*Chem. Soc.*, 1027.
Parham, W. E., Gordon, I., and Swalen, J. D. (1952), *J. Amer. Chem. Soc. 74,* 1824.
Sinnott, M. L., and Souchard, I. J. L. (1973), *Biochem. J. 133,* 89.
Sinnott, M. L., and Viratelle, O. M. (1973), *Biochem. J. 133,* 81.
Stokes, T. M., and Wilson, I. B. (1972), *Biochemistry 11,* 1061.
Tenu, J. P., Viratelle, O. M., Garnier, J., and Yon, J. (1971), *Eur. J. Biochem. 20,* 363.
Viratelle, O., Tenu, J. P., Garnier, J., and Yon, J. (1969), *Biochem. Biophys. Res. Commun. 37,* 1036.
Wallenfels, K., and Kurz, G. (1962), *Biochem. Z. 335,* 559.
Wallenfels, K., and Malhotra, O. P. (1961), *Advan. Carbohyd. Chem. 16,* 239.
Wallenfels, K., and Weil, R. (1972), *Enzymes, 3rd Ed. 7,* 617.
Wengenmayer, F., Ueberschar, K., and Kurz, G. (1973), *Eur. J. Biochem. 40,* 49.
Woods, G. F., and Kramer, D. N. (1947), *J. Amer. Chem. Soc. 69,* 2246.

# Purification and Characterization of Two Forms of Geranyl Transferase from *Ricinus communis*†

Terrence R. Green and Charles A. West*

ABSTRACT: Two forms of geranyl transferase (I and II) were purified 970- and 645-fold, respectively, from cell-free extracts of germinating castor bean (*Ricinus communis* L.) seedlings. Both enzymes catalyze the specific formation of *trans,trans*-farnesyl pyrophosphate from isopentenyl pyrophosphate and geranyl pyrophosphate and can utilize either dimethylallyl pyrophosphate or geranyl pyrophosphate, but not farnesyl pyrophosphate, as an initial allyl pyrophosphate substrate. Transferases I and II show similar pH dependencies with an optimum of 6.8 and metal ion requirements with $Mg^{2+}$ (optimum 1–2 mM) in preference to $Mn^{2+}$. They are less sensitive to inhibition by common sulfhydryl reagents than the corresponding transferases from mammalian sources. At higher protein concentrations both transferases show nearly identical molecular weights of 72,500 ± 3000 on a calibrated G-100 Sephadex column. However, at lower enzyme concentrations, somewhat lower molecular weights of 56,000 ± 2000 and 60,000 ± 2000 were determined for transferases I and II, respectively, on the same column. The apparent $K_m$ values for substrates were also seen to vary as a function of protein concentrations; the $K_m$ values at protein concentrations of 42–56 μg $ml^{-1}$ for purified preparations of transferases I and II were found to be in the range of 30–50 μM for iPe-PP and geranyl-PP and 4–6 μM for $Me_2$allyl-PP. However, the $K_m$ values at 12–20 μg $ml^{-1}$ of the same preparations for both enzymes were found to be in the range of 2–3 μM for iPe-PP, 4–6 μM for geranyl-PP, and 1–2 μM for $Me_2$allyl-PP. These and other observations indicate that transferases I and II are capable of undergoing reversible protein–protein interactions which serve to modulate their catalytic properties.

The prenyl transferases as a group catalyze the sequential condensations of iPe-PP($C_5$)[1] with $Me_2$allyl-PP($C_5$) and longer prenyl pyrophosphates to produce pools of geranyl-PP($C_{10}$), farnesyl-PP($C_{15}$), $Ger_2$PP($C_{20}$), and in some cases cis isomers of these all-trans compounds. Longer chain polyprenyl pyrophosphates are also produced in some instances. Through sequences of enzyme-catalyzed cyclizations, alkylations, and other modifications, these prenyl pyrophosphates serve as precursors in the synthesis of a wide array of biologically significant molecules. Such isoprenoid end products include photosynthetic pigments (plastoquinones, chlorophylls, and carotenoids), mitochondrial electron transport components (ubiquinones and cytochrome oxidase), sterol and triterpene products implicated as membrane components and surfactants, growth and regulatory hormones (steroid hormones, ecdysone and juvenile hormone, gibberellins, and abscisic acid), phytoalexins (antibiotics) directed against common plant pathogens, polyprenols involved in the biosynthesis of cell walls and complex glycosides, and a wide array of other compounds, many of whose functions are obscure. The metabolic pathways leading to these diverse end products have been elucidated in many cases through isotopic tracer studies and in some instances studies of participating enzymes, but the nature of the regulation by modulation of enzyme activities and compartmentation of enzymes which must occur are still poorly understood. Hence the role of prenyl transferase in shunting the flow of carbon in this pathway between short and longer chain prenyl pyrophosphates would appear to be of considerable importance in determining the availability and distribution of starting material at branch points of the isoprenoid pathway from which such important end products arise. Our interest in prenyl transferases is in evaluating the regulatory role they may play in higher isoprenoid biosynthesis.

Lynen and coworkers (Lynen *et al.*, 1959; Grob *et al.*, 1961) first detected and partially purified prenyl transfer-

† From the Division of Biochemistry, Department of Chemistry, University of California, Los Angeles, California 90024. *Received March 19, 1974.* The investigation was supported by U. S. Public Health Service Grant GM 07065-13.

[1] Abbreviations used are: iPe-PP, isopentenyl pyrophosphate; $Me_2$-allyl-PP, dimethylallyl pyrophosphate; $Ger_2$PP, geranylgeranyl pyrophosphate; $PP_i$, inorganic pyrophosphate.

ase activity from crude yeast autolysates which catalyzed condensations between $Me_2$allyl-PP and iPe-PP to yield geranyl-PP, farnesyl-PP, and $Ger_2$PP. Since then prenyl transferases have been detected and partially characterized from a variety of plant, animal, and microbial sources (Kandutsch *et al.*, 1964; Nandi and Porter, 1964; Wells *et al.*, 1964; Benedict *et al.*, 1965; Dorsey *et al.*, 1966; Holloway and Popjak, 1967; Ogura *et al.*, 1972; Momose and Rudney, 1972). Prenyl transferases of longer chain specificity have also been partially purified and studied (Christenson *et al.*, 1969; Kurokawa *et al.*, 1971). Virtually nothing is known about the regulatory properties of these enzymes, nor of their molecular structure, in part due to poor yields from the purification procedures employed.

Because cell-free extracts of castor bean seedlings have the capacity to biosynthesize sesqui- and diterpenoid products (Robinson and West, 1970a,b; West *et al.*, 1968), such extracts are especially valuable in studying the nature of the prenyl transferase catalysts for the formation of prenyl pyrophosphates of different chain lengths. In this paper we wish to present a summary of our results on the purification and properties of two forms of geranyl transferase[2] from germinating castor beans (*Ricinus communis* L.). Evidence is presented which not only reveals the existence of two forms of geranyl transferase, but also suggests that each interconverts between species of different molecular weights, and that such changes, brought about through protein–protein interactions, alter the kinetic properties of each enzyme.

## Experimental Section

### *Materials*

(*R,S*)-[2-$^{14}$C]Mevalonate (*N,N'*-dibenzylethylenediamine salt), trilithium [1-$^{14}$C]iPe-PP, and Omnifluor were purchased from New England Nuclear Corp. Unlabeled (*R,S*)-mevalonic lactone and the disodium salt of ATP were obtained from Sigma Chemical Co. Bacterial alkaline phosphatase was purchased from Worthington Biochemical Co., Inc. Geranylgeraniol was a generous gift of Dr. Walter Adolf, Department of Chemistry, UCLA. $Me_2$allyl-PP and geranyl-PP were obtained through the generosity of Dr. George Popjak, Department of Biological Chemistry, UCLA.

### *Methods*

*Preparation of [4-$^{14}$C]iPe-PP.* [4-$^{14}$C]iPe-PP was biosynthesized from yeast autolysates from (*R,S*)-[2-$^{14}$C]mevalonate and ATP and purified on silica gel H preparative thin-layer chromatography (tlc) plates in 1-propanol–ammonia–water (6:3:1) as described previously (Green and Baisted, 1972). This substrate served as the major source of iPe-PP in later experiments due to depletion of our initial stock of [1-$^{14}$C]iPe-PP and an inability to acquire more from commercial sources.

*Prenyl Transferase Assay.* Transferase activity was measured by conventional methods using either [1-$^{14}$C]iPe-PP or [4-$^{14}$C]iPe-PP and geranyl-PP or $Me_2$allyl-PP as substrates (Benedict *et al.*, 1965; Holloway and Popjak, 1967; Ogura *et al.*, 1972). Routine assays were carried out under the following conditions: enzyme sample, 0.1 ml; $MgCl_2$, 0.8–2 μmol; Tris-maleate, 20 μmol (pH 6.8); [$^{14}$C]iPe-PP, 4.5–5.0 nmol; geranyl-PP, 41–45 nmol; and water to a total volume of 0.52 ml. All components except [$^{14}$C]iPe-PP were mixed together and equilibrated for 3 min at 30°. Assays were then initiated by the addition of [$^{14}$C]iPe-PP (10 μl) and stopped 5 min later by addition of 0.5 ml each of 6 N HCl and absolute ethanol. Allyl pyrophosphates were allowed to hydrolyze to their corresponding free alcohols by incubating the acidified assay mixture for an additional 15-min period at 30°; free alcohols were extracted in a total volume of 3–4 ml of petroleum ether, mixed with 10 ml of nonaqueous scintillation fluid (4 g of Omnifluor made up to 1 l. in toluene), and monitored for radioactivity on a Packard 2008 liquid scintillation spectrometer at a carbon-14 counting efficiency of 80%.

Since the action of iPe-PP isomerase (EC 5.3.3.2), phosphatase, and background levels of [$^{14}$C]iPe-PP in the ether extracts are all potential sources of error in estimating true transferase activity, blank assays were also carried out in the absence of geranyl-PP. Transferase activity was taken as the difference between experimental and blank assays. Interference in crude enzyme extracts by iPe-PP isomerase was eliminated by the addition of 1 mM iodoacetamide to the assay medium. One unit of enzyme activity was defined as the activity required to incorporate 1 nmol of [1-$^{14}$C]iPe-PP min$^{-1}$ into allyl pyrophosphate at 30°.

*Protein.* Protein concentration was estimated according to the method of Lowry *et al.* (1951) and from standard curves determined concurrently with bovine serum albumin.

*Preparation of Crude Enzyme Extract.* Castor beans were freed from their seed coats, sterilized in 0.01% sodium hypochlorite for 2 min, washed thoroughly in sterile water, and then germinated in sterile Petri dishes on moist cheesecloth in darkness at 30° for 60–72 hr. Whole seedlings were mixed in a Waring blender with extraction medium consisting of 50 mM citrate buffer (pH 4.8) made up in 10 mM potassium pyrophosphate ($PP_i$) and 1 mM 2-mercaptoethanol at a ratio of 150 ml/100 seedlings. Homogenization was carried out in the cold room at 4° at half-speed for 5 sec and for 25 sec at full speed. After pressing the crude slurry through six layers of cheesecloth, crude homogenate was centrifuged at 27,000*g* for 10 min. The floating lipid layer was removed by passing the cell-free supernatant through eight layers of cheesecloth. This filtrate, designated as the 27,000*g* supernatant, normally contained 7–10 mg of protein ml$^{-1}$.

*Ammonium Sulfate Fractionation.* In preliminary studies, the 27,000*g* supernatant was treated by the slow addition of crystalline ammonium sulfate to obtain the protein which precipitated between 0–30, 30–40, 40–50, 50–60, and 60–70% saturation with ammonium sulfate at 4°. Such fractionations are hereafter referred to as $F_0^{30}$, $F_{30}^{40}$, $F_{40}^{50}$, etc. Precipitated protein fractions were collected by centrifugation at 12,000*g* and redissolved in a minimal volume (20–30 ml) of 1 mM $PP_i$ (pH 7.2). More than 95% of total prenyl transferase activity in the 27,000*g* supernatant precipitated in the $F_{30}^{50}$ fraction (see Results). Consequently, the $F_{30}^{50}$ fraction was routinely prepared from the 27,000*g* supernatant, then dialyzed 24–48 hr in the cold room against several 1-l. changes of 1 mM $PP_i$ (pH 7.2). Dialyzed $F_{30}^{50}$ prenyl transferase was clarified by centrifugation and then used immediately as an enzyme source in subsequent

[2] Geranyl transferase (EC 2.5.1.1) catalyzes the condensation reaction between iPe-PP and geranyl-PP to yield farnesyl-PP and $PP_i$ as products (Popjak, 1969). The use of the term geranyl transferase is functional in the sense that it describes the chemical reaction, but no evidence has yet been presented allowing an unambiguous correlation of catalytic activity with a unique enzyme catalyzing only that portion of the condensation designated as geranyl transferase activity.

purification steps, or, alternately, stored in a freezer at −20° for future use. Protein in this fraction normally ranged between 17 and 25 mg $ml^{-1}$.

*G-100 Sephadex Chromatography.* Aliquots (15–30 ml) of dialyzed $F_{30}^{50}$ prenyl transferase were routinely sieved on a 5 × 100 cm G-100 Sephadex column (void volume ($V_0$), 550 ml) equilibrated in 1 mM $PP_i$ (pH 7.2) by upward flow using a peristaltic pump adjusted to a flow rate of approximately 50 ml $hr^{-1}$. Fractions (~10 ml) were collected on an automatic fraction collector and monitored for protein at 280 nm.

*G-25 Sephadex Chromatography.* Except for dialysis of the $F_{30}^{50}$ fraction, all enzyme preparations were desalted and reequilibrated to a desired pH and buffer system by gel chromatography on a 3.5 × 100 cm G-25 Sephadex column ($V_0$, 220 ml) previously equilibrated in the same buffer system. A flow rate of approximately 300 ml $hr^{-1}$ was maintained by downward gravity flow. Complete equilibration and recovery of enzyme activity was accomplished by pooling fractions containing protein which emerged at the void volume of the column.

*QAE Sephadex Chromatography.* Up to 1200 mg of protein solution (dialyzed $F_{30}^{50}$ or G-100 fractions containing prenyl transferase) in a volume of 40–50 ml was applied to a 1.0 × 15 cm column of QAE A-50 Sephadex preequilibrated to pH 5.0 in 5 mM citrate, 1 mM $PP_i$, and 1 mM 2-mercaptoethanol. The enzyme solution was equilibrated with the same buffer by gel chromatography before its application to the column. Unbound protein was washed from the QAE Sephadex column with an excess of starting buffer; this was followed by development with a linear gradient of 600 ml of 0–0.2 M KCl. The column was run at a constant flow rate of about 12 ml $hr^{-1}$ with the aid of a peristaltic pump. Fractions of 3–5 ml were collected on an automatic fraction collector and monitored for protein in the usual manner at 280 nm. Eluted enzyme lots were recycled by (i) pooling active fractions and concentrating on an Amicon XM-50 Diaflow Ultrafilter in the Model 202 cell to a volume of 15–25 ml, (ii) passing the concentrated sample again through G-25 Sephadex equilibrated in starting buffer for QAE Sephadex chromatography, and (iii) fractionating the sample once again as described above.

*Disc Gel Electrophoresis.* Enzyme fractions were examined for homogeneity by disc gel electrophoresis in 7% acrylamide gels at pH 7.2 in barbital buffer according to the method of Davis (1964). Inorganic pyrophosphate (1 mM) was included in all buffers used in preparing and running such gels as a stabilizing agent against the loss of enzyme activity during electrophoresis. Enzyme aliquots were layered above the "spacer gels" in 20% glycerol, then subjected to electrophoresis at 5 mA/tube at 4° for a period of 1.5 hr. Protein bands were detected by staining gels in Coomassie Blue according to the method of Vesterberg (1971). Unstained gels were monitored for enzyme activity by dividing each gel into 20 equal segments (approximately 2.5-mm segments) and then assaying each segment for prenyl transferase activity in the usual manner. Enzyme activity in the segments across the gel were compared with protein bands in the stained gels.

*Identification of End Products.* [$^{14}$C]-labeled allyl pyrophosphates, derived from the enzyme-mediated condensation of [$^{14}$C]iPe-PP with allyl pyrophosphate acceptor (*i.e.*, initially geranyl-PP), were first cleaved with alkaline phosphatase to their corresponding free alcohols (Popjak, 1969). An aliquot of the petroleum ether extract of the free alcohols was mixed with authentic samples of geraniol, farnesol, and geranylgeraniol, and the mixture was chromatographed by reverse phase tlc on silica gel G impregnated with 10% DC-200 silicone oil (Dow-Corning) in methanol–water (4:1) according to the method of Oster and West (1968). Tlc plates were scanned for radioactivity on a Packard Strip Scanner, Model 7201, to correlate the $R_F$ value of labeled alcohol with reference samples visualized by exposure to $I_2$ vapor.

End products were further identified by gas chromatography on a Varian Model 1200 equipped with a flame ionization detector. Commercial farnesol (approximately 2 mg), composed of a mixture of cis,cis, cis,trans, trans,cis, and trans,trans isomers of farnesol, was cochromatographed with an aliquot of the petroleum ether extract on a 0.25 in. × 9 ft copper column of 5% butanediol succinate polyester on Anakrom SD (80–90 mesh) at 155°. Nitrogen served as the carrier gas and was adjusted to a flow rate of 50 ml $min^{-1}$. A splitter attached at the base of the detector allowed for the diversion of effluent gas from the detector at a ratio of 7:1 in favor of the effluent port. Fractions of the gas effluent were trapped in glass "Z" tubes immersed in a Dry Ice–acetone bath and monitored for radioactivity by thoroughly rinsing each tube with benzene and counting the benzene washings in scintillation fluid as previously described. The isomeric form of farnesyl-PP thus produced by prenyl transferase was deduced by noting the retention time and mass peak response of each isomer and corresponding level of radioactivity trapped within such regions.

## Results

*Stabilization of Prenyl Transferase in Crude Cell-Free Extract.* Prenyl transferase enzyme activity was initially extracted from whole seedlings as described in Methods, but in 50 mM Tris-HCl buffer (pH 7.4) made up in 5 mM $MgSO_4$, 5 mM $MnSO_4$, 1 mM dithiothreitol, and 25% (w/v) Polyclar AT. Under these conditions prenyl transferase activity precipitated in the $F_{40}^{60}$ fraction, but was unstable. More than 70% of initial $F_{40}^{60}$ enzyme activity was lost upon dialysis overnight at 4° against Tris-HCl buffer, and approximately 60% of the initial activity was lost in 1 hr at 30°. Various combinations of salts, chelators, glycerol (up to 10%), and thiol protectants did not improve the stability of crude prenyl transferase in this buffer system.

On the other hand, initial activity in the $F_{40}^{60}$ fraction was completely stable to dialysis against potassium phosphate buffer. In 5 mM phosphate buffer (pH 7.2) less than 3% of the initial activity was lost in storing the enzyme for 1 hr at 30°. Pyrophosphate proved to be even more effective in stabilizing crude prenyl transferase activity, especially in buffers of lower pH. At pH 5.0 in 5 mM citrate buffer made up in 1 mM $PP_i$, no appreciable loss in enzyme activity was detected over a 7-day period while storing the enzyme solution in the cold room at 4°. At pH 4.2 under the same conditions the enzyme lost approximately 30% activity over a period of 3 days. Prenyl transferase activity was completely lost in a matter of hours in the absence of $PP_i$ at these lower pH values.

Polyclar AT was initially included in the homogenization medium because of its ability to remove inhibitory phenols (Loomis and Battaile, 1966). At pH's around 5 the inhibitory interactions of phenolic substances and proteins are considerably reduced, thus eliminating the necessity for Polyclar AT. By omitting Polyclar AT from the homogenizing medium, approximately twice as much tissue can be pro-

TABLE I: Extraction and Recovery of Prenyl Transferase Activity from Whole Seeds in Two Homogenization Media.

| | Stage in Purification | Protein (mg) | Enzyme Units | Units/mg of Protein | Purification | |
|---|---|---|---|---|---|---|
| | | | | | Fold | Yield (%) |
| Experiment I[a] | 27,000*g* supernatant | 1150 | 71.6 | 0.06 | 1 | 100 |
| | $F_{40}^{60}$ fraction[d] | 253 | 7.2 | 0.03 | 0.5 | 10 |
| | Dialyzed $F_{40}^{60}$, 24 hr[e] | 239 | 28 | 0.12 | 2 | 39 |
| | G-100 Sephadex | 53 | 72 | 1.36 | 23 | 100 |
| Experiment II[b] | 27,000*g* supernatant | 684 | | | | |
| | $F_{30}^{50}$ fraction[d] | 226 | 38.5 | 0.17 | 2.6 | 55[c] |
| | Dialyzed $F_{30}^{50}$ | | | | | |
| | 24 hr[e] | 212 | 48 | 0.23 | 3.5 | 67 |
| | 48 hr[f] | 200 | 71.6 | 0.36 | 6.0 | 100 |

[a] Extraction medium–50 mM phosphate–1 mM 2-mercaptoethanol (pH 7.2). Transferase activity was extracted from 200 seeds and assayed as described in Methods. [b] Extraction medium–50 mM citrate–1 mM $PP_i$–1 mM 2-mercaptoethanol (pH 4.8). [c] Based upon initial transferase activity in the 27,000*g* supernatant of experiment I. [d] Dissolved in approximately 40 ml of 1 mM $PP_i$ buffer (pH 7.2). Dialyzed overnight at 4° against 1 l. of 1 mM $PP_i$ buffer (pH 7.2). [f] Extensive dialysis against several successive 1-l. volumes of 1 mM $PP_i$ buffer (pH 7.2).

TABLE II: Purification of Geranyl Transferase from *Ricinus communis L.*

| Stage in Purification | Protein (mg) | Enzyme Units | Units/mg of Protein | Purification | |
|---|---|---|---|---|---|
| | | | | Fold | Yield |
| 1. 27,000*g* supernatant | 684 (1150)[a] | (71.6)[a] | (0.06)[a] | 1 | (100)[a] |
| 2. $F_{30}^{50}$ fraction[b] | 226 | 38.5 | 0.17 | 2.7 | 55 |
| 3. Dialyzed[c] $F_{30}^{50}$ | 200 | 71.6 | 0.36 | 5.8 | 100 |
| 4. QAE-Sephadex[d] | | | | | |
| Transferase I | 0.5 | 28.6 | 60 | 970 | 40 |
| Transferase II | 0.5 | 21.5 | 40 | 645 | 30 |

[a] Data from an equivalent set of seeds homogenized and extracted in 50 mM phosphate buffer (pH 7.2) (*cf.* Experiment I of Table I). [b] Dissolved in approximately 40 ml of 1 mM $PP_i$ buffer (pH 7.2). [c] Dialyzed against 1 mM $PP_i$ (pH 7.2.). [d] Summary of twice recycled transferases I and II on QAE-Sephadex (see text).

cessed in a single homogenization. For this reason and because of the stabilizing effect of $PP_i$, homogenizations of whole seedlings were routinely carried out in citrate–$PP_i$ buffer at pH 4.8. The yield of enzyme per unit fresh weight of tissue was equivalent to that obtained from extractions in the presence of phosphate or Tris-HCl buffers at a higher pH near 7 (Table I).

*Ammonium Sulfate Fractionation.* All of the activity initially observed in the 27,000*g* supernatant precipitated in the $F_{30}^{50}$ fraction. Although the apparent initial recovery of enzyme activity in the $F_{30}^{50}$ pellet was low, complete recovery of activity invariably occurred after dialyzing the $F_{30}^{50}$ fraction overnight against 1 mM $PP_i$ (pH 7.2) (Table II). Moreover, recovery of enzyme activity in the $F_{30}^{50}$ pellet prior to dialysis was proportional to the volume in which the pellet was dissolved. With progressively larger volumes of buffer, calculated yields approached 100% of the activity initially observed in the cell-free supernatant extract. Yields were consistently 100% after dialysis.

*G-100 Sephadex Chromatography.* Crude prenyl transferase, applied to the calibrated column at a protein concentration of approximately 25 mg $ml^{-1}$, eluted from the column as a single symmetrical peak with a $V_e/V_0$ ratio of 1.32 (Figure 1). Prenyl transferase from the G-100 Sephadex column had a specific activity of approximately 1 unit mg of $protein^{-1}$ (15–20-fold purification) and was virtually free of iPe-PP isomerase and phosphatase activity; 1% of the total activity in this peak consisted of iPe-PP isomerase (as measured in the absence of iodoacetamide). The molecular weight of crude prenyl transferase measured under the above conditions was estimated to be 72,500 ± 3000 (Figure 2).

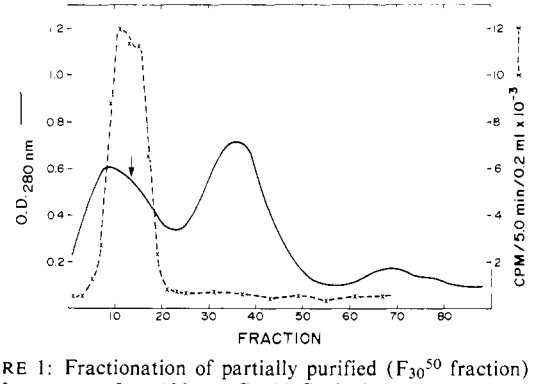


FIGURE 1: Fractionation of partially purified ($F_{30}^{50}$ fraction) prenyl transferase on a 5 × 100 cm G-100 Sephadex column ($V_0$, 550 ml) equilibrated in 1 mM $PP_i$ buffer (pH 7.2); 11-ml fractions were collected and monitored for protein (—) and prenyl transferase activity (-x-x-) beginning approximately 450 ml after application of partially purified enzyme as described in Methods.

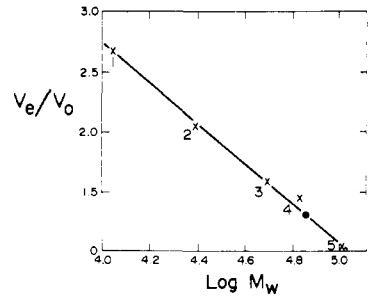


FIGURE 2: Molecular weight estimation of $F_{30}^{50}$ prenyl transferase on G-100 Sephadex. A 0.9 × 100 cm column was calibrated with (1) cytochrome *c* (mol wt 11,000); (2) monomeric and (3) dimeric α-chymotrypsin (mol wt 24,500 and 49,000); (4) bovine serum albumin (mol wt 66,500); and (5) glucose-6-phosphate dehydrogenase (mol wt 102,000) as described in Methods. Prenyl transferase activity (●) eluted as a single peak with a $V_e/V_0$ ratio of 1.32 corresponding to mol wt 72,500 ± 3000.

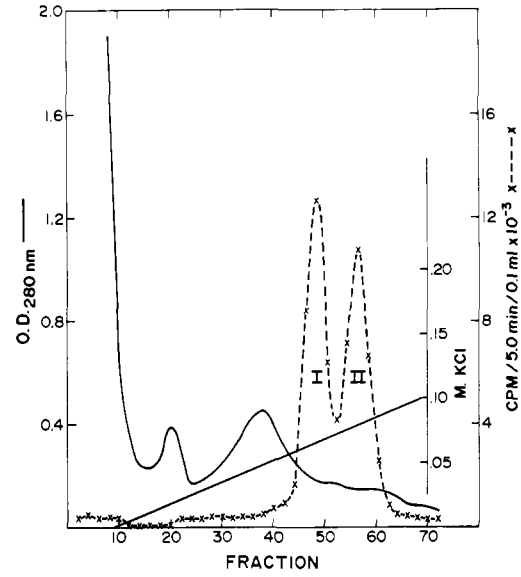


FIGURE 3: QAE A-50 Sephadex chromatography of partially purified ($F_{30}^{50}$ fraction) prenyl transferase on a 600-ml 0–0.2 M KCl linear gradient in 5 mM citrate buffer (pH 5.0) made up in 1 mM $PP_i$ and 1 mM 2-mercaptoethanol. Approximately 680 mg of $F_{30}^{50}$ prenyl transferase in starting buffer (pH 5.0) were applied to the column. Unbound protein emerged from the column between fractions 1–10. The linear gradient was begun at fraction 9. Approximately 4-ml fractions were collected and assayed for protein (—) and prenyl transferase activity (-x-x-) as described in Methods. Prenyl transferase I (peak I) and II (peak II) emerged from the column under the above conditions at 0.065 and 0.085 M KCl, respectively.

Data from a variety of ion exchange, disc gel electrophoresis, and kinetic studies, however, led us to consider the possible existence of a second molecular weight species not normally present at such high protein concentrations (see Disc Gel Electrophoresis and Kinetic Characterization). In particular, kinetic studies on prenyl transferases I and II, which were so designated after their resolution from one another on ion-exchange chromatography, implicated the existence of protein–protein interactions as a factor in modifying the properties of this enzyme.

Since these studies led us to anticipate the possible existence of a lower molecular weight species at low protein concentrations where protein–protein interactions would be less prevalent, the molecular weight of prenyl transferase was reinvestigated at such low protein concentrations. At 20 μg $ml^{-1}$, purified aliquots of prenyl transferases I and II emerged from the G-100 Sephadex column with $V_e/V_0$ ratios characteristic of proteins of lower molecular weights on the order of 56,000 ± 2000 and 60,000 ± 2800, respectively. This same protein concentration was within the range wherein kinetic measurements implicated the presence of a lower molecular weight species.

*Ion-Exchange Chromatography.* Preliminary studies of the binding of prenyl transferase to cationic resins (carboxymethyl- and phosphocellulose) suggested an isoelectric point within the range of pH 4.5–4.7. Above pH 4.7 all enzyme activity eluted from the resin with unbound protein, while enzyme remained firmly bound to resins equilibrated to pH 4.5 and lower. Cation exchange chromatography was not used in purifying enzyme, however, due to significant losses of enzyme activity upon gradient elution of bound enzyme.

Among anion exchange resins, QAE A-50 Sephadex was chosen because of its high binding capacity over a broad pH range above the apparent isoelectric point of this enzyme. The binding properties of prenyl transferase to this resin as a function of pH supported the assignment of an isoelectric point between pH 4.5 and 4.7 as had been deduced from the results obtained by cation exchange chromatography. At pH 5.0 in citrate–$PP_i$ buffer, enzyme activity was eluted from QAE-Sephadex by a linear gradient of 0–0.2 M KCl in two closely spaced peaks (Figure 3). These two forms of prenyl transferase, hereafter designated as transferases I and II, respectively, were fully resolved from one another by recycling each peak twice more through the QAE-Sephadex column. Prenyl transferases I and II were homogeneous in charge as indicated by their emergence from QAE-Sephadex as single symmetrical peaks in terms of enzyme activity, but were impure in terms of total protein as indicated by the lack of coincidence between enzyme activity and uv absorbance profiles. Despite repeated recycling of each enzyme on QAE-Sephadex, further purification was not achieved. Thus, under the conditions of our purification scheme, it would appear that a major protein contaminant (or contaminants) still copurifies with prenyl transferases I and II.

Purified transferases I and II showed no evidence of interconversion between one form and the other upon recycling on QAE-Sephadex, and were completely free of iPePP isomerase and phosphatase activities. First cycle transferases I and II were of nearly identical specific activity (20–25 units $mg^{-1}$, 340–400-fold purification) and were recovered in 100% yield with respect to total initial activity in the crude 27,000*g* supernatant. Recycled transferases I and II were recovered in a total yield of 70% (40% transferase I, 30% transferase II) with specific activities of 60 units $mg^{-1}$ (970-fold purification) and 40 units mg of $protein^{-1}$ (645-fold purification), respectively.

A summary of the enzyme purification scheme is shown in Table II. Note that the G-100 Sephadex step was excluded from the purification scheme, since this step did not significantly improve upon the direct fractionation of the $F_{30}^{50}$ enzyme lot (equilibrated to pH 5.0) on QAE-Sephadex.

*Disc Gel Electrophoresis.* Disc gel analysis of prenyl transferases I and II revealed, firstly, the presence of a major activity band unique to each enzyme form and, secondly, a small but perceptible band of enzyme activity running slightly ahead of each enzyme's major activity peak (Figure 4). Also evident in both gels was the presence of several contaminating protein bands as was to be expected on the basis of the ion-exchange results. Despite several at-

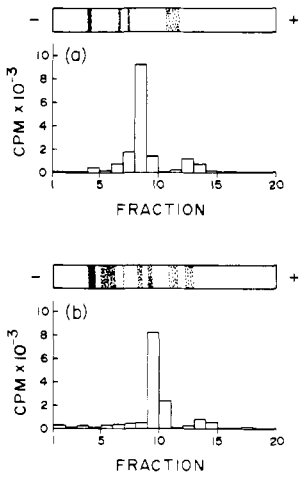


FIGURE 4: Disc gel electrophoresis of (a) geranyl transferase I and (b) geranyl transferase II at pH 7.2 in 7% polyacrylamide. Protein bands were stained and enzyme activity was assayed as described in Methods.

tempts, we were unable to convincingly correlate any visible protein bands with peak enzyme activity.

*pH Optimum.* The pH optima of prenyl transferases I and II were determined by assaying aliquots of each enzyme as a function of pH in the usual manner once the two enzymes had been fully resolved from one another by recycling on QAE-Sephadex. Both enzymes exhibited a nearly identical pH optimum of 6.8 in Tris-maleate (Figure 5).

*Metal Ion Optimum and Specificity.* Prenyl transferases I and II were both found to be highly dependent upon divalent metal ion for optimum activity (Figure 6). The two enzymes exhibited nearly identical activation profiles as a function of $Mg^{2+}$ concentration with peak activity between 1.6 and 2 mM. Enzyme activity was also partially restored by the addition of $Mn^{2+}$ to the assay medium at optimal concentrations of 0.25 and 0.50 mM for prenyl transferases I and II, respectively (Figure 6). Both enzymes were markedly inhibited by $Mn^{2+}$ at concentrations above the optimum, especially prenyl transferase I.

*End Product Specificity.* Scans of reverse phase tlc plates for radioactivity in the alcohols released from the products by phosphatase digestion revealed a single peak of radioactivity which cochromatographed with authentic carrier farnesol. Moreover, gas chromatography of labeled product revealed a predominance of label within the *trans,trans*-farnesol fraction (Figure 7) regardless of the enzyme source (*i.e.*, prenyl transferase I or II). Hence it appears that both enzymes have an absolute end product specificity for producing *trans,trans*-farnesyl-PP. Addition of $Mn^{2+}$ to the assay medium, either alone or in the presence of $Mg^{2+}$, did not alter the end product specificity of either enzyme. Based upon their product specificities, prenyl transferases I and II appear to be isoenzymic forms of geranyl transferase (EC 2.5.1.1) and will hereafter be designated as geranyl transferases I and II, respectively.

*Effect of Sulfhydryl Inhibitors.* The effects of common sulfhydryl inhibitors on a mixture of partially purified (about 400-fold) geranyl transferase I and II and on crude (27,000*g* supernatant) iPe-PP isomerase were studied by preincubating enzyme solutions for 5 min in the presence of such inhibitors prior to the addition of substrates. Geranyl transferase activity was markedly resistant to inhibition. Neither iodoacetamide nor *N*-ethylmaleimide significantly inhibited the enzyme at concentrations less than 5 mM. At a concentration of 8 mM, enzyme activity was inhibited approximately 33% in the presence of iodoacetamide and 54% in the presence of *N*-ethylmaleimide. In the presence of 1 mM *p*-hydroxymercuribenzoate, geranyl transferase activity decreased by only 25% and concentrations of *p*-hydroxymercuribenzoate in excess of 3 mM were required before complete inhibition occurred.

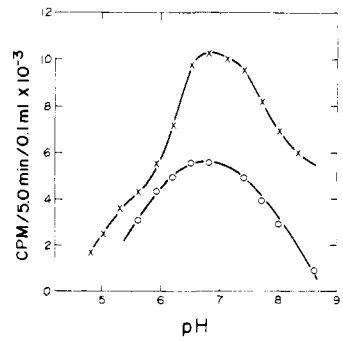


FIGURE 5: pH optima of purified prenyl transferases I (x) and II (O). Aliquots of each enzyme (0.1 ml), previously equilibrated to pH 6.8 in 5 mM phosphate buffer, were mixed with 0.4 ml of 50 mM Tris-maleate buffer, adjusted to the pH values shown, in 2 mM $MgCl_2$, and assayed for prenyl transferase activity as described in Methods.

On the other hand, iPe-PP isomerase was strongly inhibited by all such sulfhydryl inhibitors. Less than 3% of initial isomerase activity remained after preincubation of the enzyme in 0.5 mM iodoacetamide. The relative resistance of geranyl transferase to sulfhydryl inhibition, as opposed to iPe-PP isomerase, provided the means for assaying geranyl transferase in crude enzyme extracts. When interference by the latter enzyme proved to be a problem in such extracts, iodoacetamide (1 mM) was included in the assay mixture, thereby eliminating further interference from iPe-PP isomerase while assaying for geranyl transferase activity. The latter result provided a method for routinely assaying isomerase activity by omitting geranyl-PP from the assay medium and carrying out assays with [$^{14}$C]iPe-PP in the presence and absence of iodoacetamide as described by Popjak (1969).

*Kinetic Characterization.* Kinetic studies of enzyme ac-

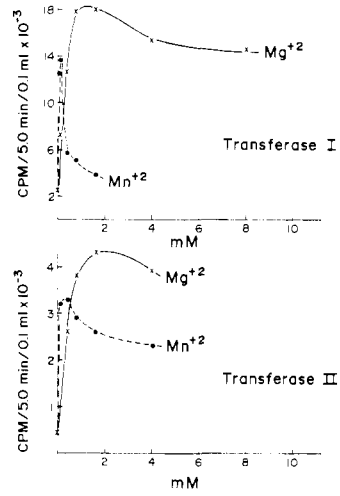


FIGURE 6: Optima for metal ion activation of purified prenyl transferases I and II at pH 6.8 in 40 mM Tris-maleate buffer. Aliquots of each enzyme (0.1 ml), previously equilibrated to pH 6.8 in 5 mM phosphate buffer, were mixed with 0.4 ml of 50 mM Tris-maleate buffer (pH 6.8) made up in either $MgCl_2$ or $MnCl_2$, such that the final concentration of $Mg^{2+}$ (x) or $Mn^{2+}$ (●) varied as shown. Prenyl transferase activity was assayed as described in Methods.

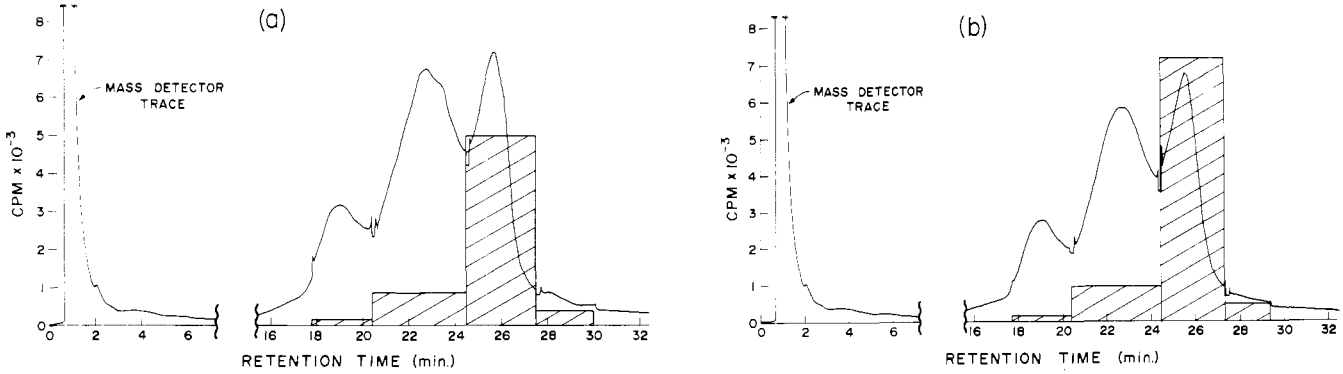


FIGURE 7: Identification of $^{14}C$-labeled end products. Petroleum ether extracts of $^{14}C$-labeled products of geranyl transferase I (a) and geranyl transferase II (b) were derived from alkaline phosphatase digests of each reaction mixture and subjected to gas chromatography on 5% butanediol succinate polyester on Anakrom SD (80/90 mesh) at 155° as described in Methods. Cis,cis, cis,trans/trans,cis, and trans,trans isomers of farnesol were identified by the order in which they emerged from the column (Bates *et al.*, 1963). Major mass peak tracings at 19.0, 22.5–23.5, and 25.7 min correspond to cis,cis, cis,trans/trans,cis (not resolved), and trans,trans isomers of farnesol. Interruptions in the mass detector response were caused while changing the $^{14}C$ trap in collecting samples from the column effluent. Hatched areas shown in the histogram beneath each mass detector tracing represent total cpm trapped within each fraction.

tivity as a function of substrate levels were carried out separately on purified preparations of geranyl transferases I and II. Immediately before attempting to characterize each enzyme in terms of substrate $K_m$ values and apparent $V_{max}$, each lot of enzyme was preequilibrated to pH 6.8 in 5 mM phosphate buffer by gel chromatography. The final concentration of orthophosphate in the assay mixture was held constant at 1 mM.

The results of initial assays of enzyme activity on partially purified preparations suggested that the $K_m$ (and possibly the $V_{max}$) values of geranyl transferases I and II vary as a function of protein concentration. In one experiment, for example, dialyzed $F_{30}^{50}$ fraction was concentrated on an XM-50 Amicon micropore filter and aliquots were removed at various stages of concentration for determination of geranyl transferase activity in the usual manner. As can be seen in Figure 8, the specific activity of this enzyme fraction decreased dramatically with increasing protein concentration in spite of an excess of available substrate at all protein concentrations tested. This decrease in specific activity was fully reversible. When aliquots of the concentrated $F_{30}^{50}$ fraction were serially diluted and reassayed, the specific activity increased accordingly. Deviations in linear rates of product formation were evident at protein concentrations in excess of 500 $\mu$g ml$^{-1}$. In a second set of experiments on much purer preparations of geranyl transferase I (about 400-fold purified), with substrate levels fixed at 10 and 90 $\mu$M for iPe-PP and geranyl-PP, respectively, the rate of product formation as a function of (i) incubation time and (ii) protein concentration was investigated. Figure 9a shows that under the usual conditions of enzyme assay, the rate of product formation at a protein concentration of 21 $\mu$g ml$^{-1}$ remained linear over a period of 16 min (up to 17% substrate consumption). On the other hand, when the assay period was held constant at 5 min and the protein concentration varied, the rate of product formation began to deviate noticeably from linearity at protein concentrations in excess of 15 $\mu$g ml$^{-1}$ even though only a fraction of the available substrate (<10%) had been consumed (Figure 9b).

Based upon these early observations, kinetic measure-

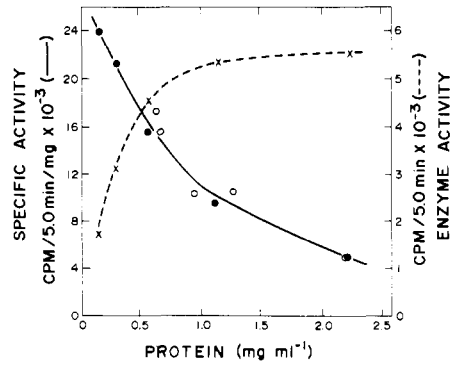


FIGURE 8: Effect of protein concentration on enzyme activity in partially purified ($F_{30}^{50}$ fraction) prenyl transferase. Approximately 27 ml of dialyzed $F_{30}^{50}$ prenyl transferase (in 1 mM $PP_i$ (pH 7.2)) was placed on an Amicon XM-50 micropore filter (mol wt exclusion limit, 30,000) and concentrated under nitrogen pressure until the volume of enzyme solution decreased to 8 ml. In the course of concentrating this solution, aliquots (0.1 ml) were removed at intervals and assayed in the usual manner for prenyl transferase activity as described in Methods. The concentrated enzyme solution was then diluted serially in 1 mM $PP_i$ buffer (pH 7.2) and reassayed. (○) specific activity as a function of protein concentration while concentrating prenyl transferase; (●) specific activity as a function of protein concentration upon diluting and reassaying concentrated prenyl transferase; (x) total enzyme activity as a function of protein concentration.

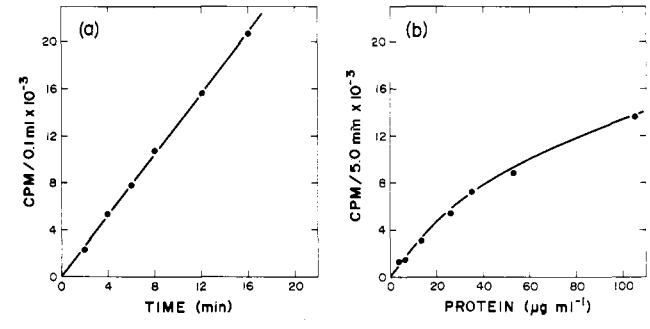


FIGURE 9: (a) Kinetics of prenyl transferase catalyzed product formation. The formation of [$^{14}C$]prenyl pyrophosphate through the catalytic action of prenyl transferase I (purified approximately 400-fold) upon [$^{14}C$]iPe-PP and $Ger_2PP$ was measured as a function of time at 30° in 40 mM Tris-maleate buffer (pH 6.8) in 1.6 mM $MgCl_2$ as described in the text. Each assay contained 10.6 $\mu$g of protein, 5 nmol of [$^{14}C$]iPe-PP (117,400 cpm), and 45 nmol of $Ger_2PP$ in a total volume of 0.52 mol. (b) Effect of protein concentration on enzyme activity in highly purified prenyl transferase I. Enzyme activity was measured as described in Methods as a function of protein concentration on prenyl transferase (purified approximately 400-fold). Aliquots of enzyme (0.1 ml) were assayed essentially as described in Figure 8, except the incubation period was held constant at 5.0 min while the protein concentration in each aliquot was varied to give the final protein concentrations shown. Each assay contained 5 nmol of [$^{14}C$]iPe-PP (117,400 cpm) and 45 nmol of $Ger_2PP$ in a total volume of 0.52 ml.

ments were made at high and low protein concentrations corresponding to the linear and nonlinear portions of the velocity curve in Figure 9b. In determining the $K_m$ for iPe-PP, geranyl-PP was fixed at a final concentration of 60 $\mu$M and iPe-PP varied from 2.2 to 33 $\mu$M. In determining the $K_m$ for $Me_2$allyl-PP and geranyl-PP, iPe-PP was fixed at 95 $\mu$M while geranyl-PP was varied from 0.8 to 48 $\mu$M and $Me_2$allyl-PP from 1.0 to 30 $\mu$M. Lineweaver–Burk double reciprocal plots were linear within the range of substrate and protein concentrations studied.

At moderately high protein concentrations ranging from 42 to 56 $\mu$g ml$^{-1}$, $K_m$ values for geranyl transferases I and II were found to be as follows: transferase I, 48 ± 10 $\mu$M (iPe-PP), 5.9 ± 1.2 $\mu$M ($Me_2$allyl-PP), and 36 ± 7.2 $\mu$M (geranyl-PP); transferase II, 32 ± 6.4 $\mu$M (iPe-PP), 4.5 ± 0.9 $\mu$M ($Me_2$allyl-PP), and 39 ± 7.8 $\mu$M (geranyl-PP). Our best estimates of $V_{max}$ values for geranyl transferases I and II within this same protein concentration range were 60 ± 6 and 40 ± 4 units mg of protein$^{-1}$, respectively.

At moderately low protein concentrations ranging from 12 to 20 $\mu$g ml$^{-1}$ both enzymes exhibited $K_m$ values on the order of 2.2 ± 0.4 $\mu$M (iPe-PP), 1.5 ± 0.3 $\mu$M ($Me_2$allyl-PP), and 5.1 ± 1.0 $\mu$M (geranyl-PP) and rather variable $V_{max}$ values ranging between 2 and 20 units mg of protein$^{-1}$. At these lower protein concentrations both enzymes were especially labile and, hence, $V_{max}$ values were particularly subject to error. Possibly this may be attributed in part to nonspecific binding and inactivation of low levels of enzyme on the glass-walled tubes used in our assays and inactivation of enzyme at the air–water interface which constitutes a significant loss of added enzyme at these lower protein concentrations.

## Discussion

To our knowledge this is the first report on the isolation and partial characterization of more than one form of geranyl transferase from a single source. Whether geranyl transferases I and II are true isoenzymes or modified forms of the same parent enzyme remains to be determined. Indirect lines of evidence have been offered to support the concept of enzyme segregation, wherein at least two functionally distinct pathways of higher isoprenoid biosynthesis operate autonomously in response to specific needs of different subcellular organelles (Goodwin and Mercer, 1963; Goodwin, 1967; Stoddart, 1969; Croteau and Loomis, 1971; Momose and Rudney, 1972). Whether the enzymes which comprise one such compartmentalized pathway are distinguishable from their counterparts in another compartment of the cell depends on the origin and subsequent fate of each class of enzymes. If the enzymes of one compartment have evolved separately from those of another through separate gene pools (*e.g.*, from nuclear, chloroplastidic, or possibly mitochondrial origin), or if such enzymes are chemically modified to meet the needs of their specific microenvironments, even though they may have originally arisen from a common gene pool, the end result would allow detection of more than one form of the same enzyme from the whole tissue.

It is also conceivable that one form of geranyl transferase arose from the other as an artifact during extraction and purification (*e.g.*, through deamidation, dephosphorylation, etc. of residues making up the native protein's backbone). However, no evidence of interconversion between these two enzymes was observed during any stage of the purification procedure. Another possibility which cannot yet be fully excluded is that one form of geranyl transferase arose as an artifact through modification of a second, yet unidentified, prenyl transferase whose product specificity in the native state initially differed from that of geranyl transferase. In the case of geranyl transferases I and II, there is as yet no evidence suggesting any functional distinction between these two enzymes.

Geranyl transferases derived from mammalian systems have somewhat different properties from geranyl transferases I and II reported here. Pork liver geranyl transferase has been reported to have a pH optimum of 7.8–8.0 (Dorsey *et al.*, 1966; Holloway and Popjak, 1967) as opposed to an optimum of 6.8–7.0 for castor bean transferase. Differences in the effect of sulfhydryl inhibitors on these enzymes are in general quite striking. Dorsey *et al.* (1966) reported 97–98% inhibition upon preincubation of pork liver enzyme in 1 $\mu$M *p*-hydroxymercuribenzoate or 1 mM *N*-ethylmaleimide and 37% inhibition in the presence of 10 mM iodoacetamide. Popjak (1969) reported 80% inhibition upon preincubating pork liver transferase with 0.5 $\mu$M *p*-hydroxymercuribenzoate, 5 $\mu$M *N*-ethylmaleimide, or 2 mM iodoacetamide. This compares to 25% inhibition in 1 mM *p*-hydroxymercuribenzoate, 15% inhibition in 5 mM *N*-ethylmaleimide, or 40% inhibition in 10 mM iodoacetamide for castor bean transferase. Hence in the main pork liver and castor bean transferase differ by several orders of magnitude in their susceptibility toward sulfhydryl inhibitors. Pork liver transferase appears to have a molecular weight of 52,000[3] (earlier estimates were of 66,000) in contrast to molecular weight estimates ranging from 56,000 to 73,000 for castor bean transferase. However, under conditions where more than one molecular species exists in equilibrium with its counterpart, as suggested in the case of castor bean transferase, a hybrid peak of activity uncharacteristic of either molecular species may be formed (Mysels and Scholten, 1962). Such a peak may thus lead to an erroneous molecular weight estimate and, hence, differences in molecular weight estimates between pork liver and castor bean transferase may be attributed to differences in the conditions under which experimental measurements were made. This possibility has not yet been resolved, since little is known about the existence of more than one molecular species of geranyl transferase, especially in the case of pork liver transferase. Other properties, such as metal ion specificity and substrate $K_m$ values (under optimum conditions) for these enzymes, appear to be quite similar.

Geranyl transferase from pumpkin seed (Ogura *et al.*, 1968) is in the main similar to castor bean transferase. The enzyme appears to have a pH optimum around 7.5 and a molecular weight of approximately 68,000. This enzyme is activated by $Mg^{2+}$ in preference to $Mn^{2+}$ and is relatively insensitive to sulfhydryl inhibition (*i.e.*, only 31% inhibition in 5 mM iodoacetamide). Optimal substrate $K_m$ values are also quite similar.

What appears to be a discrepancy between our results and those reported by Ogura *et al.* (1968), however, is with respect to the linearity of enzyme activity with protein concentration. In plotting enzyme activity as a function of protein concentration, these authors have drawn a straight line through their experimental points, indicating a linear re-

[3] A more recent determination of the molecular weight of this enzyme is 52,000 in contrast to the previously reported estimate of 66,000 (T. Parker and G. Popjak, unpublished observation).

sponse in their assay system for protein concentrations between 0 and 204 $\mu$g ml$^{-1}$. However, it can be seen by careful inspection of these data that the enzyme assay is not linear, but hyperbolic, deviating from linearity at protein concentrations just under 100 $\mu$g ml$^{-1}$ in very much the same fashion as in our experiments with castor bean transferase. This apparent drop in enzyme activity is quite pronounced at protein concentrations of 200 $\mu$g ml$^{-1}$ despite the fact that less than 6% of the available substrate has been consumed under these assay conditions (*i.e.*, substrate levels dropped from 25 $\mu$M to approximately 23.7 $\mu$M). We have calculated from their data the specific activity of the enzyme used in these studies and have found it to be on the order of 5 units mg of protein$^{-1}$. It is for this reason, in our opinion, that these authors did not recognize the effect of protein concentration on the kinetic properties of this enzyme; the actual enzyme concentration used in their assays was too dilute to detect readily the kinetic consequences of protein–protein interactions. Hence, the apparently different conclusion reached in the present study from that of Ogura *et al.* on the effect of protein concentration on enzyme activity is not in fact a discrepancy, but appears to be primarily the result of a difference in the purity of enzyme samples. The intrinsic properties of these two enzymes appear to us to be quite similar. Similar protein–protein interactions, though less thoroughly characterized, have been observed by Kandutsch *et al.* (1964) as a property of $Ger_2PP$ synthetase (farnesyl transferase) from *Micrococcus lysodeikticus*.

At relatively high protein concentrations (*e.g.*, 40–60 $\mu$g ml$^{-1}$), purified forms of geranyl transferases I and II both exhibit unusually high $K_m$ values for iPe-PP and geranyl-PP. These values (30–50 $\mu$M) are an order of magnitude higher than anticipated, based upon previous reports on the kinetic properties of geranyl transferase derived from other enzyme sources. However, the series of experiments designed to study the effect of assay period and protein concentration upon enzyme activity (Figure 9), using geranyl transferase I as the enzyme source, clearly establish a correlation between protein concentration and kinetic properties for this enzyme. These results, and our interpretation of the data of Ogura *et al.* as discussed above, caused us to reexamine the kinetic properties of this enzyme at relatively low protein concentrations (<20 $\mu$g ml$^{-1}$) where the effect of protein–protein interactions appears to be negligible (Figure 9b). Lineweaver–Burk plots of enzyme activity at protein concentrations within this linear range yield $K_m$ values on the order of 2–5 $\mu$M for iPe-PP and geranyl-PP in contrast to previously determined $K_m$ values of this enzyme at much higher protein concentrations. Similarly, though less pronounced, the $K_m$ value of these enzymes for $Me_2$allyl-PP increased approximately threefold in going from low to high protein concentrations (*e.g.*, from 1.5 to 4.5–5.9 $\mu$M).

These results thus show that protein–protein interactions are capable of modulating the kinetic properties of geranyl transferase and are consistent with the idea that (i) the enzyme exists in more than one molecular weight form and (ii) that the lower molecular weight species (*i.e.*, that which experiences a lesser degree of protein–protein interaction) has significantly lower $K_m$ values for substrates participating in the production of farnesyl-PP. The nature of these protein–protein interactions have not been determined, although it should be noted that the capacity to undergo this change in kinetic properties as a function of protein concentration is retained as the enzyme activity is purified many fold (*cf.* Figures 8 and 9b). By whatever mechanism, the high molecular weight form of this enzyme must have a modified binding or catalytic site for substrates such that the $K_m$ values are markedly increased.

The molecular weight of 72,500 initially determined for castor bean transferase by gel filtration probably represents that of the higher molecular weight species. This conclusion is based upon our kinetic studies of crude $F_{30}^{50}$ geranyl transferase, where it is assumed that deviations in the linear rate of product formation with increasing protein concentration reflect the transition between low and high molecular weight species of the same enzyme. If one assumes that crude $F_{30}^{50}$ geranyl transferase is predominantly in the high molecular weight form at protein concentrations in excess of 0.5 mg ml$^{-1}$ (*cf.* Figure 8), then it is highly probable that the molecular weight of 72,500 represents that of the high molecular weight form of this enzyme, since the enzyme eluted from the calibrated G-100 Sephadex column at a protein concentration on the order of 5 mg ml$^{-1}$. Moreover, crude $F_{30}^{50}$ transferase was originally applied to the column at a protein concentration of approximately 25 mg ml$^{-1}$.

The proposal that these geranyl transferases exist in more than one species of different molecular weights is supported primarily by the changes in kinetic properties as a function of protein concentrations as discussed above. The attempts to measure the molecular weights of the purified enzymes at low protein concentrations (<20 $\mu$g ml$^{-1}$) by gel filtration did indicate the existence of lower molecular weight species. But several problems make the interpretation of the molecular weights calculated from these data difficult. The enzymes were much more labile in the dilute solutions required during these experiments and the assay was less sensitive at these dilute protein concentrations. It is also difficult to evaluate to what extent equilibration between different forms of the enzyme influences its behavior on the gel filtration column in the course of the determinations. In addition to these indications of the existence of enzymatically active lower molecular weight species, it was noted that geranyl transferases I and II showed both major and minor peaks of activity on disc gel electrophoresis after each had been recovered as an apparently homogeneous activity peak with respect to charge from the QAE-Sephadex columns. (It should be noted that each sample was concentrated prior to application to the disc gels.)

The ability of these and possibly other prenyl transferases to interconvert between forms with different kinetic properties as suggested from these data may have possible regulatory significance. Thus, further study of this phenomenon and factors which may influence it should be of interest.

## References


Bates, R. B., Gale, D. M., and Gruner, B. J. (1963), *J. Org. Chem. 28,* 1086.

Benedict, C. R., Kett, J., and Porter, J. W. (1965), *Arch. Biochem. Biophys. 110,* 611.

Christenson, J. G., Gross, S. K., and Robbins, P. W. (1969), *J. Biol. Chem. 244,* 5436.

Croteau, R., and Loomis, W. D. (1971), *Phytochemistry 11,* 1055.

Davis, B. J. (1964), *Ann. N. Y. Acad. Sci. 121,* 404.

Dorsey, J. K., Dorsey, J. A., and Porter, J. W. (1966), *J. Biol. Chem. 241,* 5353.

Goodwin, T. W. (1967), *in* Biochemistry of Chloroplasts,

Vol. II, Goodwin, T. W., Ed., London, Academic Press, p 721.
Goodwin, T. W., and Mercer, E. I. (1963), *Biochem. Soc. Symp. 24,* 37.
Green, T. R., and Baisted, D. J. (1972), *Biochem. J. 130,* 983.
Grob, E. C., Kirschner, K., and Lynen, F. (1961), *Chimia 15,* 308.
Holloway, P. W., and Popjak, G. (1967), *Biochem J. 104,* 57.
Kandutsch, A. A., Paulus, H., Levin E., and Bloch, K. (1964), *J. Biol. Chem. 239,* 2507.
Kurokawa, T., Ogura, K., and Seto, S. (1971), *Biochem. Biophys. Res. Commun. 45,* 251.
Loomis, W. D., and Battaile, J. (1966), *Phytochemistry 5,* 423.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.
Lynen, F., Agranoff, B. W., Eggerer, H., Henning, W., and Moeslein, E. M. (1959), *Angew. Chem. 71,* 657.
Momose, K., and Rudney, H. (1972), *J. Biol. Chem. 247,* 3930.
Mysels, K. J., and Scholten, P. C. (1962), *Science 136,* 693.
Nandi, D. L., and Porter, J. W. (1964), *Arch. Biochem. Biophys. 105,* 7.
Ogura, K., Nishino, T., and Seto, S. (1968), *J. Biochem. 64,* 197.
Ogura, K., Shinka, T., and Seto, S. (1972), *J. Biochem. 72,* 1101.
Oster, M. O., and West, C. A. (1968), *Arch. Biochem. Biophys. 127,* 112.
Popjak, G. (1969), *Methods Enzymology 9,* 363.
Robinson, D. R., and West, C. A. (1970a), *Biochemistry 9,* 70.
Robinson, D. R., and West, C. A. (1970b), *Biochemistry 9,* 80.
Stoddart, J. L. (1969), *Phytochemistry 8,* 831.
Vesterberg, O. (1971), *Biochim. Biophys. Acta 243,* 345.
Wells, L. W., Schelble, W. J., and Porter, J. W. (1964), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 23,* 426.
West, C. A., Oster M., Robinson, D., Francis, L., and Murphy, P. (1968), in Biochemistry and Physiology of Plant Growth Substances, Wightman, F., and Setterfield, G., Ed., Ottawa, Runge Press, p 313.

# Comparative Properties of Rat Liver and Sea Urchin Eggs *S*-Adenosyl-L-methionine Decarboxylase†

Carol-Ann Manen and Diane H. Russell*

ABSTRACT: *S*-Adenosyl-L-methionine decarboxylase has been extensively purified from both rat liver and sea urchin eggs utilizing a new procedure which includes affinity chromatography. This has resulted in a preparation from sea urchin eggs with a very high specific activity, *i.e.,* over 500 units/mg of protein. Acrylamide gel electrophoresis indicated that a high degree of purity had been obtained; *i.e.,* over 60% of the remaining protein was *S*-adenosyl-L-methionine decarboxylase. The properties of the enzyme isolated from sea urchin eggs are very similar to those previously reported for rat liver, with the exception that metal ions have no effect on the enzyme from sea urchin eggs. The molecular weight of the enzyme isolated from both sources is very similar (approximately 50,000). Both preparations, after extensive purification, still exhibit coupling of the decarboxylation of *S*-adenosyl-L-methionine and the formation of either spermidine or spermine depending upon which amine is added as the receptor for the propylamine moiety.

It has been established that the mammalian enzyme, *S*-adenosyl-L-methionine decarboxylase, has vastly different requirements than the enzyme performing the same task in *Escherichia coli* (Tabor and Tabor, 1964; Pegg and Williams-Ashman, 1969). In the mammalian system, evidence indicated that the decarboxylation of *S*-adenosyl-L-methionine and the transfer of the propylamine moiety from decarboxylated *S*-adenosine-L-methionine to putrescine to form spermidine, or to spermidine to form spermine, might be catalyzed by one enzyme or an enzyme complex. It was not possible to demonstrate any decarboxylated *S*-adenosyl-L-methionine as a free intermediate in crude homogenates and this reaction required either putrescine or spermidine, or a similar substrate, as a receptor molecule for the propylamine moiety (Pegg and Williams-Ashman, 1969; Williams-Ashman and Schenone, 1972). While the enzyme isolated from *E. coli* required magnesium, the mammalian enzyme did not require any metal ions (Tabor and Tabor, 1964; Feldman *et al.,* 1971). The mammalian enzyme is strongly inhibited by known inhibitors of pyridoxal phosphate requiring enzymes (Pegg and Williams-Ashman, 1969; Feldman *et al.,* 1972). Also, J. Sturman and L. Kremzner (personal communication) have demonstrated recently a definite requirement for pyridoxal phosphate by *S*-adenosyl-L-methionine decarboxylase assayed in liver extracts of vitamin $B_6$ (pyridoxal phosphate) deficient rats. The enzyme isolated from *E. coli* requires covalently bound pyruvate as a prosthetic group (Wickner *et al.,* 1970).

Recently, certain investigators have reported that it is possible to separate the decarboxylase function from the propylamine transfer function (Jänne and Williams-Ash-

† From the Department of Pharmacology, University of Arizona College of Medicine, Tucson, Arizona 85724. *Received September 19, 1973.* Supported by U. S. Public Health Service Grant 1-RO1-CA-14783 from the National Cancer Institute.