INORGANIC PYROPHOSPHATASE IN LEAVES OF *CYNODON DACTYLON* PERS.

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INORGANIC PYROPHOSPHATASE (EC 3.6.1.1) is known to be present in yeast[1], mammalian tissues[2,3] and plant leaves[4,5]. Plant inorganic pyrophosphatases are studied to a less extent in comparison to their mammalian and bacterial counterparts. We have found a very high level of alkaline inorganic pyrophosphatase activity (much more than that of maize leaf[5] reported earlier) in the leaves of *Cynodon dactylon* Pers., a grass belonging to Gramineae family. Some properties of this enzyme from *Cynodon dactylon* are reported here and its role in the C_4-pathway of photosynthesis is discussed.

Methods

Fresh plant leaves were homogenized at 0° C using 50 mM tris buffer (pH 7·5) and centrifuged at 6000 g. The supernatant was assayed for the enzyme activity.

Inorganic pyrophosphatase activity was assayed according to the method of Simmons and Butler[5] in a 1 ml reaction mixture containing 1 mM tetrasodium pyrophosphate, 10 mM magnesium chloride, 100 mM tris acetate buffer (pH 9·0) and aliquots of enzyme suitably diluted. The orthophosphate produced in the reaction mixture was measured by Fiske–Subba Row method[6]. The dry weight of the leaf was determined by heating at 110° C to constant weight.

Concentration of different ion species of magnesium and pyrophosphate in the enzyme reaction mixture was determined by the method of Klemme and Gest[7]. At pH 9·0, the following equilibria take place in the reaction mixture containing magnesium and pyrophosphate, the protonated species being virtually absent at alkaline pH:

$$PP_i^{4-} \underset{K_1}{\overset{Mg^{2+}}{\rightleftharpoons}} MgPP_i^{2-} \underset{K_2}{\overset{Mg^{2+}}{\rightleftharpoons}} Mg_2PP_i.$$

The concentration of different ion species was calculated from the following conservation equations by using $K_1 = 10^{6.41}\ M^{-1}$ and $K_2 = 10^{2.31}\ M^{-1}$ as suggested by Josse[8].

Conservation equations:

$$Mg^{2+}\text{ (total)} = \text{Free } Mg^{2+} + 2Mg_2PP_i + MgPP_i^{2-}$$
$$PP_i^{4-}\text{ (total)} = \text{Free } PP_i^{4-} + Mg_2PP_i + MgPP_i^{2-}.$$

Results and Discussion

The level of alkaline inorganic pyrophosphatase activity is found to be very high in the leaves of *Cynodon dactylon* and sugarcane (199·2 and 114·5 units per g of dry tissue respectively) in comparison to other five species studied in the Gramineae family of plants (Table I). As reported earlier[9] that C_4-pathway of photosynthesis is totally absent in bamboo, rice and barley plants but exists in sugarcane and some tropical grasses, may give a possible implication of the role of alkaline inorganic pyrophosphatase in the C_4-pathway of photosynthesis. The synthesis of phosphopyruvate which is the CO_2 acceptor in this pathway is facilitated by the concurrent hydrolysis of inorganic pyrophosphate formed, to orthophosphate by inorganic pyrophosphatase as suggested by Simmons and Butler[5].

TABLE I

Distribution of alkaline inorganic pyrophosphatase in the leaves of Gramineae family of plants

Activity is expressed as μmoles PP_i hydrolysed per minute

Plant	Enzyme activity	
	Units/g wet tissue	Units/g dry tissue
Bambusa arundinaceae Willd (Bamboo)	17·5	35·90
Panicum indicum Linn. (Common grass)	12·3	56·1
Oryza sativa Linn. (Rice)	11·5	33·7
Cynodon dactylon Pers. (Dubh grass)	72·0	199·2
Saccharum officinarum Linn. (Sugarcane)	38·8	114·5
Triticum vulgare Vill. (Wheat)	10·9	33·3
Hordeum vulgare Linn. (Barley)	7·9	24·1

$$\text{Pyruvate} + \text{ATP} + P_i \xrightleftharpoons[\text{synthetase}]{\text{Phosphopyruvate}} \text{Phosphopyruvate} + \text{AMP} + PP_i$$

$$\text{Phosphopyruvate} + CO_2 \xrightarrow[\text{carboxylase}]{\text{Phosphopyruvate}} \text{Oxaloacetate} + P_i.$$

The enzyme has been partially purified to thirty-fold from *Cynodon dactylon* leaves by ammonium sulphate fractionation (50%–70% cut) and subsequent sephadex G-100 gel filtration. The partially purified enzyme shows almost negligible acid and alkaline phosphatase activity against sodium-β-glycerophosphate and *p*-nitrophenylphosphate indicating very little interference of the nonspecific pyrophosphate splitting phosphatases (present if any) in the subsequent pyrophosphatase reactions. The enzyme shows a pH

optimum at 9·0. Experiment on thermal stability of the enzyme shows that it can withstand a temperature of 50°C for 10 minutes with little change in enzyme activity (about 20% activity is lost). At 60°C only 37% of the activity is retained. The effect of metal ions on the enzyme activity shows that magnesium is absolutely required for the enzyme activity. No other metal ions can replace magnesium appreciably. Only Co^{2+} and Mn^{2+} have a little activating effects (7% and 12% respectively with respect to Mg^{2+} ion). In presence of 10 mM magnesium, other metal ions at 10 mM concentration exert inhibitory effects on the enzyme activity. The order of inhibition is $Ca^{2+} > Cd^{2+}, Zn^{2+} > Pb^{2+} > Hg^{2+} > Mn^{2+} > Ba^{2+} > Co^{2+} > Sr^{2+} > Cu^{2+}$. Fluoride severely inhibits the enzyme activity. Chromate, cyanide and azide have no inhibitory effects but tungstate and molybdate are somewhat inhibitory. Iodoacetate (10 mM) causes 40% inhibition whereas *p*-chloromercuribenzoate (10 mM) has no inhibitory effect.

For fixed concentration of tetrasodium pyrophosphate (1 mM) the enzyme activity was measured at variable concentration of magnesium chloride. The comparison of variation in the concentrations of free Mg^{2+}, free PP_i^{4-}, $MgPP_i^{2-}$ and Mg_2PP_i (determination of concentration of each ion species has been described in 'Method' portion) with the activity as a function of total $MgCl_2$ (Fig. 1) reveals that $MgPP_i^{2-}$, and not free PP_i^{4-}, is the true substrate of the enzyme in presence of free Mg^{2+} ion. Mg_2PP_i may also possibly act as a substrate as evident from Fig. 1. Effect of pH on Mg^{2+} requirement shows that the optimum pH shifts towards lower pH values with larger excess of Mg^{2+} as shown in Fig. 2.

From the findings of the present study it appears that *Cynodon dactylon* leaves give rise to a rich source of alkaline inorganic pyrophosphatase and this enzyme is very much similar to maize leaf inorganic pyrophosphatase[5].

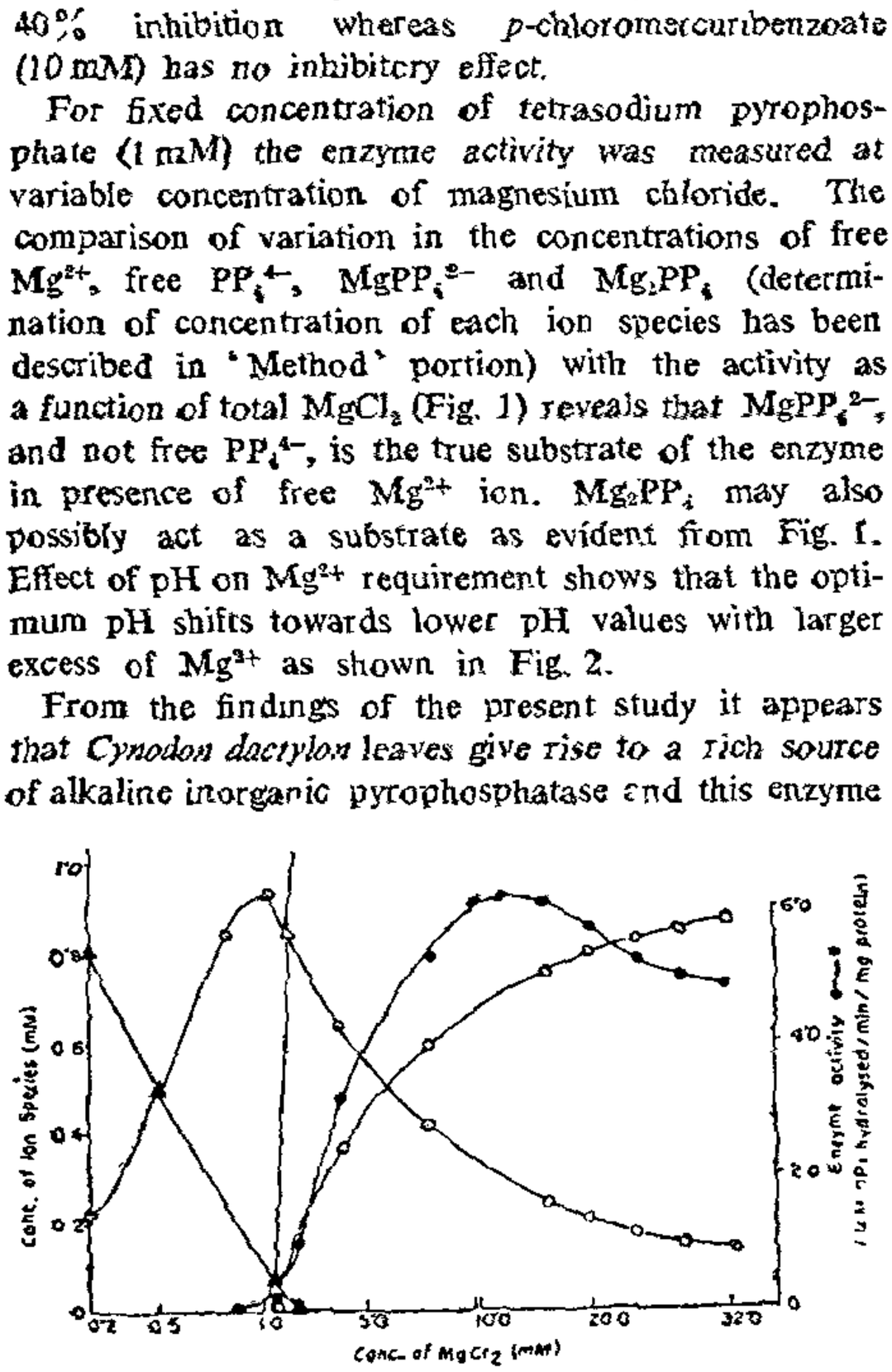

FIG. 1. Comparison of the variation in the concentration of free PP_i^{4-} (▲——▲), free Mg^{2+} (△——△), $MgPP_i^{2-}$ (⊙– –⊙), Mg_2PP_i (○——○) in *Cynodon dactylon* inorganic pyrophosphatase catalysed reaction mixture with the activity (●——●) as a function of total $MgCl_2$.

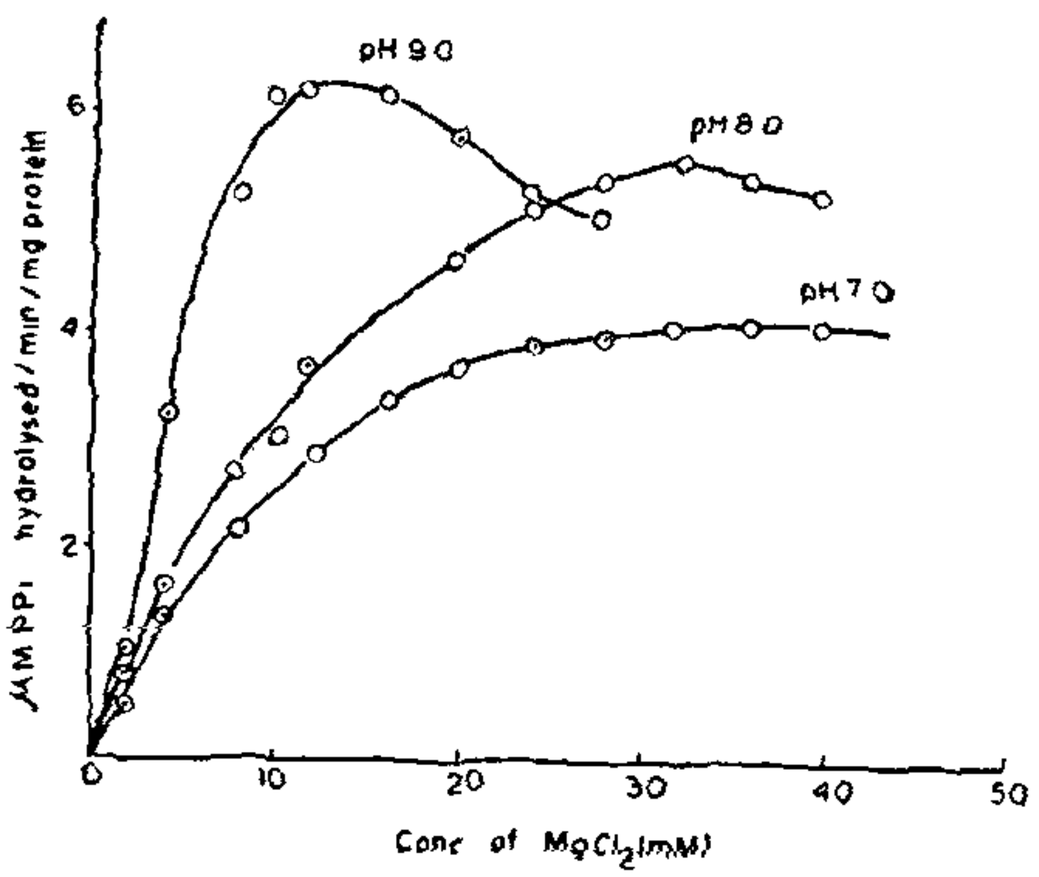

FIG. 2. Effect of the concentration of magnesium chloride on the activity of *Cynodon dactylon* inorganic pyrophosphatase at different levels of pH.

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t-BUTANOLYSIS OF LECANORIC ACID

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DEPSIDES, a class of naturally occurring compounds, almost unique to lichens, are dimeric esters of variously substituted orsellinic acids and their analogues. The cleavage of the depside link is frequently effected by alcoholysis with methanol in the presence of sodium hydroxide[1], whereby the left side acid half