Mg^{2+} is an essential activator of hydrolytic activity of membrane-bound pyrophosphatase of *Rhodospirillum rubrum*

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The substrate for the hydrolytic activity of membrane-bound pyrophosphatase is the PP_i–Mg^{2+} complex. The enzyme has no activity when the free Mg^{2+} concentration is lower than 10 μM (at 0.5 mM-PP_i–Mg^{2+}), and therefore free Mg^{2+} is an essential activator of the hydrolytic activity. The K_m for the substrate changes in response to variation in free Mg^{2+} concentration, from 10.25 to 0.6 mM when free Mg^{2+} is increased from 0.03 to 1.0 mM respectively. The K_m for Mg^{2+} depends on the substrate concentration: the K_m decreases from 0.52 to 0.14 mM from 0.25 to 0.75 mM-PP_i–Mg^{2+} respectively. The extrapolated K_m for Mg^{2+} in the absence of the substrate is 0.73 mM. Imidodiphosphate–Mg^{2+} and free Ca^{2+} were used as competitive inhibitors of substrate and activator respectively. The equilibrium binding kinetics suggest an ordered mechanism for the activator and the substrate: Mg^{2+} ions bind the enzyme before PP_i–Mg^{2+} in the formation of the catalytic complex, membrane-bound pyrophosphatase–(Mg^{2+})–(PP_i–Mg^{2+}).

INTRODUCTION

Membrane-bound pyrophosphatase (EC 3.6.1.1) of *Rhodospirillum rubrum* chromatophores catalyses not only the hydrolysis of pyrophosphate but also the synthesis of pyrophosphate with energy derived from photosynthetic electron transport (Baltscheffsky, 1978; Baccarini-Melandri & Melandri, 1978). Pyrophosphate hydrolysis in the chromatophore membrane is linked to the electrogenic translocation of protons in a fully reversible process (Baltscheffsky, 1968; Keister & Minton, 1971; Moyle *et al.*, 1972).

Most inorganic pyrophosphatases, both cytoplasmic and membrane-bound, use the PP_i–Mg^{2+} complex as the real substrate for hydrolysis; free PP_i^{4-} is an inhibitor of this reaction (Lathi, 1983).

Randahl (1979) suggested that Mg^{2+} ions modulate the properties of the enzyme. Indeed, it has been shown (Celis *et al.*, 1985) that P_i/PP_i exchange reaction and pyrophosphate hydrolysis catalysed by membrane-bound pyrophosphatase have sharply different requirements for Mg^{2+} ions. In the same way, Celis & Romero (1987) have demonstrated that the two reactions have a markedly different pH requirement.

Studies on the effect of bivalent cations on the hydrolytic reaction and P_i/PP_i exchange (Celis & Romero, 1987) show that Mn^{2+} and Co^{2+} partially support the P_i/PP_i exchange reaction (50%), whereas bivalent cations such as Zn^{2+} and Ca^{2+} do not. In the hydrolytic reaction, Zn^{2+} at low concentration can replace Mg^{2+} in the formation of the substrate. In the presence of high concentrations of substrate for the hydrolysis (PP_i–Mg^{2+}), free bivalent cations are inhibitory.

These sets of observations suggest that bivalent cations and protons regulate the catalytic properties of membrane-bound pyrophosphatase. However, it is difficult to determine the regulatory effect of free Mg^{2+} on hydrolytic activity because, as has been stated, it also forms the substrate complex. The regulatory properties and kinetics of Mg^{2+} activation of cytoplasmic pyrophosphatase have been studied previously (Klemme & Gest, 1971; Moe & Butler, 1972*a*; Barry & Dunaway-Mariano, 1987). However, to our knowledge, these properties have not been studied for a membrane-bound pyrophosphatase. Since this pyrophosphatase is coupled to the proton gradient, whereas the cytoplasmic enzyme is not, the study of the regulation of the membrane-bound enzyme is important for the understanding of the mechanism of energy transduction. In the present work, the role of free Mg^{2+} as an essential activator of the hydrolytic reaction of membrane-bound pyrophosphatase was studied. An ordered mechanism for the hydrolytic activity of the enzyme is suggested in which Mg^{2+} binds to the enzyme before PP_i–Mg^{2+} in the formation of the catalytic complex.

MATERIALS AND METHODS

Bacterial growth and preparation of chromatophores from wild-type *R. rubrum*

Wild-type *R. rubrum* A.T.C.C. 11170 was grown anaerobically in the light (tungsten lamps of 40 W at 30 cm) at 30 °C in a medium described by Cohen-Bazire *et al.* (1957). Bacterial cells were harvested in the late-exponential phase.

The cells were washed with 50 mM-KCl/50 mM-Mops buffer, pH 7.5, and chromatophores were prepared by sonication for 2 min in an MSE sonicator at full power, in 10 mM-Tris/HCl buffer, pH 7.5. After centrifugation at 26000 ***g*** for 20 min, the supernatant was collected and centrifuged at 105000 ***g*** for 90 min. The residual Mg^{2+} was eliminated from the chromatophores by washing with 5 mM-EDTA/5 mM-EGTA/10 mM-Tris/HCl buffer, pH 7.5, followed by a second washing with 10 mM-Tris/HCl buffer, pH 7.5. The final pellet was resuspended in 10 mM-Tris/HCl buffer, pH 7.5, at a protein concentration of 30–35 mg/ml. The chromatophore preparation was kept at 4 °C and used within the next 3 days. No change in the hydrolytic activity was detected within this time. Protein was determined by the method of Lowry *et al.* (1951), with BSA as standard.

Hydolysis of pyrophosphate

The hydrolytic reaction was determined in the dark with a green safety light under the conditions described in the Results and discussion section. The reactions were initiated by adding the chromatophores to the reaction medium, and were arrested with 6% (w/v) trichloroacetic acid (final concentration). Phosphate was determined in the supernatant as described by

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Fiske & SubbaRow (1925). In the initial-velocity studies, the maximum amount of PP_i–Mg^{2+} complex hydrolysed was only 15% in 1.5 min; in this period the product formation (phosphate) had no effect on the hydrolytic velocity. Initial velocities are defined as nmol of P_i produced/min per mg of protein.

Calculation of free metal ions, ligands and complex

Concentrations of metal ion complex and free ions in the reaction media were calculated with the computer program of Fabiato (1988), using published association constants for all components of the reaction media. All values were taken from Martell & Sillén (1971) except for the association constant for the formation of the Mg^{2+}–EDTA complex, which was taken from Fabiato (1988). In the computations, adjusted values of the association constants suitable to temperature and pH of reaction media were used. The program was run in an IBM AT computer with an arithmetic co-processor.

RESULTS AND DISCUSSION

Effect of free Mg^{2+} on the hydrolytic activity of membrane-bound pyrophosphatase

The substrate for the hydrolytic activity of membrane-bound pyrophosphatase is the PP_i–Mg^{2+} complex, and free Mg^{2+} has an apparent activating effect (Randahl, 1979; Celis *et al.*, 1985; Celis & Romero, 1987). In order to investigate the effect of free Mg^{2+} on the hydrolytic activity, free Mg^{2+} concentration was increased from 0.01 μM to 1.0 mM, keeping PP_i–Mg^{2+} constant at 0.5 mM (Fig. 1). It can be observed that the hydrolytic activity depends strictly on free Mg^{2+} concentration; the apparent K_m for Mg^{2+} ($K_{Mg(app.)}$) is 0.28 mM. No PP_i–Mg^{2+} hydrolysis occurs when free Mg^{2+} concentration is lower than 10 μM. Therefore Mg^{2+} could serve as an essential activator for the hydrolytic activity of the membrane-bound pyrophosphatase, given the Mg^{2+} requirement for substrate recognition by the enzyme.

Effect of free Mg^{2+} on PP_i–Mg^{2+} hydrolysis

In order to determine the effect of free Mg^{2+} on the hydrolytic activity, free Mg^{2+} was fixed at four different concentrations, and for each case the concentration of PP_i–Mg^{2+} was varied (Fig. 2*a*). An increase in free Mg^{2+} produced an enhanced hydrolytic activity, but hydrolysis depended on substrate concentration in a saturable manner; this is in agreement with the report by Schwarm *et al.* (1986) working with the membrane-bound pyrophosphatase of *Rhodopseudomonas palustris*. The data shown in Fig. 2(*a*) were replotted as Lineweaver–Burk plots. A small change in $V_{max.}$ was obtained (515 to 482 nmol of P_i/min per mg of protein at fixed Mg^{2+} concentrations of 0.03 to 1.0 mM respectively). When the free Mg^{2+} concentration was raised, the apparent K_m for PP_i–Mg^{2+} ($K_{PP_i\text{-}Mg(app.)}$) decreased. The calculated K_m values were 10.25, 2.92, 0.84 and 0.6 mM at free Mg^{2+} concentrations of 0.03, 0.1, 0.5 and 1 mM respectively. Fig. 2(*c*) shows a linear relationship between the apparent K_m for PP_i–Mg^{2+} obtained from Fig. 2(*b*) and the reciprocal fixed free Mg^{2+} concentration. The intercept on the ordinate axis (0.17 mM) gives the K_m for PP_i–Mg^{2+} at saturating concentrations of free Mg^{2+}. This is in agreement with the role of free Mg^{2+} as an essential activator for PP_i–Mg^{2+} hydrolysis.

Free Mg^{2+} activation and its dependence on PP_i–Mg^{2+} concentration

The effect of various free Mg^{2+} concentrations was studied at three concentrations of PP_i–Mg^{2+} (Fig. 3*a*). The increase in hydrolytic activity depended on free Mg^{2+}, but the hydrolytic activity also increased as the concentration of PP_i–Mg^{2+} increased. The calculated $V_{max.}$ values were 270.6, 362.9 and 367.3 nmol of P_i/min per mg of protein at 0.25 mM-, 0.5 mM- and 0.75 mM-PP_i–Mg^{2+} respectively. The apparent K_m for Mg^{2+} decreased as PP_i–Mg^{2+} concentration increased; the $K_{Mg(app.)}$ was 0.51, 0.28 and 0.12 mM for PP_i–Mg^{2+} at 0.25, 0.5 and 0.75 mM respectively. The K_m for Mg^{2+} in the absence of substrate was calculated by interpolation of the intersection point to the abscissa, and a value of 0.73 mM was obtained.

Segel (1975) has described rapid-equilibrium analysis between an essential activator and a substrate, and we have applied this approach to determine the type of mechanism. If the activator concentration is fixed and that of the substrate is varied giving rise to a competitive pattern, and if the substrate concentration is fixed and that of the activator is varied giving rise to a mixed type in the double-reciprocal plot (Figs. 2*b* and 3*b*), these patterns are characteristic of an obligated ordered mechanism for the binding of free Mg^{2+} and PP_i–Mg^{2+}, in which Mg^{2+} binds to the enzyme before PP_i–Mg^{2+}. This mechanism contrasts with the pattern described for the yeast cytoplasmic pyrophosphatase, in which the substrate binds to the enzyme before Mg^{2+} ions (Knight *et al.*, 1981).

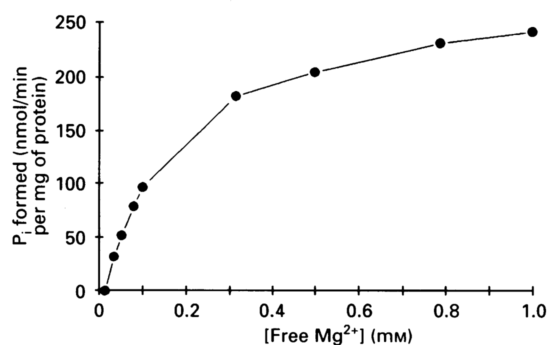


Fig. 1. Effect of free Mg^{2+} on the hydrolytic activity of membrane-bound pyrophosphatase

The necessary concentrations of sodium pyrophosphate and $MgCl_2$ were calculated to maintain the PP_i–Mg^{2+} concentration at 0.5 mM and the indicated free Mg^{2+} concentration in presence of 1.0 mM-EDTA. The incubation medium contained 50 mM-Tris/maleate buffer, pH 6.5, and 1 mg of chromatophore protein. Incubation time was 1.5 min.

Imidodiphosphate–Mg^{2+} and free Ca^{2+} as competitive inhibitors of PP_i–Mg^{2+} hydrolysis and activation by free Mg^{2+}

In order to substantiate the ordered mechanism in the formation of the catalytic complex, an imidodiphosphate–Mg^{2+} complex was used as a competitive inhibitor of PP_i–Mg^{2+} hydrolysis. In addition, the effect of Ca^{2+} ions was studied on the activator effect of free Mg^{2+}. The cross-inhibitory patterns (imidodiphosphate–Mg^{2+} versus free Mg^{2+} and free Ca^{2+} versus PP_i–Mg^{2+}) were analysed to clarify whether the binding of PP_i–Mg^{2+} and free Mg^{2+} is ordered or random and whether it is in rapid equilibrium or in a steady state (Segel, 1975; Morrison, 1979).

Imidodiphosphate has been reported to inhibit the cytoplasmic pyrophosphatase of yeast (Kelly *et al.*, 1973). The effect of imidodiphosphate–Mg^{2+} on the hydrolytic activity was analysed by fixing its concentration at 0.5 mM and 1.0 mM while that of the PP_i–Mg^{2+} was varied at fixed free Mg^{2+} concentration (1.0 mM). Fig. 4(*a*) shows imidodiphosphate–Mg^{2+} as a competitive inhibitor of PP_i–Mg^{2+} hydrolysis, since the K_m for PP_i–Mg^{2+} increased from 0.68 mM to 2.44 mM, whereas $V_{max.}$ did not change (331 nmol of P_i/min per mg of protein). These data clearly

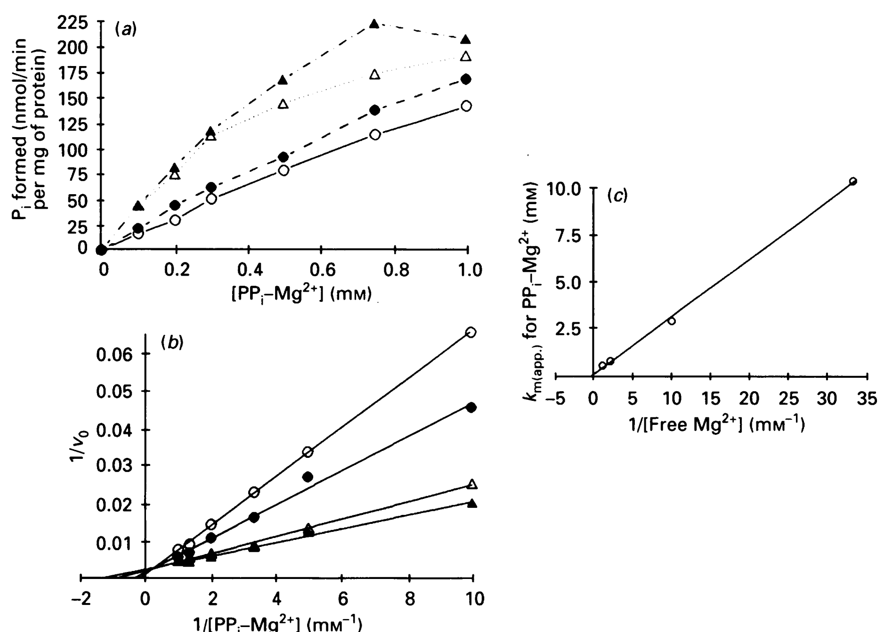


Fig. 2. Effect of free Mg^{2+} ions on the hydrolysis of the PP_i–Mg^{2+} complex

(*a*) The experimental conditions were as in Fig. 1, but the concentrations of sodium pyrophosphate and $MgCl_2$ were calculated to have four different concentrations of free Mg^{2+}: 0.03 mM (○), 0.1 mM (●), 0.5 mM (△) and 1.0 mM (▲). The apparent K_m for the substrate obtained in (*b*) was replotted in (*c*).

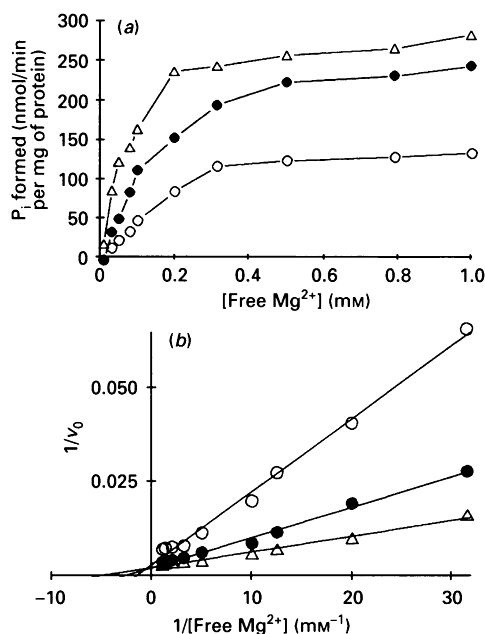


Fig. 3. Dependence of PP_i–Mg^{2+} concentration on the activation of free Mg^{2+}

(*a*) The experimental conditions were as in Fig. 1. The concentrations of sodium pyrophosphate and $MgCl_2$ were calculated to give three fixed concentrations of PP_i–Mg^{2+}: 0.25 mM (○), 0.5 mM (●) and 0.75 mM (△). (*b*) Lineweaver–Burk plot of the results from (*a*).

indicate that imidodiphosphate–Mg^{2+} competes with PP_i–Mg^{2+} for binding to the enzyme–Mg^{2+} complex.

Ca^{2+} is an inhibitor of several types of pyrophosphatase (Baykov *et al.*, 1989; Moe & Butler, 1972*b*). The effect of free Ca^{2+} on PP_i–Mg^{2+} hydrolysis was analysed by using fixed free Ca^{2+} concentrations while that of free Mg^{2+} was varied and PP_i–Mg^{2+} was kept at 0.5 mM (Fig. 4*b*). Lineweaver–Burk analysis shows that free Ca^{2+} acts as a competitive inhibitor of Mg^{2+} activation, since the K_m for Mg^{2+} increased 7-fold, from 0.25 to 1.71 mM, whereas the $V_{max.}$ did not change (237 nmol of P_i/min per mg of protein). Free Ca^{2+} binds to the enzyme to form an enzyme–Ca^{2+} complex, competing with free Mg^{2+} binding. The PP_i–Ca^{2+} complex was present in the reaction medium at concentrations ranging from 0.77 mM to 0.007 mM when free Ca^{2+} was fixed at 0.05 mM, and from 0.15 mM to 0.015 mM when free Ca^{2+} was fixed with 0.1 mM. Since a clearly competitive effect of free Ca^{2+} for the activation of free Mg^{2+} is observed, and a mixed-type inhibition is not present, an effect of PP_i–Ca^{2+} on the enzyme can be ruled out.

To complete the analysis, the effect of imidodiphosphate–Mg^{2+} on Mg^{2+} activation and free Ca^{2+} on the substrate was studied. Imidodiphosphate–Mg^{2+} concentration was fixed at 0.1 and 0.15 mM and free Mg^{2+} concentration was varied (at PP_i–Mg^{2+} 0.5 mM). Fig. 4(*c*) shows a change in $K_{Mg^{2+}}$ from 0.23 to 0.074 mM and a decrease in $V_{max.}$ (201 to 150 nmol of P_i/min per mg of protein) showing uncompetitive inhibition. This inhibition pattern is in agreement with rapid equilibrium for the ordered mechanism, indicating that PP_i–Mg^{2+} binds to the enzyme after free Mg^{2+}.

Free Ca^{2+} concentration was fixed at 0.05 and 0.1 mM while that of the substrate was varied (with free Mg^{2+} fixed at 1.0 mM). The inhibition pattern again is uncompetitive (Fig. 4*a*). The $K_{PP_i\text{–}Mg^{2+}}$ decrease changed from 0.73 to 0.19 and the $V_{max.}$ also decreased from 433 to 118 nmol of P_i/min per mg of protein. These data are not consistent with the predicted inhibition pattern (Table 1); they indicate that free Ca^{2+} binds to the enzyme–substrate complex, but that the PP_i–Ca^{2+} is formed in the reaction medium at a concentration 70 times lower than the substrate at a free Ca^{2+} concentration of 0.05 mM, and 33 times lower than that of PP_i–Mg^{2+} at a free Ca^{2+} concentration of 0.1 mM. For this reason binding constants relevant for Ca^{2+} inhibition in the presence of PP_i–Ca^{2+} are difficult to analyse (Baykov *et al.*, 1989).

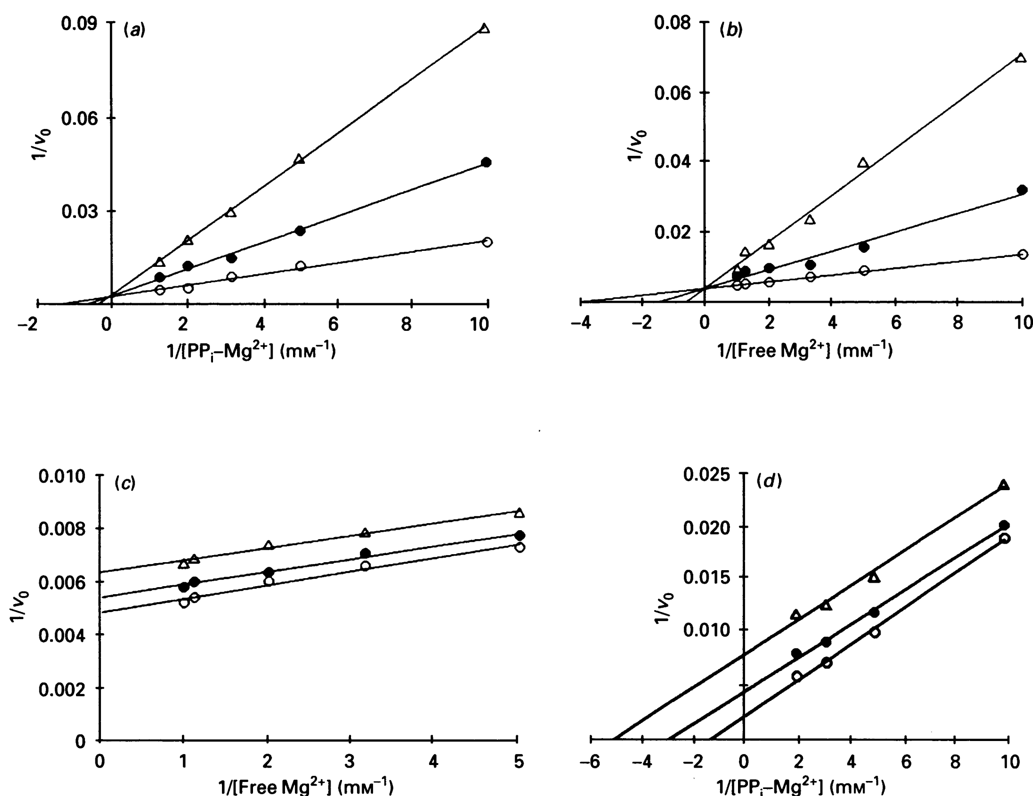


Fig. 4. Inhibition patterns for imidodiphosphate–Mg^{2+} and free Ca^{2+} as inhibitors for the substrate and the activator effect of free Mg^{2+}

The experimental conditions were as in Fig. 1. (*a*) Lineweaver–Burk plot of initial velocity versus [PP_i–Mg^{2+}] at imidodiphosphate–Mg^{2+} concentrations of 0 mM (○), 0.5 mM (●) and 1.0 mM (△). (*b*) Lineweaver–Burk plot of initial velocity versus free Mg^{2+} at free Ca^{2+} concentrations of 0 mM (○), 0.05 mM (●) and 0.1 mM (△). (*c*) Lineweaver–Burk plot of initial velocity versus [free Mg^{2+}] at imidodiphosphate–Mg^{2+} concentrations of 0 mM (○), 0.1 mM (●) and 0.15 mM (△). (*d*) Lineweaver–Burk plot of initial velocity versus [PP_i–Mg^{2+}] at free Ca^{2+} concentrations of 0 mM (○), 0.05 mM (●) and 0.1 mM (△).

Table 1. Predicted and observed inhibition patterns from the data of Fig. 4

Types of inhibition: competitive (C), uncompetitive (UC) and non-competitive (NC).

			Predicted inhibition patterns			
Substrate whose concentration was varied	Inhibitor	Observed inhibition pattern	Rapid equilibrium random	Steady-state random	Rapid equilibrium ordered	Steady-state ordered
PP_i–Mg^{2+}	Imidodiphosphate–Mg^{2+}	C	C	C	C	C
Mg^{2+}	Ca^{2+}	C	C	C	C	C
PP_i–Mg^{2+}	Ca^{2+}	UC	C	NC	C	UC
Mg^{2+}	Imidodiphosphate–Mg^{2+}	UC	C	NC	UC	NC

Effect of free PP_i on hydrolytic activity

Free PP_i is present in the reaction medium, and it is an inhibitor of pyrophosphatases (Ridlington & Butler, 1972; Lathi, 1983). The concentration of free PP_i was fixed at 0.03, 0.1, 1.0 and 5.0 mM, and that of PP_i–Mg^{2+} was varied for each case (Fig. 5*a*). An increase in free PP_i has an apparent inhibitory effect on the hydrolytic activity. When free PP_i concentration was kept constant and that of PP_i–Mg^{2+} was varied, there was a decrease in free Mg^{2+} concentration in a constant ratio described by eqn. (1):

$$K_{PP_i\text{–}Mg^{2+}} = \frac{[Mg][PP_i]}{[PP_i\text{–}Mg^{2+}]} \qquad \frac{K_{PP_i\text{–}Mg^{2+}}}{[PP_i]} = \frac{[Mg^{2+}]}{[PP_i\text{–}Mg^{2+}]} \tag{1}$$

There were no apparent inhibitory effects at 0.03 and 0.1 mM-PP_i, but the corresponding free Mg^{2+} concentrations were enough for the enzyme to recognize the substrate ($K_{Mg^{2+}}$ at 0.75 mM substrate is 0.12 mM). At 1.0 and 5.0 mM free PP_i, the corresponding concentrations of free Mg^{2+} were at the lower limit for substrate recognition by the enzyme (Table 2). Although there was a high concentration of free PP_i (5.0 mM), the enzyme activity increased in response to variations in substrate concentration. Therefore free PP_i seems to be a weak inhibitor. Since free Mg^{2+} is essential for hydrolytic activity, its decrement produces an inhibitory effect.

The inverse of PP_i–Mg^{2+} concentrations from Fig. 5(*a*) were squared, and a modified Lineweaver–Burk plot was obtained (Fig. 5*b*). The latter data indicate an apparent non-competitive inhibition. This effect is not consistent with the previously

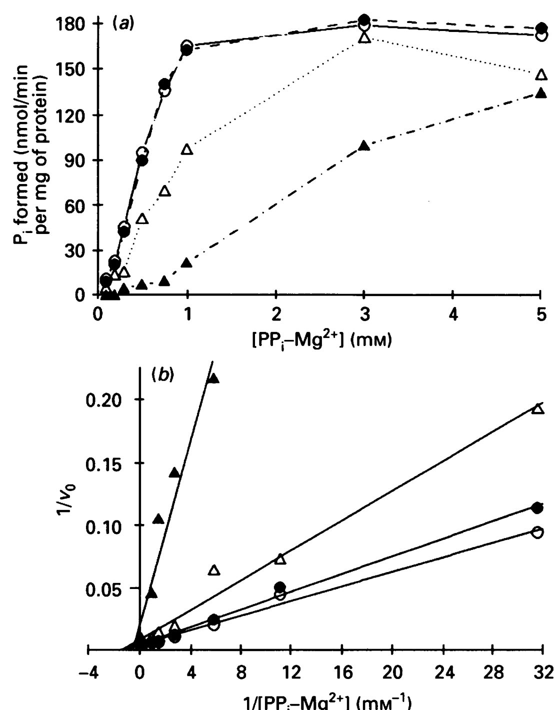


Fig. 5. Effect of the free PP_i on the hydrolytic activity

The experimental conditions were as in Fig. 1. (*a*) Concentrations of sodium pyrophosphate and $MgCl_2$ were calculated to give four concentrations of free PP_i: 0.1 mM (○), 0.5 mM (●), 1.0 mM (△) and 5.0 mM (▲). (*b*) Double-reciprocal plots of the results from (*a*).

Table 2. Decrease in Mg^{2+} concentration in the experiment of Fig. 5(*a*)

Constant PP_i (mM)	Variation in PP_i–Mg^{2+} (mM)	$C = \frac{[Mg^{2+}]}{[PP_i–Mg^{2+}]}$	Variation in free Mg^{2+} (mM)
0.03	0.1 to 5.0	3.3×10^{-2}	0.003 to 0.165
0.1	0.1 to 5.0	1×10^{-2}	0.001 to 0.05
1.0	0.1 to 5.0	1×10^{-3}	0.0001 to 0.005
5.0	0.1 to 5.0	2×10^{-4}	0.00002 to 0.001

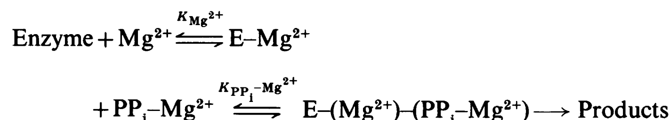
reported competitive inhibition for other pyrophosphatases (Josse, 1966). The observed inhibition is probably due to a decrease in Mg^{2+} concentration, although an additional inhibitory effect by free PP_i cannot be ruled out.

CONCLUSION

Free Mg^{2+} is an essential activator for the hydrolytic activity of membrane-bound pyrophosphatase of *R. rubrum*. The catalytic complex is enzyme–(Mg^{2+})–(PP_i–Mg^{2+}). The binding of Mg^{2+} modifies the enzyme's affinity for the substrate; a similar effect was described from cytoplasmic pyrophosphatase of yeast (Knight *et al.*, 1981) and for cytosolic and mitochondrial pyrophosphatase (Unguryte *et al.*, 1989). In the above-mentioned pyrophosphatases, three bivalent cations per active site are required for activity (Cooperman, 1982; Unguryte *et al.*, 1989). One of these metal ions binds to PP_i to form the substrate. For *R. rubrum*, the number of Mg^{2+} ions that bind to the enzyme is not known.

The binding of the substrate (PP_i–Mg^{2+}) produces an increase in free Mg^{2+} affinity for the enzyme (Fig. 3*a*). The K_m for Mg^{2+} is 0.73 mM in the absence of substrate. The rapid-equilibrium treatment between the essential activator and the substrate suggests an ordered mechanism in which free Mg^{2+} binds to the enzyme before the substrate. For the cytoplasmic pyrophosphatase of yeast, Barry & Dunaway-Mariano (1987) proposed an ordered mechanism in which the substrate binds to the enzyme before Mg^{2+}. In the case of slow substrates such as $Cr(H_2O)PP_i$, the binding steps are in rapid equilibrium, whereas for PP_i–Mg^{2+}, the binding steps are in a steady state. In contrast, in *R. rubrum*, when PP_i–Mg^{2+} is used as the substrate, the binding steps are in rapid equilibrium. Studies of competitive inhibition suggest an ordered mechanism which may be rapid equilibrium or steady state. Formulation of a complete model should be the goal of future research.

The proposed model for rapid-equilibrium kinetics for the hydrolytic activity of membrane-bound pyrophosphatase of *R. rubrum* is:



$$\text{Enzyme} + Mg^{2+} \underset{}{\overset{K_{Mg^{2+}}}{\rightleftharpoons}} E\text{–}Mg^{2+}$$

$$+\,PP_i\text{–}Mg^{2+} \overset{K_{PP_i\text{–}Mg^{2+}}}{\rightleftharpoons} E\text{–}(Mg^{2+})\text{–}(PP_i\text{–}Mg^{2+}) \longrightarrow \text{Products}$$

In a different way from Randahl's (1979) model, the enzyme–(PP_i–Mg^{2+}) complex is ruled out, since it predicts that, in the absence of free Mg^{2+}, the K_m for the substrate approaches infinity. Indeed, all other routes for catalytic complex-formation were eliminated from our model.

Randahl (1979) proposed PP_i inhibition of pyrophosphatase of *R. rubrum*. We suggest an additional strong inhibitory effect due to the decrease in free Mg^{2+} in experimental conditions; direct determination of the inhibition constant for PP_i is impracticable.

Both membrane-bound and cytoplasmic pyrophosphatases of several organisms require free metal for activation (Rapoport *et al.*, 1972; Unguryte *et al.*, 1989), and their substrate is a metal ion–PP_i complex. In spite of similar requirements for all kinds of pyrophosphatases, specific differences in their kinetic properties could be important in their regulation. Since the membrane-bound pyrophosphatase provides the simplest model for the study of hydrolysis and synthesis of phosphoanhydro bonds, the characteristics and properties of this enzyme are relevant in the mechanism of energy transduction.

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