Characterization of Ca²⁺-ATPase Activity in *Streptomyces griseus*

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Ca²⁺-ATPase activity has been characterized in *Streptomyces griseus*. The enzyme has a pH optimum of 8.5 at 37 °C. Its Ca²⁺ requirement can be substituted by Cd²⁺, Zn²⁺ and Mn²⁺. Mg²⁺ inhibits the enzyme non-competitively.

**INTRODUCTION**

Adenosine triphosphatase (ATP phosphohydrolase, EC 3.6.1.3) activity requiring Ca²⁺ or Mg²⁺ has been reported in a large number of Gram-positive and Gram-negative bacteria (Abrams & Smith, 1974; Machtiger & Fox, 1973). Other microbial ATPases are stimulated by Na⁺ and/or K⁺ (Abrams et al., 1960; Hayashi & Uchida, 1965). There is ample evidence that bacterial ATPase activities are associated with the cell membrane structures and have a functional role in the transport of ions across the cell membrane. Recently, a very active Mg²⁺-(Ca²⁺)-stimulated ATPase has been demonstrated in *Mycobacterium smegmatis* (Grover et al., 1978), but there have been no reports on ATPase systems in the Streptomycesaceae. The present communication describes the existence and partial characterization of a Ca²⁺-ATPase in *Streptomyces griseus*.

**METHODS**

*Organism, growth and preparation of cell-free extracts. Streptomyces griseus* (127-2) was grown aerobically for 5 d at 27 °C as described by Talwar & Khuller (1977), then harvested by filtration, washed three times with chilled 0.85% (w/v) NaCl and dried between filter papers. The organisms were stored at −20 °C. Cell suspension (1 g wet wt per 3 ml) was ultrasonically disrupted in 0.05 M-Tris/HCl buffer (pH 7.4) for 10 min keeping the sample in ice. The homogenate was centrifuged at 800g for 10 min at 4 °C, the pellet was discarded and the supernatant was recentrifuged for 40 min at 20000g at 4 °C. This supernatant exhibited nearly 90% of the activity of the original enzyme preparation. It was stored at −20 °C.

*Enzyme assay.* ATPase activity was measured by determining the release of inorganic phosphate from ATP using a slightly modified version of the procedure of Evans (1969). The assay mixture contained (in 1 ml): Tris/HCl buffer (pH 8.5), 100 mM; ATP (Sigma), 4 mM; CaCl₂, 4 mM; cell-free extract. After 15 min at 37 °C, the reaction was stopped by adding 1 ml of chilled 10% (w/v) trichloroacetic acid and the tubes were left in ice for 15 min. Samples were centrifuged at 3000g for 5 min and inorganic phosphate was determined in the supernatant according to Tausky & Shorr (1953). Protein was estimated by the method of Lowry et al. (1951). Enzyme activities are expressed as μmol inorganic phosphate (P_i) liberated per mg protein in 15 min under the standard assay conditions.

**RESULTS AND DISCUSSION**

The enzyme preparation obtained from *S. griseus* contained an active Ca²⁺-activated ATPase [specific activity, 0.68 μmol P_i (mg protein)⁻¹ (15 min)⁻¹]. No inorganic phosphate was liberated in the absence of Ca²⁺. A linear relationship between enzyme activity and
substrate concentration was apparent up to 4 mM-ATP, but above 8 mM-ATP enzyme activity was severely depressed. The $K_m$ for ATP, calculated from a double reciprocal plot, was 14.4 mM with a $V_{max}$ of about 2.5 \( \mu \text{mol P}_i \) (mg protein)$^{-1}$ (15 min)$^{-1}$.

At room temperature, the enzyme activity was completely lost within 48 h. At $-20 \, ^\circ C$ the enzyme retained only 30% of the original activity over 4 d, whereas at $4 \, ^\circ C$ about 50% of the original activity remained after 4 d. The enzyme preparations kept at $4 \, ^\circ C$ and at $-20 \, ^\circ C$ had lost all activity within 10 d. The enzyme had a pH optimum of 8.5 which is slightly higher than the optimum pH reported for ATPase from other bacteria (Grover et al., 1978; Gross & Coles, 1968). Its optimum temperature under the conditions of assay was 40 °C.

**Effect of monovalent and divalent metal ions on ATPase activity**

Preliminary experiments established that for maximum enzyme activity, the optimal ratio of Ca$^{2+}$ to ATP was 1:1. This was identical to that reported for the ATPase system in *Streptococcus faecalis* (Abrams et al., 1960; Abrams, 1965) though in *Bacillus megaterium* (Greenwalt et al., 1962) the optimal Mg$^{2+}$ to ATP ratio is 1:2. The enzyme from *S. griseus* exhibited 80 to 120% of its activity with Ca$^{2+}$ when Ca$^{2+}$ was replaced by Mn$^{2+}$, Zn$^{2+}$ or Cd$^{2+}$ at the same concentration. However, Ba$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Fe$^{2+}$ and Hg$^{2+}$ (each at 4 mM) strongly inhibited enzyme activity. Mg$^{2+}$, which normally stimulates ATPase activity (Greenwalt et al., 1962; Grover et al., 1978), strongly inhibited the ATPase of *S. griseus* both in the presence and absence of Ca$^{2+}$. Thus, the ATPase activity present in this micro-organism has a strict requirement for Ca$^{2+}$ indicating it to be a true Ca$^{2+}$-ATPase system and thus similar to those characterized in various other micro-organisms (Evans, 1969; Munoz et al., 1969).

The type of inhibition exerted by Mg$^{2+}$ on the Ca$^{2+}$-ATPase was shown by Dixon plots (Fig. 1) to be non-competitive, i.e. its site of interaction differs from that of Ca$^{2+}$. Similar results have been observed in *Escherichia coli* (Evans, 1969; Farris et al., 1972) and in *Aspergillus nidulans* (Selvam & Shammugasundram, 1974). The inhibition constant ($K_i$) for Mg$^{2+}$, calculated from Fig. 1, was 0.42 mM. This inhibition might be of some significance in the regulation of the cation transport across the cell membrane.

Na$^+$ and K$^+$ (up to 140 mM) had no effect on enzyme activity. However, Cu$^+$, Li$^+$, Ag$^+$ and NH$_4^+$ (each at 4 mM) were strongly inhibitory.

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REFERENCES


