08321 - Apresentação de Cartaz

E - 035 - Obtainment and Characterization of Hsp90 Domains of Plasmodium falciparum

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INTRODUCTION

Malaria is the most common parasitic disease in the world. It is caused by the Plasmodium ssp protozoa, that is transmitted by the Anopheles spp fly. Both vector and parasite have developed resistance to treatments. Once molecular chaperones and co-chaperones play an important role in the adaptation and survival of the protozoan, they became interesting therapeutic targets. Hsp90 is a dimeric heat shock protein of about 90 kDa. Each protomer has three distinct domains: N-terminal (N), middle (M) and C-terminal (C). These domains are responsible for the interaction with ATP and its hydrolysis, the interaction with client proteins and the dimerization, respectively. Hsp90 works in protein folding, protein signaling, protein translocation and targeting for degradation, among others and is essential mainly under stress conditions such as faced by the Plasmodium falciparum adaptation and development.

OBJECTIVES

Structural and functional characterization of PfHsp90NM and PfHsp90M aiming at interaction analysis with co-chaperones.

MATERIALS AND METHODS

DNA coding for PfHsp90M and PfHsp90NM were amplified by PCR from pET28a::PfHsp90 expression vector and cloned into pET28a vector. Recombinant proteins were expressed in Escherichia coli BL21(DE3) strain. Then, the proteins were purified by Ni^{2+} affinity and size exclusion chromatographies, respectively. Spectroscopic analysis were performed by Circular Dichroism (CD) and intrinsic tryptophan Fluorescence techniques. In addition, Analytical Size Exclusion Chromatography (aSEC) and Differential Scanning Calorimetry (DSC) were also performed.

DISCUSSION AND RESULTS

The proteins were obtained folded and with minimum 95% purity. CD experiments showed a majority of α-helix content for both proteins, in higher proportion for the NM domain construction. Both proteins were monomeric according to aSEC. Besides, PfHsp90M and PfHsp90NM have different chemical and thermal stabilities.

CONCLUSION

This study provides information about individual domains, allowing to evaluate and mapping the interaction of Hsp90NM e Hsp90M with Hsp90 co-chaperones. This work will contribute to the understanding of domain function when compared to the full-length protein.

Keywords: Malaria, molecular chaperone, Hsp90