Molecular dynamics evidence of the biological complex between PhzM-PhzS enzymes.

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Abstract

Thermal shift assay along with molecular dynamics studies suggest that the PhzS-PhzM complex might be formed at pH 6.0 instead of pH 8.0.

Introduction

Pyocyanin is a virulence factor that can be targeted for drug development efforts. Although several enzymes of this pathway have kinetic and structural data, no inhibitors of this virulence factor production have been described. Drug development towards the final steps of pyocyanin biosynthesis is particularly challenging due to fact that PhzM is active only in the presence of PhzS.[1,2] Herein we report the first evidence that PhzS-PhzM complex formation might be influenced by pH.

Results and Discussion

Parson and coworkers have reported that PhzM only converts its substrate in the presence of PhzS, but they did not found structural evidence of this complex formation at pH 8.0. Thermal shift assay carried out in our lab suggests that both enzymes are more stable at pH 6.0 than at pH 8.0 (figure 1). Furthermore, electrostatic potential surface calculations with APBS (figure 2) show poor charge complementarity at pH 8.0, whereas attractive electrostatic interactions are seen nearby the active sites at pH 6.0.

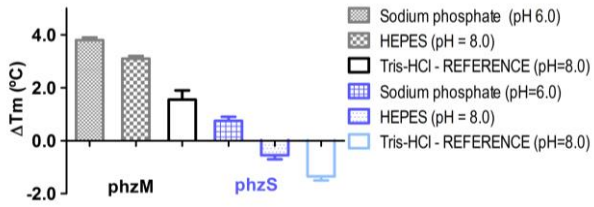

Figure 1. Thermal stability of PhzS and PhzM at pH 6.0 and 8.0

In order to further investigate this matter, protein-protein docking was carried out with the CLUSPRO[3] server and the best ranking pose was employed as the initial structure for molecular dynamics simulations (MD).

Aiming to analyze the pH influence over the complex stability, the simulations were carried out at both pH (6.0 and 8.0) with GROMACS 4.6.5[4].

Although the structures show similar RMSD (0.40±0.03 vs 0.41±0.02) at both pHs, the RMSF for residues 180-225 of phzM, located in the putative complex interface, is lower at pH 6.0 (Chain-A) (Figure 3).

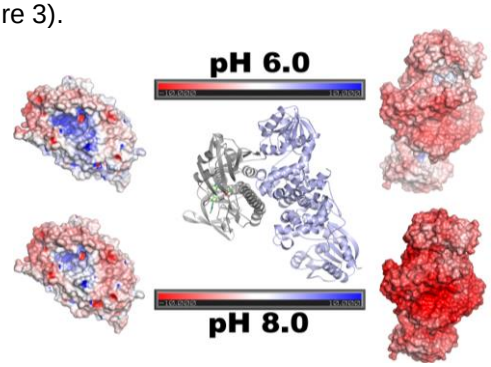

Figure 2. Electrostatic potential surface of PhzM (left) and PhzS (right) at two pH values.

Moreover, at this pH the chains´ center of mass distance between PhzS (Chain-C) and phzM (Chain-A) increases in the productive phase (Figure 3).

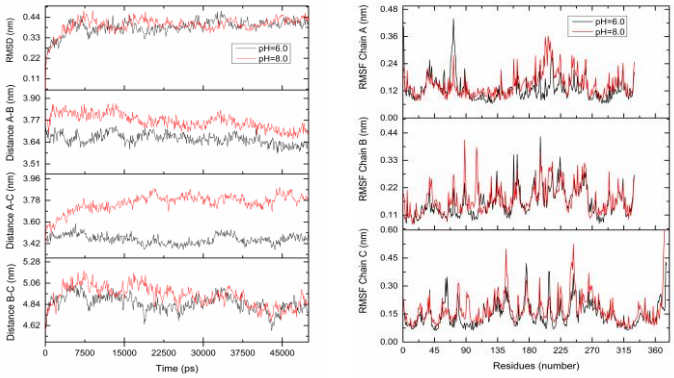

Figure 3. PhzS-PhzM complex molecular dynamics simulation parameters at different pHs

Conclusion

Thermal shift assays and molecular simulation studies suggest that the biological active complex formation is pH dependent. However, the influence of substrate and cofactor presence over the complex stability remains to be clarified

Acknowledgments

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