5-PHOSPHORIBOSYL PYROPHOSPHATE SYNTHETASE FROM EHRLICH ASCITES-TUMOUR CELLS: EFFECT OF MAGNESIUM AND ATP CONCENTRATION ON THE ENZYMIC ACTIVITY*

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Received October 23, 1967

The enzyme 5-phosphoribosyl pyrophosphate (PRPP) synthetase (ATP: D-ribose-5-phosphate pyrophosphotransferase EC 2.7.6.1) catalyses the reaction:

$$\text{ATP} + \text{ribose-5-phosphate} \longrightarrow \text{PRPP} + \text{AMP}.$$

PRPP is involved in the biosynthesis of purine, pyrimidine and pyridine nucleotides and of histidine and tryptophan and it has been suggested that the activity of the enzyme from bacterial sources is regulated by cumulative feedback inhibition (Switzer, 1967; Atkinson and Fall, 1967). Atkinson and Fall (1967) have shown that the activity of PRPP synthetase from *Escherichia coli* is also controlled by the [ATP] / [ADP] + [AMP] ratio.

As part of a study of the regulation of PRPP synthetase from Ehrlich ascites-tumour cells the effects of Mg^{2+}, ATP and ribose-5-phosphate concentration on the enzymic activity have been examined. The results show that $Mg\text{-}ATP^{2-}$ is the true substrate for the tumour cell enzyme and that

* This work was supported by grants from the University of Adelaide Anti-Cancer Foundation, the Australian Research Grants Committee and the National Health and Medical Research Council.

activity is stimulated by free Mg^{2+}† and inhibited by free ATP.

MATERIALS AND METHODS

Substrates. ATP labelled with P^{32} in the γ-position was prepared as described by Glynn and Chappell (1964), neutralized with *N*-ethyl-morpholine and made to a final concentration of 10 m*M* with the *N*-ethylmorpholine salt of unlabelled ATP. The preparations of P^{32} contained about 0.008 mol. prop. of ADP and had initial specific activities of approx. 0.5 µmole/µC.

Concentrations of Mg^{2+} were determined as described by Vogel (1962) using Eriochrome Black as indicator.

Enzyme preparation. Acetone-dried powders of ascites cells were made as described by Reichard and Sköld (1963). 1 Gram of powder was extracted for 30 min. at 2° in 20 ml. of 20 m*M*-phosphate-10-m*M*-glutathione (Na^+, pH 8.0) and centrifuged at 10,000*g* for 10 min. The supernatant was adjusted to pH 5.0 with about 2 ml. of 1*M*-acetate (K^+, pH 5.0) and after 10 min. was centrifuged at 10,000*g* for 10 min. The precipitate was dissolved in 10 ml. of 20 m*M*-*N*-ethylmorpholine-10 m*M*-glutathione (adjusted to pH 8.0 with HCl) and the solution was made 0.1 m*M* with respect to EDTA. The extract was dialysed against 1 l. of water for 30 min. and then against 100 ml. of 20 m*M*-*N*- ethylmorpholine-10 m*M*-glutathione pH 8.0 for 3 hr. The dialysed extract was used as the source of PRPP synthetase which was stable for at least 1 week when stored at -15°.

Assay. The assay was based on the procedure described by Kornberg, Lieberman and Simms (1955). Reaction mixtures contained 10 µmoles of *N*-ethylmorpholine, pH 8.0, 1 µmole of glutathione (neutralized with *N*-ethylmorpholine), 0.4 µmole of ribose-5-phosphate, 20 µmoles of sodium phosphate, pH 8.0 and $MgCl_2$ and ATP^{32} as required. The reactions were started with 0.05 ml of extract (containing approx. 0.2 mg. of protein);

† Free Mg^{2+} is defined as Mg^{2+} not complexed with ATP. Phosphate was present in the assays and part of the free Mg^{2+} would be present as magnesium phosphate.

the final volume of the assays was 0.4 ml. After incubating for 5 min. at 37° reactions were stopped with 1 ml. of 3.5% perchloric acid and cooled in ice. The reaction mixtures were thoroughly mixed with 0.1 *g*. of charcoal ('Norit Sx2'), allowed to stand 5 min. at 2° and filtered through Whatman No. 541 paper. Portions (0.15 ml.) of the filtrate were dissolved in 5 ml. of Bray's solution (Bray, 1960) and counted in an automatic liquid scintillation spectrometer. Extracts contained an ATPase activity and the true rate of PRPP synthesis was obtained by subtracting activity in the absence of ribose-5-phosphate from that in the presence of ribose-5-phosphate.

RESULTS AND DISCUSSION

PRPP synthetase from ascites cells showed an absolute dependence on phosphate, maximum rates being obtained at a concentration of 50 m*M*. Remy, Remy and Buchanan (1955) and Kornberg et al. (1955) assayed the synthetase by enzymic estimation of PRPP and Flaks (1963) suggested that phosphate may inhibit a divalent cation-activated decomposition of PRPP. However, lack of activity using the ATP^{32} assay indicates that phosphate has an essential function in enzymic activity.

Fig. 1 shows the activity of PRPP synthetase with varying $Mg\text{-}ATP^{2-}$ concentrations. Under the assay conditions used it can be calculated that at least 99.5% of the ATP added was present as $Mg\text{-}ATP^{2-}$ (Jackson, 1962; Martell, 1964; Keech and Barritt, 1967). It is clear that free Mg^{2+} stimulates PRPP synthetase by increasing the apparent affinity of the enzyme for $Mg\text{-}ATP^{2-}$ and that free ATP acts as a competitive inhibitor of the reaction. The concentration of $Mg\text{-}ATP^{2-}$ for half-maximal velocity was 3.5 m*M* and in the presence of 1 m*M*-$MgCl_2$, 10 m*M*-$MgCl_2$ or 2 m*M*-ATP was 1.5, 0.54 and 5.0 m*M* respectively. A range of divalent cations were tested for their ability to support PRPP synthetase activity. In the presence of 1.25 m*M*-ATP, 1.25 m*M*-$BaCl_2$, $MnCl_2$ and $CoCl_2$ gave rates 22, 16 and 15% respectively of the rate with 1.25 m*M*-$MgCl_2$. No reaction

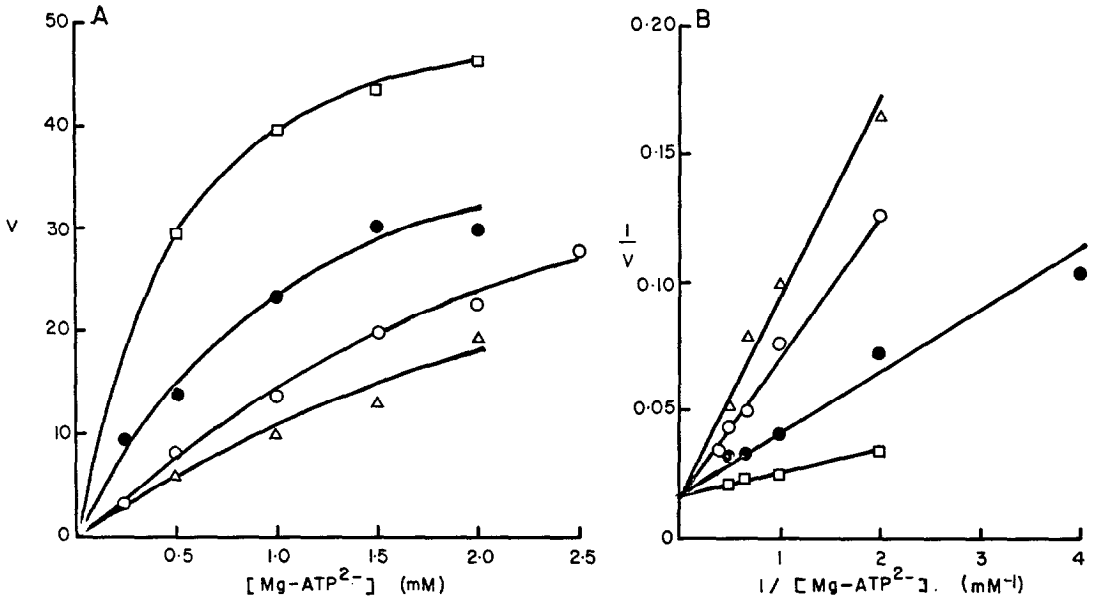

Figure 1. A. The effect of Mg^{2+} and ATP concentration on the rate of PRPP synthetase. It is assumed that the addition of equimolar Mg^{2+} and ATP resulted in the concentrations of Mg-ATP^{2-} shown in the figure (see the text). O—O , rate as a function of Mg-ATP^{2-} concentration; ●—● □—□ and Δ—Δ , rate as a function of Mg-ATP^{2-} concentration in the presence of 1 m*M*-$MgCl_2$, 10 m*M*-$MgCl_2$ or 2 m*M*-ATP respectively. The reaction velocity (v) is expressed as mμmoles of PRPP formed/min./mg. of protein. B. The data in A plotted in a double reciprocal form.

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was observed with 1.25 m*M*-$CaCl_2$, $NiCl_2$ or $ZnCl_2$.

The effect of free Mg^{2+} was also examined as the concentration of ribose-5-phosphate was varied (Fig. 2). Addition of 7.3 m*M*-$MgCl_2$ increased the maximum velocity from 45 to 67 mμmoles of PRPP formed/min./mg. of protein. This observed increase (49%) was close to the value (40%) calculated from the data in Fig. 1 as resulting from activation of the enzyme at the non-saturating concentration of Mg-ATP^{2-} (1.25 m*M*) used in this experiment. The concentration of ribose-5-phosphate giving half-maximal velocity (0.2 m*M*) was the same in the presence or absence of additional Mg^{2+}.

The results indicate that misleading kinetics of the PRPP synthetase reaction could be obtained in experiments in which the concentration of

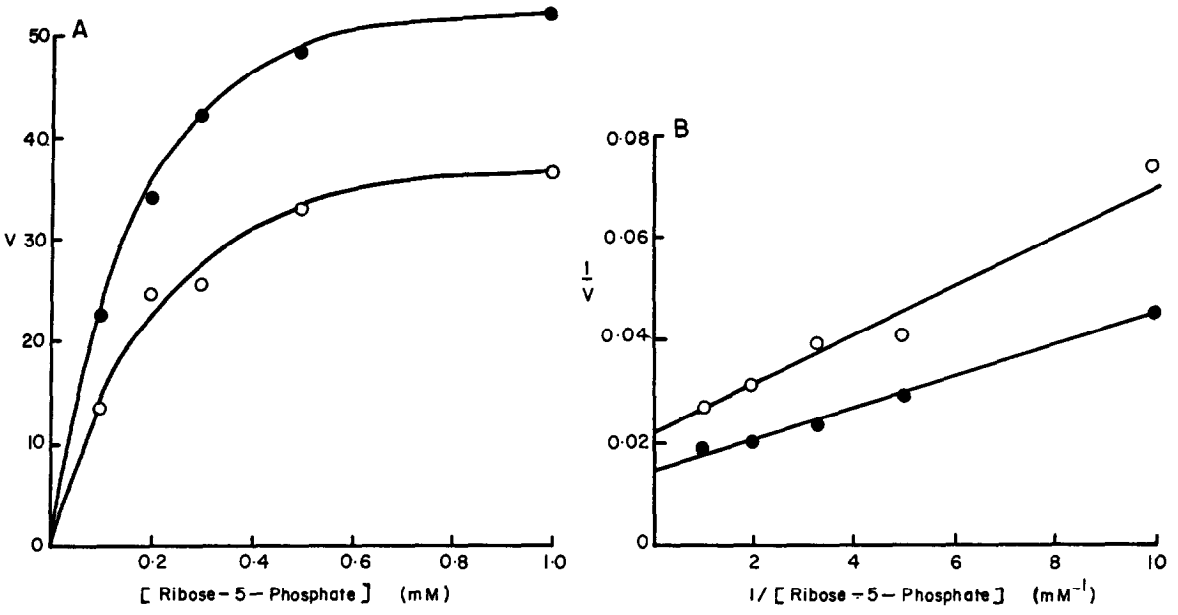

Figure 2. A. The effect of ribose-5-phosphate concentration on the rate of PRPP synthetase. Assays contained 1.25 m*M*-$MgCl_2$ and 1.25 m*M*-ATP and varying concentrations of ribose-5-phosphate in the absence (O—O) and presence (●—●) of 7.3 m*M*-$MgCl_2$. The reaction velocity (v) is expressed as mμmoles of PRPP formed/min./mg. of protein.
B. The data in A plotted in a double reciprocal form.

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one substrate was varied in the presence of constant concentrations of the other substrates. Under these conditions the concentrations of free Mg^{2+} and ATP present in assay mixtures would also vary.

The results reported in this paper may be important when determining the effects of inhibitors on the PRPP synthetase reaction; it will be necessary to determine whether inhibitory compounds affect both the reaction of $Mg\text{-}ATP^{2-}$ and the stimulation by free Mg^{2+} to the same extent. Special care in interpretation will be needed if inhibitors are used which complex with magnesium.

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