https://doi.org/10.17952/37EPS.2024.P2116

Development of a potent and specific peptide inhibitor for the *E. coli* MazF toxin

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Introduction

Antibiotics are compounds capable of interfering in active metabolic processes, such as transcription, translation, and cell wall synthesis. Therefore, in a population of pathogenic microorganisms, the cells that temporarily assume a dormant state are less susceptible to antibiotics. Cells displaying this phenotype are called persistent [1]. Although different pathways lead to this state, toxin-antitoxin systems (TAs) are assumed to play an important role in persistence [2].

TAs are abundant in the chromosome of prokaryotes, being composed of two types of protein: a toxin with cell growth inhibitory activity and a cognate antitoxin that keeps the toxin inactive under basal conditions. Through toxins, microorganisms enter a state of low metabolic activity and great resilience to different types of stress. Among the well-known TAs, a few have more prominent effects on bacterial physiology, such as the mazEF module. MazF is a 12 KDa-toxin with endoribonucleolytic action that inhibits cell growth through mRNA cleavage, while MazE is the cognate 9 KDa-antitoxin that keeps MazF inactive by blocking its catalytic site. MazEF complex, a heterohexamer, has a central role in several bacteria since it is involved in the formation of biofilm and increase of fitness in face of stress situations, such as phage infection, antibiotics, extreme temperatures and oxidative stress [3,4].

Inspired on such information, we designed this study aiming to discover a potent and specific peptide inhibitor for the toxin MazF [5].

Experimental

Briefly, peptide sequences of 12 amino acid residues interacting with *E. coli* His-MazF immobilised in a 96-well plate were selected with the Ph.D.TM-12 Phage Display Peptide Library kit (NEB, MA/USA). The best candidates were synthesized by microwave-assisted solid-phase synthesis at 60 °C using customized protocols [6], purified by RP-HPLC and characterized by ESI-MS and amino acid content. Cloning, expression, purification, and protein refolding technologies provided the recombinant *E. coli* MazE, His-MazF, and its mutant MazF(E24A). The synthetic peptides and MazE were compared in the capacity of inhibiting the endoribonuclease activity of His-MazF. Mapping of the interaction between the peptide inhibitor and the recombinant toxin or its mutant was performed by ITC, peptide analogues and NMR spectroscopy. 3D-structure analyses and calculations of toxin-P1 and toxin-antitoxin complexes by 800 MHz-NMR are in progress. Recently, effects of MazF inhibition on *E. coli* cells were also examined by bacterial growth, antibiotic surviving, oxygen consumption and resazurin assays [5,7].

Results and Discussion

The results obtained so far demonstrate that a potent and specific peptide inhibitor for *E. coli* MazF toxin (Fig. 1A) was discovered and studied. P1 contains 12-amino acid residues and acts as a reversible competitive inhibitor (Fig. 1B). Extensive scanning of P1 amino acid sequence using different techniques and a few synthetic analogues allowed mapping the interactions of the synthetic P1 with the recombinant His-MazF and comparing the points of contact with those already described for the MazEF complex. P1 proved to be also functional *in vivo* as it prevented the deterministic activation of the toxin that causes a strong metabolic inhibition with consequent acquisition of bacterial tolerance to antibiotics. Overall, these results indicated that MazF is a possible regulator of bacterial persistence.

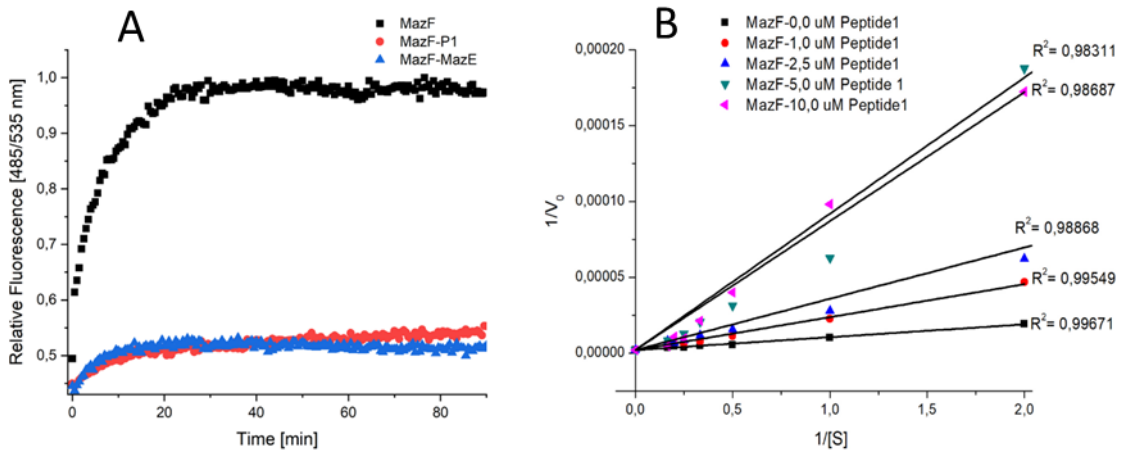

Fig. 1. I*n vitro* catalytic profiles of *E. coli* His-MazF using a specific fluorescent substrate in the absence and presence of MazE or the synthetic peptide (**A**). Plot of Lineweaver-Burk in the presence of P1(**B**).

Acknowledgments

The authors thank FAPESP (2022/01825-2) for the research grant to MTM, CNPq for research and doctoral scholarships to MTM (314470/2023-9) and LRPC (141141/2016-6), respectively, Dr. Cleber W. Liria for all mass spectrometry and amino acid analyses, Prof. German Sgro (FCFRP-USP) for discussion and help with protein cloning, expression and purification, Jose D. Rivera and A. Davalos for help with 800-MHz NMR analyses, Profs. S.R. Marana, I.M. Cuccovia, S.C. Farah, C.O. Columbano, and A. Kowaltosvski (IQ-USP) for advices and/or sporadic use of their equipments.

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