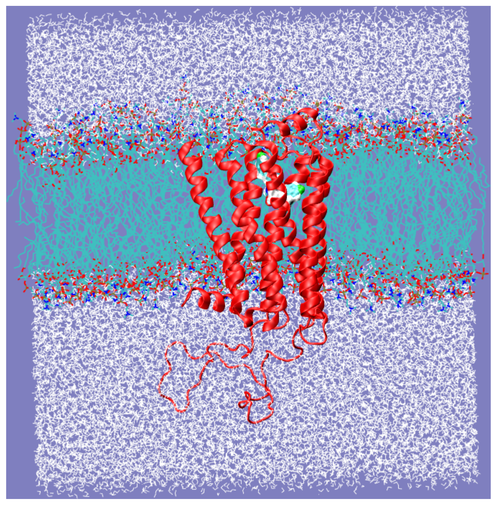
TAMPERE UNIVERSITY OF TECHNOLOGY

MOLECULAR DYNAMICS SIMULATIONS OF NEBIVOLOL COMPLEXED WITH β1 AND β2 ADRENERGIC RECEPTORS SUBTYPE SPECIFICITY STUDIES



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INTRODUCTION

Beta-adrenergic receptors (ADRBs or βRs) are the membrane proteins which belong to G-protein-coupled receptors (GPCR) family. There are two major subtypes: ADRB1 and ADRB2 which share 60% of overall sequence identity. In the trasmembrane region identity reaches ~80% - high sequence conservatism.

Beta-adrenergic antagonists (β- blockers) are the class of drugs which play an important role in the treatment of various cardiovascular diseases including hypertension, cardiomyopathy and congestive heart failure. Beta-antagonists interact with beta-adrenergic receptors. The overall effects of their action lead to lowering of blood pressure.

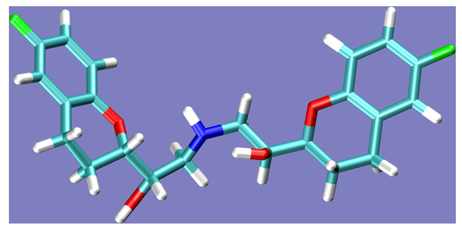
AIM OF THIS WORK

The work discussed here constitutes a part of computational studies which address the problem of ADRB1 selectivity which is of essential importance in treatment of hypertension. The specificity for subtype ADRB1 is one the key properties which contributes to the antihypertensive profile of various β-blockers. Here we are presenting the results which relate to nebivolol – the most ADRB1 selective agent among all available β-blockers.

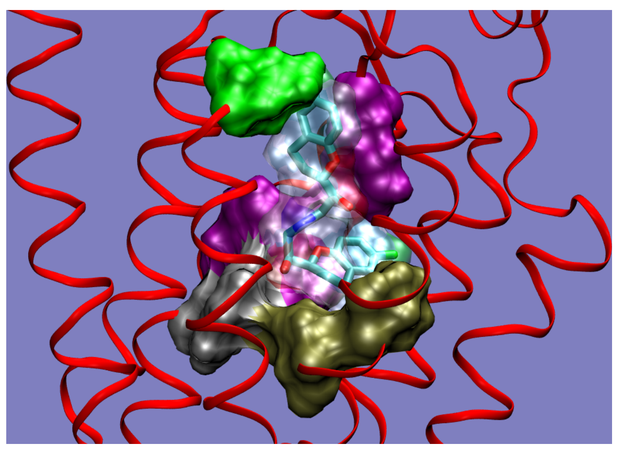
CONCLUSIONS

1. A high sequence conservatism (see sequence alignment) in the binding cavities (helix area) of ADRB1 and ADRB2 suggests a similar binding pattern.
2. Indeed a detailed analysis of the interactions confirms only a subtle differences in the binding pockets of both simulated subtypes.
3. However we assume that a higher degree of nebivolol selectivity towards ADRB1 subtype is probably caused by higher overall flexibility of ADRB1 molecule which results in more ‘’relaxed’’ conformation of the binding site.
4. Accommodation of ‘’stiff’’ drug molecule into the more spatial and flexible binding pocket of ADRB1 is easier, thus from the energetic point of view more favorable

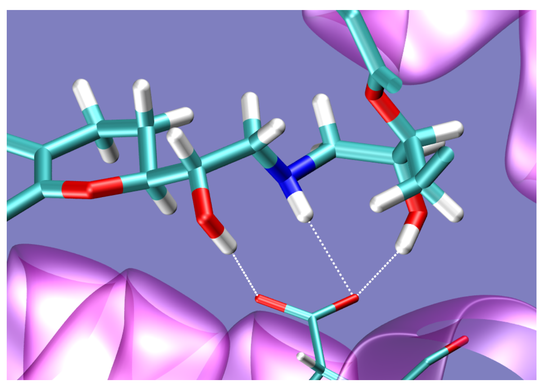
Sequence alignment of ADRB1 and ADRB2. Helices are highlighted in red and orange, loop regions in green. Conservative residues are colored in blue.



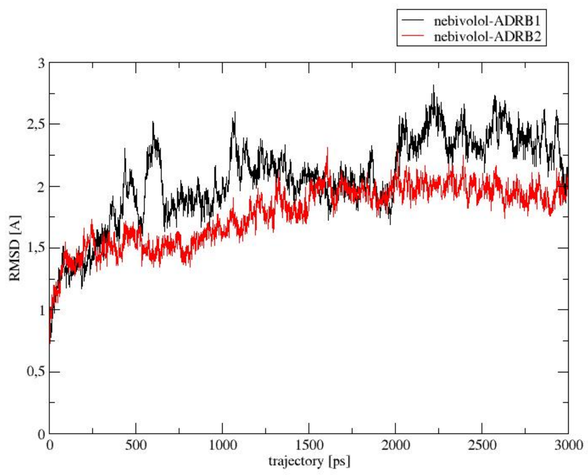
Structure of nebivolol. Carbon atoms are shown in cyan, hydrogens in white, oxygens in red, nitrogens in blue, fluorine in green.



Non-bonded interactions around the nebivolol in the binding pocket of ADRB1. PHE residues colored in purple, VAL in brown, ILE in green, TRP in silver – residues shown in surface representation. Nebivolol molecule is drawn in licorice model and also represented as transparent surface.



Hydrogen bonds between crucial ASP residue in helix 3 and donnors of nebivolol. Snapshot from molecular dynamics simulation with ADRB1.



RMSD value in simulations performed with ADRB1 and ADRB2.

METHOD

Procedure

- Nebivolol was docked into the binding sites of ADRB2 and ADRB1
- The docked complexes were energy minimized
- The lowest energy configurations of nebivolol complexed with ADRB1 and ADRB2 were submitted to molecular dynamics simulation
- 3 MD simulations of nebivolol complexed with ADRB1 and 3 MD simulations of nebivolol complexed with ADRB2 were performed

System description

- System composition: receptor protein, drug molecule, lipid, water, ions
- Size of the systems: ADRB2 – 120 000 atoms, ADRB1 – 190 00 atoms

Simulation parameters

- Software: NAMD
- Forcefield: CHARMM CMAP forcefield for protein and CHARMM 27 for lipid
- Simulation length: each repeat – 30ns
- Temperature: 310K
- Target pressure: 1bar

RESULTS

Structure stability of simulated receptors

- RMSD value (calculated for backbone atoms) in simulations performed with ADRB2 was ~2A, the complexes of ADRB1 exhibited more flexibility – RMSD was ~3A

Hydrogen bonds

- ~50% of detected h-bonds between nebivolol and receptor proteins relate to sequence conservative ASP residue in helix 3
- This residue functions as h-bond acceptor
- However a detailed analysis showed that h-bonds in the binding pocket of ADRB1 are stronger and much more stable during dynamics
- An average number of h-bonds per drug molecule was ~3.5 and ~3 respectively in simulations with ADRB1 and ADRB2

Non-bonded interactions

- ~ 80% of vDW point atom contacts and Pi-Pi stacking interactions related to these same hydrophobic and aromatic residues in the binding sites of both receptors
- However the binding pocket of ADRB1 seemed to be more flexible and spatial

Ligand conformation

- In case of both simulated receptor subtypes ligand structure was almost equally rigid