

Analysis of interaction of atropine with phospholipase A2 (1th6.pdb)

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Abstract

The toxic tropane group alkaloid atropine, used chiefly as a muscle dilator during eye surgery, and, recreationally in nerve gas exposure after a terrorist attack, has also got the potentiality to inhibit phospholipase A2 proteins which are heat-stable, calcium-dependent enzymes catalyzing the hydrolysis of the 2-acyl bond of 3-n-phosphoglycerides, and, involved in cell signaling processes, such as inflammatory response. The paper presents UCSF Chimera beta version 2199 rendering and ArgusLab v 4.0 docking results in 1th6.pdb crystal structure of 133-residue long phospholipase A2 in complex with atropine at 1.23Å resolution. The enzyme molecule comprising five helices and two sheets had an atropine (1R,5S)-8-Methyl-8-Azabicyclo[3.2.1]Oct-3-yl(2R)-3-Hydroxy-2-Phenylpropanoate: C₁₇H₂₃NO₃) interacting with active site amino acids tyr28 (Y28) and asp49 (D49) of the polypeptide chain through H-bonds of 2.686 Å⁰ and 2.629 Å⁰ respectively. Atropine ligand could well dock into the active site of the enzyme even after few mutations. In unmutated molecule, best docked position was shown by pose 9 with RMSD = 3.995034 Å⁰ and energy requirement as -6.71 Kcal/mol. The dock pose 1 with lowest energy requirement as -7.632 Kcal/mol. showed RMSD = 6.051521 Å⁰.

Introduction

Atropine, derived from *Atropa belladonna* (deadly nightshade) or some other plants of Solanaceae, is a depressant of parasympathetic nervous system with its primary role in dilating the pupil during eye surgery (<http://www.phytomedical.com/>), but, also used in treating Parkinson's disease, peptic ulcers, diarrhea, bronchial asthma, and, nerve gas poisoning (Demeyer *et. al.*, 1994; Bania *et. al.*, 2004). It is chemically 1R,5S)-8-Methyl-8-

Azabicyclo[3.2.1]Oct-3-yl(2R)-3-Hydroxy-2-Phenylpropanoate with molecular formula as $C_{17}H_{23}NO_3$.

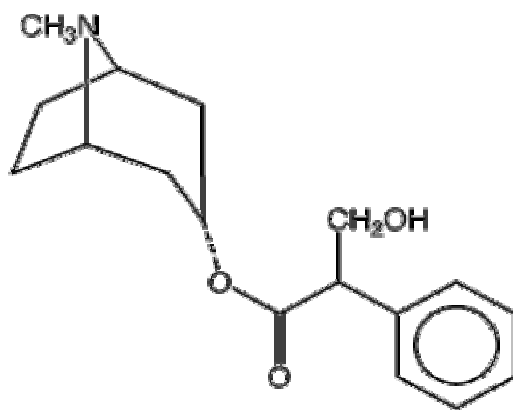


Fig.1 Atropine

Phospholipases are enzymes involved in lipid metabolism and are important probes of structure-function relationships in biological membranes. (Dawson, 1973). Phospholipase A2 represents a class of heat-stable, calcium-dependent enzymes catalyzing the hydrolysis of the 2-acyl bond of 3-n-phosphoglycerides, and, named as such to denote its 2-acyl specificity (Uthe, 1971). Phospholipase A2 has been isolated from mammalian pancreas, snake and bee venoms (Verheij *et. al.*, 1983; Chang *et. al.*, 1987; Bhat *et. al.*, 1991).

Low molecular weight phospholipase A(2)s (PLA(2)s) are now known to be involved in the release of arachidonic acid, a precursor for the biosynthesis of pro-inflammatory eicosanoids (Forst *et. al.*, 1986). Inhibition of PLA2 by lipocortins has been shown to be resulting in decrease in inflammations (Six *et. al.*, 2000). Singh *et. al.* (2006) determined the crystal structure of a complex of PLA2 with atropine at 1.23Å resolution. The high-quality observed electron densities for the two compounds allowed the accurate determination of the atomic positions in the complex. The structure revealed that the atropine bound to the enzyme at the substrate - binding cleft and its position was stabilized by hydrogen bonds and hydrophobic interactions. The PDB code of the complex is 1th6.pdb (<http://www.pdb.org/>).

Structure of *1th6.pdb*

Structural elucidation of 3D coordinates of PLA2 enzyme complexed with atropine (*1th6.pdb*) has been achieved by UCSF Chimera beta version 1 build 2199, platform: Win 32 (Pettersen *et al.*, 2004). Fig.2 presents the general 3D structure of PLA2 enzyme and atropine bound in its active site.

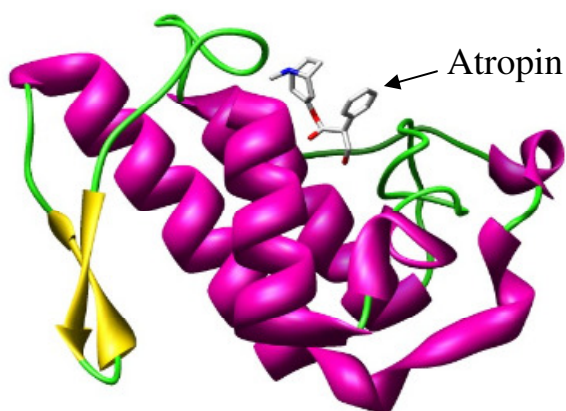


Fig.2 PLA2 and atropine (Only backbone of the enzyme shown in ribbon form)

Sequence of amino acids in the enzyme molecule was as shown below, where helices are highlighted in gray and β -sheets are highlighted in black.

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SLLEFGKMILEETGKLAIPSYSSYGCYCGWGGKGTPKDATDRCCFVHDCCYGNLPDCNPKSD  
RYKYKRVNGAIVCEKGTSCENRICECDKAAAICFRQNLNTYSKKYMLYPDFLCKGELKC133
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Fig.3 presents H-bonds that CH_2OH end of atropine has successfully formed with water molecules (HOH205, HOH212, HOH238), asp49 (D49) and tyr28 (Y28).

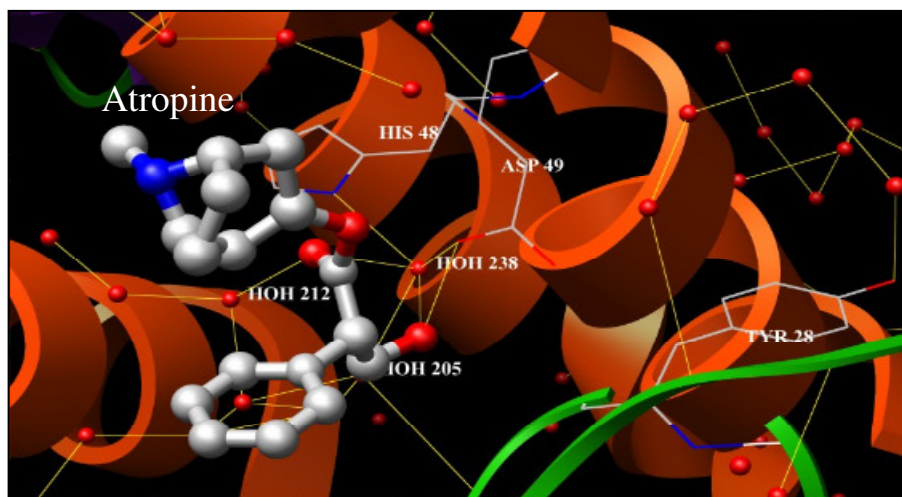


Fig.3 H-bond stabilization of atropine (Part of the enzyme's active site)

H-bonding with Tyr28 was, however, transient. His48 helped to stabilize atropine molecule by bonding with HOH238. At least, three H-bonds occurred between atropine and water molecules. H-bond length between atropine and Y28 was 2.686 \AA , and, that between atropine and D49 was 2.629 \AA . Interaction of atropine led to some alteration in its structure, but, the enzyme did not show any conformational change. Residues that formed hydrophobic interactions with these compounds are not identical because their positions do not exactly superimpose in the large substrate-binding hydrophobic channel (Singh *et. al.*, 2006).

Ligand group docking

Molecular docking software ArgusLab 4.0.1 (Thompson, 2004) was used for atropine group ligand docking exercise in *1th6.pdb* crystal structure to understand the degree of stability of ligand binding that can be used to inhibit PLA2 enzyme. Lower RMSD (Root Mean Square Deviation) at minimum required energy level is the pre-requisite for successful docking exercise.

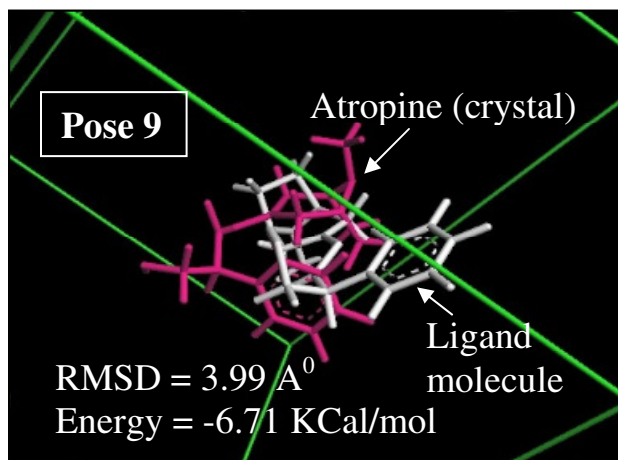


Fig.4 Candidate Pose 9 in ArgusLab docking

Grid calculations during Argusdock with scoring function as *Ascore* for flexible ligand group were as follows -

Grid min (x,y,z) : 41.5118, 28.246, -2.2952

Grid max (x,y,z) : 63.1118, 44.246, 14.5048

Grid dimensions: 55 x 41 x 43

Total number of grid points : 96965

Number of ligand torsions was 5, and, number of target torsions was 0. 150 candidate poses were attempted in 6 seconds' time, and, after clustering, 92 final unique configurations were presented by the software. Energy-wise, pose 1 was the best ligand pose with energy requirement as low as -7.632 KCal/mol (RMSD = 6.051521 Å⁰). However, pose 9 was the best in terms of RMSD (= 3.995034 Å⁰) with energy requirement as -6.71 KCal/mol.

***In silico* mutations**

DeepView / Swiss-PdbViewer Version 3.7 (SP5) (Guex et. al., 2006) was used to insert mutations in the protein by amino acid substitutions. In one such mutated molecule, His48 was substituted by Asp48. Despite D48, H-bonding occurred only with D49 as usual.

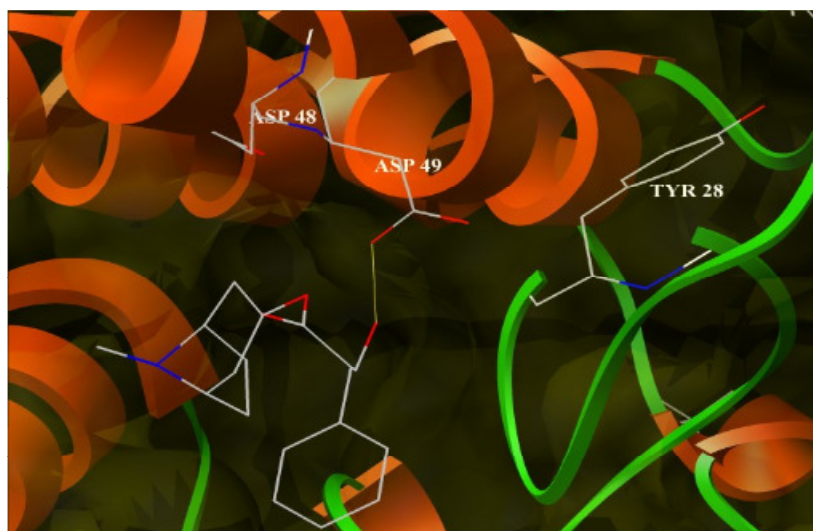


Fig.5. Atropine shows H-bonding with Asp49 and not with Asp48 (Mutated PLA2)

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