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Research Article

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Insilico Interaction of Sumo1 and Conjugating Enzyme UBC9 with Cyclodiene

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ABSTRACT

SUMoylation is a posttranslational modification occurring to a number of cellular proteins which play key roles in cellular process. This process is transient, highly regulated and is essential for normal function of cell.

Cyclodiene are the most potent family of pesticides. Residual contamination of this family of pesticides has been reported from all over the world. They are highly stable and persistent in the environment for decades. Uptake of humans leads to accumulation of these pesticides or their metabolites in tissues, mainly in adipose tissue. Evidently, this leads to neurological disorders, endocrine disruption and cancer.

Noncovalent interaction between Ubc9 and SUMO support SUMO chain formation In the present study the Cyclodienes were docked insilico to identify possible interaction with human SUMo1 and UBC9 (SUMoylation pathway enzyme, E2) by employing three different docking tools. The results were visualized in PyMol and 2D representations of the protein- ligand complexes were generated in LIGPLOT. Potential ligand binding pockets were generated for SUMO1 and UBC9 by MetaPocket2.0.

Endosulfan and, Endosulfansulfate show polar interactions at Asp 73 and Arg 70of SUMo1 (in addition non-polar interactions at lys48, pro58, Arg63, Phe 64, Ile 71 and Ala 72). The amino acids that contributed to the binding are represented in the potential binding pocket generated by Metapocket 2.0. Besides, Heptachlor, HEOM and Chlordan(which are structurally related to endosulfan) also show interaction at Arg 70.If proved experimentally, this interaction of SUMO1 at Arg 70 is noteworthy as this amino acid is required for association with E1 enzyme in SUMoylation process.

Endosulfan and, Endosulfansulfate show polar interactions at Ala 26 and Trp 16and non-polar interactionsat Thr 91, Glu 98, Arg 17, Lys 74 & Cys 93 of UBC9.

Keywords: Cyclodienes; Docking; SUMo; UBC9.

INTRODUCTION

Small ubiquitin like Modifiers (SUMo) are family of ubiquitous proteins in eukaryotes[4,8]. Monomers of SUMo proteins are of approximately of 100 amino acids in length and 12 kDa in mass (Schwartz et al., 2005). They are structurally similar to Ubiquitin, having homology of 18%. So far 4 SUMo isoforms in humans (SUMo1, 2/3 and 4), one in yeast and upto 8 isoforms in plants have been identified[27].

SUMo proteins conjugate to other cellular proteins[6], this process is called 'SUMoylation', which is a multistep process similar to Ubiquitination. Preproteins of SUMo are cleaved to their active forms by Sentrin-specific proteases (SENPs) to expose the diglycine motif at C-terminus that is essential for conjugation to targets. SUMo-activating enzyme complex (E1) transfers SUMo to a conjugating enzyme, Ubc9 (E2). SUMo is then ligated to target protein often to a lysine residue within the consensus motif ΨKXE (Ψ= aliphatic residue, X=any amino acid) or KXEXXpSP (pS= phosphoserine), by one of the several SUMo ligases (E3s)7,[11,12,19]. SUMoylation is a vital process. Desumoylation of target proteins is carried out by Sentrin-specific proteases[27]. A large number of proteins involved in key cellular processes are targets of SUMo. Thus, SUMoylation is an essential post translational modification for a number of cellular proteins.

SUMoylation is also a highly regulated process and deregulation is associated with loss of cellular homeostasis leading to diseases such as cancer, Alzheimer's and Huntington's[1,13,26]. In view of this, it is important to investigate whether any environmental pollutants/ toxins disrupt SUMoylation process.

Cyclodiene are genotoxic in nature, classified as class II b carcinogens by IARC[5,15]. These compounds are persistent and remain active in the environment for several decades. Due to their high lipid solubility and resistance to bio-degradation, biomagnification occur in organisms. Hence, contamination of the environment with these compounds is of great concern. Although, cyclodiene have been discontinued in use in many countries, there have been reports of soil, water and sediment contamination from all over the world. Mainly endosulfan is considered as Persistent organic pollutant (POP) [9,14,18]. High concentrations of endosulfan and endosufansulfate currently exist in the environment of those countries that have banned this chemical in recent years[25]. Some of the cyclodiene such as Heptachlor, chlordane are still in use for termite and pest control (NRDC2005) but banned in many countries. Cyclodiene affect major organs in human body so in an effort to identify the potential SUMoylation disruptors, we have performed docking SUMo1 and UBC9 with a number of structurally related compounds that belong to Cylodiene family.

TOOLS AND MATERIALS

For docking purpose structures of Cyclodiene were retrieved from PubChem, a repository of small molecules at NCBI. X-ray crystallographic/NMR structures of human SUMo1 and Ubc9 were retrieved from the PDB (www.rcsb.org/pdb).Docking was carried out by AutoDock4, Hex6.3 and Argus Lab. Docked complexes were visualized in Pymol and schematic 2-D representations of the complexes were generated in Lig Plot.

Hex version 6.3, an interactive protein docking and molecular superposition program.

Argus Lab is a molecular modeling, graphics, and drug design program[22].

AutoDock 4 is a reliable free open ware for molecular docking applicable in drug discovery and virtual screening designed. AutoDock4 is an improved version with enhanced accuracy with a capacity to interact with multi-CPU setups[16].

PyMol is a high quality 3D image visualization tool for small compounds as well as biological macromolecule.(http://www.pymol.org/)

Ligplotgenerates schematic 2-D representations of protein-ligand complexes and gives information about hydrogen bonds, hydrophobic interactions, atom accessibilities and their strengths[24].

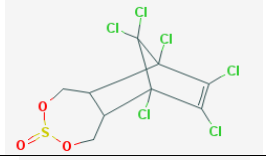
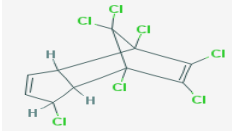
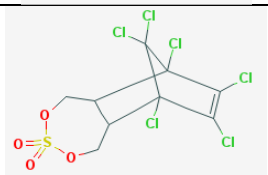
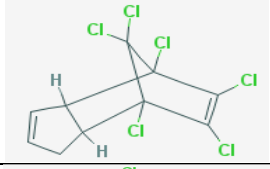
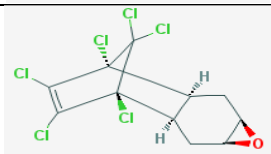
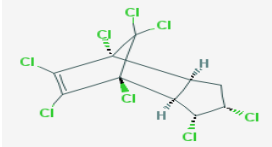
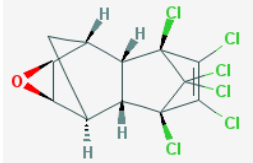
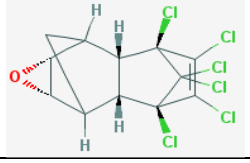
MetaPocket 2.0 is a web service that predicts drug binding sites in proteins using multiple computational approaches[28].

Methodology

Before Molecular docking, the structures of SUMo1 and UBC9 were downloaded from PDB (Protein Data Bank) and the chemical structures were retrieved from Pub chem. PDB Entries of protein molecular structures of SUMo1 (PDB ID - 1A5R) and Ubc9 (PDB ID - 1A3S) were prepared for docking by removing ligands and water molecules. PubChem entries of endosulfan, heptachlor, endosulfansulfate, dieldrin, chlordene, chlordan, HEOM, and endrin (refer to Table1.) were selected and converted into 3Dstructures in Marvin Sketch. AutoDock4 were performed by preparing receptor and ligand files in PDB format and then saved in PDBQT format. Hydrogen atoms were added to the protein molecules and selection of grid 40x40x40 Å with the distance between two connecting grid points of 0.375 Å. Grid docking is performed using Lamarckian genetic algorithm using 10 runs and lowest binding energy was noted. Additionally, Docking was carried out in Hex 6.3. Scoring values of total energy e-value and interactions of amino acids were noted. The interactions were visualized in PyMol and schematic 2D representations were generated in Ligplot. Molecular structures of all the proteins under study were also docked with each of the ligands using Argusdock. Argus Lab 4.0.1, most common and freely available software, was used for docking analysis (to calculate the binding energy requirements of different ligands with SUMo1 and UBC9). TheCyclodiene and target protein were geometrically optimized and "Argus dock" docking engine was used. Protein molecules were geometrically optimized and flexible mode is selected for the ligand (Cyclodiene).

Calculation types were set to "Dock" mode and ligand is selected as "flexible mode". Least energy represented the easy binding character of ligand and receptor energy values are shown in Table 2 and Table 4. Metapocket 2.0 was used to predict the potential protein-ligand binding sites by uploading the PDB ID and results were obtained.Metapocket 2.0 combines eight predicting methods namigly ConCavity (CON); Fpocket (FPK); GHECOM (GHE); LigsiteCS (LCS); PASS11 (PAS); POCASA (PCS); Q_SiteFinder (QSF); SURFNET (SFN).Results were shown in Table 3 and Table5.

Table1: Details of Cyclodiene selected for Docking and their Molecular properties (ref. PubChem)

Name of the compound	CID (PubChem)	Structure (PubChem)	Properties (PubChem)
Endosulfan	3324		MolWt: 406.92514 [g/mol] Molecular Formula: C9H6Cl6O3S XLogP3: 3.8 H-Bond Donor: 0 H-Bond Acceptor: 4
Heptachlor	3589		MolWt: 373.3177 [g/mol] Molecular Formula: C10H5Cl7 XLogP3-AA: 4.3 H-Bond Donor: 0 H-Bond Acceptor: 0
Endosulfansulfate	13940		MolWt: 422.92454 [g/mol] Molecular Formula: C9H6Cl6O4S XLogP3: 3.7 H-Bond Donor: 0 H-Bond Acceptor: 4
Chlordene	19519		MolWt: 338.87264 [g/mol] Molecular Formula: C10H6Cl6 XLogP3-AA: 4.2 H-Bond Donor: 0 H-Bond Acceptor: 0
HEOM (Hexachloroocta hydro- epoxy-methanonaphthalene)	6454255		MolWt: 368.89862 [g/mol] Molecular Formula: C11H8Cl6O XLogP3-AA: 3.7 H-Bond Donor: 0 H-Bond Acceptor: 1
Chlordan	12303038		MolWt: 409.77864 [g/mol] Molecular Formula: C10H6Cl8 XLogP3-AA: 4.9 H-Bond Donor: 0 H-Bond Acceptor: 0
Endrin	12358497		Molecular Weight: 380.90932 [g/mol] Molecular Formula: C12H8Cl6O XLogP3-AA: 3.7 H-Bond Donor: 0 H-Bond Acceptor: 1
Dieldrin	16211853		Molecular Weight: 380.90932 [g/mol] Molecular Formula: C12H8Cl6O XLogP3-AA: 3.7 H-Bond Donor: 0 H-Bond Acceptor: 1

RESULTS

Interactions of human SUMo1 (1A5R) with Cyclodiene.

PubChem entries of the chosen compounds were docked to SUMo1 (PDB ID -1A5R) in AutoDock 4, Hex 6.3 and Argus lab. The binding energies of all three dockings and interacting amino acids are tabulated in Table2.

As shown in Table 2, apart from unique interactions, structurally more closely related cyclodiene show common interactions at several amino acids. Chlordan, heptachlor, endosulfan and endosulfansulfate all show interactions at lys48, pro58, Arg63,Phe 64, Arg 70, Ile 71 and Ala 72 (excepting endosulfan at Arg63 and Phe 64 as well as heptachlor at Ile 71). HEOM also showed interaction with pro58, Arg63, Phe 64and Arg 70. Endrin and chlordene show interactions at Asp15, Lys 17 (except dieldrin), Gly 81, Glu83 and Met 82 apart from other interactions. Six amino acids, lys48, pro58, Arg63, Arg 70, Ile 71 and Ala 72, contribute to the binding pocket as predicted by MetaPocket 2.0 (Table3). Polar interactions were shown at Asp73 and Arg 70 with endosulfan and endosulfansulfate, respectively in docking by Hex6.3 in Figure1.

Table 2: Interactions of SUMo1 (1A5R) with Cyclodienethrough binding affinity scores and energy values

Name of the Compound	CID (PUB CHEM)	Lowest binding energy obtained in AutoDock (K Cal/Mol)	E-total (K Cal/mol)obtained in Hex and interactions in PyMol	Interactions visualised in Lig plot	Energy values obtained in Argus lab (K Cal/mol)
Endosulfan	3224	-5.9	(E-Total= -344.35) P I-Asp73 NPI-Lys45, Lys48,Arg70	Lys45,Lys 48, Pro58, Arg 70, Ile71, Ala 72, Asp73, Asp74,	Asp73=-10.2345
Heptachlor	3589	-5.5	(E-Total= -253.49) NPI-Ile71, Phe64	Lys48, Pro58, Arg63, Phe 64,Lue65, Phe66 , Arg 70, Ala72	Ile71=-9.4583
Endosulfansulphate	13940	-6.1	(E-Total= -306.20) PI-Arg70 NPI-Lys-48	Ala72,Ile71,Phe64, Arg70, Arg63, Pro58, Lys48	Arg70=-9.8786
Chlordene	19519	-5.3	(E-Total= -321.05) NPI-Asp-15, Gly-14	Leu13, lys14, Asp15	Asp15=-6.46151, Gly14=-6.69086
HEOM	6454255	-6.0	(E-Total= - 381.48) NPI-Arg63	Arg63,Phe64,Arg70,Leu62 ,Met59, Pro58,Ser61, Asn60	Arg63=-11.8396,
Chlordan	12303038	-5.5	(E-Total= -290.79) NPI-Phe -64	Phe64, Arg63, Pro58, Lys48, Al72, Arg70, Ile71	PHE-64=-13.6958
Endrin	12358497	-5.8	E-Total= -453.79 NPI-Glu-83, Met-40	Met40, Glu83, Met82, Glu20, Gly81,Asp15, Lys17, Thr41	GLu83=-6,53703
Dieldrin	16211853	-5.5	(E-Total= - 373.57) No polar and non-polar interactions	Ile22, Met40, Asp15,Met82,Gly81, Glu83, Lys17	GLy81=-7.32592

(PI- Polar Interaction, NPI- Non Polar Interaction)

Fig.1: Interaction of SUMo1 with endosulfan

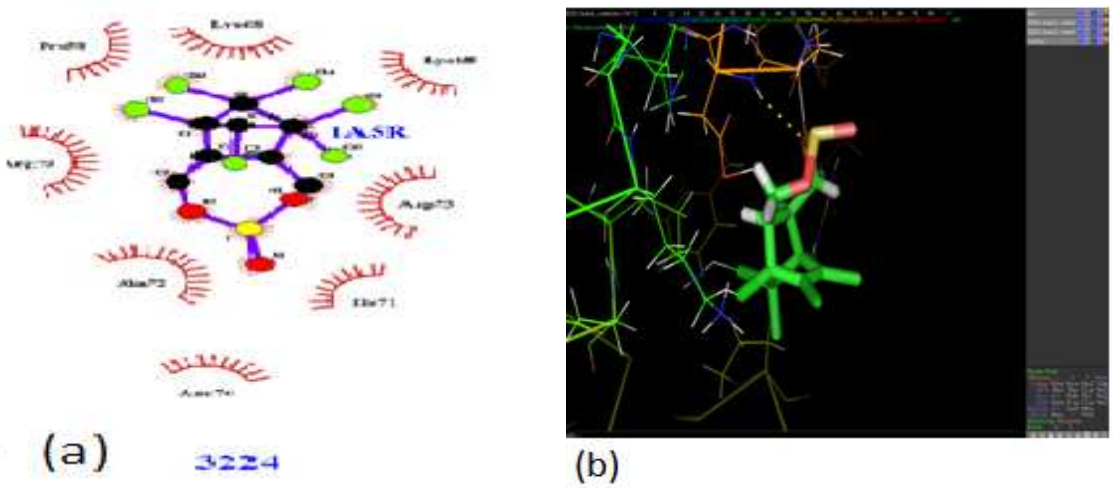


Figure 1: Visualization of SUMo1- Endosulfan complexes generated by docking using Hex.
(a) Schematic 2D representation of SUMo1- Endosulfan complex generated in Ligplot: hydrophobic contacts are indicated by quarter open circles. (b)PyMol visualization of SUMO 1-endosulfan complex: the dotted line represents polar interaction with Asp73. (Also refer table 2)

Fig. 2: Interaction of SUMo1 with endosulfansulfate

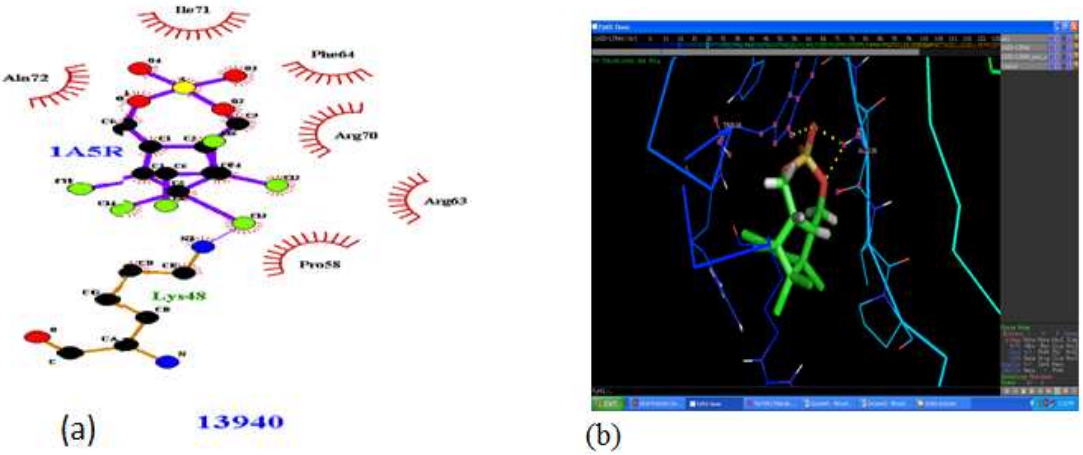


Fig2: Visualization of SUMo1- Endosulfansulfate complexes generated by docking using Hex: (a) Schematic 2D representation of SUMo1- Endosulfansulfate complex generated in Ligplot: hydrophobic contacts are indicated by open circles with radiating spokes. (b)PyMol visualization of SUMO 1-endosulfansulfate complex: the dotted line represents polar interaction with Arg70. (Also, refer Tble 2)

In Tding sites are listed. The aminoacids in red colour shows ligand –protein binding site residues predicted to interact with SUMo1 with cyclodiene (endosulfan, heptachlor, endosulfansulfate, dieldrin, chlordene, chlordan, HEOM, and endrin) with Hex 6.3 and analyzed with Ligplot and PyMol.

Table3:List of the amino acids contributing to the binding pocket of SUMo1 (1A5R), generated by "Metapocket".

HEADER binding site ID: 1					
RESI	LYS_A^48^	PRO_A^58^	ARG_A^70^	MET_A^59^	ASN_A^60^
RESI	VAL_A^57^	ILE_A^71^	ASP_A^73^	ALA_A^72^	GLN_A^53^
RESI	GLU_A^49^	SER_A^50^	ARG_A^63^	ARG_A^54^	SER_A^61^
HEADER binding site ID: 2					
RESI	GLN_A^55^	GLN_A^92^	GLN_A^94^	ARG_A^54^	GLY_A^96^
RESI	GLY_A^97^	THR_A^95^	HIS_A^98^		
HEADER binding site ID: 3					
RESI	GLN_A^53^	VAL_A^57^	PRO_A^58^	GLU_A^49^	

Interaction of human UBC9 (PDB ID-1A3S) with cyclodiene

The human UBC9 (the conjugating enzyme, E2, of SUMoylation pathway) was docked with cyclodiene as described in methodology section. PubChem entries of the chosen cyclodiene were docked to UBC9 (PDB ID -1A3S) in AutoDock 4, Hex6.3 and Argus lab. (Table 4) summarizes the binding energy values and interacting amino acids.

Endosulfan, endosulfansulfate, chlordan all show interactions with Typ16, Arg 17, Val 25, Ala 26 and Pro28. Chlordene, endrin, dieldrin, and HEOM show interactions at Pro72, Pro73, Lys 74, Tyr87, Thr 91, Val 92, Cys 93, leu 97 and Glu 98 with one /two amino acid exceptions (chlordane at pro and Cys; Dieldrin at pro 73; HEOM at Tyr87 and Thr 91). All these amino acids contribute to the binding pockets of UBC 9 predicted by MetaPocket 2.0.

Table 4: Interactions of UBC9 (1A3S) with Cyclodiene through binding affinity scores and energy values

Name of the Compound	CID (PUB CHEM)	Lowest binding energy obtained in AutoDock (K Cal/Mol)	E-total obtained in Hex and interactions shown in PyMol	Energy values obtained in Argus lab (K Cal/mol))	Interactions visualised in Lig plot
Endosulfan	3224	-5.4	-395.42 PI- Ala-26 Trp-16	Trp16=-12.8484	Val27, Val25,Arg17, Pro28,Trp16, Ala26
Heptachlor	3589	-5.8	-364.51	Leu97= -7.78 Tyr87= -14.62	Leu97, Lys74,Thr91,Tyr87, Cys93,Glu98,Val92
Endosulfansulfate	13940	-5.9	-439.64 PI -Ala-26 Trp-16	Trp16 =-12.98 Ala26 = -11.78	[P.I.(Polar interaction)-Trp16-3.15, Ala26-3.05],Val25,val27,Pro28,Arg17
Chlordene	19519	-6.0	-379.84NPI - Thr91	Thr91=-4.8	[Thr91-P.I], Glu98,Lys74,Pro73,Leu94,Val92,Leu97
HEOM	6454255	-6.1	-383.28 NPI-Glu98	Glu98=-6.53	[Glu98-P.I],Cys93,Val92,Leu97,Leu94,Lys74,Pro73,Pro72
Chlordan	12303038	-5.6	-398.07 NPI-Arg17	Arg17= -9.03	[Arg17-P.I], Arg13,Pro28,Val27,Trp16,Val25,Ala26
Endrin	12358497	-6.1	-467.73 NPI-Lys74	Lys74=-8.32	[Lys74-P.I],pro73,pro72,Leu97,Val92,Tyr87,Thr91,Cys93,Glu98
Dieldrin	16211853	-6.2	-390.57 PI-Glu98,Cys93	Glu98= -5.76 Cys-93= -6.63	Cys93-[2.64,Leu-2.99],Val92,Tyr87,Thr91,ley97,Lys74,Pro72,Glu98

(PI- Polar Interaction, NPI- Non Polar Interaction)

Fig. 3: Interaction of UBC9 with endosulfan

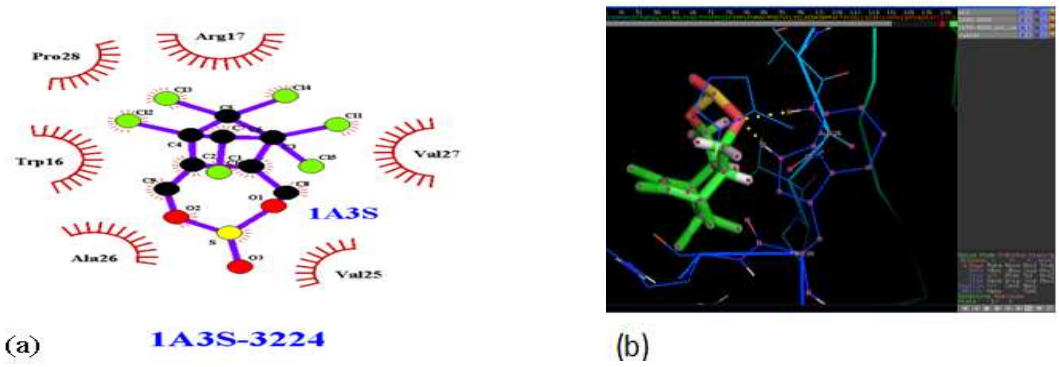


Figure 3: UBC9- Endosulfan complex docked in Hex: (a) Schematic 2D representation of UBC9-Endosulfan complex generated in Ligplot: hydrophobic contacts are indicated by quarter open circles. (b) PyMol visualization of UBC9-endosulfan complex- the yellow dotted line represents polar interaction withTrp16 (Also refer table 2)

Fig. 4: Interaction of UBC9 with endosulfansulfate

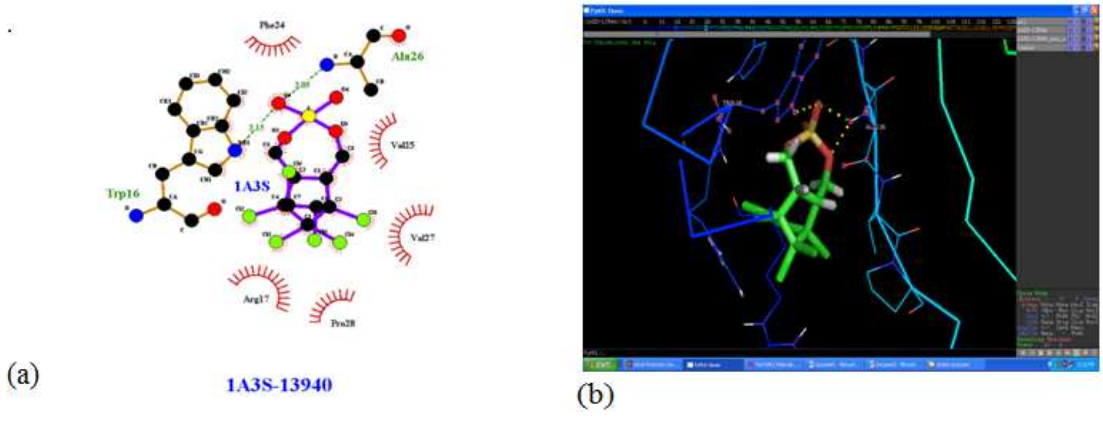


Figure 4: UBC9-endosulfansulfate complex docked in Hex. (a)Schematic 2Dof UBC9-endosulfan sulfate complex generated in Ligplot: UBC9 (1A3S) shows hydrogen bonding at Trp 16 and Ala 26 (indicated by green broken lines) with hydrogen bond length of 3.15 and 3.05, respectively. Hydrophobic contacts are indicated by quarter open circles with reddish prickles.(b)PyMol visualization of UBC9-endosulfan sulfate complexes (docked using Hex): Polar interaction seen in UBC9 with endosulfansulfate at Trp16 and Ala26.

Meta Pocket results of UBC9 (1A3S): Potential ligand-binding sites in UBC9 (1A3S) predicted by multiple computational algorithms.Predicted top three ranked binding sites (ID Nos. 1–3) and the residue numbers of amino acids that form the potential binding sites are listed. The aminoacids in red colour shows ligand –protein binding site residues predicted to interact with UBC9 with cyclodienes (endosulfan, heptachlor, endosulfansulfate, dieldrin, chlordene, chlordan, HEOM, and endrin) simulation with Hex 6.3 and analyzed with Ligplot and PyMol.

Table5: List of the amino acids contributing to the binding pocket of UBC9 (1A3S), generated by "Metapocket"

HEADER binding site ID: 1					
RESI	TRP_A^16^	HIS_A^20^	ALA_A^26^	VAL_A^27^	ARG_A^17^
RESI	VAL_A^25^	ARG_A^13^	PRO_A^28^	LYS_A^30^	TRP_A^41^
RESI	MET_A^36^	LYS_A^14^	THR_A^29^	THR_A^35^	GLU_A^12^
RESI	PHE_A^24^	GLU_A^42^	ILE_A^109^		
HEADER binding site ID: 2					
RESI	VAL_A^92^	CYS_A^93^	LEU_A^94^	SER_A^95^	LEU_A^97^
RESI	GLU_A^98^	LEU_A^63^	PHE A^64^	LYS_A^65^	TYR_A^68^
RESI	SER_A^71^	PRO_A^72^	PRO_A^73^	ASP_A^67^	PRO_A^69^
RESI	SER_A^70^	ASP_A^66^	LYS_A^74^	GLU_A^99^	CYS_A^75^
RESI	THR_A^91^	LYS_A^76^	TYR_A^87^		

DISCUSSION AND CONCLUSION

Bioaccumulation of Environmental pollutants (Cyclodiene) is known to cause endocrine disruption and diseases such as neuro-degeneration and cancer. Since SUMoylation is highly a regulated and is an important post translational modification to several cellular proteins, disruption of this is deleterious to cellular homeostasis. In view of this identification of the environmental pollutants and chemicals that can cause disruption of SUMoylation gain importance. In an effort to progress in this direction, we performed insilico docking of compounds with X-ray crystallographic structures of human SUMo1and the SUMoylation pathway enzyme, UBC9.

As described in the results section, several amino acids of human SUMo1 (PDB entry 1A5R) have shown consistent interaction with cholrdan, heptachlor, endosulfan and endosulfansulfate. Of the notable is the interaction at amino acid Arg70 and Asn 60. The Arg70of SUMo1 is shown to be one of the amino acids that interact with Sae2 (a protein that form E1 enzyme complex along with Sae1)[12]. Endrin, dieldrin and aldrin (structurally closely related compounds) show binding (in Hex) with Sumo 1 at Gly81andGlu83 amino acids.X-ray crystallographic studies of Ubc-9 - Sumo-1 complexes revealed that SUMo1 and Ubc9 interact at several amino acids which include Gly81 and Glu83of sumo1.

Crystallographic studies by Duda et al and Knipscheer et al revealed that Ubc9's α-helix1, particularly Arg13 and Arg17, interacts noncovalently with Sumo 1 as well as with Uba2. Mutation at Arg17 is lethal for survival. It is interesting to observe that in our docking studies chlordane shows polar interaction at Arg17andEndosulfan and chlordane show non polar interactionsat Arg17.

The present observations gain significance, if proven experimentally. The severe consequences observed in humans on exposure to endosulfan may be due to multiple effects, one of which might be interaction with sumo1 in cells where bioaccumulation occurs.

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