ATPase Activity in Phytohemagglutinin[1] (37628)

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Phytohemagglutinin (PHA) from the red kidney bean (*Phaseolus vulgaris*) has been prepared in two forms depending on the extraction procedure (1). The saline extracted PHA-M contains more carbohydrate than the acid extracted PHA-P (2). Both forms, however, have the same *in vitro* biological actions, which include the transformation of lymphocytes and the agglutination of both erythrocytes and leucocytes (2).

Since preparations of phytohemagglutinin have been shown to be heterogeneous (3, 4), the nature of the active substance(s) is as yet unresolved. Yachnin *et al.* (4) have demonstrated five active mitogenic substances in PHA-P, all of which have agglutinating activities. We are reporting the partial characterization of an ATPase activity found in commercial preparations of PHA-M.

Materials and Methods. PHA-M and PHA-P were purchased from Difco (Detroit, Mich.) and Gibco (Grand Island, N.Y.). All substrates used were obtained from Sigma (St. Louis, Mo.) as sodium salts.

PHA-M was dissolved in 5 ml of distilled water containing 25 m*M* EGTA and 25 m*M* EDTA (pH 7.0) and dialyzed overnight at 4° against two changes of 8 liters of glass-distilled water.

The assay for phosphohydrolase activity was performed at 37° in a total vol of 2 ml containing 50 m*M* Tris–HCl (pH 7.5 at 37°), 10 μg of PHA-M (dialyzed), 3 m*M* of substrate (routinely ATP), and varying amounts of metal cations. The reaction tubes and necessary blanks were preincubated for 10 min at which time freshly prepared substrate was added to initiate the reaction. After 5 min the reaction was terminated by the addition of 0.5 ml of 30% cold trichloroacetic acid and 2 ml aliquots were analyzed for inorganic phosphate using the method of Fiske and Subbarow (5). Protein was determined by the method of Lowry (6), using bovine serum albumin as a standard.

Results and Discussion. The velocity of the ATPase reaction was proportional to the concentration of PHA-M in the presence of calcium (Fig. 1). This proportionality was observed with PHA-M from two commercial sources, with both having a significant ATPase activity. Dialyzed PHA-M showed a marked stimulation in the presence of calcium and an inhibition by magnesium (Fig. 2). PHA-P had no ATPase activity either in the presence or absence of divalent cations. This lack of ATPase activity in PHA-P is probably due to the acid extraction during its preparation (1).

Although the PHA-M used was dialyzed, the possibility still exists that tightly bound

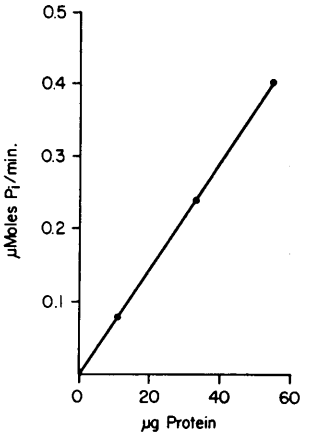

FIG. 1. Proportionality of dialyzed PHA-M (Difco) vs rate of ATP hydrolysis in the presence of 3 m*M* $CaCl_2$.

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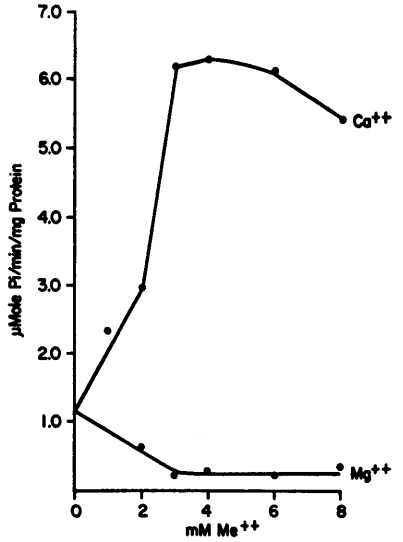

FIG. 2. Effect of divalent cations on ATPase activity of PHA-M.

metal ions were not readily removed. In an attempt to exclude effects of remaining divalent cations on PHA-M ATPase activity, the reaction was performed in the presence of metal chelators with no additional divalent cations (Fig. 3). Both the nonspecific chelator EDTA and the calcium specific chelator EGTA stimulated the ATPase activity. Since the sodium salts of the chelators were used, it seemed possible that the chelator effect was due to either a specific sodium stimulation or that changes in ionic strength were influencing the activity. By using three different salts over the same ionic strength range (Fig. 4), it became apparent that there is a specific stimulatory effect up to a concentration of 0.5 *M* in the absence of divalent cations.

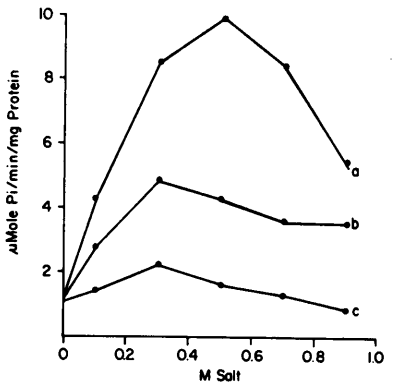

FIG. 4. Effect of salts on ATPase activity of PHA-M. (a) sodium chloride, (b) ammonium acetate, (c) potassium chloride.

In the presence of 0.5 *M* NaCl, both calcium and magnesium ions inhibited PHA-M ATPase activity (Fig. 5). This could be due to ATP binding the divalent cations, thus becoming the less preferred substrate or the divalent cations may be competing for sodium binding sites on the enzyme.

A preliminary examination of substrate specificity indicates that nucleosidetriphosphates are favorable substrates in the presence of 0.5 *M* NaCl and no divalent cations (Table I).

The lack of ATPase activity in PHA-P

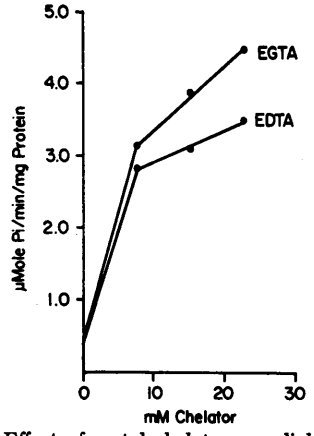

FIG. 3. Effect of metal chelators on dialyzed PHA-M ATPase activity.

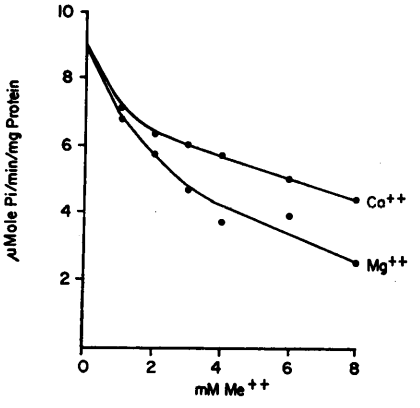

FIG. 5. Inhibition of PHA-M ATPase activity by divalent cations in the presence of 0.5 *M* NaCl.

TABLE I. Substrate Specificity.

Substrate (3 m*M*)	Relative activity[a]
ATP	100
CTP	24
GTP	55
ITP	42
UTP	90
dATP	47
ADP	0.04
AMP	0.02
CMP	0.1
UMP	0
Glucose 6-phosphate	0.02
3-phosphoglyceric acid	0
PP_i	0.04

[a] Based on ATP as 100% activity.

does not exclude the presence of a substance having nucleotide or calcium binding activity. There is a quantitative difference between the biological activities of PHA-M and PHA-P (1) which may be attributable to the presence of an ATPase of high specific activity in PHA-M but absent in PHA-P.

Summary. The plant mitogen, phytohemagglutinin (PHA-M), has been found to contain an ATPase activity which is stimulated by calcium ions and inhibited by magnesium ions in the absence of NaCl. Sodium ions have been found to be stimulatory in the absence of divalent cations. Both calcium and magnesium ions become inhibitory in the presence of NaCl.

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