TITLE: PEPTIDES DERIVED FROM THE 43-kDa GLYCOPROTEIN (GP43) FORM AMYLOID FIBRILS IN SOLUTION: IMPLICATION FOR PARACOCCIDIOIDOMYCOSIS

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ABSTRACT:
Paracoccidioides brasiliensis (*P. brasiliensis*) is one of the causative agents of Paracoccidioidomycosis (PCM), a systemic and progressive mycosis. The 43-kDa glycoprotein (gp43) is the main antigen secreted by *P. brasiliensis* and it has been considered one of the evasion mechanisms of this pathogen. Peptides derived from this glycoprotein (P4, P10 and P23) have different functions and present immunogenic activity making their study relevant. P10 peptide, a T-cell epitope, induces protective response while P4 and P23 peptides inhibit both, macrophage functions and inflammatory reaction, thus facilitating infection. Recently, it was described that *P. brasiliensis* is able to induce Neutrophil Extracellular Traps (NETs), and these structures were identified in tegumentary lesions of patients. However, the mechanisms involved in this process have not yet been fully clarified. As it has already been shown that amyloid fibrils trigger neutrophil extracellular traps (NETs) release, in the present study, we used algorithms that predict the aggregation propensity of a given protein sequence to address whether P10 (QTLIAIHTLAIRYAM), P4 (NLGRDAKRHLSKHWDTFITEDDFKNIAAAGL) and P23 (AFEVGAGWYFWTWKTEGAPGWDMQD) have tendency to aggregate and form amyloid fibers. Aggrescan and ZipperBD predicted a strong aggregation propensity for P10 and P23, but not for P4. *In vitro* studies with the three peptides corroborated our bioinformatics analyses: P10 and P23 aggregated in minutes, when incubated in aqueous solution, while P4 did not form aggregates. Interestingly, P10 and P23 form amyloid fibrils as visualized by electron microscopy and tioflavin T binding. Besides, when small pieces of amyloid fibrils composed of P10 were added to soluble P4, this peptide also formed amyloid fibril. These structures have been implicated in several human diseases, such as Alzheimer´s and Parkinson´s diseases as well as in biofilm production. Thus, our results showed the ability of these peptides (P10 and P23) to aggregate and form amyloid fibers, and we envision that if they are formed upon gp43 proteolysis, amyloid fibril can be produced, and this process could be related to NETs release in PCM. Further studies are being conducted to elucidate these mechanisms.

KEYWORDS: *Paracoccidioides brasiliensis*, GP43, Peptides P10, Peptides P4, Peptides P23, Amyloidosis

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