Structural Characterization of the Recombinant Protein Pb27 from *Paracoccidioides brasiliensis*.

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The systemic mycosis Paracoccidiodomycosis (PCM), caused by the thermal-dimorphic fungus *Paracoccidioides brasiliensis,* causes lesions in lungs and can disseminate to other tissues. There is no standardized diagnostic or treatment protocol for this disease. The recombinant 27-kDa antigen has been used with high sensibility and specificity in diagnosis of PCM but its biological function in the fungus is unknown and no other similar protein has been described in databases. The Pb27 gene was subcloned into pET-DEST42 vector with a C-terminus His-tag. Pb27r-CHis was expressed and purified using a metal-affinity chromatography. Crystallization of Pb27r-CHis was performed with commercial kits and automated tests screens using the vapour diffusion technique. But, no protein crystals have been observed. In the mean time, the Pb27 gene was also subcloned into pDEST17 vector with N-terminal His-tag (Pb27r-NHis). This protein was expressed and purified but the initial crystallization attempts did not produce crystals. Dynamic light scattering and circular dichroism experiments showed that the proteins, although partially polydisperse in solution, were structured, with about 40% of a-helix. In an attempt to facilitate the formation of crystals by minimizing the entropy of the Pb27 surface, site directed mutation was held to exchange two theoretically surface residues. This protein was cloned into pDEST17 but became insoluble. To remove more flexible protein regions that could be hindering crystals formation, Pb27r-CHis and Pb27r-NHis were subjected to limited proteolysis with trypsin. A region of approximately 25 kDa remained constant when analyzed by SDS-PAGE and mass spectrometry. The study of the three-dimensional structure of this protein will enable a more detailed understanding of this molecule and could probably help to identify its biological function.

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