Characterization of Ca^{2+} -ATPase Activity in *Streptomyces griseus*

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Ca^{2+} -ATPase activity has been characterized in *Streptomyces griseus*. The enzyme has a pH optimum of 8.5 at 37 °C. Its Ca^{2+} requirement can be substituted by Cd^{2+} , Zn^{2+} and Mn^{2+} . Mg^{2+} inhibits the enzyme non-competitively.

INTRODUCTION

Adenosine triphosphatase (ATP phosphohydrolase, EC 3.6.1.3) activity requiring Ca^{2+} or Mg^{2+} 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 Mg^{2+} -(Ca^{2+})-stimulated ATPase has been demonstrated in *Mycobacterium smegmatis* (Grover *et al.*, 1978), but there have been no reports on ATPase systems in the Streptomycetaceae. The present communication describes the existence and partial characterization of a Ca^{2+} -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 800 g for 10 min at 4 °C, the pellet was discarded and the supernatant was recentrifuged for 40 min at 20 000 g 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; CaCl_2 , 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 3000 g 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 Ca^{2+} -activated ATPase [specific activity, $0.68 \mu\text{mol P}_i$ (mg protein) $^{-1}$ (15 min) $^{-1}$]. No inorganic phosphate was liberated in the absence of Ca^{2+} . A linear relationship between enzyme activity and

Fig. 1. Plots according to the method of Dixon (1953) showing that the inhibition of Ca^{2+} -ATPase by Mg^{2+} is non-competitive. Activities were assayed as described in Methods with Mg^{2+} between 0 and 10 mM, Ca^{2+} at 4 mM, and ATP at 4 mM (●) or 8 mM (○).

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 $2.5 \mu\text{mol P}_i (\text{mg protein})^{-1} (15 \text{ min})^{-1}$.

At room temperature, the enzyme activity was completely lost within 48 h. At -20°C the enzyme retained only 30% of the original activity over 4 d, whereas at 4°C about 50% of the original activity remained after 4 d. The enzyme preparations kept at 4°C and at -20°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

- ABRAMS, A. (1965). The release of bound adenosine triphosphatase from isolated bacterial membranes and the properties of the solubilized enzyme. *Journal of Biological Chemistry* **240**, 3675-3681.
- ABRAMS, A. & SMITH, J. B. (1974). Bacterial membrane adenosine triphosphatase. In *The Enzymes*, vol. 10, pp. 395-429. Edited by P. D. Boyer. New York: Academic Press.
- ABRAMS, A., MCNAMARA, P. & JOHNSON, F. B. (1960). Adenosine triphosphatase in isolated bacterial membranes. *Journal of Biological Chemistry* **235**, 3659-3662.
- DIXON, M. (1953). The determination of enzyme inhibitor constants. *Biochemical Journal* **55**, 170-171.
- EVANS, D. J., JR (1969). Membrane adenosine triphosphatase of *Escherichia coli*: activation by calcium ions and inhibition by monovalent cations. *Journal of Bacteriology* **100**, 914-922.
- FARRIS, R. N., LONDERO, L. & TRUCCO, R. E. (1972). Effect of membrane lipid composition on the allosteric inhibition by sodium of the (Ca^{2+}) adenosine triphosphatase from *Escherichia coli*. *Journal of Bacteriology* **109**, 471-473.
- GREENWALT, J. W., WEIBULL, C. & LOW, H. (1962). The hydrolysis of adenosine triphosphatase by cell fractions of *Bacillus megaterium*. *Journal of Biological Chemistry* **237**, 853-858.
- GROSS, R. & COLES, N. W. (1968). Adenosine triphosphatase in isolated membranes of *Staphylococcus aureus*. *Journal of Bacteriology* **95**, 1322-1326.
- GROVER, G., DHARIWAL, K. R. & VENKITASUBRA-
MANIAN, T. A. (1978). Studies on Mg^{2+} -(Ca^{2+} -) activated adenosine triphosphatase from *Mycobacterium smegmatis* CDC 46. *Journal of General Microbiology* **105**, 343-345.
- HAYASHI, M. & UCHIDA, R. (1965). A cation-activated triphosphatase in cell membranes of halophilic *Vibrio parahaemolyticus*. *Biochimica et biophysica acta* **110**, 207-209.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- MACHTIGER, N. A. & FOX, C. F. (1973). Biochemistry of bacterial membranes. *Annual Review of Biochemistry* **42**, 575-600.
- MUNOZ, E., SALTON, M. R. J., NG, M. M. & SCHOR, M. T. (1969). Membrane adenosine triphosphatase of *Micrococcus lysodeikticus*. *European Journal of Biochemistry* **7**, 490-501.
- SELVAM, R. & SHAMMUGASUNDRAM, K. R. S. (1974). Adenosine triphosphatase of *Aspergillus nidulans*. Activation by calcium ion and inhibition of this effect by magnesium ions. *Indian Journal of Biochemistry and Biophysics* **11**, 83-85.
- TALWAR, P. & KHULLER, G. K. (1977). Effect of age on the major phospholipids of *Streptomyces griseus*. *Indian Journal of Biochemistry and Biophysics* **14**, 185-186.
- TAUSKY, H. H. & SHORR, E. (1953). A micrometric method for the determination of inorganic phosphorus. *Journal of Biological Chemistry* **202**, 675-685.