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Prenylation of proteins in *Trypanosoma brucei*

Kohei Yokoyama [a], Ying Lin [a], Kenneth D. Stuart [b,c], Michael H. Gelb [a,*]

[a] *Departments of Chemistry and Biochemistry, Box 351700, University of Washington, Seattle, WA 98195–1700, USA*
[b] *Department of Pathobiology, Box 357238, University of Washington, Seattle, WA 98195–7238, USA*
[c] *Seattle Biomedical Research Institute, Seattle, WA 98109–1651, USA*

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Abstract

Prenyl modification of proteins by farnesyl and geranylgeranyl isoprenoids occurs in a variety of eukaryotic cells. Culturing of *Trypanosoma brucei* in the presence of [^{3}H]mevalonolactone (which is hydrolyzed in cells to give mevalonic acid, the precursor of protein prenyl groups) and an inhibitor of mevalonic acid biosynthesis leads to the radiolabeling of a specific set of proteins when analyzed by gel electrophoresis. *T. brucei* proteins were also labeled when cells were cultured in the presence of [^{3}H]farnesol or [^{3}H]geranylgeraniol, and each prenol labels a distinct set of proteins. Unlike mammalian cells, only a few *T. brucei* proteins of molecular weights similar to those of the mammalian Ras superfamily of GTPase (20–30 kDa) were labeled with [^{3}H]farnesol or [^{3}H]geranylgeraniol. When the 0–55% ammonium sulfate fraction of *T. brucei* cytosol was fractionated on anion exchange chromatography, protein farnesyltransferase (PFT) and protein geranylgeranyltransferase-I (PGGT-I) activities were detected and elute as two distinct peaks. Partially purified *T. brucei* PFT and PGGT-I display partly different specificities toward prenyl acceptor substrates from those of mammalian protein prenyltransferases. As shown previously, rat PFT utilizes proteins ending in CVLS and CVIM as efficient prenyl acceptors and rat PGGT-I utilizes proteins ending in CVLL and CVIM in vitro. On the contrary, *T. brucei* PFT farnesylates a protein ending in CVIM but not CVLS or CVLL, and *T. brucei* PGGT-I preferentially geranylgeranylates a protein ending in CVLL. © 1997 Elsevier Science B.V.

Keywords: Prenylation; Isoprenylation; *Trypanosoma brucei*; Trypanosomatidae; Farnesylation; Geranylgeranylation; Protein farnesyltransferase; Protein geranylgeranyltransferase; Mevalonic acid

Abbreviations: Biotin-Lamin-B-CAIS, biotin-CONH-$(CH_2)_5$-CO-GTPRASNRSCAIS; Biotin-γ6-CAIL, biotin-CONH-$(CH_2)_5$-CO-NPFREKKFFCAIL; [^{3}H]FOH, [1-^{3}H]farnesol; FPP, farnesyl pyrophosphate; [^{3}H]FPP, [1-^{3}H]farnesyl pyrophosphate; [^{3}H]GGOH, [1-^{3}H]geranylgeraniol; GGPP, geranylgeranyl pyrophosphate; [^{3}H]GGPP, [1-^{3}H]geranylgeranyl pyrophosphate; H-Ras-CVLS, His_6-tagged human H-Ras produced in *E. coli*; H-Ras-CVLL, a His_6-tagged human H-Ras mutant produced in *E. coli* and with a C-terminal sequence CVLL; RAS1-CVIM, a yeast RAS1 mutant produced in *E. coli* and with a C-terminal sequence CVIM; [^{3}H]MVL, RS-[5-^{3}H]mevalonolactone; PFT, protein farnesyltransferase; PGGT-I, protein geranylgeranyltransferase type I; CaaX, C-terminal signal sequence for PFT and PGGT-I substrates.

* Corresponding author. Tel.: +1 206 5437142; fax: +1 206 6858665; e-mail: gelb@chem.washington.edu

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1. Introduction

Protein prenylation is a recently discovered post-translational modification that occurs in many eukaryotic cells [1,2]. The three structural classes of protein prenylation that have been identified are C-terminal farnesylation (15-carbon isoprenoid) [3,4], C-terminal geranylgeranylation (20-carbon isoprenoid) [5,6], and C-terminal digeranylgeranylation [7]. Mammalian cells contain on the order of 100 distinct prenylated proteins, and many of these are probably small GTPases that are members of the Ras superfamily [8]. Other examples of prenylated proteins include nuclear lamins [9], heterotrimeric G protein γ-subunits [10,11], and hepatitis δ-virus large antigen [12]. Although the precise functions of protein prenyl groups remains to be established, there is general consensus that they can function to bind proteins to cell membranes.

Prenyl groups are attached to proteins via a thioether linkage to one or more cysteines near or at the C-terminus of the protein, and the attachment is catalyzed by protein prenyltransferases [13,14]. Protein farnesyltransferase (PFT) catalyzes the transfer of the farnesyl group from farnesyl pyrophosphate (FPP) to the cysteine residue in the C-terminal motif CaaX, where C is cysteine, a is usually but not necessarily an aliphatic residue, and X is glutamine, serine, methionine and probably a limited number of other residues. Protein geranylgeranyltransferase-I (PGGT-I) transfers the geranylgeranyl group from geranylgeranyl pyrophosphate (GGPP) to the cysteine of the C-terminal motif CaaL/F. Finally, protein geranylgeranyltransferase-II (also known as Rab geranylgeranyltransferase) attaches two geranylgeranyl groups to proteins with C-terminal sequences CCXX, CXC, and CC [15]. All three types of enzymes have been purified from mammalian sources, and all of the components have been cloned [14]. PFT and PGGT-I share a common α-subunit but have distinct β-subunits. Rab geranylgeranyltransferase is composed of two distinct subunits and a third subunit that delivers the Rab protein to the transferase.

Recent studies have shown that protein prenylation occurs in the parasites *Giardia lamblia* [16] and *Schistosoma mansoni* [17]. Culturing of Giardia trophozoites in media containing tritiated mevalonolactone ([^{3}H]MVL) (this compound is hydrolyzed to give radiolabeled mevalonic acid, the precursor of prenyl groups) leads to radiolabeling of several parasite proteins, some of which are small GTPases ($\lesssim$21–30 kDa). Competitive inhibitors of hydroxymethylglutaryl-CoA reductase, the enzyme that makes mevalonic acid, block parasite growth, and growth resumes upon addition of mevalonate to the medium. Incubation of schistosomes with radiolabeled mevalonate leads to the predominant labeling of 25- and 43-kDa proteins. The 43-kDa protein becomes labeled when shistosomal homogenate is incubated with labeled FPP, whereas addition of radiolabeled GGPP leads to the labeling of the 25-kDa protein. Since the prenylation of proteins is required for the function of a number of important regulators of cell growth, inhibitors of these enzymes are likely to have therapeutic potential for the treatment of parasitic diseases. In this paper, we summarize our initial findings showing that protein farnesylation and geranylgeranylation occur in *Trypanosoma brucei*, the causative agent of sleeping sickness. We have also identified and partially purified a PFT and a PGGT-I from this microorganism.

2. Materials and methods

2.1. Materials

[^{3}H]FPP and [^{3}H]GGPP (both 15 Ci mmol^{-1}) are from American Radiolabeled Chemicals, and [^{3}H]MVL (40 Ci mmol^{-1}) is from NEN. [^{3}H]FOH (3.7 Ci mmol^{-1}) was prepared as described [18], and [^{3}H]GGOH (60 Ci mmol^{-1}) was obtained from NEN. The radiochemical purity of all isoprenoids was periodically assessed by thin layer chromatography [19], and the compounds were used only if their purity was $>$80%. Saponified simvastatin was obtained as a generous gift from Professor A. Corsini (University of Milan). Biotin-Lamin B-CAIS and biotin-γ6-CAIL were prepared as described [19]. H-Ras-CVLS and H-Ras-CVLL were obtained as generous gifts

from Professor G. James (University of Texas), and RAS1-CVIM is a generous gift from Dr C. Omer (Merck). Recombinant rat PFT and PGGT-I were produced using Sf9 insect cell/baculovirus expression system and purified as described [20]. Recombinant baculoviruses that express the α- and β-subunits of rat PFT were obtained as generous gifts from Professor Y. Reiss (University of Tel-Aviv) and that which expresses the β-subunit of rat PGGT-I was obtained as a generous gift from Professor J. Goldstein (University of Texas).

2.2. Culturing of cells and in vivo radiolabeling

Trypanosoma brucei brucei EATRO 160 was cultured at 27°C in SDM79 medium containing 10% fetal calf serum (FCS). Radiolabeling experiments were carried out by culturing cells (1×10^7 cells ml^{-1} at 27°C for 24 h in 1 ml SDM79 medium/10% FCS containing 40 μM saponified simvastatin and either 2.5 μM [^{3}H]MVL (100 μCi), 10 μM [^{3}H]FOH (37 μCi), or 5 μM [^{3}H]GGOH (60 μCi). The cells were harvested by centrifugation at $6000 \times g$ for 10 min, and the pellet was washed twice with 1 ml of ice-cold phosphate buffered saline (PBS) and then suspended in 1 ml of cold lysis buffer (20 mM Tris–HCl (from a 1 M stock), pH 8.0, 50 mM NaCl, 1 mM DTT [added fresh]) containing freshly added protease inhibitors: PMSF, 1 mM (from a 0.1 M stock in isopropanol); TLCK and TPCK, 30 μM each (from 0.1 M stocks in DMF); 0.01 mg ml^{-1} each of aprotinin and leupeptin (from 10 mg ml^{-1} stocks in water); and pepstatin A, 0.01 mg ml^{-1} (from a 2 mg ml^{-1} stock in methanol). Cells were disrupted in a Branson sonicator using a microtip probe (10×6 s pulses). An equal volume of ice-cold 20% trichloroacetic acid was added, the samples were placed on ice for 30 min, and the mixtures were centrifuged for 2 min at approx. $10\,000 \times g$. The pellet was delipidated by washing three times with 1 ml of ice-cold absolute ethanol as follows. After addition of ethanol, the samples were sonicated briefly to disperse the pellet, and the protein was pelleted as above. The resulting pellet was boiled with 40 μl of Laemmli sample buffer containing 2% 2-mercaptoethanol, and 20 μl aliquots were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (12.5% Laemmli gel). The gel was subjected to fluorography as described [18].

Human skin fibroblasts were obtained from human volunteers and cultured as described [21] in 2 ml Dulbecco's modified Eagle medium (DMEM) containing 10% FCS in a 12-well plate (0.15×10^6 cells/well). Cells were cultured overnight at 37°C in a humidified atmosphere of 5% CO_2. Prior to labeling, cells were synchronized in G_0 phase by culturing at 37°C for 72 h in 2 ml medium containing 0.4% FCS. For radiolabeling, the medium was removed and replaced with fresh medium containing 10% FCS (1 ml per well), 40 μM saponified simvastatin, and either 2.5 μM [^{3}H]MVL (100 μCi), 10 μM [^{3}H]FOH (37 μCi), or 5 μM [^{3}H]GGOH (60 μCi). Cells were incubated at 37°C for 24 h. After removal of the medium, the cells were rinsed twice with 1 ml of PBS. The cells were scraped from the plate, suspended in 0.5 ml of ice-cold lysis buffer (see above), and disrupted in a 2 ml Potter–Elvehjem homogenizer. Proteins were precipitated and delipidated as above. The samples were analyzed by SDS-PAGE (12.5% Laemmli gel), and the gel was visualized by fluorography.

2.3. Partial Purification of T. brucei PFT and PGGT-I.

T. brucei cells from a 4 l culture (1×10^8 cells) were collected by centrifugation at $6000 \times g$ for 10 min at 4°C and washed with 100 ml of 20 mM sodium phosphate buffer, pH 7.9, containing 0.15 M NaCl and 20 mM glucose. The cell pellet was resuspended in 1 mM Tris–HCl, 1 mM EDTA, pH 8.0, at a cell density of 1.2×10^9 cells ml^{-1}, and the suspension was homogenized in a Dounce homogenizer with a tight fitting pestle B (five strokes). To the homogenate was added 1.75 M sucrose solution to give a final sucrose concentration of 0.25 M. The lysate was centrifuged at $15\,800 \times g$ for 10 min at 4°C, and to the resulting supernatant were added the following components at the indicated final concentrations: 20 mM Tris–HCl (from a 1 mM stock), pH 8.0, 5 mM DTT (as powder), 5 μM $ZnCl_2$, and protease inhibitors (as above). The

mixture was centrifuged at 120 000 × *g* for 80 min at 4°C. The supernatant (cytosol fraction) contained 564 mg of protein (Bio-Rad Bradford assay using bovine serum albumin as a standard) and was subjected to protein precipitation with 55% saturated ammonium sulfate at 0°C. The precipitate was dialyzed overnight against 20 mM Tris–HCl, pH 8.0, 50 mM NaCl, 5 mM DTT and 5 μM $ZnCl_2$ (buffer A, 3 exchanges, 2 liters each).

The dialyzed 0–55% ammonium sulfate fraction (92 mg of protein) was loaded onto a column of Q-Sepharose Fast Flow (Pharmacia, 2.6 × 7.5 cm) previously equilibrated with buffer A at 2 ml min^{-1} using FPLC (Pharmacia). The column was washed with buffer A at the same flow rate for 200 min and then developed with a gradient of buffer A and buffer B (buffer A containing 1 M NaCl) as follows: 0–160 min, 0–60% buffer B; 160–180 min, 60–100% buffer B; 180–220 min, buffer B. Fractions of 5 ml were collected, and the protein content was estimated from the absorbance of the fractions at 280 nm. The fractions were kept at 4°C overnight and then assayed for prenyltransferase activities.

PFT activity was assayed in buffer (30 mM potassium phosphate, 5 mM DTT, 0.5 mM $MgCl_2$, 20 μM $ZnCl_2$, pH 7.7 [adjusted after all components present]) containing 15 μM RAS1-CVIM and 1 μM [^{3}H]FPP (0.3 μCi), and 4 μl of Q-Sepharose fraction. The mixtures (20 μl total volume) were incubated at 30°C for 15 min, and the amount of radiolabeled farnesylated protein was quantified with the glass fiber filter method [22]. Assay of PFT activity was also carried out by incubating 8 μl of Q-Sepharose fraction at 30°C for 1 h with 5 μM biotin-Lamin B-CAIS and 1 μM [^{3}H]FPP (0.3 μCi). The amount of radiolabeled farnesylated peptide was quantified using the avidin-agarose method as described [19]. PGGT-I activity was assayed by incubating 8 μl of Q-Sepharose fraction in the same buffer at 30°C for 1 h with 1 μM [^{3}H]GGPP (0.3 μCi) and 15 μM H-Ras-CVLL or 5 μM biotin-Lamin B-CAIS as a prenyl acceptor substrate. The amounts of radiolabeled geranylgeranylated protein and peptide were quantified as described above for PFT assays.

3. Results and discussion

3.1. In vivo prenylation of proteins in T. brucei

The most common method to detect prenylation of proteins in eukaryotic cells is to culture them in the presence of [^{3}H]MVL, which is hydrolyzed in cells to tritiated mevalonic acid, and an inhibitor of hydroxymethylglutaryl-CoA reductase [23]. Mevalonic acid is the universal precursor to the isoprenoids, and inhibition of hydroxymethylglutaryl-CoA reductase is needed to shutdown mevalonic acid biosynthesis so that the specific radioactivity of mevalonate is not significantly reduced by dilution with unlabeled mevalonate present in cells. As shown in Fig. 1, culturing *T. brucei* in medium containing [^{3}H]MVL and the hydroxymethylglutaryl-CoA reductase inhibitor simvastatin gives rise to a specific set of radiolabeled proteins when analyzed by SDS-PAGE (proteins are delipidated prior to

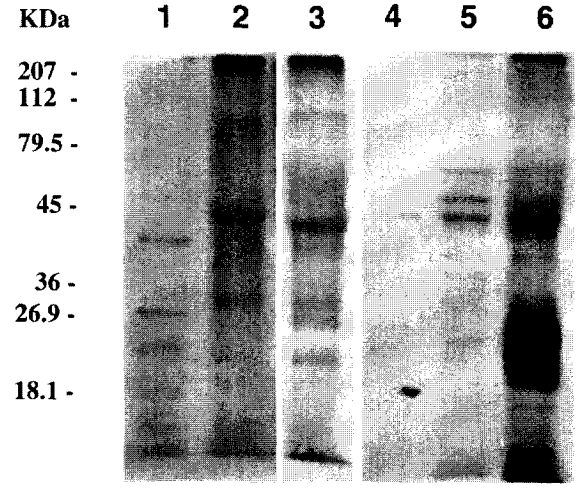

Fig. 1. In vivo prenylation of proteins in *T. brucei*. *T. brucei* cells were labeled for 24 h with either 2.5 μM (100 μCi) [^{3}H]MVL (lane 1), 10 μM (37 μCi) [^{3}H]FOH (lane 2), or 5 μM (60 μCi) [^{3}H]GGOH (lane 3) in the presence of 40 μM saponified simvastatin, as described in Section 2. Radiolabeled proteins were anaylzed by SDS-PAGE on a 12.5% gel followed by fluorography. For comparison, human skin fibroblast cells were labeled for 24 h with either [^{3}H]MVL (lane 4), [^{3}H]FOH (lane 5), or [^{3}H]GGOH (lane 6). X-ray films were exposed for 4 days (lane 3) or 2 weeks (all lanes except for lane 3).

loading onto the gel, see Section 2). We (A. Corsini, C. Farnsworth, P. McGeady, M. Gelb, J. Glomset, unpublished) and others [24,25] have shown that culturing mammalian cells with [^{3}H]FOH or [^{3}H]GGOH leads to radiolabeling of prenylated proteins. It has been hypothesized that these prenols are taken up by cells and converted to their corresponding pyrophosphates by one or more unknown kinases. As shown by SDS-PAGE analysis (Fig. 1), different patterns of radiolabeled prenylated proteins are seen when *T. brucei* was cultured in the presence of [^{3}H]FOH or [^{3}H]GGOH, and in the presence of simvastatin. These results strongly suggest that specific sets of *T. brucei* proteins undergo farnesylation or geranylgeranylation. For comparison, prenylated proteins from human skin fibroblasts labeled with either [^{3}H]MVL, [^{3}H]FOH, or [^{3}H]GGOH are shown in Fig. 1. As expected, the mammalian cells labeled with [^{3}H]GGOH yielded intense bands of MW $\lesssim$ 20–30 kDa; these represent the geranylgeranylation of a large number of GTPases that are members of the Ras superfamily [1]. Interestingly, relatively few 20–30 kDa radiolabeled prenylated proteins are seen in *T. brucei* labeled with all three isoprenoid precursors (Fig. 1). This suggests either that *T. brucei* contains few, if any, 20–30 kDa GTPases (none are found in the protein sequence data bases) or that such proteins have significantly different molecular weights from their mammalian homologues. These results represent the first demonstration of protein farnesylation and geranylgeranylation in trypanosomes.

3.2. Detection and partial purification of T. brucei PFT and PGGT-I

There is no available information in the current protein sequence data bases about potential prenylated proteins of *T. brucei* that contain the C-terminal CaaX motif. Thus, to detect protein prenyltransferases in *T. brucei*, we used known protein and peptide substrates of mammalian protein prenyltransferases such as RAS1-CVIM [26] and biotin-Lamin B-CAIS [19] for PFT and H-Ras-CVLL [27] and biotin-γ6-CA/L [19] for PGGT-I. Using biotin-Lamin B-CAIS/[^{3}H]FPP and H-Ras-CVLL/[^{3}H]GGPP to detect PFT and PGGT-I activities, respectively, in crude *T. brucei* cytosol, high levels of cpm were detected in control assays in the absence of prenyl acceptors, and no significant levels of the enzyme activity above the controls were detected (not shown). This high background radioactivity increased as a function of incubation time suggesting that unknown radiolabeled isoprenoids were formed from [^{3}H]FPP or [^{3}H]GGPP and bound non-specifically to avidin-agarose or glass fiber filters. This material seems to be a high MW or an insoluble isoprenoid(s) that elutes in the void volume of a Sephadex G-50 column. However, this material is not mainly prenylated proteins since only trace amounts of radioactive protein bands were detected on SDS-PAGE analysis. No rapid degradation of [^{3}H]FPP and [^{3}H]GGPP by phosphatases was observed during incubation with the cytosol since thin layer chromatographic analyses [19] of the reaction mixtures revealed intact prenyl pyrophosphates with only small amounts of [^{3}H]FOH, [^{3}H]GGOH, and the corresponding monophosphates. Therefore, it is likely either that the levels of protein prenyltransferases in the crude cytosol are too low to be measured with these assays, or that the chosen prenyl acceptors are not recognized by the *T. brucei* enzymes.

When crude *T. brucei* cytosol proteins were precipitated with 55% saturated ammonium sulfate and then fractionated by anion exchange chromatography on Q-Sepharose, PFT and PGGT-I activities were detected in the column fractions as separate peaks (Fig. 2). A single peak of PFT activity was detected using RAS1-CVIM and [^{3}H]FPP as substrates, and the level of activity (about 40 000 cpm) in the peak fraction was approximately 200-fold higher than minus prenyl acceptor or boiled enzyme control (about 200 cpm). Geranylgeranyl transferring activity to RAS-CVIM was not detected in the PFT peak, confirming that the farnesyl transferring activity is not due to PGGT-I activity. A similar peak of PFT activity was also detected using biotin-Lamin B-CAIS as a prenyl acceptor substrate (Fig. 2) although the activity was about 10-fold lower than that measured by the assay with RAS1-CVIM. Unexpectedly, no significant amount of

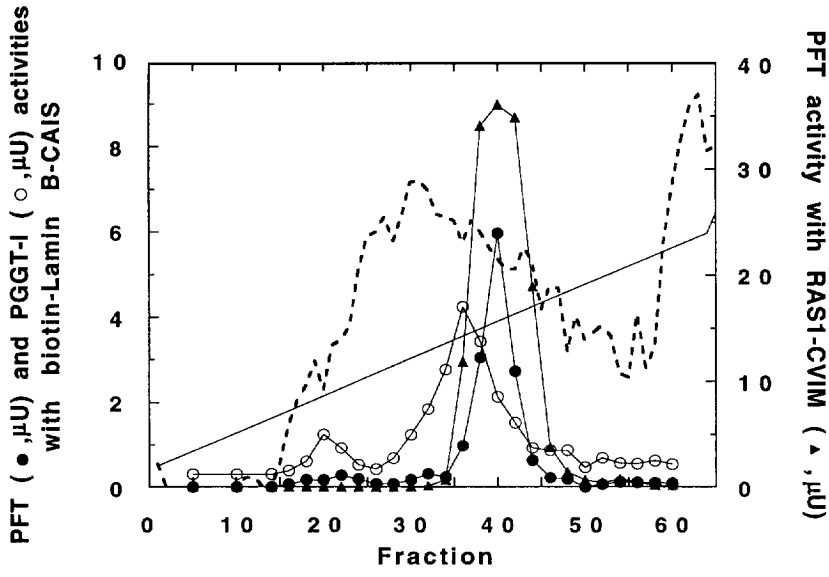

Fig. 2. Chromatography of *T. brucei* PFT and PGGT-I on Q-Sepharose. The 0–55% ammonium sulfate fraction (92 mg of protein) of *T. brucei* cytosol (from 4 l culture) was fractionated on a Q-Sepharose anion exchange column (2.6 × 7.5 cm). A gradient elution with NaCl from 0.05 to 0.6 M (——) starts from fraction 1 and ends at fraction 64. The elution profile of protein was monitored by measuring absorbance at 280 nm (— — —). PFT activity was assayed using [^{3}H]FPP/RAS1-CVIM (▲) or [^{3}H]FPP/biotin-Lamin B-CAIS (●) as substrates. PGGT-I activity was assayed using [^{3}H]GGPP/biotin-Lamin B-CAIS (○). One micro unit of the enzyme activity is defined as the amount of enzyme that produces 1 pmol of prenylated peptide or protein per minuit under the standard conditions.

PGGT-I activity was detected using biotin-γ6-CAIL which is a good substrate of mammalian PGGT-I [19]. A significant amount of PGGT-I activity (about 8000 cpm, 3-fold higher than the controls) was detected using biotin-Lamin B-CAIS and eluted in a separate peak from the PFT peak (Fig. 2). As shown previously, biotin-Lamin B-CAIS can be prenylated by both mammalian PFT and PGGT-I although it is a much more preferred substrate for the PFT over the PGGT-I [19]. H-Ras-CVLL also served as a geranylgeranyl acceptor when the same fractions were incubated with [^{3}H]GGPP but the detected activity was only about 300 cpm above controls (about 200 cpm). The relatively low level of the PGGT-I activity detected may be due to the possibility that H-Ras-CVLL and biotin-Lamin B-CAIS are poor substrates of the *T. brucei* enzyme.

Fig. 3 shows SDS-PAGE analyses of prenylated proteins produced by the partially purified *T. brucei* PFT and PGGT-I along with those produced by recommbinant rat PFT and PGGT-I for comparison. The Q-Sepharose fractions 40 and 34 (Fig. 2) were used as sources of the *T. brucei* PFT and PGGT-I, respectively. Protein substrates used are H-Ras-CVLL and H-Ras-CVLS, which are typical substrates for mammalian PGGT-I and PFT [27,28], respectively, and RAS-CVIM containing the C-terminal CaaX sequence of human K-RasB that can be both farnesylated by mammalian PFT and geranylgeranylated by mammalian PGGT-I in vitro [20,29]. The radiolabeled protein produced by incubation of *T. brucei* PFT (fraction 40) with RAS1-CVIM and [^{3}H]FPP co-migrates with the corresponding product of recombinant rat PFT, [^{3}H]farnesyl-RAS1-CVIM (Fig. 3, panel A). The product from incubation of *T. brucei* PGGT-I (fraction 34) with H-Ras-CVLL and [^{3}H]GGPP co-migrates with [^{3}H]geranylgeranyl-H-Ras-CVLL produced by recombinant rat PGGT-I (Fig. 3, panel B). It is also shown that *T. brucei* protein prenyltransferases display different prenyl acceptor specificities from those of mammalian CaaX protein prenyltrans-

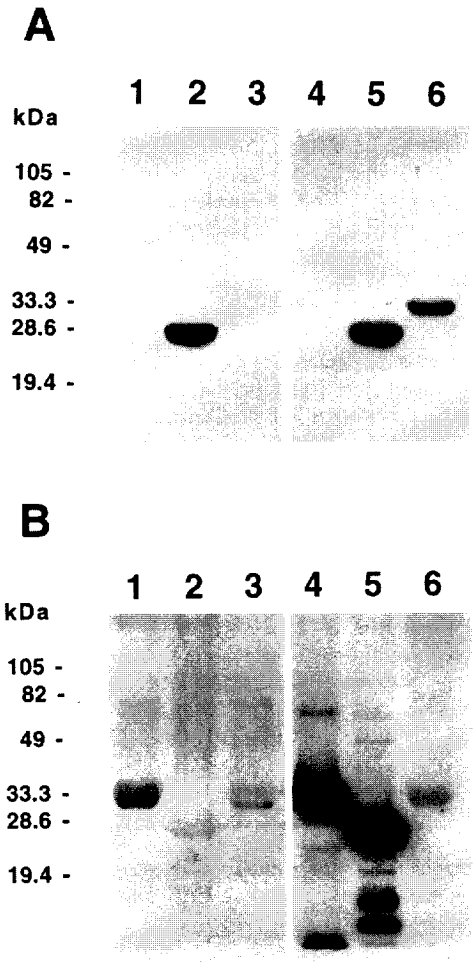

Fig. 3. SDS-PAGE analysis of prenylated proteins produced by partially purified *T. brucei* PFT and PGGT-I. A, partially purified *T. brucei* PFT (the Q-Sepharose fraction 40 [0.74 μg of protein]) was incubated at 30°C for 15 min with 2 μM (0.6 μCi) [^{3}H]FPP and either 15 μM H-Ras-CVLL (lane 1), 15 μM RAS1-CVIM (lane 2), or 15 μM H-Ras-CVLS (lane 3). Radiolabeled proteins were analyzed by SDS-PAGE on a 12.5% gel followed by fluorography. For comparison, recombinant rat PFT (0.05 μg) was incubated with [^{3}H]FPP and either H-Ras-CVLL (lane 4), RAS1-CVIM (lane 5), or H-Ras-CVLS (lane 6) under the same conditions. B, the Q-Sepharose fraction 34 (3.7 μg of protein) that contains PGGT-I activity was incubated at 30°C for 60 min with 2 μM [^{3}H]GGPP and either 15 μM H-Ras-CVLL (lane 1), 15 μM RAS1-CVIM (lane 2), or 15 μM H-Ras-CVIS (lane 3). Recombinant rat PGGT-I (0.01 μg) was also incubated with [^{3}H]GGPP and either H-Ras-CVLL (lane 4), RAS1-CVIM (lane 5), or H-Ras-CVLS (lane 6) under the same conditions.

ferses. *T. brucei* PFT farnesylates RAS1-CVIM but not H-Ras-CVLS or H-Ras-CVLL while rat PFT farnesylates both RAS1-CVIM and H-Ras-CVLS (Fig. 3, panel A). *T. brucei* PGGT-I transfers a small but significant amount of [^{3}H]geranylgeranyl group to H-Ras-CVLL but did not transfer significant amounts of the radioactivity to RAS1-CVIM and H-Ras-CVIS. In contrast, rat PGGT-I efficiently geranylgeranylates both H-Ras-CVLL and RAS1-CVIM (Fig. 3, panel B).

In mammals and *Saccharomyces cerevisiae*, the C-terminal CaaL motif-containing proteins and peptides are preferred prenyl acceptor substrates for their respective PGGT-I versus PFT [19,30–32]. Interestingly, biotin-γ6-CAIL was effectively farnesylated by *T. brucei* PFT, but it was not detectively prenylated by *T. brucei* PGGT-I (not shown). Recent studies have also shown that multiple species of PFT present in *Schizosaccharomyces pombe* can farnesylate a peptide containing the C-terminal CVIL as effectively as a C-terminal peptide of a known farnesylated protein, Ras1 ending in CVIC [33].

Further studies are underway to purify *T. brucei* PFT and PGGT-I to homogeneity and determine their precise specificities with respect to prenyl acceptors. These studies are a prelude to the possible development of trypanosome-selective protein prenyltransferase inhibitors that may have utility in the treatment of trypanosomiasis. The preliminary substrate specificity patterns exhibited by *T. brucei* PFT and PGGT-I revealed in this study provide optimism for the possibility of preparing inhibitors of these enzymes that do not block the action of their human counterparts.

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