

CORRESPONDENCE

PROTEOLYTIC ACTIVITY OF THE 43,000 MOLECULAR WEIGHT ANTIGEN SECRETED BY *Paracoccidioides brasiliensis*.

Paracoccidioidomycosis is a infection caused by the dimorphic fungus *Paracoccidioides brasiliensis*. The disease is usually initiated by inhalation of airborne conidia or mycelial fragments⁸. Most individuals exposed to the fungus develop asymptomatic infection. However, clinical manifestations are observed in some cases. The disease may affect different organs and systems, with multiple clinical features. Relatively little is known of the biochemical mechanisms involved in the pathogenicity of this fungus, including its first contact and subsequent stages of paracoccidioidomycosis development.

Filamentous fungi which successfully colonize substrates in nature typically do so by the action of specific, digestive exoenzymes².

Proteinases produced by certain fungi pathogenic in humans have been recognized as potentially important virulence factors³. It has been suggested that extracellular proteinases of such fungi may play a role in adherence and survival of the pathogen on host mucosal surfaces¹, invasion of host tissues^{9, 11}, and digestion of immunoglobulins^{10, 14}.

Mycelial and yeast forms of *P. brasiliensis* synthesize soluble antigens which comprise up to 60 components, including glycoproteins and proteins¹². One of these, the 43 kDa glycoprotein was found as of diagnostic value^{6, 13}. This fraction is easily found in culture supernatants and in sera of patients presenting the active disease⁷.

Here we report preliminary studies related to the proteolytic activity of the 43 kDa glycoprotein.

Enzymatic characteristics as inhibitors and optimal pH were determined by SDS-polyacrylamide gel electrophoresis with substrates incor-

porated into the gel⁴. The specificity of the proteolytic activity toward casein⁵ and elastin at different pH and temperatures was spectrophotometrically determined.

Extensive proteolysis (destaining) was observed when the 43 kDa fraction was submitted to electrophoresis in gelatin, gelatin-albumin or casein containing SDS-polyacrylamide gels and then incubated overnight (18h) at pH 6.0. Complete elastin solubilization and casein hydrolysis were observed at pH 6.0 and 35°C (optimal pH and temperature). Caseinolytic and collagenolytic activities were not affected by pepstatin, hydroxymercuribenzoic acid, iodoacetamide, PMSF (phenyl methyl sulfonyl fluoride) but were inhibited by EDTA. This indicates the proteolytic activity of 43 kDa fraction as metal-dependent. The digestion of tissue structural proteins eg. collagen, elastin, suggests that this fraction may play a role in the virulence of *P. brasiliensis*.

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Recebido para publicação em 22/08/90

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