

LOCALIZATION OF MYCOBACTERIAL SMEGMATIS ADENOSINE DEAMINASE

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ABSTRACT: The study was carried out to check the localization of M.smegmatic adenosine deaminase for its metabolic and clinical importance. The separation and washing of the membrane was done by using refrigerated high-speed centrifuge. The high activity was observed in crude extract while low activity in first washing of the membrane but after second washing there was no enzyme activity seen. Culture media also does not show any enzyme activity. Thus M.smegmatic adenosine deaminase may be a cytosolic enzyme and it does not excreted in to the surrounding media.

Key words: Adenosine deaminase, Mycobacterial segments, Enzyme

INTRODUCTION

Adenosine deaminase (aminohydrolase G.C.3.5,4.4) converts adenosine into inosine with removal of ammonia. The enzyme is distributed both in animal and microbial system. The deficiency of adenosine deaminase caused severe combined Immunodeficiency Syndrome (SCID) in which both thymus derived and bone marrow derived lymphocytes are sparse¹. Hence this enzyme is known as “Immunoenzyme” and considered as good marker of cell mediated immunity. Adenosine deaminase activity was studied in mycobacterial infections and ADA level was observed high in serum and peripheral blood lymphocytes of patients of pulmonary tuberculosis, leprosy and cerebrospinal fluid of tuberculous meningitis cases than normal.

Clinical laboratories are using high level of ADA as diagnostic marker of mycobacterial infection².

Mycobacterium smegmatis in non pathogenic, fast growing acid fast and saprophytic bacteria. It is strictly aerobic in nature and has resemblance with the pathogenic strain M.tuberculosis, the causative agent of human tuberculosis. SDS –PAGE analysis of cytosolic protein of M.Leprae, M.tuberculosis and M.smegmatis have shown around 30 distinct bands. Many proteins out of these were identified as antigen, when tested with serum from tuberculosis and leprosy patients. Many proteins cross react with antibodies against proteins from other mycobacterial species³.

Tests based on biochemical identification of mycobacterial products in sample specimens are performed for diagnosis of leprosy and tuberculosis. Detection of 2 ketohexyl indoline by electron capture gas chromatograph in the tuberculosis meningitis CSF was reported⁴ and tuberculosis acid by gas chromatograph⁵, Mostly bacterial cell excrete membrane bound proteins or biochemicals in surrounding media.

In the present study, we have undertaken the localization of ADA enzyme in M.smegmatis whether it is cytosolic or membrane bound as reported in micrococcussodonesis having cytosolic and also membrane bound ADA⁶. In case of membrane bound, there is possibility of the enzyme in the surrounding media, which might be useful in the diagnosis of activity mycobacterial infections.

MATERIAL AND METHODS

Material:-

Chemicals used of AR quality and purchased from M/s Sigma Chemical Co. (USA), BDH, SRL and Ranbaxy (India). *M. smegmatis* TMC 1546 used in the present study was originally obtained from the Trudeau mycobacterial culture collection, Denever, Colorado, USA.

Method:-

The cells of mycobacterium smegmatis were grown on shake culture into the Youman's and Karlson medium⁷. The first subculture was then used (0.1ml of mid log phase cells) to in culture flask, one of which was used as the source of inoculum and the other was kept as a standby culture. The cells of mid log phase were harvested by centrifugation at 5000rpm in remi refrigerated centrifuge at 4°C and washed twice with chilled distilled water. After each washing, cells were again centrifuged at 5000 rpm at 4°C and dried between suspended into 10 ml. of 50 mM. Tris HCL buffer, pH 7.6

The cell suspension was sonicated for 10 minutes by vibronic sonifier

(50 Hz, 250W) at 4°C. Cell debris was removed by centrifugation at 5000 xg in refrigerated centrifuge for 15 minutes at 4°C. supernatant containing membrane was subjected to centrifugation at 48000 xg for 45 minutes at 4°C in Beckman ultracentrifuge. Membrane plates (translucent around the edge, slightly opaque in the center) were washed twice with tris HCL buffer (pH 7.6 50 mM) to remove cytosolic protein contamination. After each washing, the centrifugation was repeated and also ADA activity and protein concentration was measured by spectrophotometric method⁸ at and lowry's methods respectively^{9,10}.

Spectrophotometric method is based on the decrease in optical density of assay mixture at 265 nm due to the conversion of adenosine into inosine per minute. The amount of product formed was calculated by taking molar extinction coefficient of adenosine as 8.5×10^3 or $(DE/AT:0.085) \times 10^4$

The assay mixture contains 10µl of buffered adenosine 5mM(pH 7.6 50mM, tris HCL buffer) enzyme 10µl of sample and 980 µl of Tris HCL buffer pH 7.6, 50 mM. One unit is defined as the amount of enzyme that converts 1µ mole of adenosine to inosine per minute under assay conditions.

DE/AT=change in optical density per minute,

U=volume of enzyme taken.

Enzyme activity in culture media

After cell growth, cell were harvested and 5ml culture media was dialyzed over night at 4°C against 100 times TRIS Hcl buffer (pH 7.6, 50mM). After dialyzed media was concentrated using polyethylene glycol (20,000). Keeping culture media in dialysis membrane at 4°C. The enzyme activity was measured by above-mentioned method.

RESULTS

Table 1 shows result of crude extract having 352.5 mg of protein and 12.345 units of total ADA enzyme activity. After first washing 34.4 mg. of protein was present in it only 2 units of membrane of enzyme. There was no ADA activity was observed in second washing of membrane but 7.48 mg. of protein of was present. The culture media also does not show any enzyme activity.

TABLE 1.Extraction of Membrane for the Detection of Membrane Bound adenosine

Extraction step	Total Protein (mg)	Specific Activity/units/mg. Per min.	Total enzyme Activity units/min.
Crude extract	352.5	0.35 unit	12.345
Membrane (First washing)	34.4	0.059	2
Membrane (second washing)	7.48	0.0	0.0

DISCUSSION

Extraction of membrane of *M. smegmatic* cell for the detection of membrane bound adenosine deaminase was done by centrifugation of supernatant containing membrane by high speed refrigerated centrifuge.

Table shows result of crud extract having protein and high enzyme activity, which indicates ADA enzyme in highly active at mid log phase of cell growth and involved in metabolic processes. After separation of membrane and its first washing enzyme activity as well as protein concentration was decreased up to six time and 10.5 times (Approx.) respectively found 34.4 mg as in case of *micrococcus sodonesiss*, it was decreased net activity was remained even after second time membrane washing and also cytosolic enzyme. But after second time membrane washing of *M. smegmatic* cells, the protein contained was obtained 7.48 mg. which might be a membrane protein and there was no enzyme activity. Hence like *ecoli*¹¹ and *Azatobacter Vinelandii*¹², *A.M. smegmatic* and might be also having cytosolic ADA having cytosolic. In view of the role of ADA in cell division and increased activity seen at mid log phase of growth of *M.smegmatic* further work by purifying and characterizing the enzyme may prove useful to understand its metabolic role. The culture media also does not show enzyme activity.

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