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The route of non-enzymic and enzymic breakdown of 5-phosphoribosyl 1-pyrophosphate to ribose 1-phosphate

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Spontaneous decomposition of 5-phosphoribosyl 1-pyrophosphate at pH 5.5 was established to occur as follows:

5-Phosphoribosyl 1-pyrophosphate→5-phosphoribosyl 1,2-(cyclic)phosphate→ribose 1-phosphate→ribose

Enzymic degradation of 5-phosphoribosyl 1-pyrophosphate by alkaline phosphatase from calf intestine and by acid phosphatases from potato and *Aspergillus niger* was found to proceed according to this pathway within the pH range 2.5–7.4 with accumulation of ribose 1-phosphate. In the case of alkaline phosphatase, Mg^{2+} ions inhibit the pyrophosphorolysis of 5-phosphoribosyl 1-pyrophosphate and stimulate the hydrolysis of ribose 1-phosphate.

INTRODUCTION

The role of 5-phosphoribosyl 1-pyrophosphate (*P*-Rib-*PP*) in the biosynthetic pathways of various nucleotides is well known. In contrast, the catabolism of this compound is poorly understood. Fox & Merchant (1974) have claimed that alkaline phosphatase is responsible for *P*-Rib-*PP* hydrolysis in human tissues. Tax & Veerkamp (1978) have reported a degradation of *P*-Rib-*PP* in mammalian haemolysates, which seems to be associated with the acid phosphatase activity. In both cases the product(s) of the enzymic degradation of *P*-Rib-*PP* have not been identified. Moreover, only the products of spontaneous decomposition of *P*-Rib-*PP* at non-physiological pH values are known: at pH > 7 *P*-Rib-*PP* is hydrolysed to inorganic phosphate (P_i) and 5-phosphoribosyl 1,2-(cyclic)phosphate [*P*-Rib-(c)*P*], which next afforded a mixture of ribose 1-phosphate (Rib-1*P*), ribose 5-phosphate (Rib-5*P*) and ribose 1,5-bisphosphate (Khorana *et al.*, 1958); at pH < 3 *P*-Rib-*PP* is split into pyrophosphate (PP_i) and Rib-5*P* (Kornberg *et al.*, 1955).

In the present paper we report the course of *P*-Rib-*PP* degradation catalysed by various phosphatases as well as of the spontaneous hydrolysis of *P*-Rib-*PP* at pH 5.5.

MATERIALS AND METHODS

Chemicals

Sources of chemicals were as follows: *P*-Rib-*PP* (tetrasodium salt) and Rib-1*P* from Sigma Chemical Co. (St. Louis, MO, U.S.A.); *P*-Rib-*PP* (dimagnesium salt), Rib-5*P*, IMP, uracil and Formycin B from Calbiochem (Los Angeles, CA, U.S.A.); hypoxanthine, inosine, Tris and Dowex 1 X8 (200–400 mesh; Cl^- form) from Serva (Heidelberg, Germany); uridine from BDH Chemicals (Poole, Dorset, U.K.); [8-^{14}C]hypoxanthine (sp. radioactivity 4.6 mCi/mmol) from Amersham International (Amersham, Bucks., U.K.); [U-^{14}C]uracil (sp. radioactivity 5.6 mCi/mmol) from Institute for Research Production and Uses of Radioisotopes (Prague, Czechoslovakia). Alkaline phosphatase [from calf intestine; 1 unit (μmol/min)/mg] was from Serva; acid phosphatase (from potato; type II) and nucleoside phosphorylase (from calf spleen) were from Sigma Chemical Co.; uridine phosphorylase (homogeneous) was kindly given by Associate Professor A. K. Drabikowska (I.B.B., P.A.N., Warsaw, Poland). Haemolysates of human erythrocytes, prepared by the procedure of Kelley *et al.* (1967), served as a source of hypoxanthine/guanine phosphoribosyltransferase (HGPRTase) and purine nucleoside phosphorylase (PNPase). Erythrocytes (HGPRTase$^+$, PNPase$^+$) from a healthy subject having both enzymes, and those (HGPRTase$^-$, PNPase$^+$) from a Lesch–Nyhan patient entirely lacking the former enzyme, were used. The inhibitors of PNPase 8-aminoguanosine and 1-β-D-ribofuranosylo-1,2,4-triazolo-3-carboxyamidine were kindly given by Professor D. Shugar (I.B.B., P.A.N., Warsaw, Poland).

Chromatography of the *P*-Rib-*PP* decomposition products

Aqueous *P*-Rib-*PP* solutions were chromatographed on Dowex 1 X8 resin (previously transformed into the formate form) by the procedure of Khorana *et al.* (1958), with the use of a Pharmacia K9/15 laboratory column (9 mm diam. × 10 cm bed height). Elution was performed successively with 0.1 M-, 0.25 M- and 0.5 M-ammonium formate buffer, pH 5.0 (see Figs. 1*a* and 1*b*). The flow rate was 1 ml/3 min. Fractions (2 ml) were examined for the presence of ribose with the orcinol reagent (Macek, 1954) and for the ability to support the synthesis of inosine and of IMP catalysed by suitable enzymes (see below), which showed the presence of Rib-1*P* and *P*-Rib-*PP* respectively. Ribose-positive fractions were freeze-dried, and dry residues were each dissolved in 0.1 ml of water, and were spotted alongside standard solutions of *P*-Rib-*PP*, Rib-1*P*, Rib-5*P* and free ribose on Eastman 13255 Cellulose Chromagram sheets. Chromatograms were developed with methanol/aq. 25% (v/v) conc. NH_3/water mixture (7:1:2, by vol.). Spots of ribose-containing compounds were located with the orcinol reagent for ribose or with the $AgNO_3$ reagent for aldoses with an unsubstituted aldehyde group (Macek, 1954). R_F values of *P*-Rib-*PP*, *P*-Rib-(c)*P*, Rib-5*P*, Rib-1*P* and ribose were 0.24, 0.35, 0.65, 0.71 and 0.82 respectively.

Enzymic synthesis of IMP and inosine from *P*-Rib-*PP* and from its decomposition products

The incubation mixture (final volume 100 μl) contained

Abbreviations used: *P*-Rib-*PP*, 5-phosphoribosyl 1-pyrophosphate; *P*-Rib-(c)*P*, 5-phosphoribosyl 1,2-(cyclic)phosphate; Rib-1*P*, ribose 1-phosphate; Rib-5*P*, ribose 5-phosphate; HGPRTase, hypoxanthine/guanine phosphoribosyltransferase; PNPase, purine nucleoside phosphorylase.

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400 μM-[8-^{14}C]hypoxanthine (3000 d.p.m./nmol), 5 mM-$MgCl_2$, 100 mM-Tris/HCl buffer, pH 7.5, 20 μl (approx. 100 μg of protein) of 20-fold diluted haemolysate, (HGPRTase$^+$, PNPase$^+$) or (HGPRTase$^-$, PNPase$^+$), and 20 μl of a *P*-Rib-*PP* solution or of the fraction obtained during *P*-Rib-*PP* column chromatography. In blanks the haemolysate was omitted. After incubation for 10 min at 37 °C, the mixture was deproteinized with 10 μl of 4 M-$HClO_4$, neutralized with 10 μl of 4 M-KOH and centrifuged. The supernatant (100 μl) was spotted on Whatman no. 3 paper with unlabelled hypoxanthine, inosine and IMP as carriers and standards, and the chromatogram was developed with aq. 5% (w/v) Na_2HPO_4 (ascending technique). Spots of separated purine compounds were located under u.v. light; after excision, their radioactivity was counted in a Beckman liquid-scintillation counter with the use of a toluene-based scintillator, with a counting efficiency of about 60%. The whole experiment was run in duplicate. The amounts of IMP and inosine synthesized during 10 min incubations were expressed in nanomoles or as percentages of the sum of radioactivities found in spots of IMP, inosine and hypoxanthine (equal to hypoxanthine radioactivity in blanks). This method enabled the presence of *P*-Rib-*PP*, Rib-1*P* and/or Rib-5*P* (because of the occurrence of phosphoribomutase in the haemolysate) to be established. The above procedure usually served for the determination of the HGPRTase activity in haemolysates for biochemical identification of the Lesch–Nyhan syndrome.

Protein in haemolysates was determined by the method of Bradford (1976), with BSA as standard.

Enzymic identification of Rib-1*P* as a product of *P*-Rib-*PP* decomposition

Uridine phosphorylase that contained no contaminating phosphoribomutase (this was confirmed by a lack of uridine synthesis in the presence of uracil and Rib-5*P*) was used. The incubation mixture (final volume 50 μl) contained: 400 μM-[U-^{14}C]uracil (10000 c.p.m./nmol), the *P*-Rib-*PP* solution examined, 200 mM-Tris/HCl buffer, pH 7.5, and 0.1 μg of uridine phosphorylase protein. After incubation for 15 min at 37 °C, the mixture was boiled for 2 min, cooled and centrifuged. The supernatant (10 μl) was spotted on an Eastman 13255 Cellulose Chromagram sheet with uracil and uridine as carriers and standards. The chromatogram was developed using a mixture of aq. 5% (w/v) KH_2PO_4 and isopentanol (2:1, v/v); in this solvent the product (uridine) migrated faster than the substrate (uracil). The radioactivity of both substances was determined as described above.

P-Rib-*PP* degradation by phosphatases

The degradation of ribose phosphates [*P*-Rib-*PP*, *P*-Rib-(c)*P* and Rib-1*P*] catalysed by three phosphatases was examined. Commercial *P*-Rib-*PP* or *P*-Rib-*PP* and *P*-Rib-(c)*P* separated on a Dowex 1 X8 column were used. The presumable intermediate product of *P*-Rib-*PP* degradation (Rib-1*P*) was identified as a substrate for inosine synthesis catalysed by PNPase. This enzyme (usually 2.0 nkat) and labelled hypoxanthine (48–200 nmol) either were added to the reaction mixture after the preincubation of the ribose phosphate (10–150 nmol) with phosphatase in a suitable buffer, or were incubated together with these substances. Preincubation and incubation times were usually 5 and 10 min respectively. After incubation the radioactivities of inosine formed and non-utilized hypoxanthine were determined as described above.

The reaction mixture contained in a final volume of 100 μl: (*a*) alkaline phosphatase from calf intestine (usually 1.13 nkat), the ribose phosphate at various concentrations, 20 μl of 1 M-Tris/HCl buffer, pH 7.4, hypoxanthine and PNPase; (*b*) acid phosphatase from potato (usually 2 nkat), the ribose phosphate and 10 μl of 1 M-citrate buffer, pH 6.0 (preincubation mixture 50 μl) plus 10 μl of 100 mM-NaF, PNPase and hypoxanthine (incubation mixture 100 μl); (*c*) acid phosphatase from *Aspergillus niger* (crude extract), *P*-Rib-*PP* or Rib-1*P* and 10 μl of 1 M-glycine/HCl buffer, pH 2.5 (preincubation mixture 45 μl), plus 10 μl of 100 mM-NaF, PNPase, hypoxanthine and 10 μl of 1 M-Hepps/NaOH buffer, pH 8.0 (incubation mixture pH 6.5, 100 μl). The blanks contained no phosphatase.

RESULTS AND DISCUSSION

Spontaneous decomposition of *P*-Rib-*PP* at pH 5.5

Chromatography on Dowex 1 X8 columns and on cellulose

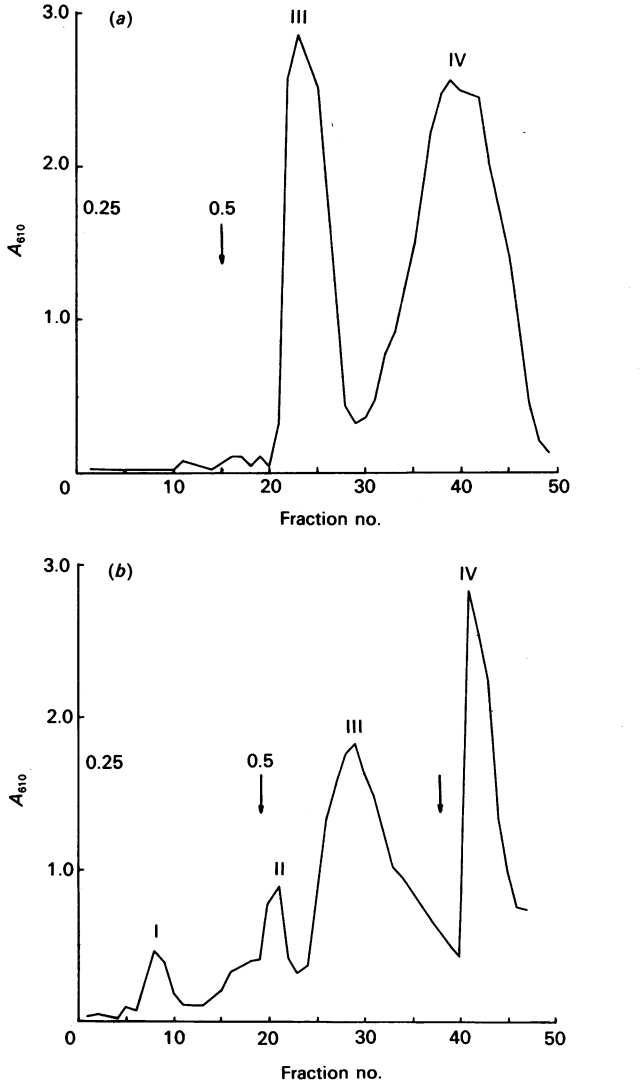

Fig. 1. Elution profiles of ribose-containing compounds present in (*a*) freshly prepared *P*-Rib-*PP* solution and (*b*) *P*-Rib-*PP* solution that had been stored for 2 weeks at 4 °C, obtained upon column chromatography on Dowex 1 X8 resin

For details see the Materials and methods section. The ordinates represent ribose content in fractions. Arrows indicate changes in eluent: (*a*) from 0.25 M- to 0.5 M-ammonium formate; (*b*) from 0.1 M- to 0.25 M- and then to 0.5 M-ammonium formate. Ribose-containing compounds in fractions were identified by chromatography on cellulose sheets and by enzymic methods. Peak I, ribose; peak II, Rib-1*P*; peak III, *P*-Rib-(c)*P*; peak IV, *P*-Rib-*PP*.

sheets as well as enzymic methods were used for elucidation of the course of *P*-Rib-*PP* decomposition.

Elution profiles obtained upon column chromatography of a freshly prepared *P*-Rib-*PP* solution or one that had been stored for 2 weeks at 4 °C (NaN_3 added) revealed the presence of two (peaks III and IV) or four (peaks I, II, III and IV) ribose-containing compounds respectively (Figs. 1*a* and 1*b*). With the freshly prepared *P*-Rib-*PP* solution the shape of peaks was better when elution with 0.1 M-formate was omitted.

In fractions corresponding to peak IV, *P*-Rib-*PP* was identified from its ability to serve, together with hypoxanthine, for IMP synthesis catalysed by haemolysate HGPRTase. The ribose-containing compound in fractions corresponding to peak III failed to support IMP synthesis, and it has been identified by Khorana *et al.* (1958) as *P*-Rib-(c)*P*. The commercial preparation of *P*-Rib-*PP* contains, according to the Sigma catalogue, up to 15% of *P*-Rib-(c)*P*. In a freshly prepared *P*-Rib-*PP* solution (Fig. 1*a*) the *P*-Rib-(c)*P* content (calculated from the contents of ribose in both peaks IV and III) accounted for 35%. In the elution profile obtained by chromatography of a stored *P*-Rib-*PP* solution, further degradation resulted in a decrease in peak IV, an augmentation of peak III and occurrence of peaks II and I (Fig. 1*b*). A ribose-containing compound in the peak II fractions, incubated with hypoxanthine and haemolysate, supported inosine synthesis; this suggested the presence of Rib-1*P* (and/or Rib-5*P*) in these fractions. Some slight synthesis of inosine was found also with the peak III fractions; the extent of synthesis increased with time of the storage of the fractions at 4 °C, probably owing to further decomposition of *P*-Rib-(c)*P*.

To verify whether Rib-5*P*, in addition to Rib-1*P*, was a product of spontaneous decomposition of *P*-Rib-*PP* at pH 5.5, the fractions corresponding to peaks II and III were chromatographed on cellulose sheets. Two ribose-containing spots were found in the case of peak III: one spot corresponding to *P*-Rib-(c)*P* (having the same R_F as one of the two spots obtained upon use of a freshly prepared *P*-Rib-*PP* solution), and another spot corresponding to the Rib-1*P* standard spot situated somewhat higher than the Rib-5*P* spot. On a chromatogram of the peak II fractions only the second spot was found. This spot gave no positive reaction with $AgNO_3$ reagent, whereas Rib-5*P* and free ribose did so. Therefore the decomposition of *P*-Rib-(c)*P* afforded only one product, namely Rib-1*P*. In the light of the greater stability of Rib-5*P* as compared with Rib-1*P*, spontaneous splitting of *P*-Rib-(c)*P* to Rib-1*P* suggests a destabilizing effect of the 1,2-(cyclic)phosphate group on the phosphate binding at position 5 of ribose.

The formation of Rib-1*P* from *P*-Rib-*PP* was definitively established by the synthesis of labelled uridine occurring in *P*-Rib-*PP* solution in the presence of [^{14}C]uracil and uridine phosphorylase that was free of phosphoribomutase activity. Under the conditions of the reaction (see the Materials and methods section), 1.9±0.15% and 12.2±0.15% of the uracil label were found in uridine after 10 min incubation with a freshly prepared *P*-Rib-*PP* solution and a stored *P*-Rib-*PP* solution respectively.

The occurrence of peak I, corresponding to free ribose, together with a decrease in inosine synthesis in *P*-Rib-*PP* solution that had been stored for 2 weeks (Table 1), testified to further decomposition of unstable Rib-1*P*.

The above findings point to the following reaction sequence during spontaneous *P*-Rib-*PP* decomposition at pH 5.5:

$$P\text{-Rib-}PP \rightarrow P\text{-Rib-(c)}P \rightarrow \text{Rib-}1P \rightarrow \text{ribose}$$

Both dimagnesium and tetrasodium salts of *P*-Rib-*PP* were hydrolysed according to this pathway (Table 1). It should be mentioned that the *P*-Rib-*PP* from four lots examined showed different susceptibilities to decomposition.

Inosine synthesis under conditions of the determination of the HGPRTase activity in haemolysate

Some amounts of labelled inosine are formed during the HGPRTase activity assay, with hypoxanthine as substrate. It could be supposed that this inosine originates from IMP synthesized by HGPRTase and next degraded by nucleotide-dephosphorylating enzymes. IMP degradation has been observed upon use of non-diluted haemolysate (Gini *et al.*, 1987) and in intact erythrocytes (Whelan & Bagnara, 1979; Bontemps *et al.*, 1986); this process seems to be enhanced in PNPase-deficient erythrocytes (Simmonds *et al.*, 1982). However, since *P*-Rib-*PP* decomposition produced Rib-1*P*, inosine could also be synthesized by PNPase occurring in haemolysates.

Examination of these two possibilities under conditions of the HGPRTase assay testifies against the former and confirms the latter. Thus inosine synthesis was not inhibited either by 150 mM-AMP (a competitive substrate for IMP-dephosphorylating enzymes) or by 3.3 mM-dTTP (an inhibitor of 5′-nucleotidase). In

Table 1. Spontaneous decomposition of *P*-Rib-*PP* to Rib-1*P* at pH 5.5 under various conditions

P-Rib-*PP* solutions were stored under the given conditions, and their ability to support IMP and inosine synthesis was examined. They were incubated with [8-^{14}C]hypoxanthine, $MgCl_2$ and haemolysate of normal human erythrocytes as a source of HGPRTase and PNPase, in Tris/HCl buffer, pH 7.5. For experimental details see the Materials and methods section. Determinations were made in duplicate; the S.D. was up to ±0.5% of hypoxanthine label incorporated into IMP or inosine.

P-Rib-*PP* solution	Storage conditions: Temperature (°C)	Storage conditions: Time (days)	Hypoxanthine label incorporated during 10 min (%): Into IMP	Into inosine
Dimagnesium salt	–	0	8.5	0.3
	−20	5	8.4	0.9
	4	5	8.9	3.4
Tetrasodium salt	–	0	10.2 0.0*	0.5 0.5*
	−20	5	10.9 0.0*	2.7 2.3*
	−20	56	4.4 0.0*	12.2 10.0*
	10	7	5.4 0.0†	38.8 62.4†
	10	14	3.9 0.0†	28.1 35.1†

* Haemolysate lacking HGPRTase and containing PNPase (Lesch–Nyhan syndrome).
† Mg^{2+} ions (necessary for HGPRTase activity) omitted, evidence that inosine was not a product of IMP degradation.

contrast, inosine synthesis occurred in the presence of haemolysate (HGPRTase$^-$, PNPase$^+$; Lesch–Nyhan syndrome) and also in the presence of haemolysate (HGPRTase$^+$, PNPase$^+$; normal erythrocytes) when Mg^{2+} ions required for the HGPRTase activity were omitted (Table 1). Moreover, inosine synthesis was inhibited by the PNPase inhibitors 1 mM-Formycin B, 2.3 mM-1-β-D-ribofuranosylo-1,2,4-triazolo-3-carboxyamidine and 2 mM-8-aminoguanosine by 20, 60 and 90% respectively. The absence of IMP dephosphorylation under the experimental conditions of the HGPRTase assay may be related to the use of 20-fold-diluted haemolysates in contrast with the undiluted ones applied in the experiments performed by Gini *et al.* (1987).

The possibility of interfering reactions in which Rib-1*P*, a product of *P*-Rib-*PP* decomposition, may be involved, should be taken into account in kinetic studies with *P*-Rib-*PP* as substrate.

Pathway of *P*-Rib-*PP* degradation by various phosphatases

Phosphatases from various sources exhibit pyrophosphorylase and monoesterase activities, but they do not split phosphate from nucleoside cyclic phosphates. Therefore they could catalyse *P*-Rib-*PP* dephosphorylation, following the sequence of reactions established for the spontaneous decomposition of *P*-Rib-*PP*. To verify this assumption, *P*-Rib-*PP* and *P*-Rib-(c)*P* were each incubated with phosphatase (alkaline or acid) in the presence of an excess of hypoxanthine and PNPase, to trap intermediate Rib-1*P* as inosine, before further splitting of Rib-1*P* into phosphate and ribose. Because of the instability of ribose phosphates and of the complex sequence of reactions, it was difficult to obtain exactly reproducible results, but the same general pattern was obtained in repeated experiments.

Alkaline phosphatase from calf intestine. During incubation with this enzyme (pH 7.4), both *P*-Rib-*PP* and *P*-Rib-(c)*P* were degraded to Rib-1*P*; the Rib-1*P* appeared also to be a substrate for this enzyme (Fig. 2*a*). Dephosphorylation to Rib-1*P* is in accord with the pyrophosphorylase activity of the enzyme and with the inability to hydrolyse nucleoside 2′,3′-(cyclic) phosphates (Hiwada & Wachsmuth, 1974; Chappelet-Tordo *et al.*, 1974). The quantity of intermediate Rib-1*P* depended on the amount of enzyme and on the reaction time, but not in a proportional manner, probably because of the complexity of the enzymic process. In the presence of 5 mM-$MgCl_2$ the degradation of *P*-Rib-*PP* decreased by 25% and that of Rib-1*P* increased by 28%, in accordance with the known effects of Mg^{2+} on inhibition of the phosphorylase activity and stimulation of the monoesterase activity of alkaline phosphatase (Fernley, 1971). Another factor (not studied in the present investigation) that could change the course of *P*-Rib-*PP* degradation is P_i, an inhibitor of alkaline phosphatase. In studies of the degradation of ATP to adenosine by this enzyme, Heppel *et al.* (1962) have found an accumulation of intermediate AMP, and explained it by the inhibitory effect of P_i accumulating in the reaction medium. Possibly changes in P_i and Mg^{2+} concentrations *in vivo* could exert some effect on the course of *P*-Rib-*PP* degradation by alkaline phosphatase.

Acid phosphatase from potato. All three ribose phosphates examined were dephosphorylated by this enzyme, with reaction rates in the order $V_{\text{Rib-1}P} > V_{P\text{-Rib-}PP} > V_{P\text{-Rib-(c)}P}$ (Fig. 2*b*). The use of NaF, a strong inhibitor of acid phosphatase, gave the opportunity to demonstrate that the accumulation of Rib-1*P* during preincubation of *P*-Rib-*PP* with this enzyme really occurs, despite the highest rate of Rib-1*P* dephosphorylation in this case. This may suggest that Rib-1*P* affinity of this enzyme is the lowest. At pH 6.0 the K_m for Rib-1*P* appeared to be 400–500 μM (at a substrate concentration range of 500–1500 μM), and the approximate value of K_m for *P*-Rib-*PP* was estimated to be about 2-fold lower. However, the latter value cannot be determined exactly because the enzyme is involved in subsequent dephosphorylations and is inhibited by P_i (Hsu *et al.*, 1966) accumulating in the reaction medium. The inhibition by P_i may contribute to Rib-1*P* accumulation, similarly to the case of alkaline phosphatase. Mg^{2+} exerted no effect on the course of

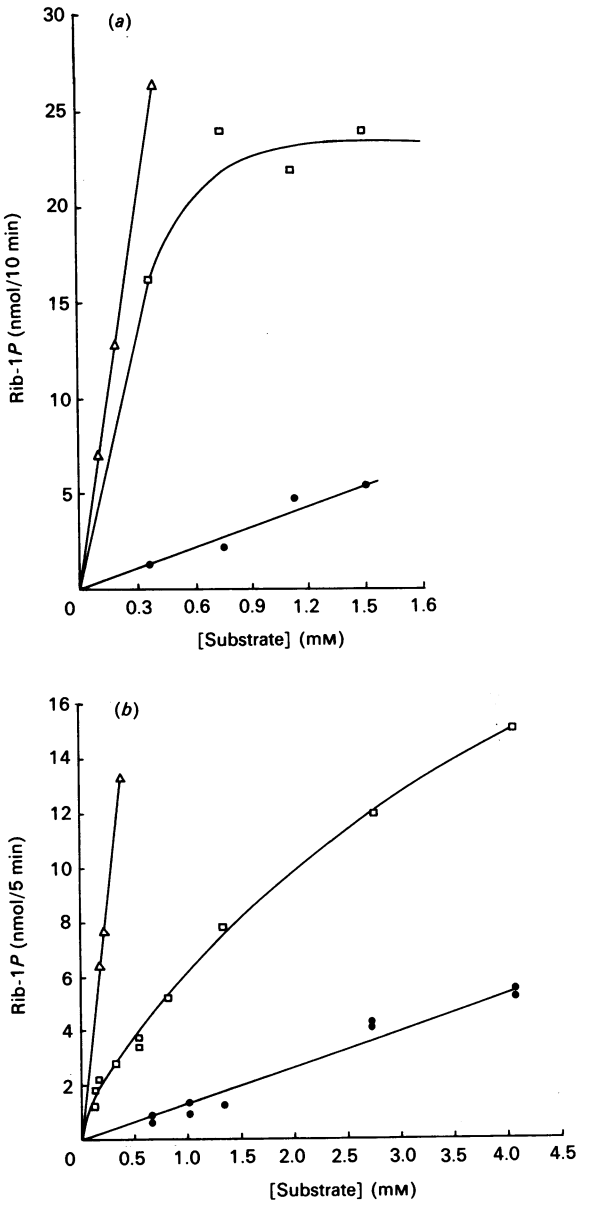

Fig. 2. Degradation of *P*-Rib-*PP*, *P*-Rib-(c)*P* and Rib-1*P* catalysed by phosphatases

(*a*) Alkaline phosphatase from calf intestine was incubated with each of the three ribose phosphates and labelled hypoxanthine plus PNPase in Tris/HCl buffer, pH 7.4. (*b*) Acid phosphatase from potato was preincubated with each of the three ribose phosphates in citrate buffer, pH 6.0; the reaction was stopped with NaF, and the reaction mixture was incubated with labelled hypoxanthine plus PNPase, used to determine intermediary Rib-1*P* as inosine. In blanks phosphatase was omitted. For details see the Materials and methods section. Concentrations of substrate on abscissae refer to each substrate used: *P*-Rib-*PP*, *P*-Rib-(c)*P* and Rib-1*P*. Ordinates represent Rib-1*P* concentration (equal to that of inosine): □, Rib-1*P* formed from *P*-Rib-*PP*; ●, Rib-1*P* formed from *P*-Rib-(c)*P*; △, substrate Rib-1*P* dephosphorylated, i.e. free ribose (difference between Rib-1*P* in blank and sample).

P-Rib-*PP* degradation. Upon a change in pH from 6.0 to 7.4, the *P*-Rib-*PP* dephosphorylation rate decreased several fold, and Rib-1*P* hydrolysis was prevented.

Acid phosphatase from *Aspergillus niger*. This enzyme can act at pH below 3.0, at which *P*-Rib-*PP* is spontaneously decomposed to Rib-5*P* and PP_i (Kornberg *et al.*, 1955). However, with this enzyme acting on *P*-Rib-*PP* at pH 2.5, Rib-1*P* was found in the preincubation mixture in amounts proportional to the enzymic activity and increasing with substrate concentration. Enzymic activity at pH 4.5 and 5.5 was 17% and 5% respectively of that at pH 2.5, assumed as 100%. Rib-1*P* was also a substrate for this enzyme.

The present results show that *P*-Rib-*PP* degradation catalysed by alkaline phosphatase or acid phosphatase proceeds within a pH range of 2.5–7.4 along the same pathway as in the case of spontaneous decomposition of *P*-Rib-*PP* at pH 5.5:

$$P\text{-Rib-}PP \rightarrow P\text{-Rib-(c)}P + P_i \rightarrow \text{Rib-1}P + 2P_i \rightarrow \text{ribose} + 3P_i$$

with accumulation of intermediate Rib-1*P*. Therefore not only phosphorolysis of nucleoside by PNPase (Gini *et al.*, 1987) but also *P*-Rib-*PP* dephosphorylation by phosphatases may be a source of Rib-1*P*, particularly when the *P*-Rib-*PP* concentration in cells is elevated. As Rib-1*P* is also a substrate for phosphatases, its re-use for nucleoside synthesis should be dependent upon the ratio of the phosphatase/nucleoside phosphorylase activities and upon the concentration of P_i, which inhibits both reactions.

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