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 1×10^{-4} M and 4×10^{-4} M, respectively. The enzyme was heat-labile: loss of activity by 80% at 45 °C and over 90% at 60 °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 $2 \text{ ADP} \rightleftharpoons \text{ATP} + \text{AMP}$ 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 1 l of the same buffer overnight. Unless specified, every procedure was carried out at 4 °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

Fig. 2

Fig. 1. Two-dimensional chromatography of AMP and ATP generated by adenylate kinase activity in cell-free extracts of *M. leprae* (50 μ g 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-KH₂PO₄ (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 μ g protein in 10 μ l) 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 μ l 20 mM-Tris/HCl buffer (pH 7.0) containing 500 nmol MgCl₂ 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 μ l 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/NH₄OH solution (50:45:5, by vol.) through a Millipore HVLP Duromembrane filter disc (pore size 0.45 μ m). 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-KH₂PO₄ (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 Mg²⁺, 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 1×10^{-4} M at a constant 2 mM concentration of $MgCl_2$, and was 4×10^{-4} M for Mg^{2+} at a constant 0.4 mM concentration of ADP. The K_m for ADP of armadillo adenylate kinase was 1×10^{-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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