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Research Article

IN SILICO MUTATION STUDIES ON THE LIPOPHILIC CHANNEL OF HUMAN OXIDO SQUALENE CYCLASE (OSC) DURING SUBSTRATE ENTRY.

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ABSTRACT
Oxidosqualene cyclase (OSC) is an eukaryotic monotopic membrane protein which converts oxidosqualene to lanosterol, forming the steroid scaffold in a single reaction. Prokaryotes posses an enzyme similar to OSC that is Squalene-Hopene Cyclase (SHC). Unlike SHC, OSC can only accept 2,3-oxidosqualene as the substrate. Both these enzymes have an lipophilic channel which leads into the active site through an narrow constriction. The lipophilic channel plays an important role in the entry of substrate into the active site. Although the architecture of the lipophilic channel is clear from the crystal structure, its role in substrate-enzyme interaction is still far being fully understood. The *insilico* mutation studies on the active site amino acid residue Cys456, of the lipophilic channel has revelead that the double mutation of Cys456Ser and active site residue Asp455Cys would block the entry of substrate into the active site. Thus leading to the loss of ligand and active site interactions.
KEYWORDS: Single mutation, double mutation, multiple mutation, insilico site-directed mutation.

INTRODUCTION

Oxidosqualene Cyclases (OSCs) and Squalene-Hopene Cyclases (SHCs) are the monotopic membrane proteins which play an major role in triterpenoid biosynthesis, where they convert acyclic isoprenoid precursors into tetracyclic and pentacyclic compounds[1]. The structural change considered to be most important in cholestrol and ergosterol biosynthesis occurs along the steroid nucleus development pathway[2]. OSCs are the taxonomic markers catalysing the conversion of 2,3-oxidosqualene into lanosterol in nonphotosynthetic organisms[3] (fungi and mammals). However cycloartenol and othertetra- and pentacyclic triterpenes are the products of 2,3-oxidosqualene conversion in photosynthetic plants. Prokaryotes posses an enzyme similar to OSCs: SHCs convert squalene into hopene or diplopterol, pentacyclic triterpene precursors of hopanoids[4]. Squalene and OS-cyclases have related sequences and should therefore have similar spatial structures and they are known for catalysing the monoenzymatic reactions[2,5]. The process is initialised by protonation of the substrate, four or five rings are formed, several chiral centres are created, hydride and methyl groups are 1,2 shifted and a proton is extracted (or a hydroxyl added, as in diplopterol formation) and the cyclic product is released. The catalytic mechanism for the polycyclization reactions of OSC involves several reaction steps. First, 2,3-oxidosqualene adopts a pre-organized chair–boat–chair conformation[6]. This enzyme catalyzes the cyclization of 2,3-oxidosqualene (OS) to lanosterol[7-12]. Surprisingly, SHC recognises squalene as well as 2,3-oxidosqualene as its physiological substrate while all the known OSCs accept only 2,3-oxidosqualene as substrate[2]. Although the architecture of the lipophilic channel was clear from the crystal structure[2], but its role in substrate–enzyme interaction is still far from being fully understood. The X-ray Crystallographic structure of OSC[3] assigns Cys456 and Cys533 to act as hydrogen-bonding partners with Asp455 and thus to contribute to the required acidity of Asp455. A critical Cystein residue is present inside the active site of all eukaryotic OSCs, characterizing the catalytic motif DCTAE that is highly conserved[3]. We present the insilico mutation analysis of Cys456 present in the lipophilic channel of OSC.

MATERIALS AND METHODS

The residues in the lipophilic channel and active site of OSC (PDBID: 1W6J) are carefully located and mutated using the software SCHRODINGER prime and maestro visualization module. In the lipophillic channel, one best active residue was reported to be Cys456, that plays a main role for the entry of substrate into the active site based on the active site intearactions with the ligand and the residue[3]. In the present work, this Cys456 residue has been mutated with all the remaining 19 amino acids. And not only their interactions at the active site but also the single point energy values for all the mutants and the native molecule are been calculated. It is observed that not all the mutation are favorable, only few single point energies would support the mutations. Later based on the structural similarity of cysteine and serine, favorable mutation of cysteine with serine was choose to be the best compatable mutation. And further Single, Multiple and Double mutations were carried out as per the follwing d esciption: In the Single mutation, Cys456 is alone mutated to Serine456 leaving behind all the cysteine residues in the sequence undisturbed (C456S). During multiple mutation, all the cysteines (C6S/ C29S/ C112S/ C231S/ C233S/ C243S/ C410S/ C449S/ C471S/ C484S/ C533S/ C567S/ C584/ C598S/ C609S/ C616S/ C636S/ C676S/ C700S) in the sequence are mutated into Serine except the Cys456. But in double mutaion, the two active residues are been mutated as D455C and C456S. Their corresponding interactions and energy values are been calculated and observed.

RESULTS

Energy calculations and active site interactions had played an major role in understanding the entry of substare into the active site through the lipophilic channel. Insilico site directed muation results show that there is not much variation in the interations like that of Native OSC molecule (Fig. 1) and the same interactions are been maintained when Cys456 is been mutated with all the other amino acid residues except the following few i.e.,C456K, C456M, C456S (Fig. 2), C456T.

Apart from interactions, the single point energy values of C456A, C456N, C456D, C456G, C456P, C456S, C456T, C456V have best minimal relative energy than other aminoacid mutations, which were found to have very high energy values. The interactions of the single mutation is been

displayed in the Fig.2 with their relative energy difference value of ΔE= 8.4668 kcal/mol and interactions of the mutiple mutation are been displayed in the Fig. 3 with an relative energy difference value of ΔE=164.5400 kcal/mol. Very high increase in relative energy difference was found with double mutation, where the interactions are displayed in the Fig. 4 and relative energy difference was found to be ΔE= 209.2082 kcal/mol (Table 1).

DISCUSSION

From the present study it is observed that the single mutation would least effect the entry of substrate into the active site through the lipophilic channel due to low increase in energy (~8.4668 kcal/mol) difference between native OSC molecule and single mutation single point energies. Due to very high increase in energy level (~164.5400 kcal/mol) the multiple mutation was observed to partially inhibit the entry of substrate. Instead double mutation would completely inhibit the entry of substrate due to loss of interaction with the ligand (R711733) and Cys456 and there is an very high increase in energy level (~209.2082 kcal/mol) between native OSC molecule and double mutation single point energies.

CONCLUSION

The present insilico site directed mutations studies in OSC have concluded that when a single Cystein456 residue present at the lipophilic channel is been mutated with the best compatible amino acid serine, could least inhibit the entry of substrate because of very little increase in the single point energy level . But when all the cysteine present in Human OSC are mutated except the lipophillic channel cysteine456 there was an partial inhibition for the entry of substrate due to high increase in the single point energy level. But when both active site residue Asp 455 and the lipophilic channel cysteine456 are mutated there was an complete inhibition for the entry of substrate because of very high increase in the single point energy level.

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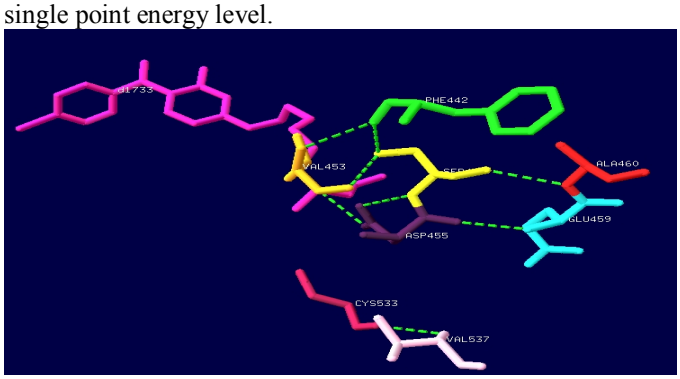

Fig. 1 : Interactions shown in the native OSC active site

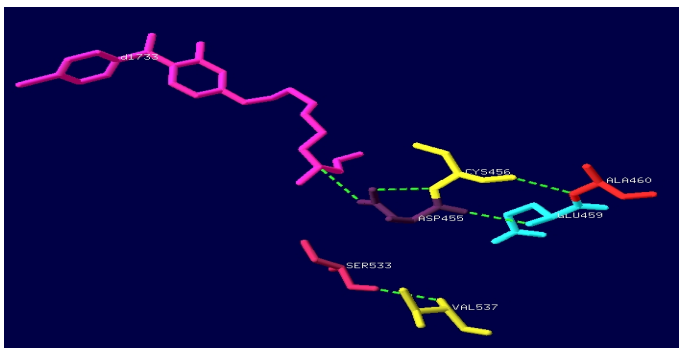

Fig. 2 : Interactions shown in the OSC active site with single mutation C456S

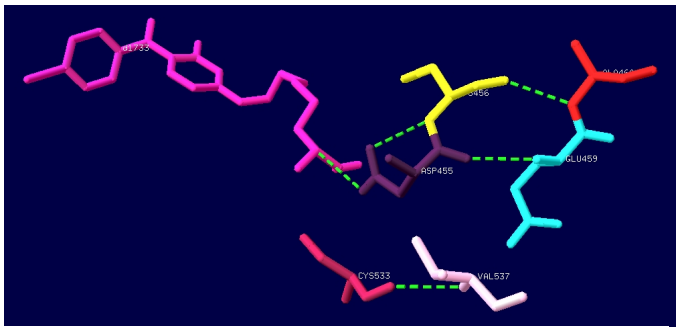

Fig. 3 : Interactions shown in the OSC active site with multiple mutations

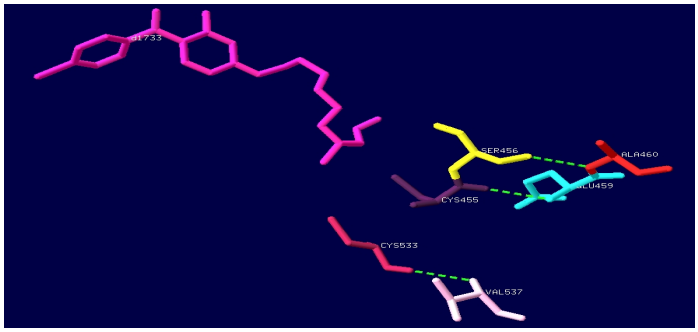

Fig. 4 : Interactions shown in the OSC active site with double mutations

Table 1 : List of interactions and energy values in native OSC and its single, mutiple and double mutants

Type of mutation	Hydrogen Bond interactions			Single Point Energy (kcal/mol)	Relative ΔE (kcal/mol)
	Residue (I)	Residue (II)	H-Bond Length (A^o)		
Native OSC	Cys 456	Ala 460	2.99	-22337.5156	0
		Asp 455	2.94		
	Asp 455	R711733	3.10		
		Glu 459	2.95		
	Cys 533	Val 537	3.00		
Single Mutation (C456S)	Ser 456	Ala 460	2.99	-22329.0488	8.4668
		Asp 455	2.94		
		Phe 422	3.08		
		Val 453	2.97		
	Val 453	Phe 422	3.10		
	Asp 455	R71173	3.10		
		Glu 459	2.95		
	Cys 533	Val 537	3.00		
Multiple Mutation	Cys 456	Ala 460	2.99	-22172.9756	164.5400
		Asp 455	2.94		
	Asp 455	R711733	3.10		
		Glu 459	2.95		
	Ser 533	Val 537	3.00		
Double Mutation (D455C/C456S)	Ser 456	Ala 460	2.99	-22128.3078	209.2082
	Cys 455	Glu 459	2.95		
	Cys 533	Val 537	3.00		

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