A KINETIC STUDY OF THE ACTIVATION OF YEAST INORGANIC PYROPHOSPHATASE BY MAGNESIUM

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Received 1 December 1971

1. Introduction

The presence of divalent cations is an absolute requirement for the activity of PP-ase* from baker's yeast (EC 3.6.1.1) [1, 2]. Mg^{2+} ion is the best activator of PP_i hydrolysis catalyzed by this enzyme [2]. The mechanism of metal ion catalysis has however not been established yet.

Earlier kinetic studies of PP-ase from other sources were essentially based on the examination of enzymatic reaction velocities as a function of total substrate and metal concentrations [3, 4]. The present analysis was done in terms of free concentrations, because this approach can provide a more detailed insight into the role of metal in a system involving substrate–metal interaction. The results obtained indicate that (i) the active $EMgPP_i$ complex can result either from direct binding of $MgPP_i$ to PP-ase or from ordered addition of PP_i and Mg^{2+} to the enzyme and (ii) this complex is most likely of a substrate bridge type.

2. Materials and methods

The first steps of the enzyme isolation, including plasmolysis with toluene, extraction with water and $(NH_4)_2SO_4$ precipitation, were done as described by Kunitz [5]. Further purification was carried out according to our procedure [6] which makes use of chromatography on DEAE-cellulose, gel filtration on Sephadex G-100 and G-150 and final chromatography on DEAE-Sephadex A-50. The activity of the preparation was 1500 E /mg (one E of activity is defined as the amount of the enzyme which catalyses the liberation of one micromole of P_i per min at 30°). The assay mixture contained: 0.02 M Tris-HCl buffer pH 7.2, PP_i and $MgSO_4$, both at the concentration of 1.7×10^{-3} M). Enzyme concentrations were measured spectrophotometrically at 280 nm where 1 mg/ml of protein has an absorbance of 1.45 [7].

All reagents, including $Na_4P_2O_7 \cdot 10\,H_2O$ and $MgSO_4 \cdot 7\,H_2O$, were analytical grade.

Kinetic measurements were made by following up the liberation of P_i due to the hydrolysis of PP_i during the first 10% of the total reaction. P_i was determined by a modified procedure of Weil-Malherbe and Green [8]. Corrections were made for PP_i spontaneous hydrolysis. All runs were performed in duplicate at 25±0.1° in 0.02 Tris-HCl buffer at pH 7.2 containing NaCl enough to ensure ionic strength of 0.1. Enzyme concentration was 0.50×10^{-6} mg/ml.

The following dissociation constants were used to calculate total concentrations of Mg^{2+} and PP_i to maintain their necessary free concentrations [6, 9]:

$$\frac{[H^+]\,[P_2O_7^{4-}]}{[HP_2O_7^{3-}]} = 10^{-9.11} \qquad \frac{[H^+]\,[HP_2O_7^{3-}]}{[H_2P_2O_7^{2-}]} = 10^{-6.36}$$

$$\frac{[Mg^{2+}]\,[P_2O_7^{4-}]}{[MgP_2O_7^{2-}]} = 10^{-5.65} \qquad \frac{[Mg^{2+}]\,[HP_2O_7^{3-}]}{[MgHP_2O_7^{-}]} = 10^{-3.75}$$

$$\frac{[Mg^{2+}]\,[MgP_2O_7^{2-}]}{[Mg_2P_2O_7]} = 10^{-2.80} \qquad (1)$$

* *Abbreviations*

PP-ase, inorganic pyrophosphatase; PP_i and P_i, pyrophosphate and phosphate, respectively.

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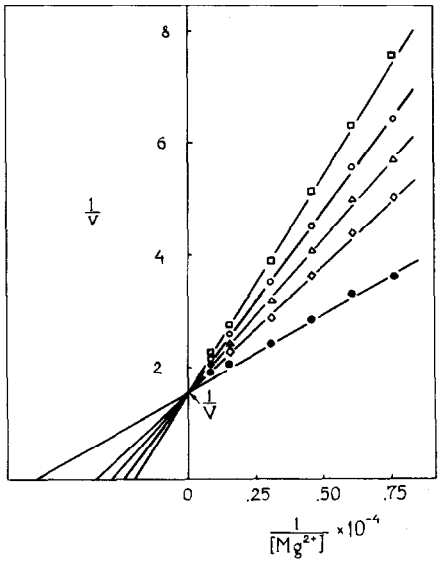

Fig. 1. The activation of yeast pyrophosphatase by Mg^{2+} at five different concentrations of free PP_i: (□–□–□): 0.4 × 10^{-5} M; (○–○–○): 0.5 × 10^{-5} M; (△–△–△): 0.7 × 10^{-5} M; (◇–◇–◇); 1.0 × 10^{-5} M; (●–●–●): 10 × 10^{-5} M. Velocity is expressed in micromoles of P_i per milligram of protein per minute.

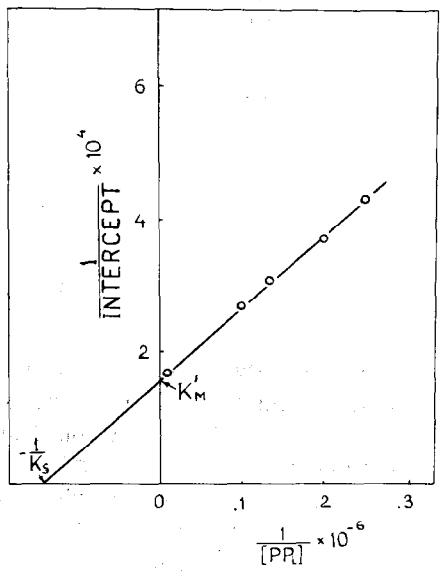

Fig. 2. Secondary plot of the reciprocals of the intercepts from fig. 1.

K_0 (see scheme), which is defined as

$$K_0 = \frac{[Mg^{2+}]\,([P_2O_7^{4-}] + [HP_2O_7^{3-}] + [H_2P_2O_7^{2-}])}{[MgP_2O_7^{2-}] + [MgHP_2O_7^{-}]}$$

was calculated at pH 7.2 using (1).

A kinetic analysis of PP-ase activation by Mg^{2+} was done in a manner similar to that of Morrison et al. [10]. It is assumed that an enzyme–Mg–PP_i complex reacts to form P_i. The mechanism may be represented as follows:

$$Mg^{2+} + PP_i \overset{K_0}{\rightleftharpoons} MgPP_i$$

$$\begin{array}{ccccc} & K_M & EMg & K'_S & \\ & \rightleftharpoons & & \rightleftharpoons & \\ E & & \rightleftharpoons K_{SM} \rightleftharpoons & & EMgPP_i \rightarrow \\ & K_S & EPP_i & K'_M & \end{array}$$

K_M, etc. are dissociation constants. The reciprocal of the initial rate equation with respect to free Mg^{2+} at various free PP_i concentrations is:

$$\frac{1}{v} = \frac{K'_M}{V}\left(1+\frac{K_S}{[PP_i]}\right)\frac{1}{[Mg^{2+}]} + \frac{1}{V}\left(1+\frac{K'_S}{[PP_i]}\right) \qquad (2)$$

3. Results and discussion

The initial velocity data for the Mg^{2+}-activated system, plotted in a double-reciprocal fashion, are shown in fig. 1 and 2. The linearity of the plots in both figures shows that over the range of the concentrations examined equation (2) is valid. Furthermore, the intersection of the lines at one point on the vertical axis in fig. 1 means that $K'_S = 0$ and $K_M = \infty$. All other constants, V, K_S and K'_M were determined as shown in the figures. K_{SM} was calculated by the relationship $K_0 K_{SM} K_S K'_M$. All the data are summarized in the table.

According to the values of the constants in the table, the active $EMgPP_i$ complex can form either as the result of enzyme interaction with a preformed metal ion–substrate complex or by ordered addition of PP_i and Mg^{2+} to the enzyme.

The fact that PP-ase does not bind Mg^{2+} implies that in the $EMgPP_i$ complex $MgPP_i$ is attached to the enzyme through the PP_i moiety. It is quite possible, however, that the EMg complex was not detected

Table 1
Summary of the data from Figs. 1 and 2

		(moles/min)
V	:	0.65×10^{-6}
K_{SM}	:	1.25×10^{-5}
K_S	:	0.85×10^{-5}
K'_M	:	1.60×10^{-4}
K'_S	:	0
K_M	:	∞
K_0	:	1.10×10^{-4}

because of its low stability and Mg^{2+} does come in contact with the protein in the $EMgPP_i$ complex. Several findings support such a supposition. Thus, Scheuch and Rapoport [11] reported Mg^{2+} to afford protection against inactivation of yeast PP-ase by heat. The interaction of the enzyme with another activator, Mn^{2+}, was shown by Cohn [12] using a proton magnetic resonance technique.

Qualitatively, the results reported in this paper are similar to those obtained by Josse [4] for *E. coli* PP-ase mentioned above. According to the mechanism proposed for the yeast enzyme one may consider $MgPP_i$ to be the real substrate of the reaction and PP_i to be a competitive inhibitor.

References

[1] K. Bailey and E.C. Webb, Biochem. J. 38 (1944) 394.
[2] M.J. Schlesinger and M.J. Coon, Biochim. Biophys. Acta 41 (1960) 30.
[3] E.A. Robbins, M.P. Stulberg and P.D. Boyer, Arch. Biochim. Biophys. 54 (1955) 215.
[4] J. Josse, J. Biol. Chem. 241 (1966) 1948.
[5] M. Kunitz, Arch. Biochem. Biophys. 92 (1961) 270.
[6] E.A. Braga, A.A. Baykov and S.M. Avaeva, Biokhimiya, in press.
[7] M. Kunitz, J. Gen. Physiol. 35 (1952) 423.
[8] H. Weil-Malherbe and R.H. Green, Biochem. J. 49 (1951) 286.
[9] S.M. Lambert and J.F. Watters, J. Am. Chem. Soc. 79 (1957) 4262.
[10] J.F. Morrison, W.J. O'Sullivan and A.G. Ogston, Biochim. Biophys. Acta 52 (1961) 82.
[11] D. Scheuch and S.M. Rapoport, Acta Biol. Med. Ger. 5 (1960) 475.
[12] M. Cohn, Biochemistry 2 (1964) 624.