Molecular mechanism of interaction of mitocurcumin-1 with Akt1 and STAT3: An In silico approach

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The bioavailability of curcumin is the limiting factor for its effective use in anti-cancer therapy. Recently, we reported a novel approach to enhance the cellular uptake by conjugating curcumin with triphenyl phosphonium, named mitocurcumin-1. We found that such conjugation significantly increased the uptake of curcumin in various cancer cells and caused cancer cell death by inducing apoptosis by decreasing the phosphorylation of Akt1 (Thr308) and STAT3 (Tyr705). In this study, a molecular mechanistic model deciphering the regulation of phosphorylation of Akt1 and STAT3 by mitocurcumin-1 was investigated and compared with curcumin. The protein structures were obtained from protein data bank data base and protein-ligand interaction studies were performed with mitocurcumin-1 and curcumin. Docking interaction studies of mitocurcumin-1 with Akt1 and STAT3 active sites showed a strong binding affinity of -60.4107 Kcal/mol and -51.1734 Kcal/mol respectively, suggesting mitocurcumin-1 interacted with the residues at the active sites of phosphorylation of these molecules. Further, a Chi rotationary root mean square deviation of 1.468 Å and 3.965 Å at the active sites in Akt1 and STAT3, respectively indicated that changes in the conformation of protein structure at the active site resulted in the inhibition of phosphorylation of these molecules. To conclude, by using molecular modeling approaches for the first time, we demonstrated the inhibition of Akt1 and STAT3 phosphorylation by mitocurcumin-1.

Keywords: Molecular modeling, Curcumin, Mitocurcumin-1, Apoptosis, Cancer cells, Akt1, STAT3

Curcumin (diferuloylmethane), a natural yellow orange colored compound derived from the rhizome of the plant Curcuma longa is widely used as a natural ingredient in food in South-East Asian countries. It has shown to have anti-cancer properties and the mechanism of action includes cell cycle arrest, induction of apoptosis through both mitochondria-dependent, as well as mitochondria-independent mechanisms. Curcumin is a potent inhibitor of the activation of various transcription factors, including signal transducer and activator of transcription (STAT3) proteins, peroxisome proliferator-activated receptor-γ (PPAR-γ) etc. It is also found to induce apoptosis in acute T-cell leukemias by inhibiting the phosphatidylinositol-3-kinase/AKT pathway and inducing G2/M arrest.

Earlier, we reported that curcumin induces cell cycle arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by modulating Akt/p38 pathways. We also found that EF24, a compound having close structural similarity to curcumin inhibits the proliferation of a variety of cancer cells by downregulating Akt and upregulating p53 and PTEN. In another study, 4-arylidene curcumin analogues have been shown to induce cancer cell death by inhibiting both NF-κB and Akt signaling. Recently, it is also reported that structurally modified curcumin analog inhibits STAT3 phosphorylation and induces apoptosis of human renal cell carcinoma and melanoma cells.

Curcumin has been shown to have limited uptake into cell and tissues, due to its hydrophobicity and rapid metabolism. In our recent study, we used a novel approach by conjugation of curcumin to lipophilic triphenylphosphonium (TPP) cation (named as mitocurcumin-1) to facilitate increased delivery of curcumin to cells and mitochondria. TPP is selectively taken up by mitochondria driven by the membrane potential by several hundred folds. Lipophilic TPP cation with highly hydrophobic structures and positive charge seems to accumulate in the mitochondria of tumor, due to the highly
negatively-charged microenvironment within the mitochondrial matrix\textsuperscript{19}. Recently, we have also observed increased uptake of mitocurcumin-1 by several-folds by cancer cells and its significant cytotoxicity to cancerous cells. Also, mitocurcumin-1 induces apoptosis by regulating Bcl2, STAT3, Akt1, ERK1/2, Cyclins, PARP cleavage etc\textsuperscript{18}. Curcumin also induces apoptosis by inhibiting the phosphorylation of Akt1 and STAT3\textsuperscript{20-23}. Several studies have shown that active site loops in these proteins containing the phosphorylation sites are the targets of the inhibitory molecules\textsuperscript{23-26}.

The PI3K/Akt pathway is highly conserved and activation of Akt1 follows a multi-step process, resulting in phosphorylation of two residues, Thr308 at activation loop and Ser473 at hydrophobic motif\textsuperscript{20,27}. Akt1 binds to PIP3 at the plasma membrane, thus allowing PDK1 (3-phosphoinositide-dependent protein kinase-1) to access and phosphorylate T308 in the “activation loop”, leading to partial activation of Akt1\textsuperscript{28}. The complete activation of Akt1 is stimulated by phosphorylation of Akt1 at S473 in the carboxy-terminal hydrophobic motif either by mTOR\textsuperscript{29} or DNA-PK\textsuperscript{30}, resulting in increase in kinase activity induced by conformational changes due to phosphorylation. Fully active PKB/Akt mediates numerous cellular functions including apoptosis. Dephosphorylation of T308 by PP2A\textsuperscript{31}, S473 by PHLPP1/2\textsuperscript{32} and conversion of PIP3 to PIP2 by PTEN\textsuperscript{33} antagonize Akt1 signaling. Recently, we have shown that mitocurcumin-1 exhibits inhibitory activity towards phosphorylation of Akt1 at T308, thus inducing apoptosis in MCF7 cancerous cell lines\textsuperscript{18}.

Signal transducers and activators of transcription3 (STAT3) is a latent cytoplasmic eukaryotic transcription factor of STAT family of proteins\textsuperscript{34}. Mammalian STATs share six structural regions: N-domain (ND), coiled-coil domain (CCD), DNA-binding domain (DBD), linker domain, SH2 domain and transcriptional activation domain. These are activated by phosphorylation of single tyrosine molecule at residues near 700 (Y705 in case of STAT3)\textsuperscript{25,35} and are activated in response to extracellular ligands associated with the cytokine receptors, JAK kinases or growth factors\textsuperscript{36}. Since STAT3 activation is through the dimerization upon phosphorylation of Tyr705, inhibition of activation by phosphorylation is seen as the major mechanism of regulation STAT3 pathway for the induction of apoptosis or anti-cancer activity\textsuperscript{34}. Modeling studies have also shown that inhibition of dimerization STAT3 results in its inhibition by curcumin and its derivatives\textsuperscript{37}.

In our recent study, we have shown that mitocurcumin-1 inhibits Akt1 and STAT3 phosphorylation more efficiently than curcumin in MCF-7 cell lines\textsuperscript{18}. In the current study, based on our previous work\textsuperscript{18} and literature\textsuperscript{20,26} molecular interaction and docking studies have been performed in order to study the molecular mechanism of action of inhibition of phosphorylation of Akt1 and STAT3 by mitocurcumin-1. Results indicate that molecular interaction of mitocurcumin-1 with Akt1 and STAT3 results in inhibiting phosphorylation with higher efficiency compared to curcumin, which is due to its higher interaction energy.

Materials and Methods

Protein structure retrieval

The protein structures of Akt1 and STAT3 for this study were retrieved from PDB/RSCB data bank as PDB files. The PDB structures taken were PDBID: 3o96 for Akt1 and 4E68 for STAT3. The crystal structures of Akt1 (3o96) contained 442 aa residues from amino acid 2 to 443 and that of STAT3 (4E68) contained 596 aa residues (from aa 127-715). The resolution of these structures was 2.7 Å and 2.56 Å, respectively.

Protein preparation (Optimization)

The retrieved structures were subjected to dehydrogenation, removal of ligands and protein-heavy metal atom bond\textsuperscript{17,37}. Protonation was done. Protein structures with active phosphokinase conformations were obtained for the use in docking studies. Energy minimization was done in five steps using CHARMM force field energy minimization algorithm. An initial RMS gradient of 70.03 for 4E68 (STAT3) and 755.9 for 3o96 (Akt1) was achieved. The software used was Accelrys Discovery studio client 2.5 version software.

Active site prediction

Prediction of active site was done using Q site server (http://www.modelling.leeds.ac.uk/qsitefinder). Out of the 10 sites, the best possible site was chosen. In order to validate the results more precisely, these sites were cross-checked with receptor binding site finder in Accelrys Discovery studio client 2.5 software and the binding site of sphere was established.

Preparation of ligands: Mitocurcumin-1 and curcumin

The structures of mitocurcumin-1 [(C\textsubscript{6}H\textsubscript{6}O\textsubscript{3}P\textsubscript{2})\textsuperscript{2+} (Cl\textsubscript{2})\textsuperscript{5}]; 1,7-Bis{3-methoxy-4-[3-(triphenylphosphonium)
propoxy]-phenyl}hepta-1,6-diene-3,5-dione dichloride] and curcumin were drawn using ACD Chem sketch software. The structures were validated and molecular weights of the compounds were obtained. The 3D conformations of these compounds were obtained using Accelrys Discovery studios 2.5 software prepare ‘ligand module’. Energy minimization steps were performed to obtain the best possible 3D conformation at physiological conditions of these molecules. pH-based ionization was used for the purpose and Lipinski filter was kept active for optimal filtration of structures. Out of the 254 3D conformations generated for curcumin and 74 3D conformations generated for mitocurcumin-1, the best conformation having the least energy and structural stability was selected for docking purpose.

Protein-ligand docking studies

The optimized protein and ligand were taken and subjected to flexible docking procedure in the C docking module. The values of input sites of sphere were established and pose cluster radius was set as 20. CHARMM force field was used for the purpose and final minimization was done to full potential. Different protein conformations were generated for the input group of residues. The number of hotspots for the purpose of docking was fixed at 100 with a tolerance limit of 0.25. The final cluster radius was adjusted to 0.5 and the root mean square deviation (RMSD) filter was kept at 2.0. Grid extension was used at 8.0. The remaining parameters were kept default and the process was run.

Results and Discussion

The structures of mitocurcumin-1 and curcumin were obtained as mol files (Fig. 1). The optimized protein structures were used for the interaction studies. Docking interactions of Akt1 molecule showed that mitocurcumin-1 interacted with the amino acid residues at the activation loop site (Fig. 2). Results showed that mitocurcumin-1 formed two hydrogen bonds with Thr160 and Ile300 aa residues and π bond interactions with Lys 156 and Lys 182 of Akt1. Total energy of the structure calculated after simulation using CHARMM force field was found to be -13941.65 Kcal/mol. The protein-ligand interaction energy and C docker energy of mitocurcumin-1 and curcumin with Akt1 are presented in Table 1. Although curcumin and mitocurcumin-1 showed binding at the same interaction site, i.e., active site loop of Akt1, due to high interaction energy, mitocurcumin-1 showed higher affinity of interaction than curcumin.

Recently, we have shown that mitocurcumin-1 inhibits the phosphorylation of Akt1. It is also earlier established that inhibitors of Akt1 binds at the active site loop inhibiting the phosphorylation site T308. In particular, the mechanism of inhibition of phosphorylation is through binding at the conserved DFG domain and inducing conformational changes within the active site. In the present study, similar mechanistic mode of interaction was observed with mitocurcumin-1 and curcumin which interacted with the conserved DFG domain at the active loop, thus inducing conformational changes, resulting in the masking of these phosphorylation sites.
Interaction of mitocurcumin-1 and curcumin with active site residues showed an overall chi-rotationary RMSD of 1.468 Å and 1.17 Å. Data pertaining to individual residues showed RMSD of 2.66 Å for D292, 0.168 Å for F293 and 0.11 Å for T308 with mitocurcumin-1 and 2.05 Å for D292, 0.324 Å for F293 and 0.11 Å for T308 with curcumin. These suggested an overall conformational change (Table 1) at the active site, resulting in blocking of phosphorylation of T308.

Earlier, it is shown that ATP competitive and allosteric inhibitors of Akt1 bind at the same site, leading to the conformation changes at DFG domain and active site, resulting in inhibition of phosphorylation\textsuperscript{24}. As phosphorylation of Akt1 and its subsequent downstream signaling are directly related to signaling process, inhibition of Akt1 activation results in apoptosis. In this study, mitocurcumin-1 was found...
to bind the active loop of Akt1 in a similar mode as of the other inhibitor molecules. Further, binding of mitocurcumin-1 induced conformational changes at active site loop residues, as evidenced from this study. Together, we concluded that mitocurcumin-1/curcumin bind in a similar manner, resulting in the inhibition of phosphorylation of Akt1, thus inducing apoptosis.

STAT3 interaction with mitocurcumin-1 (Fig. 4) or curcumin (Fig. 5) occurred near its active site of phosphorylation, Y705. Such activation resulted in the dimerization and activation of its transcription activity by binding to DNA. The protein-ligand interaction energy and C docker energy obtained from interaction of mitocurcumin-1 with STAT3 is shown in Table 1. Simulation studies indicated the overall energy of the structure as -63113.52 Kcal/mol. Results showed that mitocurcumin-1 interacted with STAT3 by the formation two H-bonds—one with Arg688 and another with Gln692.

Docking interaction studies of STAT3 with curcumin showed the same number of H-bonds i.e., one with Arg688 and the other with Ser691 along with a π bond. The protein-ligand interaction energy and C docking energy with curcumin are shown in Table 1. Simulation studies gave the overall energy of the structure as 62501.99 Kcal/mol. These results indicated an overall stable protein-ligand interaction with high affinity of the ligands i.e., mitocurcumin-1 and curcumin, towards the active site. The difference in the development of H-bonds with mitocurcumin-1 or curcumin with the active site residues of STAT3 might be due to the differences in structural constrains.

It is established earlier that phosphorylation of STAT3 at Y705 results in the activation of STAT3, thereby inducing dimerization and activation of transcription by binding to DNA promoter sites. Earlier, we have shown that mitocurcumin-1 inhibits the phosphorylation of STAT3. This could be explained using the mechanistic model of interaction, wherein mitocurcumin-1 was found to possess strong affinity towards the active site and also interact with the amino acid residues in close proximity with Y705, thereby inducing conformational changes, resulting in inhibition of phosphorylation of STAT3. This was further supported from the results of interaction studies, wherein mitocurcumin-1 produced an Chi
rotational RMSD of 3.965 Å, compared to 3.126 Å of curcumin. Individual conformations also varied with RMSD of 2.8801 Å and 2.5803 Å for Gln 692, 1.1532 Å and 0.9486 Å for Tyr 705, 0.8605 Å and 0.8034 Å for Leu 706, 1.8361 Å and 1.5532 Å for Lys 707, 2.3490 Å and 2.3634 Å for T708, 4.6564 Å and 2.8238 Å for F710, respectively. Since there was a significant change in the overall chi rotational RMSD (Table 1), the phosphorylation sites at Y705 were masked, resulting in inactivation of the protein and, therefore, blocking of the dimerization. The high affinity of mitocurcumin-1 could be explained by its high interaction energy, compared to curcumin and the conformational change at the active site resulted in the inhibition of phosphorylation of STAT3 by mitocurcumin-1.

In conclusion, the study demonstrated for the first time, the molecular interaction of mitocurcumin-1 with Akt1 and STAT3 in inhibiting phosphorylation. Further, the affinity of mitocurcumin-1 binding at the active sites was very high, indicating its higher efficiency in the inhibition of phosphorylation as compared to curcumin. Thus, this study supported our recent observation that mitocurcumin-1 not only enhances the bioavailability, but also increases cytotoxicity to cancer cells by inducing apoptosis through increased inhibition of phosphorylation of Akt1 and STAT3.

Acknowledgments

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References