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PROPERTIES AND DRUG SENSITIVITY OF ADENOSINE TRIPHOSPHATASES FROM *SCHISTOSOMA MANSONI*

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ABSTRACT: The hydrolysis of ATP was measured in the presence of schistosome homogenates and various cations. The enzyme was stimulated strongly by either Ca^{2+} or Mg^{2+}. Na^+ added to the activation by Ca^{2+}. A minor (17%) component was $Na^+ + K^+ + Mg^{2+}$–dependent and ouabain-sensitive. Praziquantel, niridazole, oxamniquine, and hycanthone had no direct effect on the ATPase activity of schistosome homogenates. When schistosomes were pretreated with these drugs in vitro, washed thoroughly, and then homogenized, hycanthone, praziquantel, and oxamniquine caused a reduction in ATPase content of the worms. Niridazole did not share this effect. These results suggest that antischistosomal drugs did not directly inhibit ATPase, but did reduce ATPase in whole worms, possibly by removing or damaging the tegument, which is thought to contain most of the ATPase activity. In vitro ATPase measurements may be a useful indicator of pharmacologic activity of some types of drugs.

The expectation that ATPases might function in the uptake of nutrients by schistosomes is supported by histological observations revealing high ATPase activity in the schistosomal tegument (Wheater and Wilson, 1976) and in the surface of schistosome sporocysts (Krupa et al., 1975). Some ATPase activity also has been localized in the muscle tissue of *Schistosomatium douthitti* (Rogers, 1976). Bueding (1962) reported the presence in schistosomes of ATPases activated by Ca^{2+} and Mg^{2+}. Although a weak dependence of schistosomal ATPase activity on Na^+ and K^+ has been reported (Ross and Jaffe, 1972), little is known of the properties or the function of this enzyme (or group of enzymes) in these parasites. Several workers have reported alkaline and acid phosphatases in schistosomes (Nimmo-Smith and Standen, 1963; Cesari, 1974; Ernst, 1976). These enzymes may be of considerable importance in the parasites' metabolism.

The interaction of ATPase and drugs also may be important because some drugs, such as hycanthone (Hillman et al., 1977) and praziquantel (Pax et al., 1978) are thought to damage the tegument of schistosomes. Therefore, effects on ATPase might be expected with these drugs, though the ATPase effect may or may not be directly related to their mechanism of therapeutic action.

In this paper we report on the effects of ions and antischistosomal drugs on in vitro ATPase activity in *Schistosoma mansoni*.

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METHODS

Tissue handling

Schistosoma mansoni was maintained in the laboratory by Drs. Sharon K. File and Jerome H. Smith of the Pathology Department at Galveston. Infected mice (ICR) were sacrificed when their infections were about 6 wk old. Worm pairs (about 20 per mouse) were recovered by dissection and placed in Fischer's cell culture medium (Grand Island Biological Co.) (FM) with added buffering and antibiotics (Hillman and Senft, 1975). Some worms were homogenized immediately. Other worms were incubated in FM for 4 hr with drugs added to the medium, then rinsed with FM, weighed on a microbalance, and homogenized.

Drug preparations

Praziquantel was obtained from Dr. Peter Andrews of Bayer Laboratories. Pfizer Limited donated oxamniquine, Lot No. R31. Hycanthone mesylate was donated by Sterling Winthrop Research Institute. Ciba Pharmaceutical contributed the niridazole. The drugs were dissolved in FM for incubation of the worms, or in water for in vitro ATPase studies.

ATPase measurements

Methods for the enzyme measurements have been described previously (Nechay and Nelson, 1970). Tissue homogenates were prepared 1:10 in sucrose solution containing tris (hydroxymethyl) aminomethane, disodium edetate (EDTA), and sodium deoxycholate. These homogenates were further diluted 1:60 with sucrose-tris solution.

The enzyme activity was measured by the rate of release of inorganic phosphate (P) from ATP at 37 C. The incubation mixture contained diluted tissue

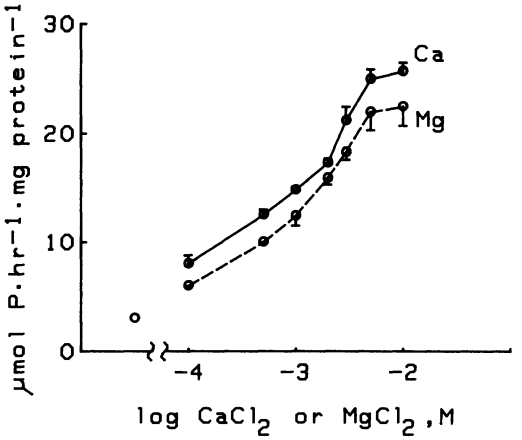

FIGURE 1. Activation of schistosomal ATPase separately by $CaCl_2$ or $MgCl_2$ with no other cations present. The data represent a mean of four experiments ± 1 standard deviation.

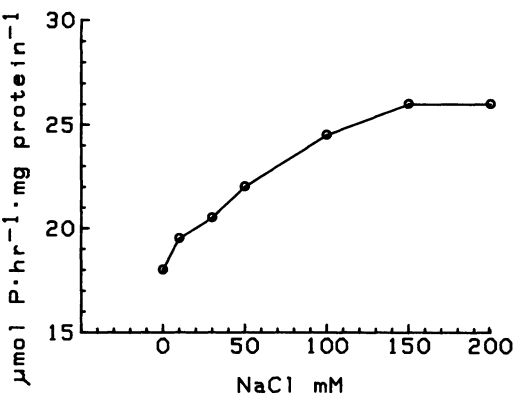

FIGURE 2. Activation of schistosomal ATPase by different concentrations of Na^+ in the presence of 3 mM $CaCl_2$. The data represent a mean of two experiments.

homogenates, NaCl, KCl, $CaCl_2$, $MgCl_2$ in various concentrations and combinations, 3 mM ATP, and histidine-imidazole buffer at pH 7.4, unless otherwise stated. The assay was started by the addition of ATP and continued for 60 min. Control experiments showed that none of the reagents or buffers used in the present study interfered with the assay.

Inorganic phosphate was determined by the method of Fiske and Subbarow (1929). Protein in homogenates of worms was measured by the method of Lowry et al. (1951), using crystalline bovine serum albumin as the standard. All determinations were performed in duplicate.

RESULTS

The survey of ATPase activities in schistosomal homogenates in various ionic environments showed that the enzyme was activated strongly either by Mg^{2+} or by Ca^{2+}. The concentration-response relationships in Figure 1 indicate that $CaCl_2$ stimulated the enzyme to a higher activity than $MgCl_2$ did. Only low activity was seen in the absence of added cations. Approximately 10^{-3} M of either $CaCl_2$ or $MgCl_2$ was required to reach 50% of maximal enzyme activity. Because the binding constant of Ca^{2+} and Mg^{2+} with ATP are $10^{3.6}$ and 10^4, respectively (Chaberek and Martell, 1959), the concentrations of free divalent cations present under conditions of enzyme stimulation were less than those of total chloride salts.

The stimulation of Ca^{2+}-dependent ATPase by Na^+ is shown in Figure 2. A maximum increase occurred at 150 mM Na^+ with one-half maximal effect at 50 mM Na^+. K^+ did not increase the enzyme activity beyond that shown in the presence of Ca^{2+} alone. Na^+ and K^+, by themselves or in combination, did not simulate the enzyme unless Ca^{2+} or Mg^{2+} were present. The maximum observed Ca^{2+}- or Mg^{2+}-ATPase activity occurred at pH 8.0 (Fig. 3).

Figure 4 shows that ouabain produced a maximum of 17% inhibition of schistosomal ATPase under optimal ionic conditions for $Na^+ + K^+ + Mg^{2+}$–ATPase activity. This maximal effect was achieved at 10^{-4} and 10^{-3} M ouabain. At 10^{-6} M ouabain a one-half maximal inhibitory effect was seen.

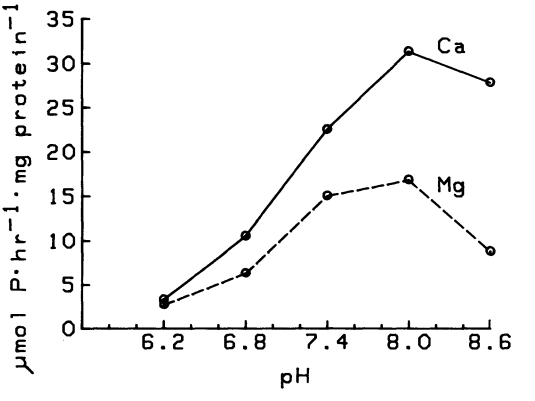

FIGURE 3. pH dependence of schistosomal ATPase activated either by 3 mM $CaCl_2$ or 3 mM $MgCl_2$. The data represent one experiment.

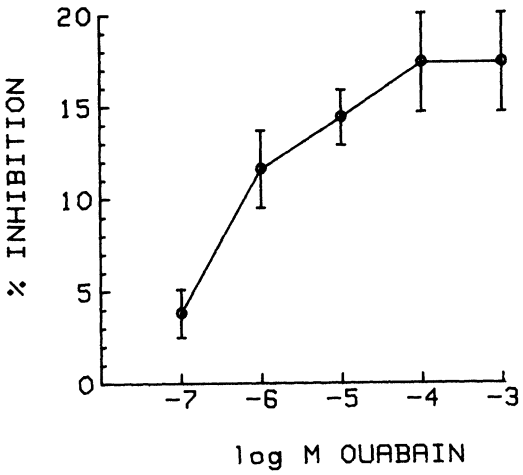

FIGURE 4. Inhibition of schistosomal ATPase by ouabain in presence of 3 mM $MgCl_2$, 100 mM NaCl, and 20 mM KCl. The data are the means of five experiments ± 1 standard deviation.

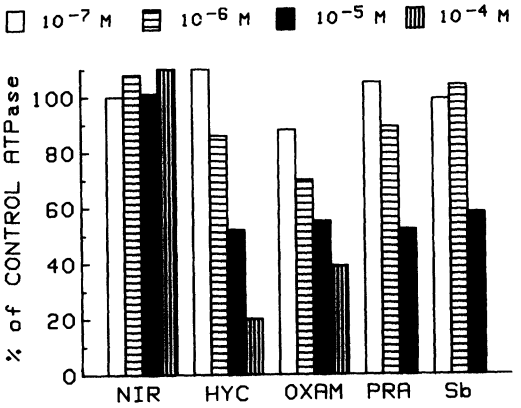

FIGURE 5. Schistosomal ATPase activity in the presence of 3 mM $CaCl_2$, 100 mM NaCl, and 20 mM KCl, after incubation of intact worms with drugs for 4 hr in Fischer' cell culture medium at 37 C. Control worms were incubated in the same medium without drugs added. The data represent one experiment, except that at 10^{-4} M drug concentration there were two experiments. NIR = niridazole; HYC = hycanthone; OXAM = oxaminquine; PRAZ = praziquantel; Sb = antimony potassium tartrate. 10^{-5} M praziquantel concentration is uncertain owing to low solubility of the compound.

Niridazole (10^{-4} M), hycanthone (10^{-4} M), oxaminiquine (10^{-5} M), praziquantel (10^{-5} M), and antimony potassium tartrate (10^{-5} M) had no direct effect on the ATPase activity of schistosome homogenates in the presence of 3 mM $CaCl_2$ + 100 mM NaCl + 20 mM KCl (2 experiments) or 3 mM $MgCl_2$ + 100 mM NaCl + 20 mM KCl (2 experiments). In all experiments, the drugs were preincubated with the enzymes plus electrolytes for 30 min and drugs were still present during the standard 60-min reaction time (data not shown). A 30-min preincubation was chosen because it produces maximum ATPase inhibition with numerous drugs.

When living schistosomes were pretreated with these drugs in vitro for 4 hr, washed thoroughly, and homogenized, hycanthone, oxamniquine, praziquantel, and antimony potassium tartrate reduced the Ca^{2+}-activated ATPase of the worms (Fig. 5). Similar results were obtained when Mg^{2+} was substituted for Ca^{2+} (data not shown).

DISCUSSION

Studies of ATP hydrolysis by schistosome tissue homogenates in the presence of various ions show that the ATPase required either Ca^{2+} or Mg^{2+} for maximum activity, but Ca^{2+} produced more activation than Mg^{2+}. Na^+ added to the activation by Ca^{2+}. About one fifth of the enzyme activity stimulated by Mg^{2+} + Na^+ + K^+ was sensitive to ouabain. Consistent with these findings, a similar fraction of the enzyme activity was stimulated above the Mg^{2+} baseline by Na^+ + K^+.

Slight ATPase activity was observed in the absence of added divalent cations. This may be the result of residual Ca^{2+} and Mg^{2+} in tissue homogenates. Activation by Cl^- was excluded by the fact that very low enzyme activity was seen in the presence of Cl^--containing imidazole-histidine buffer without added electrolytes. This negative finding is mentioned because of current interest in active Cl^- transport in various biological systems.

Of the types of ATPase present in schistosomes, the Mg^{2+} + Na^+ + K^+–activated enzyme has been studied most thoroughly in a variety of other species and organs. Such enzymes have been found to function in Na^+ and K^+ transport across cell membranes (Skou, 1965). This enzyme function is reflected in bioelectrial phenomena, osmoregulation, and salt homeostasis, as well as transport of nonelectrolytes such as glucose (Csàky, 1965; Skou, 1975; Schwartz et al., 1975; Nechay,

1977). A drastic inhibition of this enzyme by cardiac glycosides in vertebrates results in death (Möller, 1975).

There are wide species differences (as much as 1,000-fold) in sensitivity to toxic effects of cardiac glycosides which correlate well with the sensitivity of respective $Na^+ + K^+$–ATPase preparations to these drugs. The 50% inhibition of schistosomal $Na^+ + K^+$–dependent ATPase fraction by ouabain concentrations on the order of 10^{-6} M places schistosomes among species such as man, dog, cat, rabbit, and chicken that are sensitive to cardiac glycosides. Examples of resistant species are rat and toad (Repke et al., 1965).

Our results seem to be in agreement with the suggestions of Bueding (1962), who stated that Ca^{2+} and Mg^{2+}–dependent ATPases were present. We did not attempt to confirm the statement of Bueding (1962) that Ca^{2+}-dependent and Mg^{2+}-dependent enzymes could be separated by centrifugation. Our findings of high activation by Mg^{2+} agree with those reported by Ross and Jaffe (1972).

Considerably less is known about the chemistry and functions of Ca^{2+}-activated ATPase than about $Na^+ + K^+$ ATPase. In general, Ca^{2+}-dependent systems function in maintenance and restoration of low Ca^{2+} concentrations in the cytosol by active Ca^{2+} transport out of the cell and/or into sarcoplasmic reticulum and mitochondria. Thus, the Ca^{2+}-dependent ATPase participates in muscle relaxation and stabilization of epithelial and secretory cells. In red blood cells and in sarcoplasmic recticulum, where active Ca^{2+} transport is best understood, both Ca^{2+} extrusion and the Ca^{2+}-dependent ATPase require Mg^{2+} as a cofactor (Schatzmann, 1975; Korenbrot, 1977). Perhaps in schistosomes, the enzyme has a role in muscular contraction and in uptake and extrusion of nutrients and waste products.

None of the drugs tested acted by direct inhibition of ATPase, but some drugs may have caused reduction of ATPase activity by damaging or removing the schistosomal surface. In vitro measurement of ATPase could be used as an indicator of drug-induced damage to the parasite's tegument, even if loss of ATPase was not the direct mechanism of the drug's therapeutic action. Future studies of the physiological role of ATPase in schistosomes may help to predict the in vivo effects of drugs that inhibit or remove ATPase in parasites.

ACKNOWLEDGMENT

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