Piet A. van den Brandtb, Geert van Poppel A review of mechanisms underlying anticarcinogenicity by brassica vegetables Chemico-Bioiogical Interactions 103 (1997) 79- 129. (PMID:9055870).

Fahey J W, Haristoy X, Patrick Dolan, Thomas Kensle, Isabelle Scholtus, Katherine K. Stephenson, Paul Talalay and Alain Lozniewski (2002). Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene induced stomach tumors. PNAS., 99: 7610-7615. (PMID:12032331).

Gasper, A. V., Traka, M., Bacon, J. R., Smith, J. A., Taylor, M. A., Hawkey, C. J., Barrett, D. A., Mithen, R. F. (2007). Consuming Broccoli Does Not induce Genes

Associated with Xenobiotic Metabolism and Cell Cycle Control in Human Gastric Mucosa. The Journal of Nutrition. 137, 1718-24. (PMID:17585021).

Halgren, T. A. Merck molecular force field. I. Basis, form, scope, parametrization, and performance of MMFF94 Journal of Computational Chemistry 17 (5-6), 490-519 (1998).

Hazai E, Kovacs S, Demko L, Bikadi Z DockingServer (www.dockingserver.com), Virtua Drug Ltd., Budapest, Hungary.

Jin, C. Y., Moon, D. O., Moon, J. D. L., Lee, J. D., Heo, MS, Choi, Y. H., Lee, C. M., Park, Y. M., Kim, G. Y. (2007). Sulforaphane sensitizes tumor necrosis factor related apoptosis induced lignad mediated apoptosis through downregulation of ERK and AKT in Lung adenocarcinoma A549 cells. Carcinogenesis. 28, 1058-66. (PMID:17183064).

lllic, N., Normanly, J., Cohen, J. D. (1996). Quantification of Free Plus Conjugated indoleacetic Acid in Arabidopsis Requires Correction for the Nonenzymatic conversion of Indolic Nitriles. Plant Physiol. 11, 781-8. (PMID:8754680).

McDonald I K and Thornton J M Satisfying Hydrogen Bonding Potential in Proteins JMB 238, 777-793 (1994) (PMID:8182748).

Morris G M, Goodsell DS, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function Journal of Computational Chemistry 19 (14), 1639-1662 (1998).

Mrudula Donepudi, Markus G. Structure and zymogen activation of caspases Biophysical Chemistry 101 – 102 (2002) 145–153. (PMID:12487996).

Renuka DeviJ, Berla Thangam E (2012) Mechanisms of Anticancer Activity of Sulforaphane from *Brassica oleracea* in the HEp-2 Human Epithelial Carcinoma Cell Line Asian Pacific Journal of Cancer Prevention, Vol 13 (5), 2012. (PMID: 22901176).

Solis F J and Wets RJB, Minimization by Random Search Techniques Mathematics of Operations Research 6 (1), 19-30 (1981).

Steven, J. T., Jackson., Singletary, K. W, (2004). Sulforaphane: a naturally occurring mammary carcinoma mitotic inhibitor, which disrupts tubulin polymerization. Carcinogenesis, 25, 219-27. (PMID:14578157).