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Isopentenyl Pyrophosphate *cis*-1,4-Polyisoprenyl Transferase from Guayule (*Parthenium argentatum* Gray)[1]

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ABSTRACT

Electron micrographs of the mesophyll cells of guayule *Parthenium argentatum* Gray leaves show deposits of *cis*-polyisoprene (rubber) in the cytoplasm in the vicinity of mitochondria and chloroplasts and demonstrate that the rubber-synthesizing enzymes are present in guayule leaves. The terminal step in the synthesis of *cis*-polyisoprene from isopentenyl pyrophosphate (IPP) catalyzed by isopentenyl pyrophosphate *cis*-1,4-polyisoprenyl transferase has been demonstrated in crude leaf extracts by the enzymic incorporation of [^{14}C]isopentenyl pyrophosphate into the polymer and the recovery of [^{14}C]levulinic acid following ozonolysis. The rubber transferase activity in the crude extracts of guayule leaves was 5.8 nanomoles isopentenyl pyrophosphate incorporated per milligram protein per hour. This is the first description of the rubber transferase from a nonlaticiferous plant.

The specific activity (in units of nanomoles IPP converted per milligram protein per hour) of the partially purified enzyme following chromatography on diethylaminoethyl-cellulose columns was 41.7 units and contained 0.29 units of IPP isomerase activity and 0.08 units of farnesyl pyrophosphate synthetase activity. The rubber transferase requires reduced glutathione and Mg^{2+} for maximal activity. There was no incorporation of IPP into cis-1,4-polyisoprene in the absence of rubber particles as primer, and Langmuir isotherm plots showed that the specific activity of the enzyme was proportional to the concentration of the enzyme on the surface of the rubber particles. For a given rubber particle distribution, enzyme activity was proportional to time, IPP concentration, and rubber concentration. The addition of 0.4 millimolar dimethylallyl pyrophosphate to the rubber transferase reaction resulted in a 2-fold increase in the incorporation of IPP into rubber. A comparison was made of the relative activities of rubber transferase in different species of *Parthenium*, *Ficus*, and *Euphorbia*.

Recent investigations (8, 20, 22) on the chemical regulation of rubber biosynthesis in guayule (*Parthenium argentatum* Gray) suggest that rubber productivity can be improved by the use of substituted tertiary amines and could make this North American desert shrub an alternative to tropically grown *Hevea brasiliensis* as a source of natural rubber.

Application of the bioregulator DCPTA[3] to guayule plants to induce rubber formation leads to an increase in MVA kinase, IPP isomerase, geranyl transferase, and rubber transferase (8). Gene derepression is involved in the induction of terpenoids by bioregulators (13, 21).

The biochemistry of rubber formation and the properties of rubber transferase have been studied almost exclusively in preparations from the latex of *Hevea* (1–4, 15). Because of the recent interest in the regulation of rubber formation in guayule (6–9, 20, 22) it is important to describe a procedure for the isolation and assay of rubber transferase from guayule plants and to compare the biochemical properties of this enzyme to the transferase in the latex of *Hevea*.

MATERIALS AND METHODS

Chemicals. [1-^{14}C]IPP ammonium salt was purchased from Amersham Corporation. GPP and DMAPP were generous gifts of Dr. John W. Porter, Department of Physiological Chemistry, University of Wisconsin.

Plant. Guayule seed (*Parthenium argentatum* A. Gray) var 593 were soaked overnight in aerated H_2O. The seeds were germinated in small pots containing 2:1:1 (v/v/v) mixture of peat:perlite:vermiculite under fluorescent lamps at 25°C. Two weeks after germination, the seedlings were transplanted to large pots containing a 8:3:2 mixture of peat:perlite:vermiculite and grown in the greenhouse. The plants were kept well watered with distilled H_2O and fertilized every 10 d with Peters 20-20-20 H_2O soluble fertilizer and Peters S.T.E.M. Plants were sprayed with Isotox, Cygon, Plictran, and Diazanon for insect control.

Electron Microscopy. Tissue from fully expanded leaves was placed in a paraformaldehyde-glutaraldehyde fixative in a sodium cacodylate buffer pH 7.2 and sliced into 1-mm segments. After 2 h, the tissue was washed in four 15-min changes of 0.05 M cacodylate buffer pH 7.2. The tissue was then postfixed in 2% OsO_4 in a cacodylate buffer pH 7.2 for 60 min. The leaf segments were once again rinsed in four 15-min changes of 0.05 M cacodylate buffer pH 7.2. The tissue was dehydrated in ethyl alcohol followed by propylene oxide. The leaf segments were flat embedded in Epon-Araldite for 3 d in an oven at 70°C. These sections were cut with a diamond knife on an LKB Ultratone III, collected on 200-mesh copper grids, and stained in uranylacetate followed by lead citrate. Sections were photographed on a Phillips 400T transmission electron microscope.

Enzyme Extraction and Purification. Leaves from 2-year-old guayule plants were harvested, rinsed with deionized H_2O, and homogenized in a Sears blender at a liquify speed setting for 90 s in a 0.1 M Tris-HCl buffer pH 7.5 containing 0.1 mM GSH and 16% (w/v) of 100 to 200 mesh of Dowex-1-8 (chloride) resin. The Dowex-1 resin was prepared by washing with deionized H_2O until the effluent was clear, and equilibrating with Tris-HCl buffer pH 7.5 containing 0.1 mM GSH. The homogenate was filtered through four layers of cheesecloth and centrifuged at 27,000*g* for 30 min in a Sorvall refrigerated centrifuge. The

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[3] Abbreviations; DCPTA, 2-(3,4,-dichlorophenoxy)-triethylamine; MVA, mevalonic acid; DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; GPP, geranyl pyrophosphate; FPP, farnesyl pyrophosphate; *cis*-1,4-polyisoprenyl-PP, *cis*-1,4-polyisoprenyl pyrophosphate; DEAE-cellulose, diethylaminoethyl-cellulose; S.T.E.M., soluble trace element mix.

soluble supernatant was fractionated with solid $(NH_4)_2SO_4$ and the protein which precipitated between 40 and 60% of saturation was collected by centrifugation and dissolved in a minimal amount of 0.5 M Tris-HCl buffer pH 7.5 containing 0.1 mM GSH. The protein was desalted by passing it through a column (2.3 × 30 cm) of Sephadex G-25 (coarse, 100–300 μm particle size) which had been equilibrated with 0.5 M Tris-HCl buffer pH 7.5 containing 0.1 mM GSH. The protein eluted in the void volume was absorbed onto a DEAE-cellulose column (2.0 × 25 cm) which had been equilibrated with 0.1 M phosphate buffer pH 8.0 containing 0.001 M $MgSO_4$ and 0.1 mM GSH. The protein was eluted from the column by stepwise increases in phosphate buffer as shown in Figure 1. The individual tubes containing the protein eluted from the column with 0.3 M phosphate buffer were assayed for rubber transferase. The contents of the tubes were pooled and this was the source of the partially purified rubber transferase in the following experiments.

Protein Determination. Protein was determined by the method of Bradford (10). BSA was used as the standard protein for the calibration curve.

Rubber Transferase. The activity of rubber transferase in the guayule extracts was assayed by a modified procedure described by Archer and Cockbain (4). The reaction mixture contained in μmol: 50, Tris-HCl buffer pH 8.2; 3.0, GSH; 3.0 $MgSO_4$; 30 nmol [1-^{14}C]IPP containing 0.62 μCi of radioactivity; 25.0 to 28.0 mg of 'washed' rubber particles from the latex of *Ficus elastica*, and approximately 100 μg protein in a total volume of 0.5 ml. The reaction mixture was incubated at 30°C for 60 min and the reaction was stopped by the addition of 0.25 ml of 0.2 M EDTA. The contents of the reaction tubes were dried in a stream of air at 70°C. A solution of 5% acetic acid in ethanol (v/v) was added to thetubes which were gently rotated for 1 h in order to produce a rubber film on the side of the tubes. This film was washed several times with H_2O and saponified in 1 N KOH at 100°C for 60 min. After saponification, the rubber coagulate was washed several times with H_2O and repeatedly extracted with boiling ethanol over an 8-h period to ensure the removal of low mol wt isoprenoid contaminants. The rubber coagulate was air dried and dissolved in 2.0 ml of 1% TCA in toluene and an aliquot assayed for radioactivity in a scintillation spectrometer. The rate of incorporation of [1-^{14}C]IPP into rubber was calculated following correction for the residual rubber transferase activity in the washed rubber particles in the reaction tubes.

The Preparation of Washed Rubber Particles. The method of Archer and Cockbain used for *Hevea* (4) was followed to obtain washed rubber particles from the latex of *F. elastica*. *Ficus* plants were purchased from a local nursery and grown in the greenhouse. The latex was tapped from the main trunk following excision of a leaf and petiole. The latex was collected into clean tubes kept at 0°C and was centrifuged initially in a refrigerated Sorvall centrifuge at 2000*g* for 15 min to remove the sedimentable nonrubber particles (4). The supernatant fraction was diluted with 0.05 M Tris-HCl buffer pH 8.2 and centrifuged at 7000*g* for 60 min. The lower supernatant layer containing the fine rubber particles was collected with a Pasteur pipette and centrifuged at 127,000*g* for 60 min in a Beckman model L centrifuge. The suspensionof fine rubber particles separated into a top creamy layer, an upper clear serum protein layer, a middle creamy layer containing the rubber particles, and a coagulated rubber pellet. The middle layer was carefully removed with a Pasteur pipette and gently redispersed in 0.05 M Tris-HCl buffer pH 8.2. The process of centrifugation and redispersion of the rubber particles was repeated twice more.

The method of Archer *et al.* (2) was followed to determine the rubber content of washed rubber particles. A sample of washed rubber particles containing 30 to 50 mg of rubber was weighed into a tared microbeaker of 0.5 ml capacity and evaporated to dryness. One drop of benzene was added to the suspension to swell the rubber particles so that they would strongly cohere. After drying, the film of rubber was washed successively with 5% (v/v) acetic acid in ethanol and with H_2O. The film was then digested with boiling 1 N KOH for 30 min. The purified sample was washed with H_2O several times and then allowed to stand overnight to ethanol and finally dried to a constant weight of 80°C. To determine the effect of rubber content on the rubber transferase activity, samples of the top creamy layer were diluted with rubber-free serum obtained by high speed centrifugation of a mixture (1:1 w/w) of the top creamy layer with 0.1 M Tris-HCl buffer pH 8.2 so as to give a series of samples with rubber content varying from 0 to 40% as described by Archer *et al.* (2).

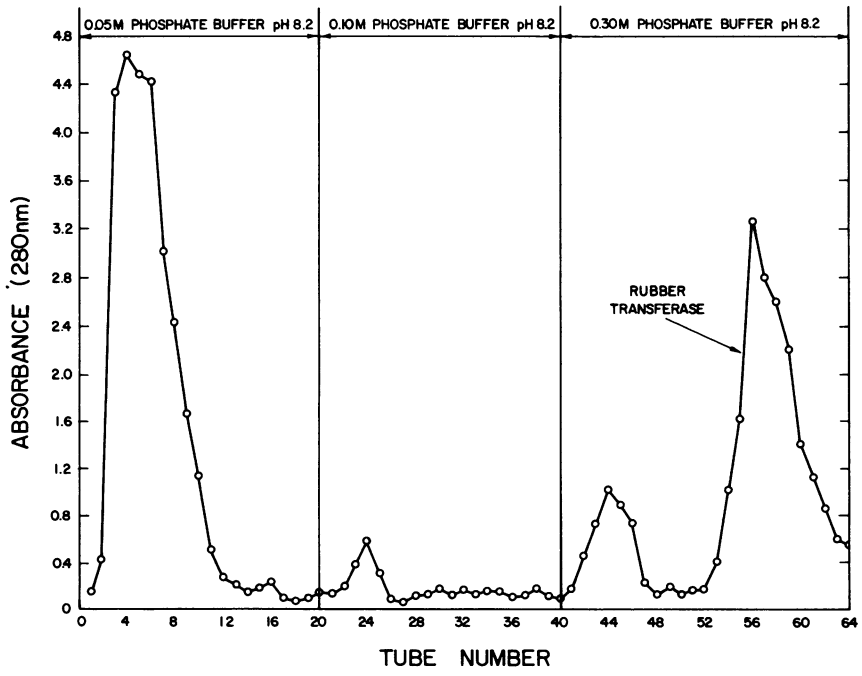


FIG. 1. The column chromatography of rubber transferase on DEAE-cellulose.

IPP Isomerase. IPP isomerase activity was determined by the procedure of Shah *et al.* (18). The reaction mixture contained in μmol: 50, Tris-HCl buffer, pH 7.9; 8, $MgCl_2$; 12, KF; 5, GSH; 27 nmol [1-^{14}C]IPP containing 0.56 μCi radioactivity; and 278 mg of washed rubber particles suspension from *Ficus* latex and/or 100 μg of guayule protein in a final volume of 1.0 ml. The reaction was incubated for 30 min at 37°C and terminated by the addition of 0.2 ml of 1 N HCl. The reaction mixture was reincubated at 37°C for 30 min and the resulting dimethylallyl alcohol and dimethylvinylcarbinol were extracted four times with 3.0 ml of ethyl ether. An aliquot of this ether extract was assayed for radioactivity.

FPP Synthetase. FPP synthetase was determined according to the procedures described by Benedict *et al.* (5) and Lynen *et al.* (14). The reaction mixture contained in μmol: 50, Tris-HCl buffer, pH 7.9; 1, $MnCl_2$; 10, KF; 19.2 nmol, GPP; 27 nmol of [1-^{14}C]IPP containing 0.56 μCi of radioactivity, and 278 mg of washed rubber particles suspension from *Ficus* latex and/or 100 μg of guayule protein in a final volume of 1.0 ml. The assay mixture was incubated for 15 min at 37°C and terminated by the addition of 0.2 ml of 1 N HCl. The reaction mixture was reincubated at 37°C for 15 min and the resulting terpenols were extracted with 10.0 ml of petroleum ether and an aliquot was assayed for radioactivity.

Ozonolysis of Radioactive Rubber. To identify the enzymic product in the rubber transferase reaction as *cis*-polyisoprene, the ^{14}C-labeled crude rubber was subjected to ozonolysis and the products chromatographed by the procedure described by Park and Bonner (16). The isolation of [^{14}C]levulinic acid was used as a criterion for the synthesis of ^{14}C-rubber from [1-^{14}C]IPP in the crude enzyme extracts of guayule leaves. The 1% TCA in toluene solution of rubber from the enzymic reaction was evaporated to dryness. The contents were dissolved in 5.0 ml of chloroform and an aliquot was assayed for radioactive crude rubber. Ozone was bubbled through this solution at 0°C. The outflowing gas was bubbled through a KI and boric acid trap and the completion of ozonization of rubber was indicated by the appearance of free I_2 in the trap. After ozonization, the chloroform was distilled off and the contents refluxed for 1 h in H_2O. After filtering the solution it was acidified with H_2SO_4 and a dilute solution of $K_2Cr_2O_7$ was added dropwise. After the appearance of a yellow color, more dichromate was added until no further change in color occurred after heating at 80°C for 10 min. Excess chromic acid was reduced with sodium sulfite. The acid solution was extracted with ether for 6 h. The extract was then chromatographed on Whatman No. 1 filter paper in a solvent of ethanol:ammonium hydroxide:H_2O (40:4:8 v/v/v). The radioactive levulinic acid at an R_F value of 0.65 was eluted from the paper and assayed for radioactivity. These results show that labeled levulinic acid obtained in these experiments arises from the degradation of rubber. The yield of [^{14}C]levulinic acid from the ^{14}C-crude rubber synthesized from [1-^{14}C]IPP in the enzyme extracts of guayule leaves corresponds to the yield of [^{14}C] levulinic acid obtained by Park and Bonner (16) following ozonolysis of the ^{14}C-crude rubber synthesized from [2-^{14}C]MVA in enzyme extracts of *Hevea*.

RESULTS

Rubber Particles in Guayule Leaves. Figures 2 through 5 are electron micrographs illustrating rubber deposits in the mesophyll cells of guayule leaves. The electron dense rubber particles in the parietal cytoplasm of the mesophyll in Figure 2 are similar to the electron dense rubber particles described in guayule stem cortical parenchyma cells (6, 7, 12) and in the latex vessels of *Hevea* (11). Figures 3 and 4 show rubber particles in the parietal cytoplasm in mesophyll cells adjacent to chloroplasts, mitochondria, and peroxisomes. Figure 5 shows several rubber particles adjacent to a chloroplast and mitochondria. The rubber particles are surrounded by a thin layer of cytoplasm and extend into the center of the cell together with strands and pieces of vacuolar membranes and cytoplasm. The electron micrographs show that rubber is synthesized in the cytoplasm of guayule leaf mesophyll cells and infer that these cells contain a functional rubber transferase enzyme.

Isolation and Partial Purification of the Rubber Transferase from Guayule Leaves. The activities of the rubber transferase resulting from the partial purification of the homogenates of guayule leaves is shown in Table I. The specific activity of the rubber transferase in the crude extracts is 5.8 nmol IPP incorporated mg^{-1} protein h^{-1} which is comparable to the specific activity of 3.6 nmol of IPP incorporated mg^{-1} protein h^{-1} for the transferase in freshly tapped latex of Hevea (1). The purification procedure resulted in a 7.2-fold increase in the transferase activity. The specific activity of the partially purified transferase was 41.7 nmol IPP incorporated mg^{-1} protein h^{-1} and the enzymic fraction eluted from the DEAE-cellulose columns contained 0.29 nmol IPP isomerized mg^{-1} protein h^{-1} of IPP isomerase activity and 0.08 nmol IPP incorporated mg^{-1} protein h^{-1} of FPP synthetase activity. In the reaction mixtures for the assay of the transferase activity from the guayule preparations there was 0.03 nmol IPP incorporated into *cis*-1,4-polyisoprene h^{-1} due to the presence of rubber transferase activity on the rubber particles from the latex of *Ficus elastica*.

Rubber Transferase Reaction. The data in Table II show that the incorporation of [^{14}C]IPP into rubber by partially purified rubber transferase requires GSH, Mg^{2+}, and rubber particles.

Protein. The rate of incorporation of IPP into *cis*-polyisoprene is not linear with increases in protein, but obeys the Langmuir isotherm equation of:

$$\frac{1}{R} = \frac{A}{P} + B$$

as established for the rubber transferase reaction from Hevea (1, 4). The data in Figure 6 show that a plot of R (specific activity^{-1}) *versus* p (protein concentration^{-1}) yields a straight line.

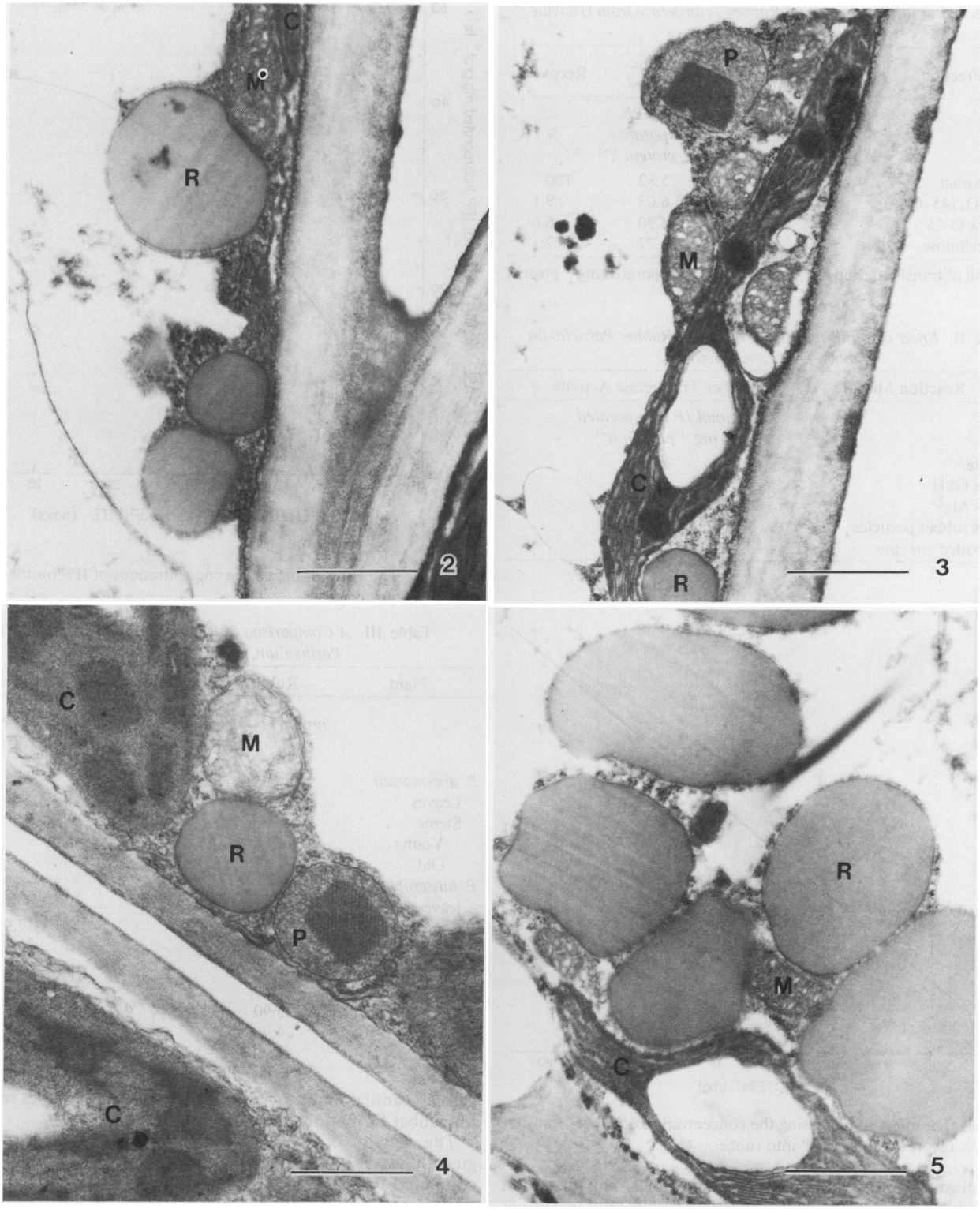
Time. Data from separate experiments have shown that the rubber transferase is linear with time up to 60 min of incubation at 30°C.

Washed Rubber Particles. The results from separate experiments show that the rubber transferase activity is linear from 0 to 20% rubber content of the washed rubber particles.

IPP Concentration. The data in Figure 7 show that the activity of the rubber transferase increases from 0 to 20 nmol IPP in the reaction mixture. The enzyme is saturated at 20 to 30 nmol IPP in the reaction mixture.

DMAPP Concentration. The results from separate experiments show that adding 200 nmol (0.4 mM) DMAPP to the reaction mixture results in a 100% stimulation of [^{14}C]IPP incorporation into rubber transferase.

Relative Rubber Transferase Activities. The data in Table III show the relative rubber transferase activity in two species of *Parthenium*, several species of *Ficus*, and one species of *Euphorbia*. The data (Table III) also show the relative abundance of the enzyme activity in the nonlaticiferous *Parthenium* species and the latex producing species of *Ficus* and *Euphorbia*. On a protein basis, the relative amount of rubber transferase activity in the extracts of guayule leaves is about equal to the activity in the stem extracts and is comparable to the enzyme activity in the latex of *F. elastica*. The relative transferase activity in the leaf extracts of *P. argentatum* exceeds the activity in the leaf extracts of *P. hysteropherus*. On a protein and per ml of latex basis, there is a variation in the relative rubber transferase activity in the species of *Ficus* and *Euphorbia*. The highest relative rubber transferase activity per ml of latex was found in *F. elastica*.



FIGS. 2–5. Cross-sections of leaf mesophyll cells from guayule plants. Figure 2, The mesophyll cell contains dense rubber particles (*R*) in the parietal cytoplasm in the vicinity of mitochondria (*M*). The magnification line represents 0.9 μm. Figures 3 and 4,The mesophyll cell contains rubber particles (*R*) in the parietal cytoplasm adjacent to chloroplasts (*C*), mitochondria (*M*), and peroxisomes (*P*). The magnification line represents 0.9 μm and 0.7 μm in Figures 3 and 4, repectively. Figure 5, The cell contains several rubber particles (*R*) adjacent to a chloroplast (*C*) and mitochondria (*M*). The magnification line represents 0.9 μm.

DISCUSSION

The electron micrographs of cross-sections of mesophyll cells of guayule leaves show the presence of electron dense rubber particles in the vicinity of chloroplasts, mitochondria, and peroxisomes in the parietal cytoplasm of the cell. A similar observation on the proximity of rubber particles to the chloroplasts were made in *Cryptostegia* leaves by Whittenberger and Kelner (19). In Figure 5 the rubber particles extend more into the center of the cell, away from the parietal cytoplasm, together with pieces

Table I. *Partial Purification of Rubber Transferase from Guayule Leaves*

Fraction	Protein	Total Activity	Specific Activity	Recovery
	mg	*units*[a]	*nmol IPP incorporated mg^{-1} protein h^{-1}*	*%*
Crude extract	3969.3	21512.5	5.82	100
$(NH_4)_2SO_4$ (45–60%)	321.7	1939.9	6.03	9.1
Sephadex G-25	169.8	1409.3	8.30	6.6
DEAE-cellulose	11.1	464.8	41.72	2.1

[a] 1 unit of transferase activity = 1 nmol IPP incorporated mg^{-1} protein h^{-1}.

Table II. *Effect of Omitting GSH, Mg^{2+}, and Rubber Particles on Rubber Transferase Activity*

Reaction Mixture	Rubber Transferase Activity
	nmol IP incorporated mg^{-1} protein h^{-1}
Complete	37.6
Minus GSH	0.9
Minus Mg^{2+}	0.3
Minus rubber particles	0.2
Plus boiled enzyme	1.2

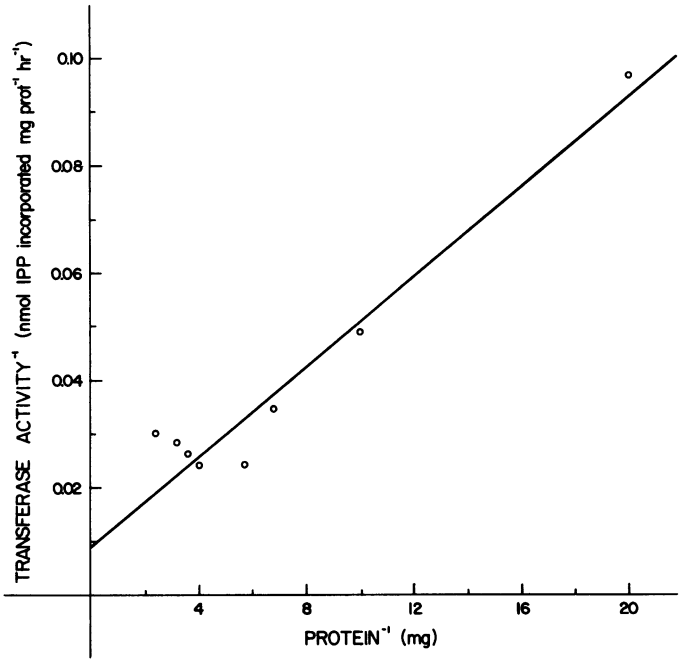


FIG. 6. The effect of increasing the concentration of rubber transferase on the incorporation of IPP into rubber.

of cytoplasm and strands of vacuolar membranes. The formation of the rubber particles in the leaf mesophyll cells may be similar to the formation of the rubber particles in the cytoplasm of stem cortical parenchyma cells. Gross *et al.* (12) have shown that developing cortical parenchyma cells are characterized by the presence of small vacuoles suspended in the cytoplasm along with rubber particles, chloroplasts, and mitochondria. As the cortical parenchyma cell matures to form a large central vacuole and parietal cytoplasm, the small vacuoles are ruptured releasing lytic enzymes which digest the central cytoplasm and vacuole membranes leaving a deposit of rubber particles in the center of the cell. The electron micrographs show that the formation of rubber particles in the mesophyll cells and cortical parenchyma

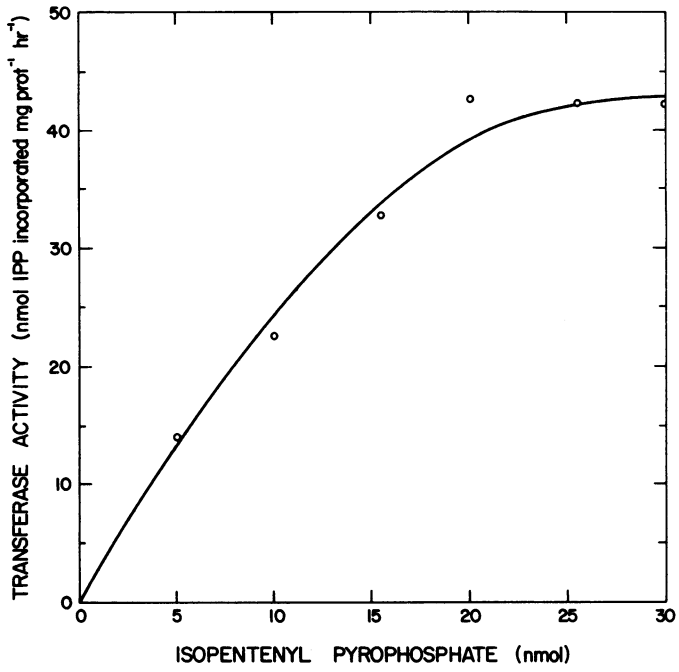


FIG. 7. The effect of increasing concentrations of IPP on the activity of rubber transferase.

Table III. *A Comparison of Rubber Transferase Activity in Parthenium, Ficus, and Euphorbia*

Plant	Rubber	Transferase	Activity
	nmol IPP mg^{-1} protein h^{-1}	*nmol IPP g^{-1} fresh wt h^{-1}*	*nmol IPP ml^{-1} latex h^{-1}*
P. argentatum			
Leaves	5.82	32.44	
Stems			
Young	4.68	14.04	
Old	4.52	17.22	
P. husterophorus			
leaves	1.28		
F. larata	2.40		7.9
F. elastica	4.36		30.52
F. benjamina	1.00		3.7
F. deltoidea	1.50		4.9
F. decora	5.90		26.21
F. indica	4.96		24.30
E. tirucalli	4.90		10.30

cells are similar and provide evidence that the enzymes required for rubber formation are present in these cells.

The activity of the rubber transferse in crude extracts of guayule leaves is 5.8 nmol IPP incorporated mg protein h^{-1} as compared to 3.6 nmol IPP incorporated mg^{-1} protein h^{-1} in freshly tapped latex from *Hevea* (1). The higher rate of transferase activity in guayule leaves may be due to the fact that all of the enzyme present in the laticifers of *Hevea* is not exuded in the tapped latex. The data do illustrate that rubber transferase can be isolated from a nonlaticiferous plant with a specific activity comparable to the enzyme isolated from *Hevea.* Previously the rubber particles isolated from the *Hevea* latex were used in the rubber transferase assay (1–4, 15). The results in this paper demonstrate that the rubber particles isolated from *F. elastica* latex can be used in the transferase assay. The purification procedures outlined in this paper result in a 7.2-fold purification of the enzyme from guayule leaves. Archer and Cockbain (4)

have reported that similar purification steps result in a 300-fold purification of the enzyme from *Hevea* latex. In the report, no purification data was given and it is not possible to compare the specific activities of the *Hevea* and guayule enzymes eluted from the DEAE-cellulose columns.

The requirements for GSH in the reaction mixture for rubber transferase activity is similar to the requirement of sulfhydryl reagents for other prenyl transferase reactions (17). The requirement for Mg^{2+} in the rubber transferase reaction is similar to the requirement of 2 atoms of Mg^{2+} per catalytic site of farnesyl pyrophosphate synthatase (17).

The rubber transferase reaction is not proportional to protein concentration, but langmuir isotherm double reciprocal plots of specific activity^{-1} *versus* protein^{-1} yields a straight line and obeys the relationship:

$$\frac{1}{R} = \frac{A}{P} + B$$

where R equals the rate of incorporation of [^{14}C]IPP into *cis*-polyisoprene, P is the concentration of the rubber transferse in the serum, A and B are constants (1, 4). The date (Fig. 6) shows the reaction is proportional to the enzyme on the surface of the rubber particle. In separate experiments it has been shown that the rubber transferase reaction increases with increases in the concentration of washed rubber particles. Presumably, increasing the concentration of the rubber particles increases the concentration of *cis*-1,4-polyisoprenyl-PP groups for reaction with IPP.

A typical enzyme substrate velocity curve results from increasing the concentration of IPP in the reaction mixture (Fig. 7). The enzyme is saturated at 20 to 30 nmol ($4–6 \times 10^{-5}$ M) IPP in the reaction mixture. In separate experiments we have shown that the addition of 0 to 0.4 mM DMAPP to the enzyme mixture stimulates the incorporation of [^{14}C]IPP into rubber. Adding 0.4 mM DMAPP increases the rate of reaction from 35 nmol IPP incorporated mg^{-1} protein h^{-1} to 78 nmol IPP incorporated mg^{-1} protein h^{-1}. Archer *et al.* (2) have shown two effects of DMAPP on the rubber transferase activity. If DMAPP is added to crude latex preparations it is inhibitory to the transferase reaction because it stimulates the formation of inhibitory amounts of FPP by the action of GPP and FPP synthetase present in the crude extracts. If this synthetase is removed from the extract, the addition of DMAPP is stimulatory to the rubber transferase reaction because it provides allylic-PP for the formation of new 'starter' rubber chains to react with the [^{14}C]IPP. The partially purified rubber transferase preparation from guayule leaves contained only 0.08 nmol IPP incorporated mg^{-1} protein h^{-1}; it is unlikely that significant amounts of FPP would be synthesized in the reaction mixtures. Therefore the addition of DMAPP would primarily stimulate the rubber transferase activity by providing new starter rubber molecules for the condensation with [^{14}C]IPP.

The data in Table III show the relative rubber transferase activities in several rubber producing plants. The term relative refers to the fact that the transferase activity is dependent on the *cis*-1,4-polyisoprenyl-PP groups on the rubber particles and the activity will vary for each preparation of rubber particles. The same preparation of washed rubber particles was used for the measurement of the enzyme activities in Table III. The data shows that the *P. argentatum* which is the species of *Parthenium* which produces the highest rubber content, has a higher rubber transferase activity than *P. hysterophorus*. The enzyme assay described in this paper can be used to assess the relative rubber transferase activity and thereby the rubber-producing potential of plants.

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