SHORT COMMUNICATION

Adenylate Kinase Activity in Mycobacterium leprae

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Adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) was detected in partially purified preparations of cell-free extracts of Mycobacterium leprae. The apparent K_m values of M. leprae adenylate kinase for ADP and Mg^{2+} were 1 \times 10^{-4} \text{ M} and 4 \times 10^{-4} \text{ M}, respectively. The enzyme was heat-labile: loss of activity by 80\% at 45 \text{ °C} and over 90\% at 60 \text{ °C} occurred within 5 min. M. leprae adenylate kinase was distinct from armadillo adenylate kinase in respect of affinity for substrate and heat-sensitivity.

INTRODUCTION

Little is known about the ways in which Mycobacterium leprae, the human leprosy bacillus, generates and stores energy. Since M. leprae has not been successfully cultured in vitro, an understanding of the energy metabolism of the organism might be expected to provide insight into its nutritional requirements and its relationship with the intracellular environment when it is grown in vivo. M. leprae can apparently generate its own ATP when incubated in vitro (Lee & Colston, 1985). In this study we demonstrate the presence of adenylate kinase, a key enzyme in adenylate energy metabolism (Criss & Pradhan, 1978), in cell-free extracts of M. leprae. Adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3) catalyses the reaction 2\ ADP \rightleftharpoons \text{ ATP} + \text{ AMP} in the presence of divalent cations. The demonstration that M. leprae has the biochemical requirements for generating its own ATP confirms the view that, unlike organisms such as chlamydia and rickettsia (Hatch et al., 1982; Winkler, 1976), M. leprae does not derive ATP from its host.

METHODS

Mycobacterium leprae. The bacteria were purified from the spleen of an infected nine-banded armadillo as described elsewhere (World Health Organization, 1980; Lee & Colston, 1985). Purified suspensions of M. leprae were treated with 0-5 M-NaOH for 1 h at room temperature, neutralized with 1 M-HEPES buffer and washed three times with buffered-Tween (0-1\% Tween 80 in 1 mM-MES, pH 6-8) in order to eliminate contamination with host tissues (Wheeler et al., 1982).

Preparation of M. leprae cell-free extracts. Suspensions of M. leprae in a total volume of 11 ml of 20 mm-potassium phosphate buffer (pH 6-9) were subjected to ultrasonic disruption (10 min at 90 W, Dawe Soniprobe, Dawe Instruments). Bacterial debris was removed by centrifugation for 20 min at 6000 g and the supernate was treated with 2\% (w/v) streptomycin sulphate (Sigma) in 20 mm-potassium phosphate buffer (pH 6-9) to give a final concentration of 1\%. The precipitate was removed by centrifugation for 20 min at 6000 g and the supernate treated with ammonium sulphate to give 75\% saturation. The resulting precipitate was collected by centrifugation for 30 min at 27000 g, resuspended in a small volume of 20 mm-potassium phosphate buffer (pH 6-9) and dialysed against 11 of the same buffer overnight. Unless specified, every procedure was carried out at 4 \text{ °C}. Dialysed preparations of cell-free extracts of M. leprae (D-CFE) containing 1 mg Lowry protein ml^{-1} were used throughout.

Abbreviation: D-CFE, dialysed cell-free extract of M. leprae.
Fig. 1. Two-dimensional chromatography of AMP and ATP generated by adenylate kinase activity in cell-free extracts of M. leprae (50 µg protein of D-CFE was used). GTP (50 nmol) was added prior to chromatography as an internal marker. Solvents were 3.3 M-ammonium formate/4.2% boric acid (pH 7.0) for the first dimension and 0.75 M-KH$_2$PO$_4$ (pH 3.4) for the second dimension. The ADP spot was due to excess ADP in the reaction mixture. This is a representative chromatogram of several experiments.

Fig. 2. Inactivation of M. leprae adenylate kinase at 45 °C (■) and 60 °C (○). D-CFE (10 µg protein in 10 µl) was incubated at 45 °C and 60 °C and assayed for adenylate kinase activity. Each point is the mean of duplicate determinations; results of a representative experiment are shown.

Assay of adenylate kinase. The formation of ATP by adenylate kinase was assayed by incubating 100 nmol ADP and an appropriate amount of D-CFE in a total volume of 250 µl 20 mM-Tris/HCl buffer (pH 7.0) containing 500 nmol MgCl$_2$ for 30 min at 35 °C. The reaction was stopped by adding 0.25 ml ice-chilled 7% (v/v) perchloric acid and 0.5 ml 0.15% (w/v) bovine serum albumin (Colowick, 1955; Criss & Pradhan, 1978). After 10 min on ice, the mixtures were centrifuged for 2 min at 12000 g (Eppendorf centrifuge) at room temperature. ATP was assayed by the firefly luciferin-luciferase system (Kimmich et al., 1975; Lee & Colston, 1985). In order to nullify the effect of perchloric acid on the luciferase activity, the supernate was diluted at least 1 in 20 with sterile distilled water before assaying.

Heat treatment of M. leprae adenylate kinase. Samples of D-CFE were placed in a water bath at either 45 or 60 °C. After incubation for the time specified, samples were immediately chilled on ice and assayed.

Chromatography of ATP and AMP. The formation of ATP from ADP was carried out as above except that the reaction mixture was incubated in 1 ml 20 mM-Tris/HCl, and the reaction was stopped by adding 100 µl 0.2 M-EDTA. Nucleotides in the reaction mixture were adsorbed onto acid-washed charcoal and eluted from the charcoal with 2 ml of an ethanol/water/NH$_4$OH solution (50:45:5, by vol.) through a Millipore HVLP Duromembrane filter disc (pore size 0.45 µm). The filtrate was concentrated to dryness under vacuum at room temperature overnight. The residue was resuspended in a minimum volume of distilled water and was spotted onto polyethyleneimine-cellulose F thin-layer plastic plates (Merck) and chromatographed first with 3.3 M-ammonium formate/4.2% (w/v) boric acid (pH 7.0) and then with 0.75 M-KH$_2$PO$_4$ (pH 3.4) as described by Lee & Colston (1985).

RESULTS AND DISCUSSION

In order to demonstrate that D-CFE of M. leprae possessed adenylate kinase activity, ADP was incubated with D-CFE in the presence of Mg$^{2+}$ and the resulting mixture analysed by chromatography (Fig. 1). The results demonstrated that ADP was converted into ATP and AMP, confirming the presence of adenylate kinase.

The properties of M. leprae adenylate kinase were quite different from those of armadillo adenylate kinase. The bacterial enzyme was much more sensitive to heat, losing 80% and more
than 90% of its activity when incubated for 5 min at 45 °C and 60 °C, respectively (Fig. 2); the armadillo enzyme lost only 25% of its activity even after 1 h at 60 °C. The $K_m$ of *M. leprae* adenylate kinase for ADP was $1 \times 10^{-4}$ M at a constant 2 mM concentration of MgCl$_2$, and was $4 \times 10^{-4}$ M for Mg$^{2+}$ at a constant 0-4 mM concentration of ADP. The $K_m$ for ADP of armadillo adenylate kinase was $1 \times 10^{-3}$ M, and this enzyme was totally inactivated by treatment with NaOH.

Although *M. leprae* has not yet been grown in vitro, the availability of large numbers of bacteria from infected armadillo tissue has permitted studies on the metabolism of the bacillus. Information has been obtained about carbohydrate and purine-pyrimidine metabolism (Wheeler, 1983, 1984; Khanolkar, 1982; Khanolkar & Wheeler, 1983), but little is known about the way in which *M. leprae* generates and stores energy. Recently we presented evidence that *M. leprae* generates its own ATP rather than taking ATP from surrounding host tissue, and that *M. leprae* can synthesize ATP when incubated in vitro (Lee & Colston, 1985). The results presented here, by demonstrating adenylate kinase activity in cell-free bacterial extracts, provide further evidence that *M. leprae* possesses a functional adenylate energy system.

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**REFERENCES**


