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Differential Prenyl Pyrophosphate Binding to Mammalian Protein Geranylgeranyltransferase-I and Protein Farnesyltransferase and Its Consequence on the Specificity of Protein Prenylation*

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Kohei Yokoyama‡, Karen Zimmerman§, Jeffrey Scholten§, and Michael H. Gelb‡¶

From the ‡Departments of Chemistry and Biochemistry, University of Washington, Seattle, Washington 98195-1700 and §Warner-Lambert, Ann Arbor, Michigan 48106-1047

Protein geranylgeranyltransferase-I (PGGT-I) and protein farnesyltransferase (PFT) attach geranylgeranyl and farnesyl groups, respectively, to the C termini of eukaryotic cell proteins. *In vitro*, PGGT-I and PFT can transfer both geranylgeranyl and farnesyl groups from geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) to their protein or peptide prenyl acceptor substrates. In the present study it is shown that PGGT-I binds GGPP 330-fold tighter than FPP and that PFT binds FPP 15-fold tighter than GGPP. Therefore, *in vivo*, where both GGPP and FPP compete for the binding to prenyltransferases, PGGT-I and PFT will likely be bound predominantly to GGPP and FPP, respectively. Previous studies have shown that K-Ras4B and the Ras-related GTPase TC21 are substrates for both PGGT-I and PFT *in vitro*. It is shown that TC21 can compete with the C-terminal peptide of the γ subunit of heterotrimeric G proteins and with the C-terminal peptide of lamin B for geranylgeranylation by PGGT-I and for farnesylation by PFT, respectively. K-Ras4B competes in both cases but is almost exclusively farnesylated by PFT in the presence of the lamin B peptide competitor. Rapid and single turnover kinetic studies indicate that the rate constant for the PGGT-I-catalyzed geranylgeranyl transfer step of the reaction cycle is 14-fold larger than the steady-state turnover number, which indicates that the rate of the overall reaction is limited by a step subsequent to prenyl transfer such as release of products from the enzyme. PGGT-I-catalyzed farnesylation is 37-fold slower than geranylgeranylation and is limited by the farnesyl transfer step. These results together with earlier studies provide a paradigm for the substrate specificity of PGGT-I and PFT and provide information that is critical for the design of prenyltransferase inhibitors as anti-cancer agents.

Modification of the C termini of specific eukaryotic proteins by attachment of either 15-carbon farnesyl or 20-carbon geranylgeranyl groups is required for their proper membrane targeting and functional activation (1–9). Two closely related enzymes protein farnesyltransferase (PFT)[1] and PGGT-I transfer prenyl groups from prenyl pyrophosphates to proteins that contain a C-terminal Caa*X* motif (C is cysteine, a is usually an aliphatic amino acid, and *X* is a variety of amino acids) (10–13). The *X* residue of this motif plays a major role in recognition by these two enzymes (14). PFT preferentially transfers a farnesyl group to the cysteine residue of the Caa*X* motif when *X* is serine, methionine, glutamine, or cysteine, and possibly other residues. PGGT-I preferentially geranylgeranylates proteins having a C-terminal leucine or phenylalanine. PFT and PGGT-I consist of a common α subunit and distinct β subunits (15–18). A third enzyme, protein geranylgeranyltransferase-II, also known as Rab geranylgeranyltransferase, transfers the 20-carbon prenyl group to both cysteines of Rab proteins that have C-terminal sequences C*X*C, CC, or possibly CC*XX* (19, 20). Although the C-terminal Caa*X* tetrapeptides are sufficient to be recognized and prenylated by PFT and PGGT-I, there appears to be additional determinants present in certain protein substrates that are important recognition features. The polylysine domain near the C terminus of K-Ras4B-CVIM and regions of γ subunits of heterotrimeric G proteins upstream of the Caa*X* sequence influence prenylation patterns *in vitro* and in cell lines over-expressing these proteins (21, 22). Rab geranylgeranyltransferase does not detectably utilize short peptides as substrates but has a unique subunit called Rab escort protein that binds to Rab proteins via contacts with regions that lie far away from the C-terminal site of prenylation (23, 24).

Steady-state kinetic analyses of PFT and PGGT-I show that both enzymes can operate by a random sequential mechanism in which either prenyl donor or acceptor binds first to the enzyme followed by binding of the other substrate to form a ternary complex that goes on to products. However, operationally, both enzymes show a strong tendency to bind prenyl pyrophosphate first (25–28). Both PFT and PGGT-I have been shown to tightly bind both FPP and GGPP (12, 29, 30). This result plus the observation that H-Ras-CVLS protein can be covalently cross-linked to the β subunit of PFT suggest that the common α subunit binds prenyl pyrophosphate and the distinct

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¶ To whom correspondence should be addressed: Depts. of Chemistry and Biochemistry, Box 351700, University of Washington, Seattle, WA 98195-1700. Tel.: 206-543-7142; Fax: 206-685-8665; E-mail: gelb@chem.washington.edu.

[1] The abbreviations used are: PFT and PGGT-I, rat protein farnesyltransferase and protein geranylgeranyltransferase-I, respectively, produced as recombinant proteins in a baculovirus/Sf9 cell system; biotin-lamin B-CAIS, biotinylated peptide from the C terminus of human lamin B except that the C-terminal methionine has been changed to serine (biotin-CONH-$(CH_2)_5$-CO-GTPRasNRSCAIS); biotin-γ_6-CAIL, biotinylated peptide from the C terminus of the γ_6 subunit of bovine brain heterotrimeric G proteins (biotin-CONH-$(CH_2)_5$-CO-NPFREKK-FFCAIL); FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; H-Ras-CVLS, human H-Ras produced in *E. coli*; K-Ras4B-CVIM, epitope-tagged human K-Ras4B with its native C-terminal sequence CVIM; RhoB-CKVL, six histidine-tagged rat Rho B protein with its native C-terminal sequence CKVL; TC21-CVIF, glutathione *S*-transferase fusion protein of human TC21 with its native C-terminal sequence CVIF; DTT, dithiothreitol; PA, prenyl acceptors.

β subunits bind prenyl acceptors (29). However, recent studies show that photoaffinity analogs of FPP and GGPP exclusively label the β subunits of PGGT-I and PFT (26, 31, 32), and cross-linking studies with photoreactive peptide prenyl acceptors suggest that prenyl acceptors bind to an α subunit/β subunit interface (33).

PFT and PGGT-I show mixed specificity under certain conditions. Purified PGGT-I can transfer both geranylgeranyl and farnesyl groups to protein or peptide substrates ending with leucine and can geranylgeranylate substrates ending with serine (26, 34). PFT can transfer a geranylgeranyl as well as a farnesyl group to substrates having a C-terminal methionine (25). The small GTP-binding protein Rho B exists as a mixture of farnesylated and geranylgeranylated forms when produced in transfected COS cells or in a reticulocyte lysate translation mixture (35). Rho B is a poor substrate for PFT in the presence of FPP or GGPP but can be efficiently geranylgeranylated or farnesylated by PGGT-I in the presence of GGPP or FPP, respectively (30). Some prenyl acceptors seem to be substrates for both prenyltransferases. The oncogenic proteins K-Ras4B-CVIM and the Ras-related GTP-binding protein TC21-CVIF (also known as R-Ras2) can be farnesylated by PFT and geranylgeranylated by PGGT-I *in vitro* (21, 36). Trueblood and co-workers (37) showed that over-expression of the β subunit of PFT or PGGT-I in yeast can partially overcome the inactivating effect of mutation in the β subunit of the other enzyme (PGGT-I or PFT, respectively). This suggests that under forcing conditions, both PFT and PGGT-I can prenylate proteins that are normally prenylated by a single prenyltransferase.

In this paper we report studies aimed at understanding the specificities of PFT and PGGT-I in detail with particular focus on determining the precise affinities of PFT and PGGT-I for both FPP and GGPP. These results, when combined with our earlier studies (26), provide a comprehensive description of the features of these enzymes that dictate their specificities. The results are important for predicting the consequences of inhibiting PFT or PGGT-I on protein prenylation in cells. This is of considerable interest because inhibitors of these enzymes are being developed as potential anti-cancer drugs due to their ability to block prenylation and thus transforming activity of oncogenic RAS proteins (38, 39). We also report kinetic studies of PGGT-I which probe the nature of the rate-limiting step for the prenyl transfer reactions, and our results are compared with the analogous study carried out with PFT (40).

EXPERIMENTAL PROCEDURES

Materials—Biotin-γ_6-CAIL and biotin-lamin B-CAIS were synthesized and analyzed by mass spectroscopy as described previously (34). [^{3}H]FPP and [^{3}H]GGPP (15 Ci/mmol, labeled on carbon-1) and unlabeled FPP and GGPP were purchased from American Radiolabeled Chemicals. The chemical and radiometric purities of these prenyl pyrophosphates were routinely monitored by thin layer chromatographic analysis as described (34), and compounds were not used if either purity was less than 90%. Recombinant baculoviruses that express the α and β subunits of rat PFT (Professor Y. Reiss, Tel-Aviv University) and that express the β subunit of rat PGGT-I (Professor J. L. Goldstein, Southwestern Medical Center) were obtained as gifts from the indicated individuals. RhoB-CKVL was obtained as a generous gift from Professor J. L. Goldstein. H-Ras-CVLS produced in *Escherichia coli* was prepared as described (41). K-Ras4B-CVIM was obtained from the cytosol of baculovirus-infected Sf9 cells as described (42). TC21-CVIF was obtained as a generous gift from Dr. V. Manne (Bristol-Myers Squibb, Princeton, NJ).

Production and Purification of Recombinant Rat PGGT-I and PFT—Sf9 cells were cultured in IPL-41 medium supplemented with 10% fetal bovine serum, 4 mg/ml yeastolate, 0.1% Pluronic F-68, and 50 μg/ml gentamycin (all from Life Technologies, Inc.). A 6-liter culture of Sf9 cells (log phase, about 1.5×10^6 cells/ml) was coinfected with recombinant viruses expressing the α subunit of PFT/PGGT-I and either the β subunit of PGGT-I or the β subunit of PFT at multiplicities of infection of 1. All subsequent steps were carried out at 4 °C unless otherwise noted. For PGGT-I production, the cells were harvested 72 h after infection by centrifugation and washed once with 20 mM Tris-HCl, 1 mM DTT, 0.13 M NaCl, pH 8.0. The cell pellet was frozen at −80 °C. The frozen cells were thawed on ice in 300 ml of 20 mM Tris-HCl, 1 mM DTT, and 0.05 M NaCl, pH 8.0 (buffer A) containing freshly added protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 30 μM tosyl-lysine-chloromethyl ketone, 30 μM tosyl-phenylalanine-chloromethyl ketone, and 10 μg/ml each of aprotinin, leupeptin, and pepstatin A, all from Sigma). The cells were disrupted by six strokes in a Dounce homogenizer. The lysate was centrifuged for 10 min at 10,000 × *g*, and the supernatant was centrifuged for 1 h at 100,000 × *g*. The resulting supernatant was fractionated on a column (2.6 × 20 cm) of Q-Sepharose (Pharmacia Biotech Inc.) equilibrated in buffer A as described previously (12). A linear gradient (4-liter total volume) of NaCl concentrations from 0.05 to 0.6 M was applied, and PGGT-I was eluted at about 0.3 M NaCl. The enzyme peak fractions (assayed with 5 μM biotin-γ_6-CAIL and 1 μM [^{3}H]GGPP as described below) were combined, dialyzed against buffer A, and then chromatographed on a Mono-Q HR10/10 column (Pharmacia) as described previously (12). The yield of PGGT-I is 90 mg, and the purity was more than 90% as judged by SDS-polyacrylamide gel electrophoresis. The specific activity is 4.7 microunits/μg, where 1 microunit is the amount of enzyme that produces 1 pmol of product per min at 30 °C using 2 μM [^{3}H]GGPP and 5 μM biotin-γ_6-CAIL peptide as substrates. PGGT-I was stored at −80 °C.

For purification of PFT, infected Sf9 cells (3.6-liter culture) were harvested 2–3 days after infection by centrifugation, and all subsequent steps were carried out at 4 °C. The cells were washed twice with phosphate-buffered saline, and the cell pellet was taken up in 300 ml of 20 mM Tris-HCl, 50 mM $MgCl_2$, 20 μM $ZnCl_2$, 1 mM DTT, pH 7.4. The cell suspension was transferred to a pre-chilled cavitation bomb (in three 100-ml portions); the bomb was charged with N_2 to 600 p.s.i., and after 30 min, the sample was drained from the bomb through a high pressure outlet valve. The cell lysate was centrifuged at 100,000 × *g* for 45 min. To the supernatant was added solid ammonium sulfate to give 55% saturation, and the suspension was centrifuged at 18,000 × *g* for 20 min. The protein pellet was taken up in 31 ml of buffer B (20 mM Tris-HCl, 50 mM NaCl, 20 μM $ZnCl_2$, 1 mM DTT, pH 7.5), and the sample was dialyzed twice against 2 liters of buffer B. The sample was centrifuged at 18,000 × *g* for 20 min, filtered through a 0.22-μm filter cartridge, and loaded onto a Mono-Q HR10/10 column. The column was washed with 15 ml of buffer B and then developed with a linear gradient of 0.05–1 M NaCl in buffer B (100 ml total). PFT (assayed as described below) elutes at about 0.5 M NaCl and was stored at −80 °C. The specific activity of the final PFT preparation is 138 microunits/μg based on the assay with 10 μM H-Ras-CVLS and 2 μM [^{3}H]FPP, and the purity was greater than 90% as judged by SDS-polyacrylamide gel electrophoresis.

The absolute mole amount of [^{3}H]GGPP-binding capacity of PGGT-I was determined as the moles of PGGT-I·[^{3}H]GGPP that eluted in the void volume of a spin gel filtration column (12) when a stock solution of PGGT-I was incubated with an excess of [^{3}H]GGPP of known specific radioactivity. The specific radioactivity of [^{3}H]GGPP, diluted with unlabeled GGPP, was determined from the mass of GGPP measured by phosphate analysis (43) and from the cpm measured by scintillation counting. In one case, the mass of [^{3}H]GGPP was determined by integration of ^{1}H NMR resonances obtained from a sample containing [^{3}H]GGPP and a known amount of dimethyl sulfoxide as an internal standard; the mass determined in this way is in agreement with the phosphate analysis. The absolute mole amount of [^{3}H]FPP-binding capacity of PFT was determined similarly using [^{3}H]FPP of known specific radioactivity. The absolute mole amount of PGGT-I was determined from the absorbance at 280 nm using the extinction coefficient of 134 mM^{-1} cm^{-1}, which was calculated from the amino acid sequence using the published formula (44).

PGGT-I and PFT Assays—To assay the geranylgeranylation activity of PGGT-I, 1 μM [^{3}H]GGPP (15 Ci/mmol) and 5 μM biotin-γ_6-CAIL were incubated with 0.1 μg of rat PGGT-I in a total volume of 20 μl containing 30 mM potassium phosphate, 5 mM DTT, 0.5 mM $MgCl_2$, 20 μM $ZnCl_2$, pH 7.7. After 5 min at 30 °C, the reaction was terminated by boiling for 3 min, and 40 μl of avidin-agarose suspension (50% aqueous suspension, Pierce) was added to the mixture to measure radioactivity transferred to the biotinylated peptide as described previously (12). For assaying the farnesylation activity of PGGT-I, 1 μM [^{3}H]FPP (15 Ci/mmol) and 20 μM biotin-γ_6-CAIL were used as substrates. The standard assay for PFT was carried out with 10 μM H-Ras-CVLS and 3.3 μM [^{3}H]FPP in a total volume of 20 μl containing 30 mM potassium phosphate, 5 mM DTT, 10 mM $MgCl_2$, 20 μM $ZnCl_2$, 25 mM NaCl, pH 7.7.

Mixtures were incubated at 30 °C for 10 min and terminated by the addition of 200 μl of 10% HCl in ethanol. The amount of radiolabeled farnesylated H-Ras-CVLS was quantified using the glass fiber filter binding method (25). The enzymatic activities of the PGGT-I·[^{3}H]GGPP and PGGT-I·[^{3}H]FPP binary complexes isolated by spin column gel filtration (see below) were assayed using the same conditions as above except that the radioactive prenyl pyrophosphate was omitted, and the incubation time was 15 or 60 s.

Assay for the Formation of Prenyltransferase·Prenyl Pyrophosphate Binary Complexes—The standard assay mixture contains 2–20 nM PGGT-I or PFT, 1 nM to 40 μM [^{3}H]GGPP or [^{3}H]FPP, 30 mM potassium phosphate, 1 mM DTT, 0.1% *n*-octylglucoside, pH 7.7, and 2 μl of 70% ethanol, 30% 0.25 M NH_4HCO_3 (prenyl pyrophosphate stock solution solvent) in a total volume of 50 μl. The mixture was incubated at 30 °C for 15 min and applied to a 0.5 ml-column of Sephadex G-50 pre-equilibrated with 30 mM potassium phosphate, 1 mM DTT, 0.1% *n*-octylglucoside, pH 7.7. The column was spun for 2 min at 2,500 rpm in an HB-4 swinging-bucket rotor and then washed once with 50 μl of the same buffer by spinning in the same way. These two eluants that contain protein but negligible amounts of unbound prenyl pyrophosphate were combined, and the mixture was submitted to scintillation counting. Control experiments without enzyme were also carried out for each assay, and the amount of cpm eluted in the first two eluants was typically 10–50 cpm, and this value was subtracted from that measured in the presence of enzyme.

Rapid Quench Kinetic Analysis—Pre-steady-state kinetic measurements were made using a KinTek Quench Flow apparatus Model RQF-3. Three syringes were driven by a step motor. PGGT-I or PFT alone or together with [^{3}H]GGPP or [^{3}H]FPP (Syringe 1) was rapidly mixed with biotin-γ_6-CAIL alone or together with nonradioactive FPP or GGPP, or [^{3}H]GGPP (Syringe 2). Each set of samples (45-μl aliquot each) was incubated at 30 °C for a series of time periods (0.25 to 50 s), and the transfer reaction was terminated by rapid mixing with stop solution (230 μl of 1.5 M $MgCl_2$, 0.2 M H_3PO_4, 0.5% bovine serum albumin, Syringe 3). Aliquots (150 μl) of the quenched mixtures were mixed with 100 μl of scintillant proximity assay beads suspension (20 mg/ml in phosphate-buffered saline, Amersham Corp.) and subjected to scintillation counting in a β counter (Wallac microbeta 1450 counter). Binding of the radioactive, prenylated and biotinylated peptide to streptavidin on the the beads places the radioactive prenyl group in close proximity to the scintillant bead. Each set of data obtained was well fit to the first-order equation, and the reaction rate constants were calculated based on the first-order equation. Additional details are given in Table II.

Substrate Specificity Studies with Competing Prenyl Acceptors—Reaction mixtures contained 0.05 μg of PGGT-I or PFT, 2 μM [^{3}H]GPPP or [^{3}H]FPP, 2 μM protein substrate (see Table III), 2 μM peptide substrate (see Table III) in 30 μl of 30 mM potassium phosphate, 5 mM DTT, 0.5 mM $MgCl_2$, 20 μM $ZnCl_2$, pH 7.7. After 15 min at 30 °C, a 10-μl aliquot was quenched with 2 × Laemmli loading buffer. After boiling, the sample was analyzed by SDS-polyacrylamide gel electrophoresis (12.5% gel). The gel was prepared for fluorography, and the radiolabeled bands were visualized using x-ray film (26). Gel slices containing radiolabeled proteins were solubilized in 0.5 ml of 30% H_2O_2 at 60 °C overnight, and the samples were submitted to scintillation counting. The cpm data were converted to pmol of prenylated protein by correcting for quenching. This was carried out by mixing a known amount of [^{3}H]GGPP with a blank gel slice and determining the cpm as described above. To quantify the amount of prenylated peptide, a 10-μl aliquot of the same prenylation reaction mixture was mixed with 200 μl of methanol; the sample was applied to a disposable anion exchange column (SpeN+, 1 ml bed, Baker) to remove [^{3}H]prenyl pyrophosphate, and the column was washed with five 200-μl portions of methanol. The eluant was collected in a single fraction, and solvent was removed in a Speed-vac concentrator (Savant Instruments). The residue was taken up in methanol and spotted onto a 20 × 20-cm plate of silica gel 60 (EM Science). The plate was developed with *n*-propanol:concentrated NH_4OH:water (6:3:1 by volume) and sprayed with EN^{3}HANCE (DuPont NEN) prior to exposure to x-ray film. The region of the plate that contained the radioactive prenylated peptide was scraped, and the silica was submitted to scintillation counting. Counting efficiency was determined by counting a known amount of [^{3}H]GGPP together with blank silica gel.

RESULTS

Differential Binding of GGPP and FPP to PGGT-I and PFT—We have previously shown with *in vitro* reactions that purified bovine PGGT-I is able to transfer both geranylgeranyl and farnesyl groups to substrates containing C-terminal CaaL motifs such as biotin-γ_6-CAIL (26, 34). Steady-state kinetic studies of PGGT-I showed that the K_M values for the interaction of GGPP and FPP with PGGT-I in the presence of a saturating amount of biotin-γ_6-CAIL are similar (0.6 μM) (26). In the presence of saturating amounts of biotin-γ_6-CAIL and prenyl donor, k_{cat} for geranylgeranylation and farnesylation are similar (26). Nevertheless, addition of 1 μM nonradiolabeled GGPP to PGGT-I reaction mixtures containing 1 μM [^{3}H]FPP and 2 μM biotin-γ_6-CAIL nearly abolished the transfer of the radiolabeled farnesyl group to peptide (Table I and Ref. 34). In addition, in PGGT-I reaction mixtures containing equimolar amounts of FPP and [^{3}H]GGPP, the amount of radioactive geranylgeranylated peptide formed was the same as in reaction mixtures that did not contain FPP (Table I and Ref. 34). This suggests that PGGT-I has different affinities for GGPP and FPP.

TABLE I
PGGT-I- and PFT-catalyzed farnesylation and geranylgeranylation of K-Ras4B-CVIM, TC21-CVIF, and RhoB-CKVL

Enzyme	Prenyl acceptor[a]	Prenyl donor(s) present[b]	Radioactivity incorporated into prenyl acceptor[c]
PGGT-I	Biotin-γ_6-CAIL	[^{3}H]GGPP	30,450 cpm 100%
		[^{3}H]GGPP, FPP	126%
		[^{3}H]FPP	98%
		[^{3}H]FPP, GGPP	12%
PGGT-I	K-Ras4B-CVIM	[^{3}H]GGPP	60,929 cpm 100%
		[^{3}H]GGPP, FPP	122%
		[^{3}H]FPP	11%
		[^{3}H]FPP, GGPP	0.1%
PFT	K-Ras4B-CVIM	[^{3}H]FPP	88,862 cpm 100%
		[^{3}H]FPP, GGPP	80%
		[^{3}H]GGPP	6.5%
		[^{3}H]GGPP, FPP	1.5%
PGGT-I	TC21-CVIF	[^{3}H]GGPP	18,617 cpm 100%
		[^{3}H]GGPP, FPP	94%
		[^{3}H]FPP	18%
		[^{3}H]FPP, GGPP	0.6%
PFT	TC21-CVIF	[^{3}H]FPP	7764 cpm 100%
		[^{3}H]FPP, GGPP	70%
		[^{3}H]GGPP	2.6%
		[^{3}H]GGPP, FPP	3.1%
PGGT-I	RhoB-CKVL	[^{3}H]GGPP	7470 cpm 100%
		[^{3}H]GGPP, FPP	104%
		[^{3}H]FPP	24%
		[^{3}H]FPP, GGPP	2.1%
PFT	RhoB-CKVL	[^{3}H]GGPP	158 cpm 100%
		[^{3}H]GGPP, FPP	127%
		[^{3}H]FPP	85%
		[^{3}H]FPP, GGPP	68%

[a] The concentration of all prenyl acceptors (CaaX) is 2 μM.
[b] The concentration of all prenyl pyrophosphates is 1 μM.
[c] The cpm of prenyl group transferred is given for the preferred prenyl donor ([^{3}H]FPP *versus* [^{3}H]GGPP) in the absence of the competing prenyl donor, and the percent cpm transferred in the other reactions are given as a percentage of the given cpm. Assays with PGGT-I/K-Ras4B-CVIM, PFT/K-Ras4B-CVIM, PGGT-I/TC21-CVIF, and PGGT-I/RhoB-CKVL were analyzed by submitting the assay mixture to SDS-polyacrylamide gel electrophoresis and determining the amount of cpm in the protein band. All other assays were analyzed with the glass fiber filter binding method.

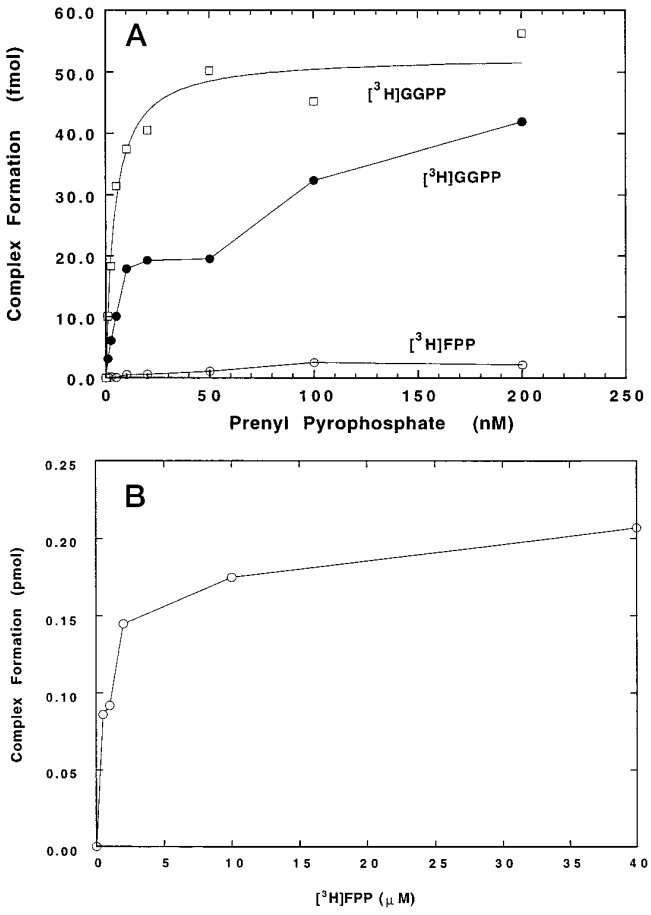

FIG. 1. **Binding of [^{3}H]GGPP and [^{3}H]FPP to PGGT-I.** *A*, PGGT-I (2 nM) was incubated with the indicated concentrations of [^{3}H]GGPP (●) or [^{3}H]FPP (○) in a 50-μl mixture containing 30 mM potassium phosphate, 1 mM DTT, 0.1% *n*-octylglucoside, pH 7.7. After 15 min at 30 °C, the mixture was applied to a 0.5-ml column of Sephadex G-50 to isolate the PGGT-I·[^{3}H]prenyl pyrophosphate complex. The curve for [^{3}H]GGPP binding to PGGT-I that had been pretreated with 5 mM DTT is shown by the *squares* (□). *B*, the same assays were carried out as in *A* except that higher concentrations of PGGT-I (20 nM) were used and [^{3}H]FPP was added (see figure). FPP concentrations higher than 5 μM were made by adding unlabeled FPP to [^{3}H]FPP (15 Ci/mmol), and the amount of complex was calculated based on the specific activity of each diluted [^{3}H]FPP mixture.

To determine equilibrium dissociation constants (K_d) for the PGGT-I·GGPP and PGGT-I·FPP complexes, a very low concentration of PGGT-I was incubated with various concentrations of either [^{3}H]GGPP or [^{3}H]FPP, and the binary complexes were isolated by spin column gel filtration. As shown in Fig. 1*A*, a biphasic profile of [^{3}H]GGPP binding to PGGT-I was observed. This measurement was carried out with 2 nM PGGT-I, the minimum that could be used to obtain a reliable amount of cpm in the column eluant. From the hyperbolic response in the range 0–50 nM [^{3}H]GGPP, it can be seen that the K_d is approximately 3 nM. When the PGGT-I·[^{3}H]GGPP complex obtained with 20 nM PGGT-I and 20 nM [^{3}H]GGPP was isolated by spin column gel filtration and then mixed with 5 μM biotin-γ_6-CAIL, 49 ± 2% (1, 100 cpm) of the bound cpm was transferred after incubation periods of 10, 30, and 180 s. Thus the transfer reaction is completed in less than 10 s. When this transfer experiment was repeated in the presence of 4 μM unlabeled GGPP added together with biotin-γ_6-CAIL, 48 ± 2% of the enzyme-bound cpm was transferred. The similarity in the percent geranylgeranyl group transferred in these two experiments indicates that both peptide binding to PGGT-I·[^{3}H]GGPP and subsequent prenyl transfer occur before [^{3}H]GGPP dissociates from both the PGGT-I·[^{3}H]GGPP binary complex and the PGGT-I·[^{3}H]GGPP·biotin-γ_6-CAIL ternary complex. These experiments were repeated with the same amount of PGGT-I but with a higher concentration of [^{3}H]GGPP (1.3 μM) so that the second binding process shown in Fig. 1*A* is saturated. After spin column gel filtration, 37 ± 3% (2,900 cpm) of the bound cpm was transferred to biotin-γ_6-CAIL after incubation periods of 10, 30, and 180 s. Again, the cpm transferred to peptide was not reduced if 4 μM unlabeled GGPP was added along with peptide.

It was initially thought that the lower affinity binding of [^{3}H]GGPP to PGGT-I (Fig. 1*A*) is due to nonspecific interaction of this isoprenoid with the enzyme. However, this is not the case. If it were, 1,100 cpm would be transferred to peptide in the presence of excess unlabeled GGPP, *i.e.* the same amount of radioactivity that is transferred from the high affinity PGGT-I·[^{3}H]GGPP to peptide. The fact that a total of 2,900 cpm is transferred from the complex prepared from 1.3 μM [^{3}H]GGPP indicates that the lower affinity PGGT-I·[^{3}H]GGPP complex is capable of prenyl transfer to peptide, and furthermore this transfer occurs without exchange of [^{3}H]GGPP with the pool of non-bound GGPP. For both the high and low affinity PGGT-I·[^{3}H]GGPP complexes, only a portion of the bound [^{3}H]GGPP is transferred to peptide, 49 and 32%, respectively. The reason for this is not known with certainty; however, it is probably due to partial dissociation of [^{3}H]GGPP from the PGGT-I·[^{3}H]GGPP binary complex or from the PGGT-I·[^{3}H]GGPP·biotin-γ_6-CAIL ternary complex that occurs before prenyl transfer.

As shown in Fig. 1*A*, PGGT-I that had been incubated with 5 mM DTT for 30 min at 30 °C at neutral pH leads to a form of the enzyme that only shows that high [^{3}H]GGPP binding component and up to 56 fmol of [^{3}H]GGPP can be bound. This suggests that two forms of PGGT-I exist that differ in their patterns of disulfides; the β-chain of PPGT-I contains 15 cysteines throughout its length (16). As will be shown by single turnover kinetic studies presented below, the rate of transfer of the geranylgeranyl group from GGPP to peptide acceptor catalyzed by these two forms of PGGT-I are the same within experimental error.

As shown in Fig. 1*A*, only a small amount of PGGT-I·[^{3}H]FPP is formed in the presence of 0–200 nM [^{3}H]FPP. Significant amounts of PGGT-I·[^{3}H]FPP were formed with 20 nM PGGT-I and concentrations of [^{3}H]FPP higher than 0.2 μM (Fig. 1*B*). From this curve a K_d of 1 μM is obtained for the PGGT-I·[^{3}H]FPP complex, and thus it is concluded that GGPP binds about 330-fold tighter to PGGT-I than does FPP.

Binding of [^{3}H]FPP and [^{3}H]GGPP to PFT was also studied by spin column gel filtration. From the binding curves shown in Fig. 2, *A* and *B*, values of K_d of ≈2 nM and 30 nM for [^{3}H]FPP and [^{3}H]GGPP, respectively, were obtained. Thus, [^{3}H]FPP binds about 15-fold tighter than [^{3}H]GGPP to PFT.

In contrast to the results with PGGT-I·[^{3}H]GGPP, only 4% of the labeled farnesyl group was transferred to biotin-γ_6-CAIL in a reaction mixture containing 16 nM PGGT-I·[^{3}H]FPP and 20 μM biotin-γ_6-CAIL after 15 s, and no transfer of radioactivity to the peptide occurred if the reaction mixture also contained excess unlabeled FPP or GGPP (10 μM). Presumably the small amount of labeled farnesyl group transferred to the peptide in the absence of unlabeled FPP or GGPP is due to the fact that the concentration of PGGT-I·[^{3}H]FPP used, 16 nM, is far below the K_d for this binary complex (1 μM), and thus most of the [^{3}H]FPP dissociated from the enzyme after the spin column but prior to the addition of peptide. Indeed, when the transfer reaction was carried out with 500 nM PGGT-I·[^{3}H]FPP, 35% of the radioactivity was transferred to biotin-γ_6-CAIL after 15 s. Only 1.5% of the radioactivity was transferred if this reaction

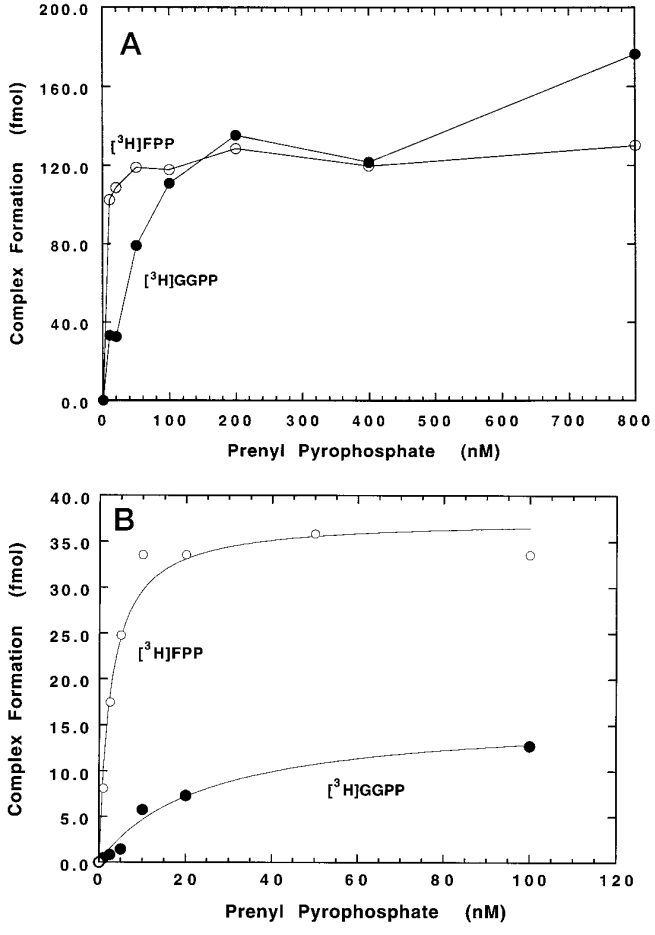

FIG. 2. **Binding of [^{3}H]FPP and [^{3}H]GGPP to PFT.** *A*, complex formation with 5 nM PFT and [^{3}H]FPP (○) or [^{3}H]GGPP (●) at concentrations ranging from 10 to 800 nM. *B*, complex formation with 2 nM PFT and [^{3}H]FPP (○) or [^{3}H]GGPP (●) at concentrations ranging from 1 to 100 nM.

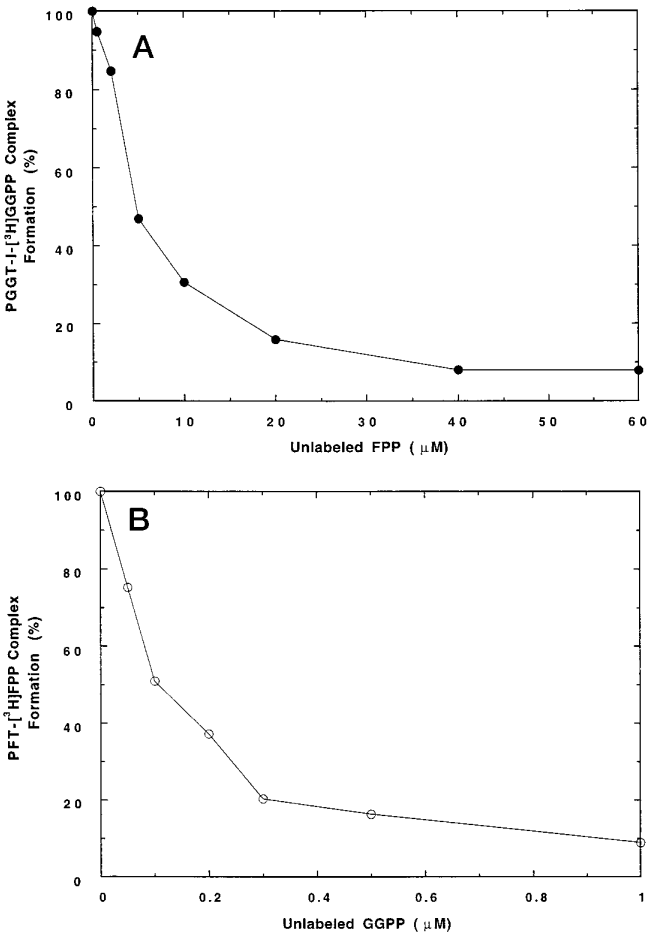

FIG. 3. **Competition between GGPP and FPP for binding to PGGT-I and PFT.** *A*, PGGT-I (20 nM) was incubated with 20 nM [^{3}H]GGPP and the indicated concentration of unlabeled FPP. After 1 h at 30 °C, the amount of PGGT-I·[^{3}H]GGPP complex was measured using the spin column method. Data are shown as relative amounts of the radioactive complex formed in which 100% is the amount of radioactivity (1328 cpm) obtained in the assay without unlabeled FPP. *B*, inhibition of PFT·[^{3}H]FPP complex formation by unlabeled GGPP. PFT (5 nM) was incubated at 30 °C for 1 h with 20 nM [^{3}H]FPP and the indicated concentration of unlabeled GGPP, and the amount of PFT·[^{3}H]FPP was determined by the spin column method.

mixture also contained 50 μM unlabeled FPP, and no detectable radioactivity was transferred in the presence of 50 μM unlabeled GGPP. These observations indicate that within a 15-s period, most of the [^{3}H]FPP bound to PGGT-I has exchanged with FPP or GGPP present in the non-enzyme-bound pool.

Values of K_d determined as described above were confirmed by competitive binding studies. As shown in Fig. 3*A*, addition of increasing amounts of unlabeled FPP to a mixture of 20 nM PGGT-I and a fixed amount of [^{3}H]GGPP (20 nM) led to a decrease in the amount of enzyme-bound radioactivity as detected by spin column gel filtration. Since [^{3}H]GGPP dissociates slowly from PGGT-I, it is important to verify that binding exchange has reached equilibrium. PGGT-I was incubated with a mixture of [^{3}H]GGPP and unlabeled FPP at 30 °C for 30 or 60 min, and the amount of enzyme-bound [^{3}H]GGPP was the same for both incubation periods (data not shown). Thus, the 60-min incubation period used in the experiments shown in Fig. 3*A* is sufficient to attain equilibrium. At equilibrium, the ratio of binary complexes is given by Equation 1.

$$\frac{[\text{PGGT} - I \cdot \text{FPP}]}{[\text{PGGT} - I \cdot \text{GGPP}]} = \frac{[\text{FPP}]\ K_d^{\text{GGPP}}}{[\text{GGPP}]\ K_d^{\text{FPP}}} \qquad \text{(Eq. 1)}$$

This ratio is unity when [FPP] is 5 μM and [^{3}H]GGPP is 20 nM (Fig. 3*A*), and thus K_d for PGGT-I·[^{3}H]GGPP (K_d^{GGPP}) is 500-fold smaller than K_d for PGGT-I·FPP (K_d^{FPP}), which is in close agreement with the results of the direct binding titrations (Fig. 1). Fig. 3*B* shows the analogous experiment done with PFT, [^{3}H]FPP, and GGPP. Unlabeled GGPP at a concentration of 100 nM is needed to displace 50% of the label from the PFT·[^{3}H]FPP complex (20 nM), and thus the $K_d^{\text{GGPP}}/K_d^{\text{FPP}}$ ratio is 9, which agrees reasonably well with the 15-fold difference in dissociation constants obtained from the direct binding studies (Fig. 2).

Single Turnover Rates of PGGT-I-catalyzed Geranylgeranylation and Farnesylation—Rapid quench experiments were carried out to measure the rate of transfer of the geranylgeranyl or farnesyl group to biotin-γ_6-CAIL catalyzed by PGGT-I, and results are summarized in Table II. A solution of 200 nM PGGT-I·[^{3}H]GGPP was rapidly mixed with a solution of 5 μM biotin-γ_6-CAIL, and the reaction mixture was quenched with acid at various times after the mixing. The results in Fig. 4*A* show that under these single turnover conditions, the geranylgeranyl group is transferred to the peptide with a first-order rate constant of 0.49 ± 0.06 s^{-1}. Essentially the same rate constant was obtained when 200 nM PGGT-I·[^{3}H]GGPP was mixed with 20 μM biotin-γ_6-CAIL instead of 5 μM, which indicates that prenyl transfer is slower than binding of peptide to PGGT-I·[^{3}H]GGPP, and thus the rate of prenyl transfer from the ternary complex being measured is not limited by peptide binding. Essentially the same observed rate constant was seen

TABLE II
Single turnover rate constants of geranylgeranyl and farnesyl transfer to biotin-γ_6-CAIL catalyzed by PGGT-I

Syringe 1 sample	Syringe 2 sample	Observed first-order rate constant
		s^{-1}
200 nM PGGT-I + 200 nM [^{3}H]GGPP	5 μM biotin-γ_6-CAIL	0.49 ± 0.06
200 nM PGGT-I + 200 nM [^{3}H]GGPP	20 μM biotin-γ_6-CAIL	0.56 ± 0.08
200 nM PGGT-I	5 μM biotin-γ_6-CAIL + 200 nM [^{3}H]GGPP	0.44 ± 0.05
1.5 μM PGGT-I + 1 μM [^{3}H]FPP	40 μM biotin-γ_6-CAIL + 10 μM GGPP	0.015 ± 0.004

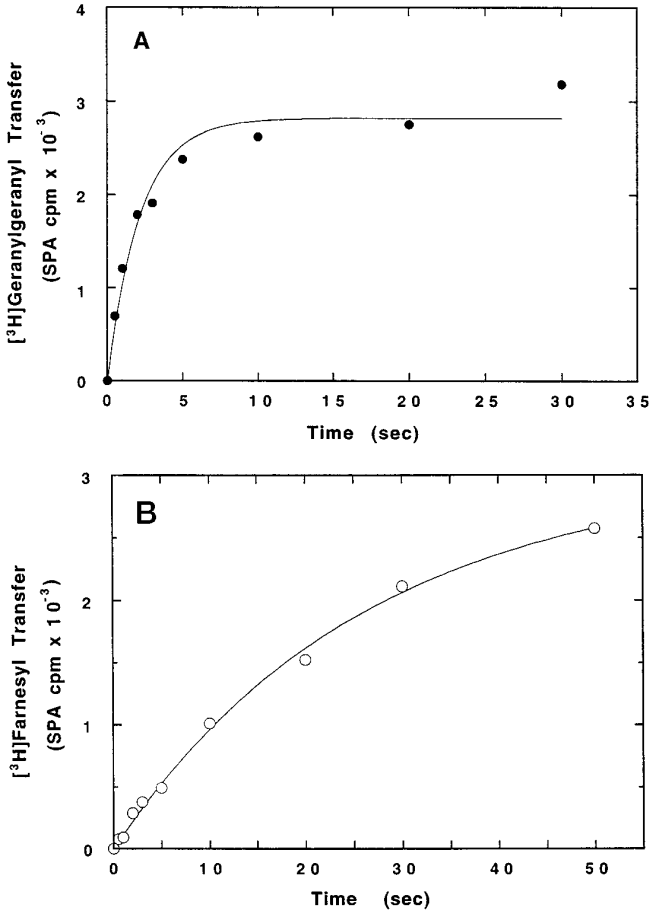

FIG. 4. **Single turnover prenylation reactions catalyzed by PGGT-I and PFT.** *A*, a solution of PGGT-I (200 nM) and [^{3}H]GGPP (200 nM) was mixed with a solution of biotin-γ_6-CAIL (5 μM) with a rapid quench apparatus. At the indicated times, the reaction mixture was quenched with acid, and the amount of radiolabeled geranylgeranylated peptide was measured as described under "Experimental Procedures." *B*, a solution of PGGT-I (1.5 μM) and [^{3}H]FPP (1 μM) was mixed with a solution of biotin-γ_6-CAIL (40 μM) and GGPP (10 μM), and at the indicated times, the reaction was quenched, and the amount of radiolabeled farnesylated peptide was determined as described under "Experimental Procedures."

when a solution of 200 nM PGGT-I was mixed with a solution containing 5 μM peptide and 200 nM [^{3}H]GGPP, which indicates that binding of [^{3}H]GGPP to PGGT-I occurs faster than prenyl transfer. When PGGT-I·[^{3}H]GGPP was mixed with a solution containing both 5 μM biotin-γ_6 and a 20-fold excess (4 μM) of unlabeled GGPP, the amount of radioactive geranylgeranyl transfer at the end of the reaction was only 10% lower than that transferred in the absence of unlabeled GGPP, which indicates that the radiolabeled geranylgeranyl group was transferred to peptide without significant release from the enzyme, and thus the observed prenyl transfer rate reflects single turnover of the ternary complex.

Rapid quench experiments with the complex formed from 1.5 μM PGGT-I and 1 μM [^{3}H]FPP mixed with a solution of 40 μM biotin-γ_6-CAIL and 10 μM GGPP yielded an observed first-order rate constant of 0.015 ± 0.004 s^{-1} (Fig. 4*B*, Table II), which is 37-fold smaller than the rate constant for geranylgeranyl transfer. In these experiments, higher concentrations of reactants were used since [^{3}H]FPP binds about 330-fold weaker to PGGT-I than does [^{3}H]GGPP. In addition, 10 μM unlabeled GGPP was included so that single turnover from the PGGT-I·[^{3}H]FPP·biotin-γ_6-CAIL complex is being measured owing to the fact that once [^{3}H]FPP dissociates from the enzyme, it will be irreversibly replaced by GGPP.

Rapid quench experiments were also performed with PFT acting on biotin-TKCVIM. A solution of PFT (150 nM) and [^{3}H]FPP (110 nM) was mixed with biotin-TKCVIM (4 μM). The farnesyl group is transferred to the peptide with a first-order rate constant of 1.9 ± 0.7 s^{-1} (not shown). The rate was essentially the same when 2 μM unlabeled FPP or GGPP was present in the peptide solution (1.8 ± 0.4 s^{-1} and 3.3 ± 0.3 s^{-1}, respectively). The rate constant for farnesyl transfer is similar to the value of 0.44 s^{-1} previously reported for PFT-catalyzed farnesylation of biotin-GLPCVVM (40).

Prenyl Acceptor Specificity Studies—In vitro prenylation studies with the prenyl acceptors K-Ras4B-CVIM, RhoB-CKVL, and TC21-CVIF have shown that these proteins can be farnesylated or geranylgeranylated (21, 30, 35, 36), and these reactions were studied in more detail in the present study. The relative affinities of PGGT-I and PFT for GGPP and FPP described above are preserved in the presence of these protein substrates. This is shown in Table I. For example, the geranylgeranylation of K-Ras4B-CVIM by PGGT-I in the presence of 1 μM [^{3}H]GGPP is not inhibited if 1 μM FPP is also present, but farnesylation of K-Ras4B-CVIM by PGGT-I with 1 μM [^{3}H]FPP is abolished if 1 μM GGPP is also present. Likewise, the farnesylation of the same protein, K-Ras4B-CVIM by PFT in the presence of 1 μM [^{3}H]FPP is not inhibited if 1 μM GGPP is present, but the geranylgeranylation reaction in the presence of 1 μM [^{3}H]GGPP is inhibited by 77% if 1 μM FPP is also present. Similar results are obtained with RhoB-CKVL and TC21-CVIF (Table I).

Competition between prenyl acceptors for geranylgeranylation by PGGT-I·[^{3}H]GGPP and for farnesylation by PFT·[^{3}H]FPP was also studied, and the results are summarized in Table III.

DISCUSSION

The results in this study clearly show that both PGGT-I and PFT display binding selectivity for their respective prenyl pyrophosphate substrates. The 330-fold higher affinity of GGPP *versus* FPP for PGGT-I does not contradict the published data showing that micromolar amounts of PGGT-I are capable of binding both GGPP and FPP (30) (also confirmed in our lab). Since the K_d values for the complexes of PGGT-I with GGPP and FPP are 3 nM and 1 μM, respectively, micromolar amounts of enzyme can be titrated with micromolar amounts of GGPP or FPP. It is only when the concentration of PGGT-I is near the K_d = 3 nM for GGPP, as is the case in the present study, that the very high affinity of PGGT-I for GGPP can be detected. The same applies to PFT (29), and in the present study using low concentrations of PFT it is found that FPP binds about 15-fold tighter to PFT than does GGPP.

TABLE III
Competition between prenyl acceptors

Enzyme/prenyl donor	Competing prenyl acceptors[a]	Protein:peptide mole ratio	Prenylated protein: prenylated peptide mole ratio
PGGT-I/[^{3}H]GGPP	K-Ras4B-CVIM *vs.* biotin-γ_6-CAIL	1	0.11
		2	0.36
PFT/[^{3}H]FPP	K-Ras4B-CVIM *vs.* biotin-lamin B-CAIS	1	37.1
		0.1	2.4
PGGT-I/[^{3}H]GGPP	TC21-CVIF *vs.* biotin-γ_6-CAIL	1	0.13
		2	0.28
PFT/[^{3}H]FPP	TC21-CVIF *vs.* biotin-lamin B-CAIS	1	0.09
		2	0.21

[a] The concentration of all protein prenyl acceptors is 2 μM.

As shown previously, values of k_{cat} are similar for PGGT-I-catalyzed geranylgeranylation and farnesylation of biotin-γ_6-CAIL under conditions where both prenyl donor and acceptor are saturating (26). However, this *in vitro* situation is not relevant to the situation that exists inside the cell where both GGPP and FPP are present and compete for binding to PGGT-I. Under competitive conditions, geranylgeranylation is greatly preferred over farnesylation unless the concentration of FPP in the cell is more than 330-fold higher than the concentration of GGPP, which seems unlikely. Even if there is a small amount of PGGT-I·FPP in the cell, the K_M for the interaction of this binary complex with biotin-γ_6-CAIL is 29-fold higher than the K_M for the interaction of PGGT-I·GGPP with the same peptide (26), and this would disfavor the farnesylation reaction. In the presence of saturating biotin-γ_6-CAIL, values of K_M for FPP and GGPP are similar (26). However, this situation is also not relevant to the reaction in the cell or *in vitro* since although prenyl acceptor can bind before prenyl donor, the PGGT-I-catalyzed reaction cycle involves the preferential binding of GGPP prior to prenyl acceptor (26). The same is true for PFT, which preferentially binds FPP before prenyl acceptor (25), and binds FPP 15-fold tighter than GGPP. Since the selectivity of PFT for binding FPP *versus* GGPP is not so rigid, it is difficult at this point to say with confidence that PFT will be loaded only with FPP *in vivo*, especially since the relative sizes of the FPP and GGPP *in vivo* pools are not known.

For the reasons given above, it seems likely that in the cell PFT and PGGT-I are fully loaded with their respective prenyl pyrophosphate, and one may now ask which prenyl acceptors do these binary complexes choose. Such a selection is dictated by steady-state Equation 2.

$$\frac{\nu_{PA1}}{\nu_{PA2}} = \frac{\left(\frac{k_{cat}}{K_M}\right)_{PA1}[PA1]}{\left(\frac{k_{cat}}{K_M}\right)_{PA2}[PA2]} \qquad \text{(Eq. 2)}$$

Equation 2 relates the ratio of velocities, $(\nu_{PA1})/(\nu_{PA2})$, for prenylation of two competing prenyl acceptors, PA1 and PA2, respectively, to the ratio of concentrations of the prenyl acceptors and the ratio of k_{cat}/K_M values for PA1 and PA2. Equation 2 applies to both the PGGT-I·GGPP and PFT·FPP complexes. In this equation, k_{cat} is the turnover number under conditions in which both prenyl donor and prenyl acceptor are saturating, and K_M is the Michaelis constant for the interaction of the prenyl acceptor with the prenyltransferase·prenyl pyrophosphate binary complex. In the case of PGGT-I-catalyzed geranylgeranylation of biotin-γ_6-CAIL, which has a C-terminal leucine, *versus* the biotin-γ_6-CAIL analog in which the C-terminal leucine is changed to serine, the former is calculated to be preferentially geranylgeranylated by 121-fold using the published kinetic data (26). This preference is due to a 10-fold larger k_{cat} and a 12-fold lower K_M for the reaction of the peptide ending in leucine *versus* the one ending in serine, and thus there is k_{cat} and K_M selectivity for the geranylgeranyl acceptor.

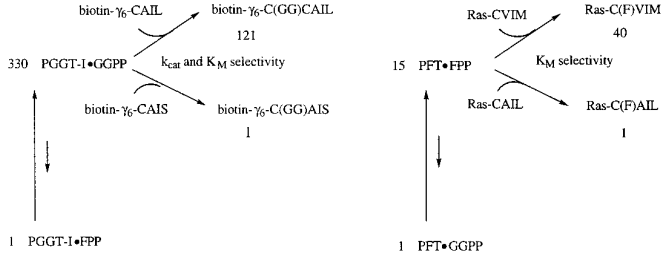

FIG. 5. **Specificity schemes for PGGT-I and PFT.** *Left*, the 330-fold preference for GGPP *versus* FPP binding to PGGT-I is shown. After initial binding of GGPP, the PGGT-I·GGPP chooses biotin-γ_6-CAIL over biotin-γ_6-CAIS by a factor of 121. *Right*, PFT binds FPP 15-fold tighter than GGPP, and the PFT-I·FPP complex chooses H-Ras-CVIM over H-Ras-CAIL by a factor of 40.

Kinetic data for PFT-catalyzed farnesylation of Ras-CVIM (farnesyl acceptor) and Ras-CAIL (geranylgeranyl acceptor) are available (25). According to Equation 2 and the published data, the PFT·FPP complex prefers Ras-CVIM over Ras-CAIL by a factor of 40 (relative k_{cat}/K_M values). Interestingly, the k_{cat} for farnesylation of Ras-CVIM is 2.5-fold smaller than the k_{cat} for farnesylation of Ras-CAIL. However, the 80-fold lower K_M for Ras-CVIM compared with Ras-CAIL more than offsets the difference in k_{cat} values, and thus Ras-CVIM is the preferred substrate based on K_M selectivity. These specificity features of PGGT-I and PFT are summarized in Fig. 5.

As described in the Introduction, the GTPases RhoB-CKVL, TC21-CVIF, and K-Ras4B-CVIM, which are members of the Ras superfamily of proteins, have been reported to undergo both farnesylation and geranylgeranylation. The presence of these proteins in reaction mixtures did not influence the preference of PFT and PGGT-I in binding FPP and GGPP, respectively (Table I). Thus, the prenylation selectivity of these proteins will be dictated by their k_{cat}/K_M values in comparison to those of other prenyltransferase substrates present in the cell according to Equation 2. When K-Ras4B-CVIM competes against an equal concentration of biotin-γ_6-CAIL (the γ_6 protein is known to be geranylgeranylated *in vivo* (45)) for geranylgeranylation by PGGT-I·GGPP, the peptide is preferred by 8.7-fold. Competition between K-Ras4B-CVIM and biotin-lamin B-CAIS (the lamin B protein is known to be farnesylated *in vivo* (46)), for farnesylation by PFT-I·FPP leads to almost exclusive farnesylation of the Ras protein. These results are consistent with those of James *et al.* (21) who showed that the K_M for the interaction of K-Ras4B-CVIM with PFT·FPP is about 10-fold smaller than the K_M for the interaction of the same protein with PGGT-I·GGPP; the k_{cat} for these two reactions are similar. These results give only the relative velocities for the pair of prenyl acceptors studied. In the cell K-Ras4B-CVIM is competing with many other substrates, and the relative k_{cat}/K_M values for these other substrates are not known. Nevertheless, the results suggest that K-Ras4B-CVIM will nor-

mally become farnesylated in cells, but if a PFT-selective inhibitor is used, K-Ras4B-CVIM can effectively compete with geranylgeranyl acceptors for geranylgeranylation by PGGT-I. This point has obviously important consequences for the design of prenyltransferase inhibitors as anti-cancer drugs. Indeed, James *et al.* (47) showed that a PFT-selective inhibitor does block Ras-dependent activation of the mitogen-activated protein kinase cascade when the cell is activated by oncogenic H-Ras. In contrast, when this cascade is regulated by endogenous Ras, the PFT inhibitor has no effect. K-Ras4B-CVIM may be the endogenous activator of mitogen-activated protein kinase in the cells studied, and inhibition of PFT may cause K-Ras4B-CVIM to become geranylgeranylated by PGGT-I with restoration of its signaling function (47).

TC21-CVIF, which is a Ras-related protein with oncogenic potential, has been shown *in vitro* to be farnesylated by PFT and geranylgeranylated by PGGT-I (36). In the present study, it is shown that TC21-CVIF can be a substrate for PGGT-I and PFT because it competes as well with biotin-γ_6-CAIL for geranylgeranylation by PGGT-I as it does with lamin B for farnesylation by PFT (Table III), although the peptide substrates are 10-fold preferred in both cases. The Caa*X* sequence of TC21 is unusual in that it has a C-terminal phenylalanine. However, this is probably not the reason that TC21 is a substrate for both PGGT-I and PFT because another GTPase CDC42, which also has a C-terminal phenylalanine, is a much better substrate for PGGT-I than for PFT (34). Interestingly, the transformation of cells by oncogenic TC21-CVIF is not inhibited by a selective PFT inhibitor, and the present results support the suggestion of Carboni and co-workers (36) that TC21-CVIF may be geranylgeranylated in cells treated with PFT inhibitors. The type of prenyl group attached to TC21-CVIF in cells treated or not treated with prenyltransferase inhibitors remains to be determined.

Furfine and co-workers (40) measured the single turnover rate of conversion of the PFT·FPP·peptide-CVVM complex to products and obtained a value of 0.06 s^{-1}. Interestingly, this value is 7.3-fold smaller than the k_{cat} for the steady-state reaction when both prenyl donor and acceptor are saturating. This implies that a step after the conversion of the ternary complex to the enzyme product complex is rate-determining for steady-state turnover. The slow step may be release of prenylated peptide from the enzyme (40). The same appears to be true for the PGGT-I·GGPP·biotin-γ_6-CAIL complex, which undergoes conversion to product with a single turnover rate constant of 0.56 s^{-1} under conditions where binding of GGPP or peptide to the enzyme is not rate-determining (Table II). With the same preparation of PGGT-I, the k_{cat} for steady-state turnover in the presence of saturating amounts of both biotin-γ_6-CAIL and GGPP is 0.032 s^{-1}. Thus the single turnover geranylgeranyl transfer rate constant is 14-fold faster than the steady-state turnover number, which establishes that a step after prenyl transfer, such as product release, is rate-determining. The conversion of PGGT-I·FPP·biotin-γ_6-CAIL to products occurs with a 37-fold smaller rate constant (0.015 s^{-1}) than the analogous geranylgeranylation reaction, and this value is similar to the steady-state k_{cat} of 0.012 s^{-1} measured with saturating amounts of FPP and biotin-γ_6-CAIS. Thus, the rate of PGGT-I-catalyzed farnesylation is probably limited by the chemical step of prenyl transfer. Thus, the similar observed values of k_{cat} for PGGT-catalyzed geranylgeranylation and farnesylation (26) is not due to similar rate constants for the prenyl transfer step.

The biphasic binding of [^{3}H]GGPP to PGGT-I purified in the presence of 1 mM DTT (Fig. 1*A*) was unexpected. The weaker binding component is not due to nonspecific adsorption of [^{3}H]GGPP to PGGT-I since [^{3}H]GGPP bound in this weaker complex is transferred to peptide without exchange with free GGPP in the buffer. Since the PGGT-I used in this study is recombinant and originates from plaque-purified baculoviruses expressing the α and β subunits of this enzyme, the low and high affinity binding of [^{3}H]GGPP is an intrinsic property of this enzyme and is not due to contamination by another prenyltransferase. This enzyme can be converted into a form that shows only monophasic, high affinity binding of GGPP by incubating it with higher amounts of DTT (5 mM). PGGT-I does not seem to exist as a mixture of multimers since enzyme handled with 1 mM DTT or treated with 5 mM DTT elute in the same volume when applied to a gel filtration column, and the elution volume is reasonable for a heterodimer of ≈90 kDa. PGGT-I contains many cysteines, and the two forms of PGGT-I may have a different pattern of intramolecular disulfides. Prenyl transfer catalyzed by these two forms occurs with similar kinetics since no evidence for a multi-exponential process was seen in the rapid quench experiments (Fig. 4, *A* and *B*).

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