TITLE: Biophysical analysis of *Paracoccidioides brasiliensis* **14-3-3 recombinant protein**

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ABSTRACT:
Paracoccidioides spp. are dimorphic fungi that cause paracoccidioidomycosis, an endemic mycosis in Latin America with high prevalence in Brazil. In order to establish in the host environment *Paracoccidioides* spp. synthesize molecules to adhere and invade host cells and to evade host immune system. The 14-3-3 protein from *P. brasiliensis* (Pb14-3-3), is involved in yeast-mycelium transition and ergosterol biosynthesis. During infection Pb14-3-3 is highly expressed, acting as an adhesin and promotes the induction of apoptosis and rearrangement of cytoskeleton in host cells, being an interest therapeutic target. The Pb14-3-3 belong to 14-3-3 protein family which are conserved eukaryotic proteins widely study as drug target and diagnosis biomarker due their involvement in neurological diseases, cancer (human isoforms) and host-parasite interaction (parasites isoforms). The aim of this study was to perform a biophysical analysis of *P. brasiliensis* 14-3-3 recombinant protein (Pb14-3-3r) and its *in silico* structure prediction. For this, the Pb14-3-3 coding sequence was cloned into *NcoI* and *XhoI* sites from pet28a vector followed by transformation in *E. coli* BL21DE3 Rosetta. The recombinant protein was purified using affinity chromatography and size exclusion chromatography. The estimate of the secondary structure content was obtained by deconvolution of the spectra obtained by circular dichroism (CD) using CDNN Deconvolution program. Analytical size exclusion chromatography (aSEC) was performed with different concentrations of the Pb14-3-3r to estimate the apparent molecular mass. Thermal stability of the Pb14-3-3r was evaluated by CD_{222nm} with a range of temperature between 15° to 90°C. The prediction of the tridimensional structure was performed using MODELLER program and PROCHECK program to validate. The biophysical analysis by CD and aSEC indicates that the secondary structure is composed of 63% α-helix, 7% β-sheets, 11% turns and 19% coils and in solution has one oligomeric state with apparent MM of 144,26 kDa. The thermal stability analysis showed that Pb14-3-3 maintains its secondary structure until ±50°C and the loss of the structure due the temperature is irreversible. The tridimensional structure obtained *in silico* shows that Pb14-3-3 is mainly composed by α-helix structure, elongated shape with some conserved binding sites. These findings expanded our knowledge of Pb14-3-3 structure and functions.

KEYWORDS: *Paracoccidioides brasiliensis*, adhesins, 14-3-3 protein

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