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A Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity in subcellular fractions from *Schistosoma mansoni*

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A Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity was found in subcellular fractions from *Schistosoma mansoni*. Its specific and relative activities were higher in the heterogeneous cuticle fraction and in the microsomal fraction. The $K_{0.5}$ for ATPase activation by free Ca^{2+} was 0.2–0.5 μM. This is the first description of an ATPase activity stimulated by Ca^{2+} in the micromolar range in *S. mansoni*.

(Ca^{2+} + Mg^{2+})-ATPase; Ca^{2+}; Subcellular fraction; Microsome; (*Schistosoma mansoni*)

1. INTRODUCTION

In mammals, Ca^{2+}-stimulated, Mg^{2+}-dependent ATPases are responsible for calcium uptake by sarcoplasmic reticulum and are part of the Ca^{2+} transport system present in plasma membranes [1]. In contrast to extensive studies in mammals, the information available on Ca^{2+}-ATPase activity in *Schistosoma mansoni* is limited to a few descriptions of ATPases activated by high concentrations of Ca^{2+} or Mg^{2+} [2–4]. Recently we also reported the presence of ATPase activity in the tegument of *S. mansoni* that required calcium or magnesium and had a $K_{0.5}$ of 0.32 mM for either of the metal-ATP complexes [5]. This activity was attributed to the presence of a Ca^{2+} or Mg^{2+}-dependent ATPase similar to the basic ATPase of sarcoplasmic reticulum from skeletal muscle [5,6]. The aim of the present work was to look for a Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity in subcellular fractions from *S. mansoni* that could be responsible for the transport of Ca^{2+} in vivo. We report here the first evidence for the presence in *S. mansoni* of such a Mg^{2+}-dependent ATPase stimulated by Ca^{2+} in the micromolar range.

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2. MATERIALS AND METHODS

Adult male worms of *S. mansoni* were obtained from infected mice as previously described [7]. Male cercariae were obtained from snails previously infected with a single miracidium. About 2000 worms were homogenized in a Dounce homogenizer at 4°C in a 0.25 M sucrose solution buffered to pH 7.4 with 5 mM Tris-HCl using 3 sequences of 10 passes of the pestle. The homogenate was centrifuged according to the method of Smithers et al. [8] to obtain four pellets (P_1, P_2, P_3 and P_4) sedimenting respectively at 300 × g_{av} (5 min), 1000 × g_{av} (10 min), 8000 × g_{av} (10 min) and 100000 × g_{av} (60 min). The pellets were resuspended in buffered sucrose solution and stored at − 15°C until use. The protein concentration was determined by the method of Lowry et al. [9] with bovine serum albumin as a standard.

ATPase activity was determined by measuring the P_i liberated according to the method of Fiske and Subbarow [10], slightly modified. The enzyme (6–40 μg protein) was incubated for 1 h at 37°C in 0.5 ml of 50 mM Hepes-Tris buffer, pH 7.4, containing 10 mM NaN_3, 5 mM Tris-ATP, 5 mM Tris-EGTA and 4 mM $MgCl_2$ in the presence or absence of various concentrations of $CaCl_2$. Under this condition, it was calculated that the MgATP concentration ranged from 3.5 mM in the absence

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of added $CaCl_2$ to 3.8 mM in the presence of the highest $CaCl_2$ concentration used.

The concentration of free Ca^{2+} was adjusted by utilizing EGTA and the concentrations of free Ca^{2+}, free Mg^{2+}, CaATP and MgATP were calculated as described by Fabiato and Fabiato [11]. For the measurements of the calcium- or magnesium-requiring ATPase activity reported earlier [5], 1 mM EGTA and millimolar concentrations of calcium were used.

Values of $K_{0.5}$ (concentration of Ca^{2+} required for half-maximal activation of ATPase) were obtained from regression lines in Hill plots [12]. Tris-ATP was prepared from vanadate-free Na_2-ATP by passage through a cation-exchange resin (Dowex 50W).

For electron microscopy, pellets of the fractions were fixed for 1 h in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), washed in buffer, post-fixed for 1 h in 1% OsO_4 in 0.1 M phosphate buffer, dehydrated in ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Jeol 100 CX electron microscope.

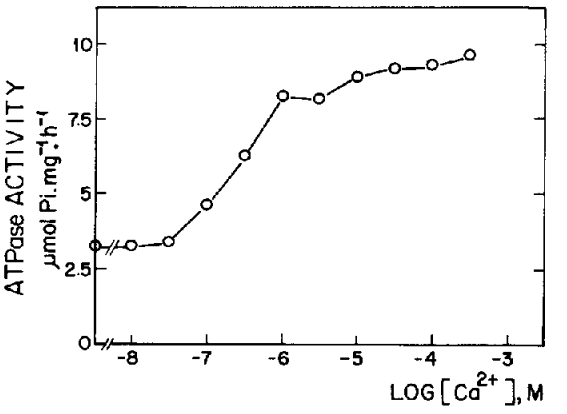

Fig.1. Concentration dependence on free Ca^{2+} of the Mg^{2+}-dependent ATPase activity of the P_4 fraction from *S. mansoni*.

3. RESULTS

In preliminary experiments, we observed an ATPase activity in the presence of relatively high concentrations of Mg^{2+} or Ca^{2+}, in the absence of other cations and in all four P_1–P_4 fractions. This activity apparently similar to that described in the tegument of *S. mansoni* [5], was referred to as basic ATPase activity. The $K_{0.5}$ values were 0.4–1 mM for either of the metal-ATP complexes.

In other experiments, ATPase activity was measured in the presence of a constant, saturating MgATP concentration with or without added Ca^{2+}. Activity measured in the absence of Ca^{2+} was referred to as the basic ATPase activity while the Ca^{2+}-stimulated ATPase activity was calculated as the difference between the activities measured in the presence and absence of Ca^{2+} [6,13]. Fig.1 shows the concentration dependence on free Ca^{2+} of the Mg^{2+}-dependent ATPase activity in a typical experiment with the P_4 fraction. Table 1 indicates the individual values of the four parameters (V_{max} of the basic ATPase; V_{max}, $K_{0.5}$ for free Ca^{2+} and Hill coefficient (h) of the Ca^{2+}-stimulated ATPase) measured for the P_1–P_4 fractions obtained from two different preparations of *S. mansoni*. The Ca^{2+}-stimulated ATPase activity was present in all four fractions but was enriched in P_1 and P_4 as indicated by the higher specific activity in these fractions (table 1). In these fractions the $K_{0.5}$ for free Ca^{2+} was 0.2–0.5 μM. The morphological analysis of the fractions showed that P_1 contained pieces of unbroken worm tissue, tegument with spines, nuclei and some vesicles (fig.2A). Fraction P_2 consisted mainly of nuclei although other structures such as mitochondria, dense bodies and vesicles were seen (fig.2B). Fraction P_3 consisted mainly of mitochondria, with a few vesicles and dense bodies also present (fig.2C). Fraction P_4 consisted mainly of free vesicles or large multivesicular structures and particles that may correspond to ribosomes. A few mitochondria were seen in this fraction (fig.2D).

Table 1

Basic and Ca^{2+}-stimulated ATPase activities of P_1–P_4 fractions from *S. mansoni*

Fraction	Basic ATPase	Ca^{2+}-stimulated ATPase		
	V_{max}	V_{max}	$K_{0.5}$	h
P_1 a	1.5	3.6	0.37	0.55
b	4.0	9.4	0.36	0.72
P_2 a	3.8	1.3	–	–
b	4.5	1.8	–	–
P_3 a	3.2	0.6	–	–
b	1.9	1.2	0.46	0.75
P_4 a	6.1	5.8	0.21	0.87
b	3.0	5.7	0.52	0.74

a and b refer to experiments performed with two different preparations. (–) Indicates that the ratio of V_{max} (μmol $P_i \cdot mg^{-1} \cdot h^{-1}$) for Ca^{2+}-stimulated and basic ATPase was so weak that it was not possible to determine the $K_{0.5}$ (μM, free Ca^{2+}) and h values in these experiments

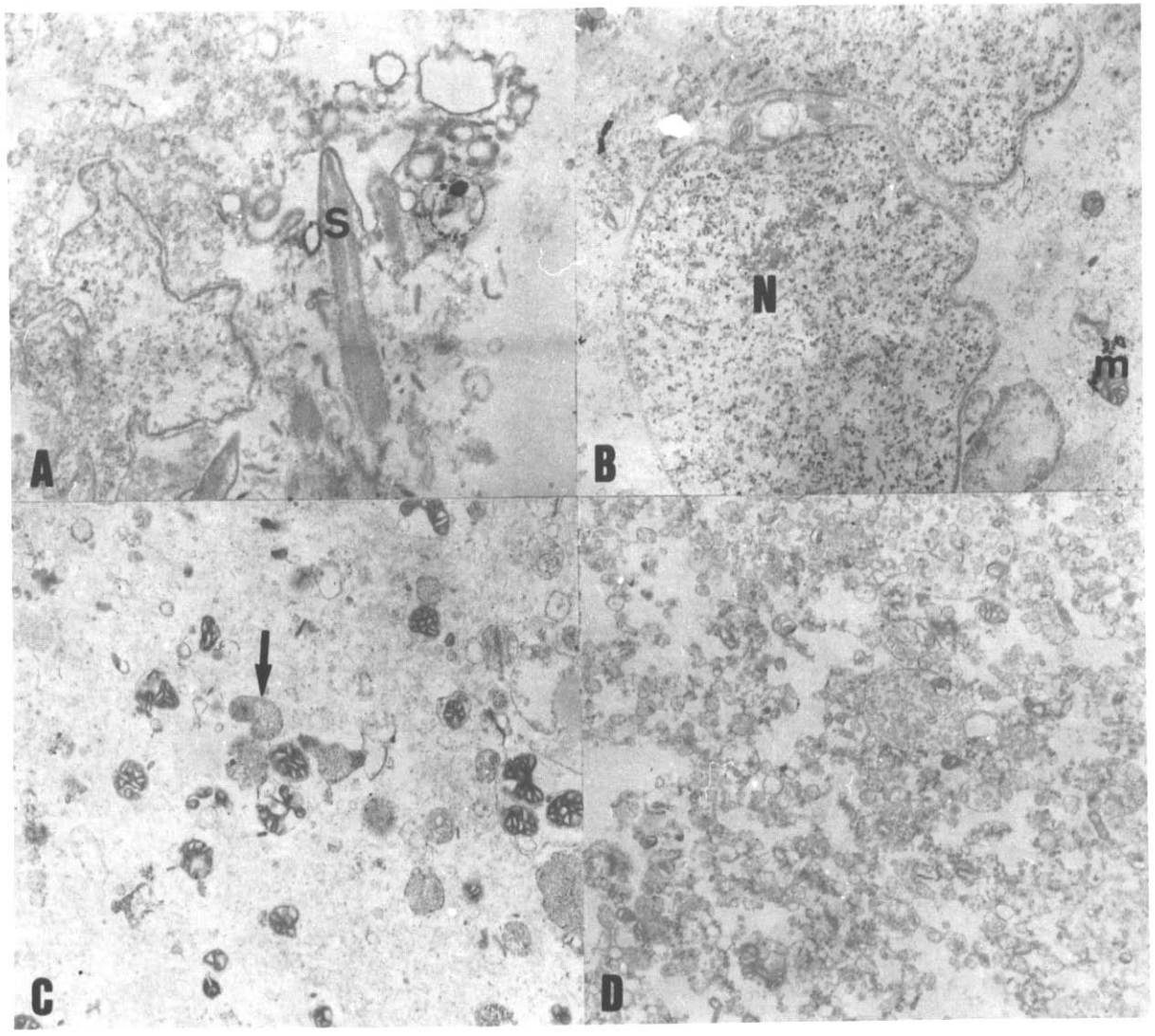

Fig.2. General view of the P_1 (A), P_2 (B), P_3 (C), and P_4 (D) fractions. Pieces of the tegument with spines (S) are seen in the P_1 fraction. The P_2 fraction contains nuclei (N), some mitochondria (m) and vesicles. Fraction P_3 contains a large number of mitochondria, some dense bodies (arrow) and vesicles. Fraction P_4 mainly consists of vesicles. × 10500.

4. DISCUSSION

Previous data on the presence of Ca^{2+}-ATPase activity in *S. mansoni* consist of reports on the presence of ATPases activated by high concentrations of Ca^{2+} or Mg^{2+} [2–5] and of histochemical observations revealing ATPase activity in the tegument [14,15]. These ATPase activities referred to basic ATPases are probably not coupled to Ca^{2+} transport. We report here the first experimental evidence for the presence of a Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase in adult worms of *S. mansoni*. This ATPase is stimulated by micromolar concentrations of free Ca^{2+} as is the case for the classical (Ca^{2+} + Mg^{2+})-ATPases present in sarcoplasmic reticulum [13] and plasma membrane [16] of mammalian cells, where they are coupled to Ca^{2+} transport. For that reason, we postulate that the Ca^{2+}-stimulated ATPase activity present in subcellular fractions from *S. mansoni* is related to a physiologically active Ca^{2+} pump in this worm. This Ca^{2+}-stimulated ATPase is

somewhat more evident in the P_1 and P_4 fractions. P_1, the most heterogeneous fraction, is regarded as the cuticle fraction since it contains large pieces of the tegument ([8] and fig.2A). However, the Ca^{2+}-stimulated activity of the present report is absent from isolated partially purified tegumental material [5], and therefore should probably be assigned to other cellular or subcellular structures. P_4, the microsomal fraction ([8] and fig.2D), is better defined but might contain plasma as well as reticulum membrane. For these reasons, the subcellular localization of the ATPase activity described here cannot yet be assigned.

Experiments in progress aim to characterize better this enzyme activity and to determine if the vesicles formed in the P_4 fraction are able to pump $^{45}Ca^{2+}$.

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