XIX SBQT
Simpósio Brasileiro de Química Teórica 2017
12 a 17/Nov, 2017, Águas de Lindóia/SP, Brasil

Docking, molecular dynamics and ensemble docking in the study of petrobactin biosynthesis inhibition in *Bacillus anthracis*

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Abstract: *Bacillus anthracis* is a large, gram-positive, aerobic, rod-shaped bacterium responsible for a systemic, acute and lethal infection known as anthrax. Under adverse environmental conditions, these bacteria are capable of forming dormant spores that are resistant to drying, cold, radiation, heat, and disinfectants, remaining viable for centuries. These spores can be found in soils all over the world and can enter mammals through either cutaneous, gastrointestinal or respiratory routes.[1] Inhalational anthrax has a lethality superior to 80% in humans, what makes *B. anthracis* a well-known bioweapon with a high potential of use as a bioterrorism agent.[2]

Three factors play a primary role in the pathogenesis and lethality of *B. anthracis*: a capsule; production of two exotoxins (lethal and edema); and its ability to quickly achieve high microbial concentrations in infected hosts.[3] This high virulence is related with the bacteria's capacity to acquire iron, an essential micronutrient, through the release of high-affinity iron chelating molecules named siderophores. Petrobactin has shown to be the first siderophore released by the bacteria in iron depletion conditions.[4] It is not recognized by siderocalin, a siderophore sequestrating enzyme found in the immune system, and has an affinity for iron 100 times higher than transferrin, the iron-carrier protein found in blood.[5]

The biosynthetic pathway of petrobactin involves six enzymes (AsbA-F), and within these, the *asbF* gene encodes a dehydroshikimate dehydratase responsible for the synthesis of the 3,4-dihydroxybenzoic acid groups that serve to coordinate iron within the siderophore itself.[6] Kinetic parameters of the enzyme have been elucidated and a high resolution crystal structure of AsbF has also been published.[7] This, coupled with the fact that this enzyme has no known homologues in humans makes it an attractive target for antibiotic intervention.

In this work, a set of compounds with experimentally known inhibitory potential against AsbF were studied through molecular docking and molecular dynamics (MD) simulations aiming a deeper understanding about the processes and mechanisms related with the enzymatic inhibition, which can guide the rational design of new compounds with improved potency, selectivity and with possible application as new therapies against anthrax.

Dockings with Autodock Vina[8] was able to reproduce the binding mode for 3,4-DHB as found in the crystal structure with a RMSD of 2,2 Å. This binding mode is

shared by several compounds with inhibitory activity and include important hydrogen bonding between carboxylate moiety in the ligands and active site residues like Arg102, Tyr70 and Tyr217 as well as between hydroxyl groups and residues like His144, His175 and Phe211.

Molecular dynamics simulations were performed with Gromacs[9] 4.5, with Amber03 force field for receptor and GAFF with AM1-BCC charges for ligands. DM results showed that hydrogen bonding with Arg102 and His175 are the major anchors for ligands in the active site and that the inhibitory potential can be related with the maximization in the number of interactions with active-site residues while keeping these two main hydrogen bonds.

Furthermore, the MD simulations were also used to obtain a set of distinct structures of AsbF, which are representative of the different conformational "substates" this enzyme can achieve in solution as well as to sample conformational changes induced by the presence of ligands.

The inclusion of receptor's flexibility through docking with these structures (ensemble docking) allowed an improvement in the linear correlation between docking scores and experimental free energies of binding, from r^2 of 0.13 (PCC=0.36) for docking in the crystallographic structure to r^2 of 0.34 (PCC=0.58) for the ensemble docking when all the experimental ligands are considered and from r^2 of 0.57 (PCC=0.75) to a r^2 of 0.86 (PCC=0.93) when considering the 10 most active compounds. This allowed the development of a model for the quantitative prediction of inhibitory potency and for the virtual screening of new compounds, the current step of this research.

Key-words: petrobactin, anthrax, enzyme inhibition, docking, molecular dynamics
Support: This work has been supported by CAPES and CNPq

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