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Rapid Communication

Purification and Characterization of Phosphoribosylpyrophosphate Synthetase from Rubber Tree Latex

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Phosphoribosylpyrophosphate synthetase (PRS; EC 2.7.6.1) from *Hevea brasiliensis* Müll. Arg. latex was located in the cytosol. After purification, its apparent molecular weight under nondenaturing conditions was estimated at 200,000 ± 10,000; a single band at 57,000 ± 3,000 was detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The enzyme seemed to be a homotetramer. Its affinity constants were estimated at 200 ± 30 μM for adenosine triphosphate and 40 ± 2 μM for ribose-5-phosphate. The purified enzyme proved to be functional in a paraphysiological medium (cytosol deproteinized by ultrafiltration). Optimum pH was 7.5 in buffer and 6.5 in a paraphysiological medium. No PRS activity was detected in the absence of the Mg^{2+} ion. Of the numerous compounds tested, only Mn^{2+}, phosphoribosylpyrophosphate, and inorganic phosphate affected the enzymatic reaction. Mn^{2+} (inhibitor constant = 20 μM) and phosphoribosylpyrophosphate (inhibitor constant = 30 μM) were inhibitors. PRS responded allosterically (Hill's coefficient = 2.3) to ribulose-5-phosphate in the presence of a physiological concentration of inorganic phosphate (10 mM). These results are set in the physiological context of laticifers.

Hevea brasiliensis Müll. Arg. latex is the cytoplasm of specialized cells forming the laticifer system. The latter are differentiated rhythmically in single-cell layers (latex vessel rings) by the cambium. The cells in the same ring anastomose, forming a network or paracirculatory system within the secondary phloem (Dickenson, 1965). When the soft bark is cut (tree tapping) the laticifer contents are expelled. Most of the latex volume (up to 50%) is made up of rubber particles (*cis*-1,4-polyisoprene) and lutoids (20%), which are acknowledged to be a polydispersed vacuolysosomal system (Pujarniscle, 1968). Latex flow and regeneration before the next tapping are the main factors likely to limit rubber production (d'Auzac et al., 1989). Applying an ethylene generator (Ethrel, Rhône Poulene, France) to the tapped bark increases rubber production (Abraham et al., 1968; d'Auzac and Ribailler, 1969).

An increase in the adenylate pool (AMP, ADP, and ATP) is considered to be one of the first biochemical modifications induced in the cytosol by ethylene (Amalou et al., 1992). It has been shown that biochemical energy availability, primarily reflected by the overall adenine nucleotide content, is a major factor in the physiological control of intralaticiferous material regeneration between two tappings (d'Auzac et al., 1989). De novo synthesis and regeneration of the adenylate pool is therefore an important aspect in controlling latex production. A study of the enzymatic pathways involved in adenylate biosynthesis within latex was initiated with the characterization of adenine phosphoribosyltransferase (EC 2.4.2.7) (Gallois et al., 1996), which catalyzes AMP formation from adenine and PRPP.

It has been clearly shown that PRPP is a major factor controlling adenine phosphoribosyltransferase activity. Moreover, this compound is an essential substrate for several metabolic pathways, including purine, pyrimidine, and nicotinamide nucleotide de novo synthesis and salvage pathways, but also for Trp and His biosynthesis (Wasternack, 1982). The essential function of PRPP has already been highlighted in the animal (Becker et al., 1979) and plant (Hirose and Ashihara, 1983; Le Floc'h, 1984; Dancer and Rees, 1989) kingdoms. The enzyme that catalyzes PRPP formation from ATP and Rib-5-P is PRS (EC 2.7.6.1). To our knowledge, this key enzyme has only been studied in plants in the hypocotyls of mung bean (Ashihara and Komamine, 1974), the leaves of spinach (Ashihara, 1977), and the tubers of Jerusalem artichoke (Le Floc'h, 1984), and its purification to homogeneity has only been carried out in animals (Becker et al., 1979) and microbes (Jensen, 1983). Purification of a plant PRS to apparent homogeneity (SDS-PAGE) is reported here, for the first time to our knowledge, along with several biochemical characteristics of the enzyme and the mechanisms regulating its activity within latex cytosol.

MATERIALS AND METHODS

The GT 1 genotype of *Hevea brasiliensis* Müll. Arg. from the Ivory Coast was used in the present work. A group of 20 rubber trees was selected for homogenous growth (circumference) and rubber production. The latex, collected on ice, was centrifuged at 16,000*g* (GSA RC-5, Sorvall) for 15 min at 4°C. The pellet, composed of lutoids and Frey-Wyssling

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Abbreviations: PRPP, phosphoribosylpyrophosphate; PRS, phosphoribosylpyrophosphate synthetase.

particles (chromoplast-like organelles), was washed in buffer (50 mM Tris-HCl, pH 7, and 0.4 M mannitol) and freeze-dried for storage at −30°C before analysis. The supernatant was centrifuged to separate rubber particles from the cytosol, which was freeze-dried and stored at −30°C. Lyophilized cytosol was resuspended (50 mg mL^{-1}) in buffer (25 mM Tris-HCl, pH 8.3, containing 5 mM $MgCl_2$, 5 mM DTT, and 0.02% NaN_3 [buffer A]). After centrifugation (20,000*g* for 10 min at 4°C), the suspension was passed through a prefilter (Millex AP 20, Millipore) and designated as the crude extract. Lyophilized lutoids were resuspended in buffer A (100 mg mL^{-1}) containing 0.1% Triton X-100 and homogenized (Potter, Millville, NJ). After centrifugation (20,000*g* for 10 min), ammonium sulfate was added to a final concentration of 80%. Precipitated proteins were pelleted by centrifugation (20,000*g* for 10 min) and resuspended in buffer A. This extract was used to determined PRS activity in the bottom fraction.

Enzyme Assay

PRS activity was determined by enzymatic measurement of AMP after incubation performed at 30°C in 250 μL of buffer A (pH 7.5 instead of pH 8.3) with enzymatic extract. The reaction was started by adding 1 mM ATP and 1 mM Rib-5-P. The incubation was stopped after 5 min by adding 10% (v/v) perchloric acid. The suspension was neutralized with a buffer containing 1.2 M Tris, 10% (v/v) KOH, and 175 mM KCl. AMP was determined after centrifugation (15,000*g* for 10 min). PRS activity was also measured with a coupled spectrophotometric assay (direct kinetic) of AMP or PRPP, as described below. PRS activity was expressed in nanokatals, and PRS specific activity was expressed in nanokatals per milligram of protein.

AMP and PRPP Measurement

After incubation AMP was determined by coupled enzymatic assay, as previously described by Gallois et al. (1996). K_m values for ATP were estimated by measurement of AMP produced by PRS activity after incubation, using different concentrations of ATP: 0.1 to 2 mM. K_m values for Rib-5-P were estimated by direct measurement (direct kinetic) of AMP produced by PRS activity, as previously described by Braven et al. (1984). The PRPP formed by PRS was determined by direct measurement as described by Dancer and Rees (1989). The mixture used for AMP measurement was equilibrated at 30°C; Rib-5-P was added at different concentrations from 10 to 250 μM.

ATP and Protein Measurement

The ATP formed by reverse reaction of PRS was determined by chemiluminescence using a biocounter (model M2500, Lumac, Landgraaf, The Netherlands) after incubation, or with direct measurement using hexokinase, as described by Lamprech and Trautschold (1974). Proteins were determined as described by Bradford (1976), with BSA as a standard.

PRS Activity and Rib-5-P Measurement in Filtered Cytosol

The cytosolic serum, also called the crude extract, was obtained as described above. The filtered cytosol (paraphysiological medium) was obtained after ultrafiltration of the crude extract through a 10K membrane (Filtron, Northborough, MA), as described by Jacob et al. (1983). The filtrate can be used to estimate the activity of a purified enzyme without serious interference from other enzymes. Rib-5-P from latex cytosol was estimated using an AMP measurement system and PRS (direct kinetic). The latter enzyme was used to initiate the reaction. Filtrate pH was modified by adding 6 N HCl or 1 N KOH.

PRS Specificity

The enzyme assay was made by direct measurement of PRPP produced, with 1 mM ITP, UTP, GTP, or CTP instead of ATP.

PRS Effectors

The following were added during the enzyme assay: K^+, Na^+, Pi, and SO_4^{3-} (1–250 mM); Mg^{2+} and Mn^{2+} (5–20 mM); PPi (0.1–1 mM); Zn^{2+}, Fe^{3+}, and Cu^{2+} (5–50 μM); and Ca^{2+} (50–500 μM). All compounds were as Cl^- salts, except Zn^{2+}, which was as a sulfate. Seven organic acids (α-ketoglutarate, pyruvate, citrate, malate, fumarate, succinate, and oxaloacetate) were used at 1 and 10 mM. Effectors were also sought among sugars and amino acids. Glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, Glc-1-P, Glc-6-P, Fru-6-P, Fru-1,6-bisP, and Rib were tested between 0.1 and 2 mM; Suc from 1 to 20 mM; Glu, Gln, Asp, and Ala from 5 to 50 mM; and Trp and His at 1 mM. The effects of bases (orotate, guanine, cytosine, adenine, and xanthine), nucleosides (adenosine, cytidine, guanosine, and uridine), and nucleotides (UMP, UDP, UTP, GMP, GDP, GTP, AMP, ADP, and ATP) were studied at a concentration of 1 mM.

Validity of the Enzymatic Assay

It was verified that 0.1% Triton X-100, PRPP (10–100 μM), Rib-5-P (1 mM), and compounds listed in "PRS Specificity" did not interfere with the enzymatic system used for assaying the PRS activity by direct measurement. These precautions made it possible to validate the enzymatic assay method used for the measurement of PRS activity.

Enzyme Purification

A crude extract was prepared from 3 g of freeze-dried latex cytosol as described above. Proteins precipitated in 50% saturated ammonium sulfate were collected by centrifugation (20,000*g*, 10 min) and resuspended in buffer A. Protein extract was applied on a 100- × 1-cm column (Sephacryl S300 HR, Pharmacia) equilibrated with buffer A and eluted with same buffer at 42 mL h^{-1}. Fractions containing PRS activity were pooled and applied on a 10- × 1-cm column (Protein-Pak DEAE 40 HR, Waters) equili-

brated with buffer A. Elution was made at 90 mL h^{-1} with 30 mM KCl prepared in the same buffer.

Biochemical characteristics of PRS were determined on active fractions obtained after this step. For further purification active fractions were applied on Blue Ultrogel (IBF, Villeneuve la-Garenne, France). Proteins not fixed were first eluted by 1.5 volumes of buffer A containing 300 mM KCl. PRS was then eluted by 400 mM KCl. All steps of Blue Ultrogel were performed at 0.2 mL min^{-1}. All purification steps described were at 10°C. Estimations of K_m for ATP and Rib-5-P were made with the purified PRS obtained after Blue Ultrogel affinity chromatography.

Electrophoresis

SDS-PAGE was carried out on the gradient-ready gel (4–20%, Mini-Protean II, Bio-Rad), with samples and buffer prepared as described by Laemmli (1970). Pharmacia molecular mass markers were used. Gels were stained by silver nitrate as described by Wray et al. (1981).

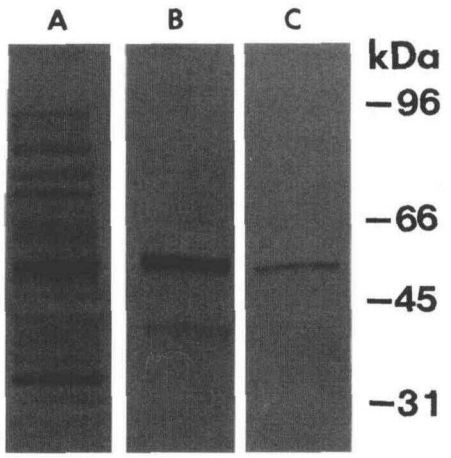

Figure 1. Analysis of PRS purification by SDS-PAGE (4–20%). Lane A, S300 HR; lane B, DEAE; and lane C, Blue Ultrogel. Proteins were detected by silver nitrate.

RESULTS

Activity and Purification

PRS was detected in *H. brasiliensis* latex cytosol in the presence of ATP, Rib-5-P, and a cytosolic protein extract (see "Materials and Methods") by measuring the AMP and the PRPP formed. No activity was detected in the sedimentable fraction of latex primarily containing vacuolysosomes and a few Frey-Wyssling particles (chromoplasts) (Dickenson, 1965).

The purification procedure is summarized in Table I. After the final step, affinity chromatography on Blue Ultrogel and SDS-PAGE on an acrylamide gradient of 4 to 20%, a single protein band with an apparent M_r of 57,000 ± 3,000 appeared (Fig. 1). Under nondenaturing conditions (filtration on Sephacryl S300 HR) the M_r of the enzyme was 200,000 ± 10,000.

Biochemical Characteristics and Enzyme Activity Effectors

Several compounds (ITP, UTP, GTP, and CTP) were tested as diphosphate group donors, but PRPP was detected only in the presence of ATP.

The effect of pH on PRS activity was determined in a 25 mM Tris-maleate buffer with a pH range of 6.0 to 8.0. Optimum activity was obtained at a pH of 7.25 (Fig. 2). Enzymatic activity could only be maintained with 5 mM Mg^{2+} and 5 mM DTT. No other sulfhydryl group protector (Cys or GSH) or antioxidant (ascorbate) could effectively replace DTT. Moreover, none of the other cations tested (Co^{2+} or Mn^{2+}) could effectively replace Mg^{2+}. The apparent K_m of the enzyme for Mg^{2+} was 1.5 mM. The K_m of PRS with respect to ATP and Rib-5-P was estimated at 200 ± 30 and 40 ± 2 μM, respectively.

Of all the ions and metals (K^+, Na^+, Pi, SO_4^{3-}, Mn^{2+}, PPi, Zn^{2+}, Fe^{3+}, Cu^{2+}, and Ca^{2+}) tested at concentrations specified in "Materials and Methods," only Mn^{2+} and Pi (Fig. 3B) had a notable effect on PRS activity. The K_i of Mn^{2+} was 20 μM, with complete inhibition above 400 μM. Pi either inhibited or activated PRS, depending on the amount of Rib-5-P in the medium. In the presence of 1 mM Rib-5-P, the curve for PRS activation by Pi was sigmoidal (Hill coefficient = 4.9), with maximum activity occurring from 10 mM; however, in the presence of 20 μM Rib-5-P, Pi inhibited PRS (Fig. 3B). This characteristic is shown in Figure 3A during a measurement of PRS affinity for Rib-5-P with or without 10 mM Pi. In the presence of a physiological concentration of Pi and increasing quantities of Rib-5-P (0–100 μM), PRS activity varied in a sigmoidal curve (Hill coefficient = 2.3).

Under our experimental conditions none of the phosphate esters or sugars (glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, Glc-1-P, Glc-6-P, Fru-6-P, Fru-1,6-bisP, Rib, or Suc), amino acids (Glu, Gln, Asp, Ala, Trp, or His), or organic acids (α-ketoglutarate, pyruvate, citrate, malate, fu-

Table I. *Purification of PRS from the latex cytosol of H. brasiliensis*
PRS activity was determined by measuring AMP formed.

Steps	Total Protein	Total Activity	Specific Activity	Purification	Recovery
	mg	*nktal*	*nkat mg^{-1}*	*-fold*	%
Crude extract	322.80	10.40	0.032	1	100
45/50%	29.23	3.50	0.12	3.7	33.7
S300 HR	10.60	2.20	0.21	6.5	21
DEAE 40 HR	0.60	1.51	2.5	78.6	14.5
Blue Ultrogel	0.070	0.85	12.2	379	8

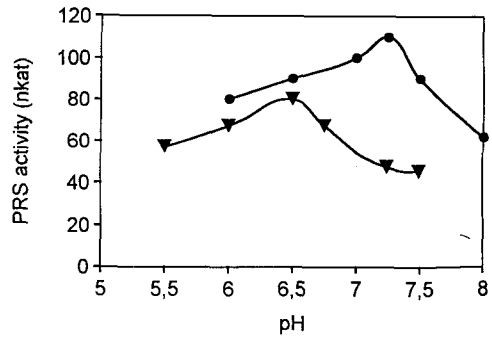

Figure 2. Effect of pH on PRS activity in a buffer medium and in a paraphysiological medium. The purified enzyme was incubated in a buffer medium (●) or in paraphysiological serum (▼), with the pH adjusted previously to the desired value. PRS activity was measured by monitoring the AMP formed after incubation with 1 mM ATP and 1 mM Rib-5-P.

marate, or succinate) tested had any effect on PRS activity. None of the nucleotides (UMP, UDP, UTP, GMP, GDP, GTP, AMP, ADP, or ATP), nucleosides (adenosine, cytidine, guanosine, or uridine), or bases (adenine, orotate, guanine, cytosine, or xanthine) studied had any notable effect on PRS activity. Some results are represented in Table II.

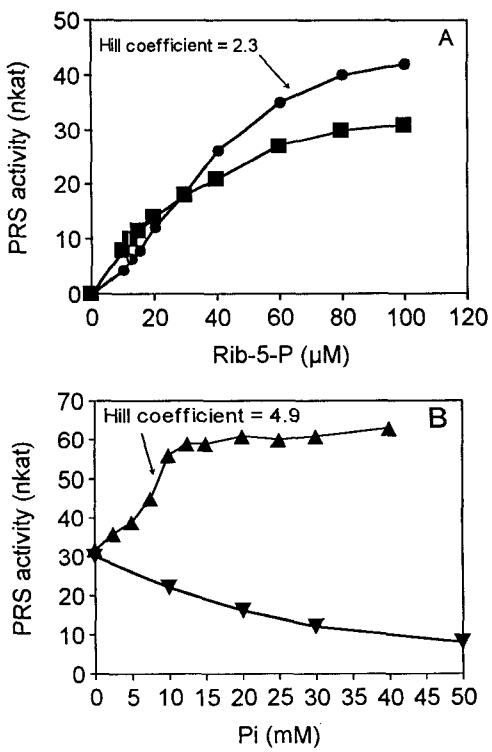

Figure 3. PRS activity related to Pi concentration. A, PRS activity was determined by direct measurement of the AMP formed after the addition of 1 mM ATP and 10 mM Pi (●), or no addition (■) and increasing concentrations of Rib-5-P (0–100 μM). B, PRS activity was measured by monitoring the AMP formed in the presence of 1 mM ATP and 1 mM Rib-5-P (▲), or 20 μM Rib-5-P (▼) and increasing concentrations of Pi (0–50 mM).

Table II. *Effect of some compounds on PRS activity*

Purified enzyme was incubated with 1 mM Rib-5-P, 1 mM ATP, and 1 mM of different compounds. PRS activity was determined by measuring the PRPP formed.

Effector	PRS Activity	Effector	PRS Activity
	% control		*% control*
AMP	95	Guanosine	94
ADP	93	Uridine	106
ATP	100	Adenine	84
UMP	106	Xanthine	95
UDP	95	Cytidine	106
UTP	98	Cytosine	100
GMP	106	Orotate	100
GDP	93	Guanine	100
GTP	102	Trp	100
Adenosine	105	His	98

Of the PRS effectors, only PRPP, a product of the reaction, was able to inhibit the enzyme with a K_i of 30 μM (Fig. 4).

PRS Activity in Deproteinized Cytosol

The pH of the paraphysiological serum obtained by filtering the latex cytosol was 6.7. The Pi concentration was previously estimated to be around 10 mM (d'Auzac et al., 1989). PRS proved to be functional in this medium. Through its activity, it was possible to measure cytosolic Rib-5-P contents varying between 20 and 40 μM, depending on the serum studied. In this medium, optimum PRS activity was obtained at a pH of 6.5, as opposed to 7.25 in the buffer medium. Overall PRS activity was also lower in the paraphysiological medium (Fig. 2).

DISCUSSION

Like spinach PRS (Ashihara, 1977), the PRS detected in *H. brasiliensis* was cytosolic under the conditions used; no activity was observed in the sedimentable fraction of the latex containing vacuolysosomes and a few Frey-Wyssling particles (chromoplasts).

Latex PRS was purified 379 times to apparent homogeneity after SDS-PAGE with silver-staining (Fig. 1). To our knowledge, no highly purified PRS has ever been obtained

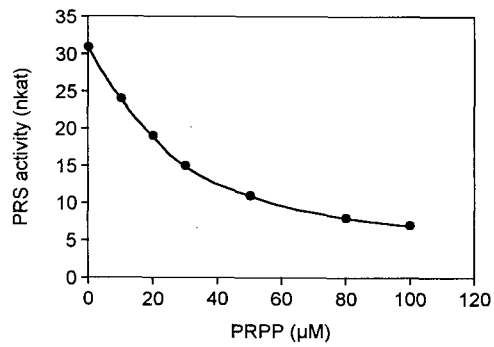

Figure 4. Effect of PRPP on PRS activity. The purified enzyme was incubated with 1 mM ATP, 1 mM Rib-5-P, and increasing quantities of PRPP. Activity was determined by monitoring the AMP formed.

from plants. Under denaturing conditions, the M_r of the PRS was 57,000 ± 3,000, as opposed to 200,000 ± 10,000 under nondenaturing conditions. PRS is probably in tetrameric form in latex cytosol. Studies on PRS of animal and microbial origin (Becker et al., 1979; Jensen, 1983; Kita et al., 1989) concluded that there were varying degrees of polymerization (2, 4, 5, 6, 8, 10, 12, 16, or 32 subunits). It would seem that the complexity of the quaternary structure is therefore a characteristic of PRS. This particularity may be related to the fine regulation mechanisms to which key enzymes of the metabolism are subjected, as is the case for most nucleotide synthesis enzymes (Smith, 1995).

Latex PRS was active only in the presence of Mg^{2+}; no other divalent cation such as Mn^{2+} or Co^{2+} could effectively replace it. These results correspond with those obtained by Ashihara (1977) concerning spinach leaf PRS, in which the substrate appeared to be the ATP-Mg^{2+} complex. Because the apparent K_m of the enzyme with respect to Mg^{2+} was 1.5 mM and its cytosolic content was 8 mM (Jacob et al., 1993), this cation should not be limiting for enzyme functioning.

As with the other PRS of plant origin, the *H. brasiliensis* enzyme functioned only in the presence of a reducing agent, thus suggesting the existence of an essential sulfhydryl group on the enzyme. This point was verified by Roberts et al. (1975), who demonstrated the existence of four Cys residues per catalytic subunit on *Salmonella typhimurium* PRS. The latex enzyme used only ATP as the diphosphate group donor, unlike the enzyme of spinach, which also uses ITP and, to a lesser degree, UTP and CTP (Ashihara, 1977).

Although it does not have any physiological significance, the reverse reaction of PRS was sought by incubation or direct kinetics (see "Materials and Methods"). Under our conditions, the latex enzyme functioned exclusively in the direction of PRPP production, which is not the case with the enzyme extracted from *S. typhimurium* (Switzer, 1971).

Two methods were used to estimate the affinity of latex PRS for Rib-5-P: product measurement after incubation or direct kinetics (see "Materials and Methods"). A K_m of 40 ± 2 μM was determined in both cases. Therefore, the enzyme demonstrated a strong affinity for Rib-5-P, like other enzymes of plant origin (K_m between 10 and 40 μM), apart from the enzyme of *Catharanthus roseus*, with a K_m of 250 μM (Hirose and Ashihara, 1983). For other biological materials, affinities from 30 to 290 μM were reported for mammals (Becker et al., 1979) and 60 μM was reported for *Escherichia coli* (Jensen, 1983).

PRS affinity for ATP estimated by enzymatic incubation was 200 ± 30 μM. This value was similar to that obtained for other plants, except spinach, which has an enzyme with a stronger affinity (K_m = 36 μM). This value is higher than the 14 μM obtained in animals (Becker et al., 1979) and lower than the 60 μM obtained in *E. coli* (Hove-Jensen et al., 1986).

The biochemical characteristics of *H. brasiliensis* latex PRS are therefore similar to those of mung bean (Ashihara and Komamine, 1974), spinach (Ashihara, 1977), and Jerusalem artichoke (Le Floc'h, 1984).

Furthermore, the affinity constants of the enzyme for its substrates are similar to the cytosol contents of these compounds. Nevertheless, since the Rib-5-P contents (between 20 and 40 μM) are less than or equal to the K_m of the enzyme for this compound (40 ± 2 μM), it is probable that the availability of the latter will be a factor in controlling PRS activity. This hypothesis was already put forward by Hirose and Ashihara (1984) during a study on *C. roseus* cells and by Boer and Sperling (1995) for human cell cultures.

In latex, Rib-5-P is synthesized from molecules produced by glycolysis and pentose phosphate pathways. Therefore, an increase in glycolytic activity in the laticifer cell following ethylene stimulation (d'Auzac et al., 1989) could also induce an increase in Rib-5-P content, thereby resulting in increased PRS activity. Likewise, the cytosolic ATP content, between 180 and 220 μM, is similar to the K_m of the enzyme for ATP (200 ± 30 μM). ATP availability may therefore also play a role in regulating enzymatic activity. After ethylene stimulation, the cytosolic ATP content can reach 1.2 to 1.8 mM (Amalou et al., 1992). This would seem to induce increased PRPP synthesis, thereby enabling increased synthesis of the purine and pyrimidine nucleotides required for latex regeneration (d'Auzac et al., 1989).

The optimum pH in buffer was 7.25; this value was comparable to that obtained for the enzyme extracted from spinach (Ashihara, 1977). In filtered cytosol, i.e. under conditions similar to those existing in situ, optimum activity occurred at a more acid pH of 6.5. In latex ethylene stimulation induces cytosol alkalinization (pH change = 0.2–0.4). This factor controls certain cytosolic enzymes such as invertase or PEP carboxylase (d'Auzac et al., 1989), but does not seem to be the case with PRS, since in this context, the drop in its activity did not exceed 20% (Fig. 2).

Among the PRS activity effectors, the PRPP produced by the reaction considerably inhibited it at a very low concentration (K_i = 30 μM). This inhibition is also seen in the enzyme of human cells (K_i = 50 μM) (Fox and Kelley, 1971). This negative retrocontrol of PRS activity by the reaction product could be a way of regulating in situ PRPP synthesis. In this way, its production would be directly linked to its use. Substantial inhibition of PRPP synthesis would explain why no trace of this compound was detected in latex cytosol (Gallois et al., 1996) and why very low PRPP contents were obtained in other species (Hirose and Ashihara, 1983).

The very low Mn^{2+} content of latex, less than 2 μM (J.L. Jacob and J.C. Prévôt, personal communication), and the PRS K_i of 20 μM did not point to its being a physiological inhibitor. Some bases, nucleosides, and nucleotides inhibited the PRS from mung bean (Ashihara and Komamine, 1974), but this did not seem to be the case with PRS (Table II).

The role of Pi in regulating PRS activity is the subject of much debate. For instance, in animal or microbe cells, several studies have indicated a change in PRS activity caused by this ion (Becker et al., 1979). In these cases Pi was essential for enzyme activity and it acted like an allosteric activator. In plants Le Floc'h (1984) also obtained substantial stimulation of PRS activity by Pi. However, Ashihara (1977), using spinach leaves, and Ukaji and Ashihara (1987), using PRS from *C. roseus* cells, found substantial inhibition of enzymatic activity by Pi. The results obtained on *H. brasiliensis* latex PRS would seem to explain these apparently contradictory results. In fact, Figure 3 clearly

indicates that the inhibiting or activating effect of Pi on PRS depends on the amount of Rib-5-P in the medium. With 20 μM Rib-5-P, Pi strongly inhibited enzymatic activity, whereas with 1 mM Rib-5-P, Pi acted as an allosteric PRS activator (Hill coefficient = 4.9). In latex cytosol the Pi content is around 10 mM (d'Auzac et al., 1989). Figure 3A demonstrates that PRS activity increases rapidly between 20 and 40 μM Rib-5-P, the amount measured in cytosol; therefore, under physiological conditions the Pi content (10 mM) makes it an allosteric regulator of PRS (Hill coefficient = 2.3). This characteristic of latex PRS is a major element in controlling its activity.

From the results presented in this study, it can be concluded that PRS is functional in latex. Thus, this enzyme could form the PRPP essential for salvage and de novo synthesis of adenine nucleotides, provided that the ATP and Rib-5-P content is adequate. Furthermore, the feedback caused by PRPP would control PRS activity as a function of its in situ concentration, and therefore of its use. Our further studies on this subject will consider the action of ethylene, which is used to stimulate latex production and laticifer metabolism. An effect of ethylene on PRS will be sought, since it may also be one of the factors likely to control adenylate synthesis, as in the case of Gln synthetase (Pujade-Renaud et al., 1994).

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