Bovine β-lactoglobulin: Interaction studies with Norfloxacin

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Introduction

Molecular docking is an efficient computational tool to predict the structures of protein-ligand complex. This kind of simulation is of fundamental importance for interpretation of numerous biochemical phenomena, providing useful information on the preferred binding sites of ligands, and therefore in rational drug design.
Bovine β-lactoglobulin (BLG) is a small extracellular protein belonging to the lipocalin superfamily. Lipocalins have been classified as transport proteins with the remarkable ability of binding small hydrophobic molecules within the central cavity also known as calyx [1]. Because of its stability, abundance and easiness of preparation BLG, has been frequently studied to clarify its structural and binding features.
Several studies suggest that more than one binding site exists [2], thus the aim of this work is to investigate the existence of other sites, in addition to the calyx one, and to verify if BLG can interact and play the role of carrier of drugs. We considered the particular case of Norfloxacin which is a broad-spectrum antibiotic used in treatment of urinary tract infections.

Methods

The X-ray structure of BLG was downloaded from the Protein Data Bank (code 1GX9). Before starting simulations, hydrogens where added to the structure.
Localisation of binding sites, classical molecular mechanic and molecular dynamics simulations where carried out with the program Moe [3]. No crystal structure of Norfloxacin is avaible so it was modelled using insightII [4] program. The structure was optimized by means of classical molecular mechanic (MM) and further refined with quantum mechanic with MOPAC PM3 semi-empiric method [5] .
The docking simulations where performed with Autodock version 3.0 [6]. Protein and ligand where treated according to the rigid bodies approximation.
To refine the characterization of binding mode of the best docked conformations of Norfoxacine in all the different binding sites, molecular flexibility was taken into account by means of molecular dynamics simulations (MD) of 3ns. The solvent was treated implicitly adding the electrostatic constant of water (ε=54), see Ref [7].

Discussion

1. First site: calyx

Different runs of docking simulations generated several structures among which we considered only the most energetically favourable. The best docking energy of Norfloxacin is -6.79 kcal/mol. The interactions are overall of hydrophobic nature which is in agreement with the characteristic of the central cavity. In order to evaluate the reability of the computed binding energy, we compare it with that one of an in vitro ligand of BLG. A docking experiment was performed applying the same protocol as before with retinoic acid; in this case the energy was of -8.03 kcal/mol. We can thus conclude that, at least in the rigid docking approximation, the interaction BLG-Norfloxacin is comparable with that of retinoic acid-BLG. To investigate more accurately this hypothesis, we analysed the conformation of Norfloxacin after dynamic and found that the position and the residues that interact with Norfloxacin are substantially the same.

2 Second and Third site

In these two hypothetical binding sites located on the surface of the protein, the computed best docking energies of Norfloxacin where -7.2 and -10.2 kcal/mol respectively. This strong interaction can be explained as due to the formation of hydrogen bonds and hydrophobic contacts between ligand and protein.
In the second site, after the dynamic simulation of 3ns (see above), the interactions between protein and ligand drastically decrease in number and in intensity, the distances between atoms involved in hydrogen bonds increase, and the number of hydrophobic interactions decrease. On the contrary, in the third site, most of the interactions are conserved during the whole simulation .

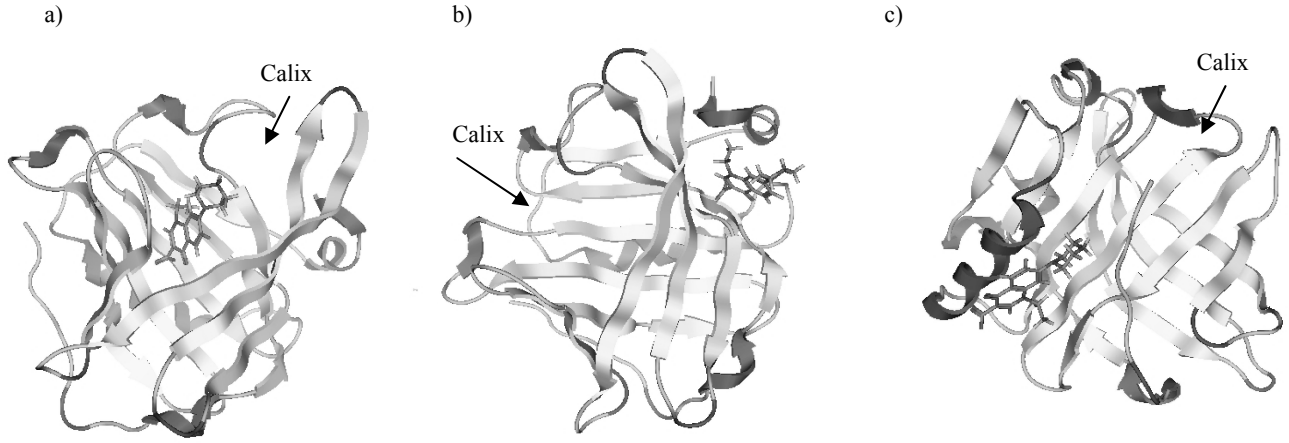

***Figure 1 : Norfloxacin docked in a) the calyx b) and c) the second and thirds sites*.**

Conclusions

The present investigation shows that BLG can be a good receptor for Norfloxacin both in the central cavity and in the third site. The calyx, although characterized by an interaction with Norfloxacin lower than the others two sites , can be considered as stable receptor site as highlighted by the dynamic simulation. The second site seems to be excluded as hypothetical binding site just from MD experiments in spite of its relatively high docking energy. As for the third site, we suggest that it could be considered as competitive binding site. However final indication will be possible after further investigations, in particular dynamics simulations carried out in the presence of explicit solvent which should affect the affinity of the protein surface for ligands.

References

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