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Gelatinase Activity in *Mycobacterium bovis* Protein Extract[1]

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Leprosy and tuberculosis are still public alth problems in many parts of the world. number of molecules obtained from my- bacteria have been characterized ([21]). The st proteins identified were intracellular at shock proteins that could play a role in ycobacterial immunopathology. Another oup of proteins comprises secreted anti- ns, with a major component being com- ex 85, which presents fibronectin-binding nctions and shows extensive homology tween *Mycobacterium tuberculosis* and *ycobacterium leprae* ([11]).

Several molecular components with pro- olytic activity have been ignored in myco- acterial studies ([2]). Moreover, there is little formation in the literature concerning my- bacteria proteinase detection using classi- al biochemical techniques.

Proteases have many roles in bacteria, nging from turnover and modification of ellular proteins, to virulence factors in pathogens. Importantly, in recent years bacterial and viral proteases have been found to interact with the cytokine network ([8]). Consequently, the targeting of proteolytic enzymes is a strategy which shows promise for the control of other bacterial pathogens ([13, 20]).

In order to understand the biology of some mycobacterial proteins, and due to the advantage of being able to cultivate *M. bovis in vitro*, we have continued with the identification of mycobacterial proteins, especially those that present proteinase activities. Therefore, in the present study, gelatinase activity from total proteins (cytosolic and membrane proteins) of *Mycobacterium bovis* (MbSA) was investigated. Our objective was to analyze some biochemical properties of the proteinases present in MbSA, including susceptibility to classical inhibitors and pH dependency of activity.

MATERIALS AND METHODS

Mycobacteria. Danish BCG strain 1331 was cultured in serum free Sauton medium ([16]). The strain was incubated at 37°C for six weeks. The soluble *Mycobacterium bovis* protein extract (MbSA) was obtained by successive passage through a French pressure cell using 10,000 lbs/in^2 ([4]), followed by centrifugation at 27,138 *g* (Sorvall RC-5B, rotor SS-34) at 4°C for 1 hr in PBS pH 7.2 to eliminate bacillary debris, and finally filtered through a 0.22 μm diameter pore size membrane. The protein concentration was determined by the BCA method (Bicinchoninic acid, Pierce-Endogen, Rockford, Illinois, U.S.A.) ([19]).

Zymography (gelatinase activity). Proteinase activity was investigated by SDS-PAGE using gelatin as the proteinase substrate, as previously described ([9]). Activity was studied in 100 μg of protein from a *M. bovis* soluble extract (MbSA). The samples were dissolved 1 hr at room temperature in pH 6.8 Laemmli sample buffer (62 mM

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Abbreviations used are as follows: PMSF = phenylmethylsulfonylfluoride; p-APMSF = p-Amidinophenylmethyl sulfonylfluoride; TLCK = Nα-Tosyl-Lys :hloromethyl Ketone; TPCK = N-tosyl-L-phenylalaine chloromethylketone; AEBSF = p-Aminoethylbenzenesulfonylfluoride; OPA = ortho-phenantroline; E-64 = trans-epoxysuccinyl-L-leucylamido-[4-guanidino]butane; PCMB = p-chloromercurybenzoate; Pepst A = Pepstatin A; 3,4-DCI = 3,4-dichloroisocoumarin; Lact = Lactacystin; HEPES = N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid; CAPS = 3-[cyclohexylamino]-1-propane-sulfonic acid; DTT = dithiothreitol; DMSO = dimethylsulfoxide; 2-ME = 2-mercaptoethanol; SDS = sodium dodecyl sulfate; and SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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