Molecular Dynamics Simulations and MM-PBSA Binding Free Energy Estimation in GK241-sPLA$_2$ GIIA complex

Sofia Vasilakaki[a], Georgios Leonis[b], Manthos G. Papadopoulos[b], Thomas Mavromoustakos[a], Georgios Kokotos[a]

[a]Department of Chemistry, University of Athens, Panepistimiopolis, Athens 15771, Greece;

[b]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., Athens 11635, Greece

Introduction

Inflammation is the complex biological response of an organism immune system to conditions such as cell damage and infection by pathogens. Potent mediators of inflammation are eicosanoids arising from arachidonic acid. Phospholipases A_2 are a superfamily of enzymes that play a key role in the hydrolysis of *sn*-2 bond of glycerophospholipids, producing mainly arachidonic acid and lysophospholipids (1). Selective inhibition of enzymes belonging to this family has been a consisted target for pharmaceutical purposes.

Kokotos and coworkers have developed a novel α-ketoamide derivative **GK126** (Table 1) which has shown inhibition against sPLA$_2$ (IC_{50}=0.30±0.06μM) (2). Extensive modifications of its structure have been carried out, aiming to improve its inhibitory activity. **GK241** -which was generated by **GK126** by changing the α-amino acid group from leucine to valine- showed an improved IC_{50} value and therefore, further computational techniques were applied in order to understand its binding properties.

GK289 was generated from **GK126** by replacing the long aliphatic chain with a four carbon chain bearing an aromatic ring and according to *in vitro* results showed a very low inhibitory activity against sPLA$_2$. Thus, we decided to run computational calculations on **GK289** -sPLA$_2$ complex and use the results for comparison with the **GK241** -sPLA$_2$ complex. This would allow us to elucidate the influence of the structural changes on their binding motif.

Table 1: 2-Oxoamide derivatives.

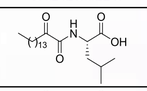
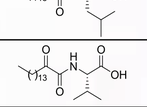
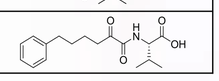
Compound	Structure
GK126	
GK241	
GK289	

Table 3: Energetic analysis for GK241 and GK289 complexes as obtained by MM-PBSA.

Energy (kcal mol $^{-1}$)	GK241	GK289
ΔG_{vdW}	-36.85 ± 0.23	-27.96 ± 0.23
ΔE_{elec}	-527.39 ± 0.81	-515.92 ±0.71
ΔG_{PB}	519.11 ± 0.77	505.90 ±0.73
$\Delta G_{MM-PBSA}$	-49.57 ± 0.28	-41.37 ± 0.41

Table 2: Interactions between the inhibitors GK241 / GK289 and the active site of sPLA$_2$

Drug Interaction	Occurrence (%)
GK241@ -COO$^-$ … Gly31@NH	85
GK241 @ -COCONH / -CONH … Gly29@NH	65
GK241@ -COO$^-$ … Lys62@NH	10
GK289@ -COO- … Gly31@NH	75
GK289@ -CONH … Gly31@NH	28
GK289@ -COCONH … Gly29@NH	6

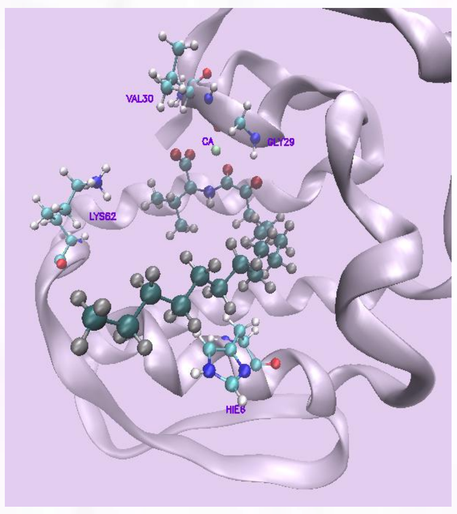


Figure 1. GK241 at the active site of sPLA$_2$ close to Lys62 .

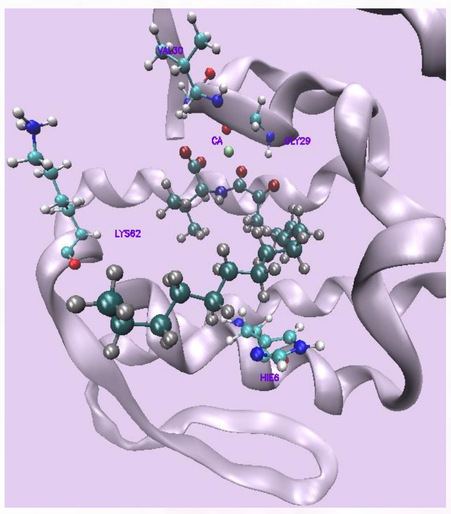


Figure 2. GK241 at the active site of sPLA$_2$ away from Lys62. Interactions between the key residues of the active site and the functional groups of GK241 are presented most of the simulation time.

Methods

Sybyl 8.0 molecular sketcher by TRIPOS was used for the design and optimization of the structures. Powell energy minimization algorithm was applied in order to carry out energy optimization of the structures and simulated annealing was used in order to investigate their conformational space. Furthermore, we used Gold 5.2 to dock the structures and generate possible binding motifs at the active site of GIIA sPLA$_2$.

For the molecular dynamics simulations, AMBER11 (3, 4) was used for the calculation of the atomic point charges (using AM1-BCC model). We created the parameter and coordinate files of the structures using tleap of AMBER11 and the general AMBER GAFF forcefield. The topology and coordinate files for both complexes were also generated by tleap and the ff99SB forcefield was applied in order to assign bond lengths, bond angles, force constraints and van der Waals parameters. Bonds between Ca^{2+} and His27, Gly29 and Gly31 were assigned using BOND command and the TIP3P water model in octahedral periodic boundary conditions was used to solvate the protein-inhibitor system.

Extensive minimization of the solvated neutralized complexes was carried out in five steps using SANDER program. In the first step, we kept the protein backbone fixed and then we removed the constraints. In the next three minimization steps we gradually reduced force constant to 8 kcal mol^{-1} Å^{-2}. Then, the systems were gradually heated up to the temperature of 300K. The systems were then equilibrated removing constrains and keeping the temperature at 300K with Langevin thermostat. Finally, unrestrained molecular dynamics simulations were run for 60ns using particle mesh Ewald molecular dynamics method (PMEMD).

Ptraj module was used for the calculation of distances, RMSD and H bonds on the resulting trajectories. Binding free energy was also calculated with MM-PBSA methods.

Results and Discussion

C*α*-based RMSD calculations have shown that both systems reached a stable state after the first 5 ns of the MD simulation. RMSD values for sPLA$_2$**-GK241** complex fluctuates around 2.5Å and sPLA$_2$**-GK289** complex slightly fluctuates approximately 1.7Å. Although it bears a long aliphatic chain, **GK241** did not undergo significant structural changes (RMSD ≈ 1.8Å) and as a result the distances between Ca^{2+} and carboxylate / amide group remained low (≈ 2Å), forming electrostatic interactions that occurred extensively during the MD time (Figures 1, 2). In comparison, **GK289** showed a major structural change after the first 18 ns (RMSD ≈ 2.4Å) by altering the orientation of the phenyl group. As a result the distance between Ca^{2+} and amide group was increased (≈5.5Å) causing the loss of the electrostatic interaction between the calcium ion and the carbonyl group of the amide (Figures 3, 4).

Gly31 forms a hydrogen bond with the carboxylate group of **GK241** that was observed for 85% of the simulation time, while in the case of **GK289** this bond exists during the first 30ns and then is replaced by a bond formed between Gly31 and the amide group (Table 2). Gly29 forms a hydrogen bond with either the carbonyl or amide group of **GK241** and it is present almost through out the simulation (up to 65% concurrency). In contrast, Gly29 of the sPLA$_2$ - **GK289** complex forms a hydrogen bond with carbonyl group of **GK289** in a low percentage. Lys62 is located near the active site and is quite flexible because of its side chain which make it able to form a hydrogen bond with the carboxylate group of **GK241** periodically.

Moreover, the calculation of the binding free energy was predicted applying the MM-PBSA methods on both complexes. Results are summarised in Table 3 . Although both **GK241** and **GK289** show ability to bind at the active site on the sPLA$_2$, **GK241** has a lower ΔG (up to 10 kcal mol $^{-1}$) which makes it more stable upon binding. Furthermore, the contribution of van der Waals energie had the greatest impact in the $\Delta G_{MM-PBSA}$ differences between complexes, comparing to the other contributions.

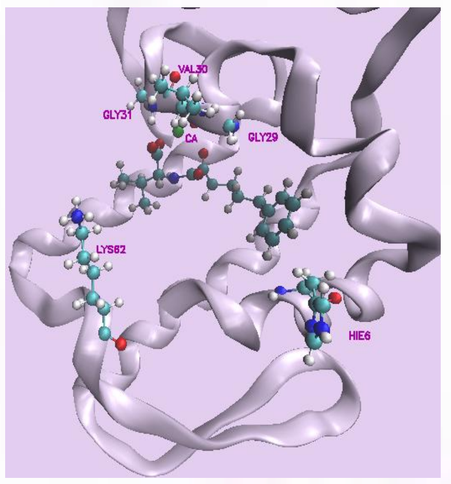


Figure 3. GK289 at the active site of sPLA$_2$ close to Lys62 and the phenyl group orientated towards His6.

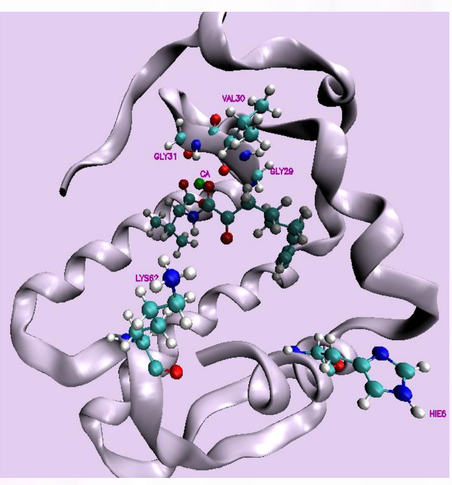


Figure 4. GK289 at the active site of sPLA$_2$ away from Lys62 and the phenyl group orientated inside the active site. 2-oxoamide group has moved away from Ca^{2+}.

Conclusion

Inhibition of secreted PLA$_2$ represents a promising approach to reduce inflammation. The results of molecular dynamics simulations and energy calculations showed that potential inhibitors should interact with calcium ion in a bidentate fashion for a better binding motif. Further computational studies on **GK241** and **GK289** are in progress aiming to lead us towards structural modifications that result in improved binding affinity.

References

1. For a review, see: E. A. Dennis, J. Cao, Y.-H. Hsu, V. Magrioti, G. Kokotos, *Chem. Rev*. **2011**, *111*, 6130–6185.
2. V. D. Mouchlis, V. Magrioti, E. Barbayianni, N. Cermak, R. C. Oslund, T. M. Mavromoustakos, M. H. Gelb, G. Kokotos *Bioorg. Med. Chem*. **2011**, *19*, 735-743.
3. R. Salomon-Ferrer, D.A. Case, R.C. Walker. An overview of the Amber biomolecular simulation package. *WIREs Comput. Mol. Sci.* **2013**, *3*, 198-210.
4. D.A. Case *et al* (2010), AMBER 11, University of California, San Francisco.

This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.