Molecular dynamics simulations of MurA enzyme in complex with reaction products

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INTRODUCTION

MurA(UDP-N-acetylglucosamine enolpyruvyl transferase, EC 2.5.1.7) is an essential enzyme in the biosynthesis of the peptidoglycan layer of the bacterial cell. The x-ray structure of the C115S mutant of Enterobacter cloacae MurA depicts the product state of the enzyme with enolpyruvyl-UDP-N-acetylglucosamine (EP-UNAG) and inorganic phosphate (PO4) trapped in the active site. Kinetic analysis revealed that the Cys-to-Ser mutation results in an enzyme that appears to perform a single turnover of the reaction[1]. Opposing the common view of Cys115 as a key residue in the chemical reaction of enolpyruvyl transfer[2], the wild-type cysteine is essential for product release only. Thus, the aim of this work was to employ molecular dynamics simulations of both native and C115S MurA in complex with PO4 and EP-UNAG in order to understand the molecular differences on release or not the reaction products.

METHODS

The Amber 12 suite of programs was employed using the ff12SB force field to run a total of 21ns MD simulations. The trajectories with 2100 snapshots were used to compute the binding free energy of the PO4 ligand at the active site of MurA enzyme along the simulation using the MM-GBSA method as implemented in amber 12.

RESULTS AND DISCUSSION

The results show that the native enzyme release the PO4 product in the first steps of the simulation. In contrasts, MurA C115S shows retaining the PO4 molecule at the active site along the simulation. MMGB-SA calculations show an increase in free energy of PO4 binding along the MD simulations of the native enzyme, which suggest repulsion interactions with the active site residues as supposed by experimental analysis[1]. Both complexes show stable values of binding free energy after 0,5ns. However, as can be seen in Figure 1, the mutant form appears with smaller binding free energy values, whereas the main interactions seen in the beginning of the simulation were maintained.

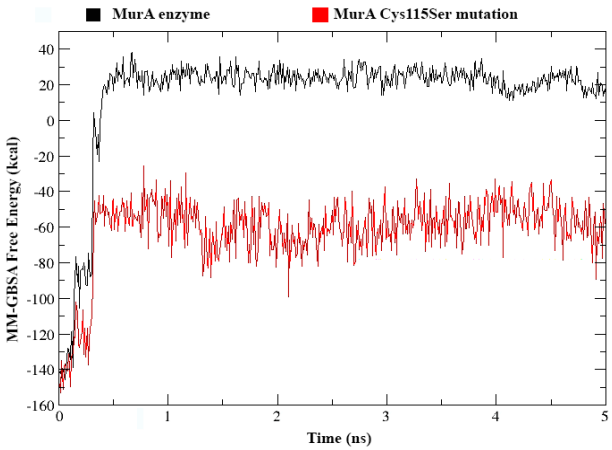

Figure 1. Free-energy profile for the first 5ns of MD simulations.

CONCLUSIONS

The simulations performed in this work are in agreement with experimental data analysis that reveal PO4 releasing in MurA native form . Once we have serine instead of the wild-type cysteine in position 115, the products are trapped in the active site. Per-residue decomposition analysis are being carried out in order to investigate which residues contribute to the instability that leads to phosphate releasing.

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