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Molecular Dynamics Simulations of Ribosome-oxazolidinone Complexes Reveal Structural Aspects for Antibiotics Design

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We present an investigation of the determinants of binding of oxazolidinone antibiotics, in particular linezolid, its derivative radezolid, and the structurally related oral anticoagulant drug rivaroxaban in complex with the large ribosomal subunit from *Haloarcula marismortui* using molecular dynamics simulations. Our results are in agreement with available experimental data that show radezolid as the most potent inhibitor compared to linezolid and rivaroxaban. Furthermore, the structural and dynamical insights obtained from this study will provide ability to design improved antibiotics in the future.

1 Introduction

The ribosome is a large ribonucleoprotein complex that carries out protein synthesis in all kingdoms of life. It is composed of a large and a small subunit, which are denoted as 50S and 30S in bacteria. Peptide synthesis can be inhibited by antibiotics that bind to three functionally different sites on the ribosomal structure, namely the decoding site, the peptidyl-transferase center (PTC), and the protein exit tunnel[1]. High resolution crystal structures of large and small ribosomal subunits in complex with antibiotics[2,3] have revolutionized our understanding of their binding sites, binding modes, and mechanisms of action. Such information provides opportunities for rational structure-based drug design approaches to improve existing or obtain novel antibiotics helpful in combating bacterial resistance[4]. However, structural determination by X-ray crystallography only provides static views of the binding processes but does not reveal the dynamics involved with antibiotics binding, or the energetic determinants of binding. Theoretical and computational approaches such as molecular dynamics (MD) simulations in combination with free energy calculations[5] are suitable to fill this gap.

In the present study, we aim at investigating the determinants of binding of the oxazolidinone class; the only synthetic antibiotic class to have entered the market during the last 40 years[6]. Structures of linezolid, the first approved oxazolidinone antibiotic, in complex with the large ribosomal subunits from *H. marismortui* (H50S)[2] and *D. radiodurans* (D50S)[3] have been solved recently. Here, we investigate linezolid, its derivative radezolid, and the structurally related oral anticoagulant drug rivaroxaban in complex with H50S by means of MD simulations to obtain insights into the determinants of binding of this antibiotic class (Fig. 1).

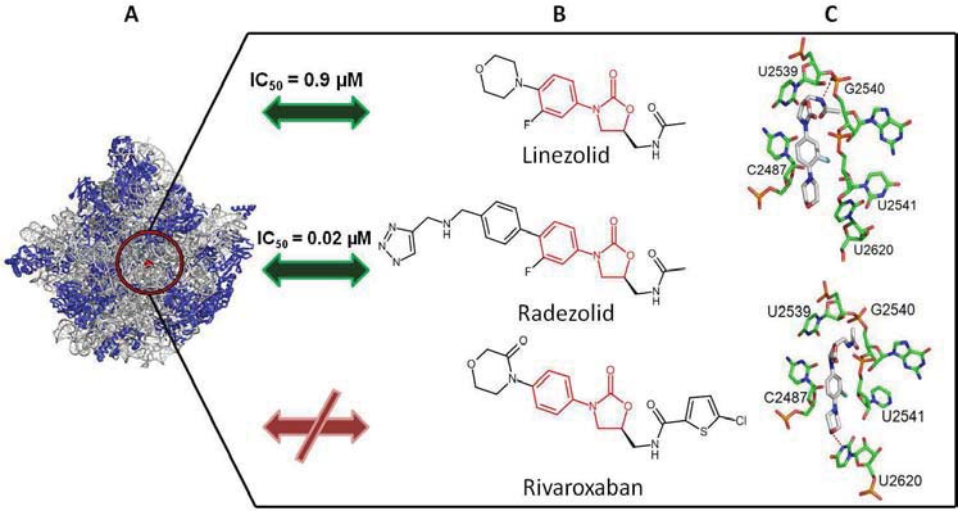

Figure 1. The binding site of oxazolidinones inside the large ribosomal subunit. (A) Crown view of H50S; proteins are shown in blue and RNAs in light grey. (B) The chemical structures of linezolid (top), radezolid (middle), and rivaroxaban (bottom); the phenyl-oxazolidinone core is colored in red. (C) Enlarged view of the linezolid binding site with key residues and respective hydrogen bonds formed (dotted lines) in H50S (top) and D50S (bottom).

2 Methods

The starting structures used for the MD simulations were derived from the 2.7 Å resolution X-ray structure of linezolid bound to H50S (PDB code 3CPW)[2] by structurally modifying linezolid to radezolid and rivaroxaban, respectively. All MD simulations (around 800,000 atoms each) were performed using the AMBER 10 suite of programs with the ff99SB force field and the TIP3P water model. All three simulations reached a length of 50 ns of which snapshots saved at 20 ps intervals were used for analysis.

3 Results

As a first step we investigated the local interactions of the oxazolidinone derivatives formed within the binding site. This analysis showed that the hydrogen bond between the acetamide NH of radezolid and the phosphate group of G2540 (H50S numbering used throughout) persists during the whole simulation, whereas it breaks in the case of linezolid after 4 ns and only forms for a few nanoseconds in the case of rivaroxaban (between 28 and 32 ns) (Fig. 2, (1)).

In a second step, the aromatic stacking interactions between the nucleobase of U2539 and the oxazolidinone core (Fig. 2, (2)) as well as between C2487 and the phenyl rings of the oxazolidinone derivatives (Fig. 2, (3)) were investigated. The aromatic interactions persist during the MD trajectories in both the radezolid and linezolid complexes but break after 30 ns in the case of the rivaroxaban complex. This finding is in agreement with

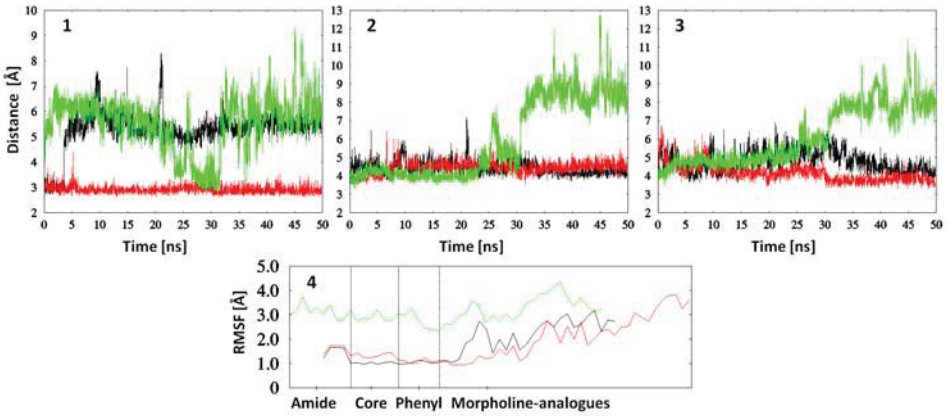

Figure 2. Time-course of non-covalent interactions: (1) Hydrogen bond formation between the ligands' acetamide NH and the phosphate group of G2540 and of aromatic interactions between (2) the oxazolidinone core and the nucleobase of U2539 as well as (3) between the phenyl ring and the nucleobase of C2487, respectively. (4) Atomic fluctuations (RMSF) of the atoms of oxazolidinones during the MD trajectories of H50S complex structures with linezolid (black), radezolid (red), and rivaroxaban (green).

the observed ligand movement inside the binding site (Fig. 2, (4)): While the core of rivaroxaban shows large fluctuations (RMSF $\sim$ 3.0 Å), the core of linezolid and radezolid are largely immobile (RMSF $<$ 1.5 Å).

Finally, the fluctuations of the binding site residues during the MD trajectories were investigated. Again, a similar behavior was observed in the case of the linezolid and radezolid simulations, where both binding sites undergo moderate movements (RMSF $\sim$1 Å). In contrast, the binding site of the rivaroxaban complex shows much more pronounced fluctuations (Fig. 3) that reflect the movement observed for the ligand. In that case, the largest movement is observed for the nucleobase of U2620.

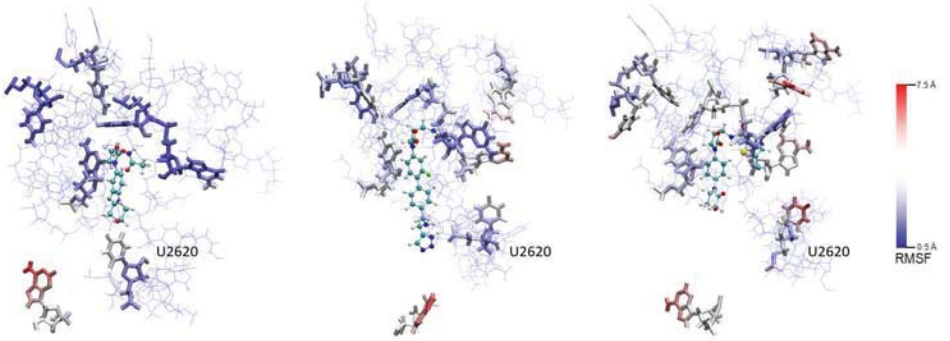

Figure 3. Color-coded representation of atomic fluctuations obtained from the MD trajectories for the binding site residues of the H50S complex structures with linezolid (left), radezolid (middle), and rivaroxaban (right). Red color indicates high and blue color low RMSF values.

4 Discussion

The present study underlines the importance of computational studies to analyze antibiotics binding to the ribosome. While certain aspects of binding of linezolid to H50S can be understood based on the available complex crystal structures, information about the binding determinants of radezolid and rivaroxaban to H50S has remained elusive so far.

To investigate the determinants of binding of antibiotics at the ribosomal structures, we have performed all-atom MD simulations of large ribosomal subunits in explicit solvent. The analysis of hydrogen bonds and aromatic interactions implicates a stronger binding of radezolid over linezolid, which is in agreement with the experimental data. Surprisingly, while the chlorothiophene moiety of rivaroxaban can be well accommodated in the H50S crystal structure, the MD simulations reveal a pronounced movement of this ligand, which is accompanied by a loss of the initial hydrogen bonding and aromatic interactions. This may be the reasons why no inhibition of protein synthesis was detected so far for rivaroxaban.

In contrast, the binding of linezolid and radezolid is mainly stabilized by the acetamide moiety as it is involved in making hydrogen bonding interactions with the RNA. In agreement, all recent linezolid derivatives preserve this moiety, which shows that any change in the group can be detrimental to the affinity. However, the morpholino group does not form apparent interactions and can be substituted with other groups without leading to a loss in inhibitory activity. This is supported by SAR studies on linezolid and radezolid by others. The triazole moiety for both linezolid and radezolid forms weak hydrogen bond interactions with the nucleobase of U2620. This interaction stabilizes the conformation of U2620, thus arresting it in a nonproductive conformation, and thereby prevents the correct positioning of the P-tRNA. In the case of rivaroxaban, U2620 undergoes large movements during the MD simulation and forms no stable interaction.

By means of MD simulations, we were able to identify key structural features required for the strong binding of radezolid to H50S. Also, we were able to identify structural reasons why rivaroxaban does not act as an antibiotic. Currently, we are in the process of investigating the contributions of the binding site residues to the relative binding free energies of the three oxazolidninone derivatives. Together with the structural information gained so far, this information will be used to further understand antibiotics selectivity and resistance. Furthermore, we expect our findings to provide a basis for designing improved antibiotics.

Acknowledgments

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