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The effects of protein farnesyltransferase inhibitors on trypanosomatids: inhibition of protein farnesylation and cell growth

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Abstract

Attachment of the prenyl groups farnesyl and geranylgeranyl to specific eukaryotic cell proteins by protein prenyltransferases is required for the functioning of a number of cellular processes including signal transduction. In this study it was found that previously reported inhibitors of mammalian protein farnesyltransferase (PFT) [those that mimic the substrate farnesyl pyrophosphate and those that mimic the protein acceptor of the farnesyl group (CaaX mimetic)] inhibit in vitro farnesylation catalyzed by partially purified *Trypanosoma brucei* (*T. brucei*) PFT. The most potent PFT inhibitors at concentrations of 3–10 μM inhibit the growth of insect (procyclic) and bloodstream forms of *T. brucei*. One of the PFT inhibitors was found to block the incorporation of radiolabeled mevalonic acid (the precursor of prenyl groups) into specific *T. brucei* proteins. This study also shows that protein prenylation occurs in

Abbreviations: CaaX, C-terminal signal sequence of PFT and protein geranylgeranyltransferase-I substrates (C is cysteine, a is usually but not always an aliphatic residue, and X is a variety of amino acids); Farnesyl-NH-PMM, amide formed between 2-phosphonomethylmalonic acid and farnesyl amine [farnesyl-NHCOCH(COO^-)($CH_2PO_3^{2-}$)]; Farnesyl-O-NH-PA, Farnesyl-O-NH-phosphonylacetyl [farnesyl-O-NHCOCH$_2$PO$_3^{2-}$]; Farnesyl-O-NH-PA ester, $CH_2OCOC(CH_3)_3$ ester of farnesyl-O-NH-PA [farnesyl-O-NHCOCH$_2$PO(O-)(OCH$_2$OCOC(CH$_3$)$_3$]; FPP, Farnesyl pyrophosphate; [^{3}H]FPP, [1-^{3}H]farnesyl pyrophosphate; HMG-CoA, hydroxymethylglutaryl coenzyme A; *L. mexicana*, *Leishmania mexicana*; [^{3}H]MVL, *RS*-[5-^{3}H]mevalonolactone; RAS1-CVIM, a yeast RAS1 mutant produced in *E. coli* and with C-terminal sequence CVIM; rCrVFM, reduced cysteinyl-reduced valyl-phenylalanyl-methionine [*N*-[2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3-methylbutyl-Phe -Met]; PFT, protein farnesyltransferase; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; *T. brucei*, *Trypanosoma brucei*; *T. cruzi*, *Trypanosoma cruzi*.

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the protozoan parasites *Trypanosoma cruzi* (*T. cruzi*) and *Leishmania mexicana* (*L. mexicana*). The growth of *T. cruzi* intracellular form (amastigote) is also sensitive to PFT inhibitors, whereas the insect form (epimastigote) is considerably more resistant to inhibition of protein farnesylation. On the other hand, growth of 3T3 fibroblast cells (host cells for amastigote growth) was not affected by up to 100 μM PFT inhibitors. The growth of *L. mexicana* insect form (promastigote) is modestly inhibited by protein farnesyltransferase inhibitors. These results suggest the potential for the development of PFT inhibitors for treating trypanosomiasis and leishmaniasis.

Keywords: Prenylation; Isoprenylation; *Trypanosoma brucei*; *Trypanosoma cruzi*; *Leishmania mexicana*; Trypanosomatidae; Farnesylation; Geranylgeranylation; Protein farnesyltransferase; Mevalonic acid; Simvastatin; Parasites; Anti-parasite therapeutics

1. Introduction

Post-translational modification of proteins by the attachment of 15-carbon farnesyl or 20-carbon geranylgeranyl groups to the C-termini of specific eukaryotic cell proteins is a recently discovered process [1,2]. Examples of prenylated proteins include nuclear lamin B [3], many of the small GTPases that are members of the Ras superfamily [4], heterotrimeric G protein γ-subunits [5,6], and hepatitis δ virus large antigen [7]. Although the precise functions of protein prenyl groups remains to be established, there is general consensus that they function to bind proteins to cell membranes and they may direct protein–protein interactions.

Mammalian 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 [8]. Mammalian protein geranylgeranyltransferase-I transfers the geranylgeranyl group from geranylgeranyl pyrophosphate to the cysteine of the C-terminal motif CaaL/F [9,10]. Finally, protein geranylgeranyltransferase-II (also known as Rab geranylgeranyltransferase) attaches two geranylgeranyl groups to proteins with C-terminal sequences CCXX, CXC, and CC [11].

Recent studies have shown that protein prenylation occurs in the parasites *Giardia lamblia* [12], *Schistosoma mansoni* [13], and *T. brucei* [14,15]. To date none of these prenylated parasite proteins have been identified with certainty. Candidate parasite prenylated proteins include the γ-subunits of heterotrimeric G proteins, which have been detected or inferred to exist in *T. cruzi* [16–21], *T. brucei* [22], and *Leishmania donovani* [23]; however, protein sequences are not yet available for any of these protozoan G proteins. Rab GTP-binding proteins have been cloned from *T. brucei* [24–26], *Leishmania major* [27], and *Leishmania amazonensis* [28], and these have C-terminal double cysteine moieties (DSCC, GGCC, CAC), which suggests that they are doubly geranylgeranylated by Rab geranylgeranyltransferase [11]. To the best of our knowledge no other candidate prenylated proteins in trypanosomatids have been identified. Thus, sequences of CaaX-containing proteins from trypanosomatids are not yet available. *T. brucei* PFT has been detected and partially purified from the procyclic form [15].

In the present study it is shown that protein prenylation also occurs in *T. cruzi* and in *L. mexicana*, and that previously reported inhibitors of mammalian PFT also inhibit *T. brucei* PFT in vitro and block the prenylation of specific proteins in vivo. Studies also show that PFT inhibitors block trypanosomatid cell growth.

2. Materials and methods

2.1. Materials

[^{3}H]FPP (15 Ci mmol^{-1}) and [^{3}H]MVL (15 Ci mmol^{-1}) are from American Radiolabeled Chemicals. The radiochemical purity of [^{3}H]FPP was periodically assessed by thin layer chromatography [29], and the compound was used only if its purity

was >80%. Saponified simvastatin was obtained as a generous gift from Professor A. Corsini (University of Milan). *T. brucei* PFT used for in vitro inhibition studies was obtained by partial purification from the cytosolic fraction of procyclic parasites (through the Q-Sepharose step, and the enzyme was assayed with RAS1-CVIM/[^{3}H]FPP [15]). Recombinant rat PFT was obtained as described [30]. PFT inhibitors were obtained from the following sources: rCrVFM (Bachem); farnesyl-NH-PMM, farnesyl-*O*-NH-PA, and farnesyl-*O*-NH-PA ester (all from CalBiochem).

2.2. Growth of T. brucei procyclic and bloodstream forms and radiolabeling studies

The procyclic form of *Trypanasoma brucei brucei* strain IsTar 1.7 was cultured in upright 25 cm^2 tissue culture flasks (Corning) in 10 ml SDM-79 medium containing penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), and 10% heat inactivated fetal bovine serum (Atlantic Biologicals) at 27°C with shaking [31]. The bloodstream form, Strain 427, was cultured in HMI-9 medium containing penicillin, streptomycin and 10% fetal bovine serum in stationary flasks at 37°C with 5% CO_2 [32]. Growth curve experiments were carried out by culturing cells ($\sim 4 \times 10^6$ cells ml^{-1} for procyclic form or $\sim 5 \times 10^4$ cells ml^{-1} for bloodstream form) for up to 120 h in 24-well tissue culture plates in 1 ml of media with or without simvastatin or PFT inhibitors (added from aqueous stock solutions). Cells were counted daily using a hemacytometer. For phase contrast microscopy to monitor cell shape, $1-5 \times 10^5$ cells were diluted with an equal volume of PBS, and the suspension was centrifuged ($700 \times g$, 10 min). Pellets were washed once in PBS and resuspended in 100 μl. Each sample (10 μl) was placed on a 14-well (5 mm) slide, and cells were incubated for 10 min in a moist chamber. Cells were fixed for 5 min in 4.5% paraformaldehyde, 100 mM potassium phosphate, pH 7. After washing three times (5 min each) with PBS, cells were photographed with a Nikon microphot-FX microscope using metamorph Imaging System (Universal Image).

For metabolic labeling experiments with [^{3}H]MVL, the [^{3}H]MVL solution (400 μCi in 0.4 ml of ethanol) was evaporated to dryness with a stream of N_2, and the residue was dispersed in 80 μl fetal bovine serum by sonication for 2 min in a bath sonicator. An aliquot of the solution was counted to assure that most of [^{3}H]MVL was dispersed. *T. brucei* bloodstream form (1×10^7 cells) was incubated for 24 h at 37°C with or without 25 μM farnesyl-*O*-NH-PA ester under 5% CO_2 in 1 ml HMI-9 medium/10% fetal bovine serum containing 6.7 μM [^{3}H]MVL (100 μCi in 20 μl of fetal bovine serum) and 40 μM saponified simvastatin. The cells were harvested by centrifugation at $2000 \times g$ for 5 min. After washing twice with 1 ml of ice-cold PBS, the cell pellet was suspended in 1 ml of cold lysis buffer (20 mM Tris–HCl, pH 8.0, 50 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 30 μM each of tosyl-lysine chloromethylketone and tosyl-phenylalanine chloromethylketone, and 10 μg ml^{-1} each of aprotinin, leupeptin, and pepstatin A). To lyze cells, the suspension was frozen at −80°C and then quickly thawed at 37°C, and 130 μl of ice-cold 100% trichloroacetic acid was added to the lysate. The mixture was placed on ice for 30 min and centrifuged for 10 min at 10,000 × g. The pellet was delipidated by washing three times with 1 ml of ice-cold absolute ethanol as described [15]. The resulting pellet was dispersed in 20 μl H_2O by sonication and boiled with Laemmli sample buffer (40 μl total volume) containing 2% 2-mercaptoethanol. Aliquots (20 μl) of the samples were analyzed by SDS-PAGE on a 12.5% gel, and the gel was subjected to fluorography as described [30].

2.3. Growth, radiolabeling, and inhibition studies with T. cruzi epimastigotes and L. mexicana promastigotes

The Tulahuen strain of *T. cruzi* was provided by S. Reed (Infectious Diseases Research Institute, Seattle, WA), and the CL strain was provided by H. Eisen (Fred Hutchinson Cancer Research Institute, Seattle, WA). The Tulahuen strain has been stably transfected with the *E. coli* β-galactosidase gene (*LacZ*) so that intracellular parasite growth could be monitored with a colorimetric assay [33]. Epimastigotes were grown in liver infusion tryptone broth with 10% heat inactivated fetal bovine

serum, penicillin, and streptomycin as described [34]. Trypomastigotes/amastigotes were grown on monolayers of mouse 3T3 fibroblasts in DMEM containing 10% fetal bovine serum, penicillin, and streptomycin as described [34]. *L. mexicana*, strain M379, was kindly provide by B. Ullman (Oregon Health Sciences University, Portland, OR). Promastigotes were grown in Medium 199 (BioWhitacker) containing 10% fetal bovine serum, penicillin, streptomycin, and 25 mM HEPES, pH 7.4.

For radiolabeling studies, *T. cruzi* epimastigotes (wild type CL strain) or *L. mexicana* promastigotes (10^7 cells, taken from a mid-log culture) were cultured for 24 h in 1 ml of medium containing 100 μCi (6.7 μM) [^{3}H]MVL and 40 μM simvastatin. Protein from cells was delipidated and submitted to SDS-PAGE as described above for *T. brucei*.

Growth inhibition studies with *T. cruzi* amastigotes were carried out using the β-galactosidase assay as described [33]. Growth inhibition studies with *T. cruzi* epimastigotes and *L. mexicana* promastigotes were carried out by counting cells microscopically with a hemacytometer. Inhibitors were added to culture medium from stock solutions in water. For all studies with *T. cruzi*, cell numbers were obtained 7 days after addition of inhibitor. *L. mexicana* was cultured for 4 days with inhibitors prior to cell quantification.

2.4. Inhibition of partially purified T. brucei PFT

In vitro PFT inhibition studies with CaaX and FPP analogs were carried out with partially purified *T. brucei* PFT and also with recombinant rat PFT for comparison. Inhibition assays were carried out with scintillation proximity assay technology (Amersham). Reactions (100 μl) contained 30 mM potassium phosphate, pH 7.7, 0.5 mM $MgCl_2$, 10 μM $ZnCl_2$, 5 mM dithiothreitol, 200 nM substrate peptide Biotin-CONH$(CH_2)_5$CONH-TKCVIM (synthesized as described in [29]), 134 nM [^{3}H]FPP and PFT. Inhibitors were added from a stock solution in DMSO so that the amount of solvent did not exceed 5% in the assay (controls were done with DMSO alone). After incubation at 30°C for 30 min, reactions were quenched by adding 150 μl of stop reagent (prepared by diluting scintillation proximity assay beads (20 mg ml^{-1} in PBS/0.05% NaN_3) with 10 volumes of 1.5 M magnesium acetate, 200 mM phosphoric acid, 0.5% bovine serum albumin). Samples were submitted to scintillation counting.

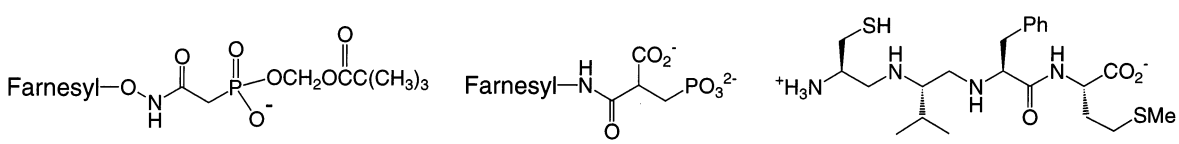
Table 1
Inhibition of partially purified *T. brucei* and rat PFTs by FPP and CaaX analogs

Inhibitor	IC_{50} (μM)	
	T. brucei PFT	Rat PFT
Farnesyl-NH-PMM	10	30
Farnesyl-O-NH-PA	0.6	0.5
rCrVFM	0.07	0.004

3. Results and discussion

3.1. Inhibition of T. brucei PFT by CaaX and FPP analogs

A large number of inhibitors of mammalian PFT have been described [35]. In this study it was found that the CaaX analog rCrVFM and the FPP analog farnesyl-O-NH-PA significantly inhibited *T. brucei* PFT, whereas the FPP analog farnesyl-NH-PMM was much less potent (Table 1). The structures of all inhibitors studied are given in Fig. 1. Farnesyl-O-NH-PA ester is the pro-drug form of farnesyl-O-NH-PA and was used only for cell culture studies.



Farnesyl-O-NH-PA ester Farnesyl-NH-PMM rCrVFM

Fig. 1. Structures of the PFT inhibitors farnesyl-O-NH-PA ester, farnesyl-NH-PMM, and rCrVFM.

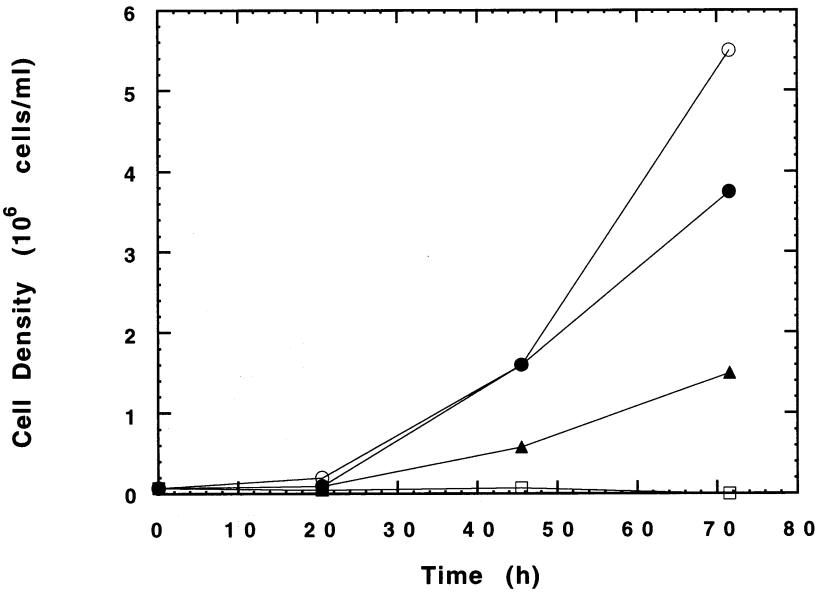


Fig. 2. Growth inhibition of bloodstream form *T. brucei* by the PFT inhibitor farnesyl-O-NH-PA ester. Bloodstream form *T. brucei* (7.5×10^4 cells ml^{-1}) was cultured with 0 (○), 1 (●), 10 (▲), or 25 μM (□) farnesyl-O-NH-PA ester. Cells were counted daily.

Inhibition data for rat PFT is also included in Table 1. IC_{50} values of these FPP analogs for *T. brucei* PFT are comparable to those for rat PFT, whereas rCrVFM is about 18-fold more potent against the mammalian enzyme.

3.2. Effect of PFT inhibitors and simvastatin on T. brucei growth

All inhibitors studied have been shown to block protein farnesylation in mammalian cells [35], which argues that they are cell permeable (it is somewhat suprising that the anionic FPP analogs penetrate cell membranes). IC_{50} values for growth inhibition by PFT inhibitors are summarized in Table 2. Fig. 2 shows that farnesyl-O-NH-PA ester causes inhibition of growth of *T. brucei* bloodstream form in a dose-dependent manner, and 25 μM PFT inhibitor completely inhibits cell growth. As shown in Fig. 3, 25 μM farnesyl-*O*-NH-PA ester causes noticeable cell deformation after 6 h, and virtually all cells are lyzed after 15 h (micrographs taken at 24 and 48 h look indistinguishable from that taken at 15 h). Similar progression of cell lysis occurs with rCrVFM (not shown). These micrographs show that PFT inhibitors are cytotoxic rather than cytostatic.

For each of the three FPP analogs tested, values of IC_{50} for growth inhibition of procyclic and bloodstream forms were similar. Procyclic form was about 10-fold more sensitive to rCrVFM than was bloodstream form. The most potent inhibitors of growth were farnesyl-O-NH-PA and rCrVFM, which were effective at low micromolar concentrations. Farnesyl-NH-PMM, which was 17-fold less potent than farnesyl-O-NH-PA and rCrVFM as an inhibitor of PFT in vitro, displayed 5- to 10-fold lower potency in growth inhibition assays. The pro-drug farnesyl-O-NH-PA ester was only slightly more potent than farnesyl-O-NH-PA at inhibiting growth of cultured parasites, which suggests that both drugs penetrate the parasite cell membrane. The fact that growth was inhibited by both a CaaX analog and FPP analogues suggests that *T. brucei* protein prenyltransferases, probably PFT, is the target for the growth inhibition by these compounds.

The HMG-CoA reductase inhibitor simvastatin, which is known to block the production of prenyl pyrophosphates including FPP and isoprenoid pathway end products such as cholesterol in mammalian cells, was also found to inhibit parasite growth. Interestingly, bloodstream form *T. brucei* was approximately 100-fold more sensi-

Table 2
Growth inhibition of *T. brucei*, *T. cruzi*, and *L. mexicana* by PFT inhibitors

Inhibitor	IC_{50} (μM)					
	3T3 fibroblast	*T. brucei* insect form	*T. brucei* mammalian blood-stream form	*T. cruzi* mammalian intracellular form	*T. cruzi* insect form	*L. mexicana* insect form
Farnesyl-NH-PMM	>100	40	50	50	>50	100
Farnesyl-*O*-NH-PA	>100	5	7	10	>100	>100
Farnesyl-*O*-NH-PA ester	>100	3	5	20	>100	>100
rCrVFM	>100	5	50	20	>100	30
Simvastatin	not determined	150	2	not determined	>100	50

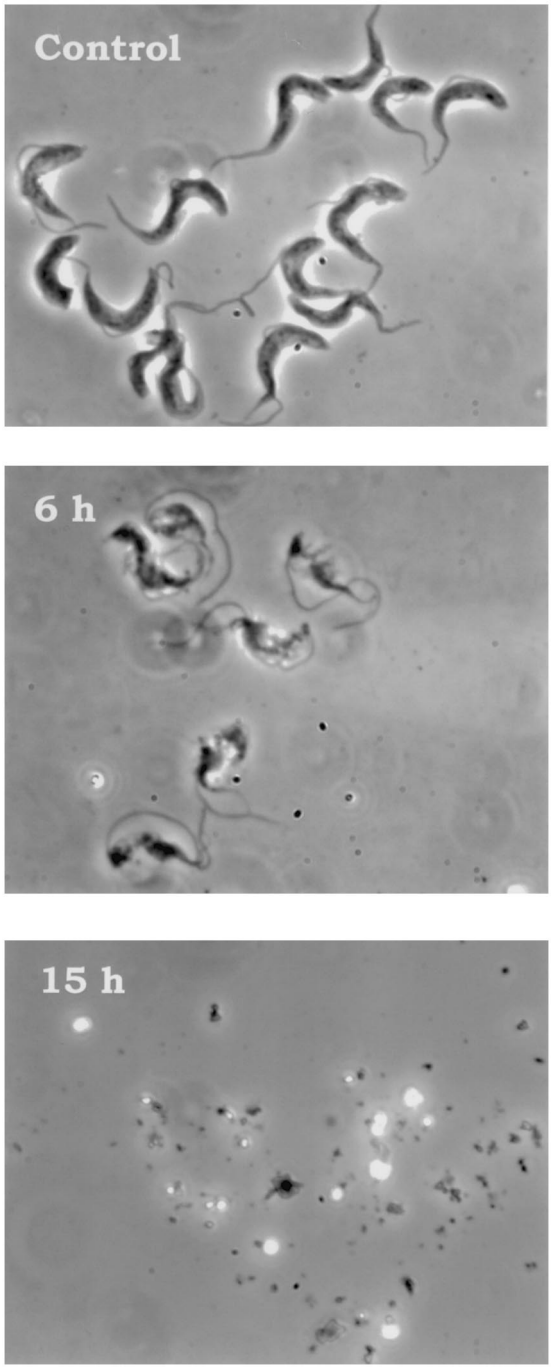


Fig. 3. Phase contrast microscopic images of bloodstream form *T. brucei* treated with farnesyl-O-NH-PA ester. Bloodstream form *T. brucei* was incubated without inhibitor (*Top*), with 25 μM farnesyl-O-NH-PA ester for 6 h (*Middle*), or 15 h (*Bottom*). Cells were fixed and visualized as described in Section 2.

tive than procyclic form to growth inhibition by this compound (Table 2). Field and coworkers recently reported that another HMG-CoA reductase inhibitor, compactin, also preferentially inhibits bloodstream form growth [14].

3.3. Effect of farnesyl-O-NH-PA ester on in vivo protein prenylation in T. brucei bloodstream form

As shown previously, a specific set of procyclic *T. brucei* proteins became radiolabeled when cells were grown in media containing [^{3}H]MVL [14,15]. Simvastatin was also added to media to prevent isotopic dilution of [^{3}H]mevalonic acid by endogenously produced mevalonic acid. As shown in Fig. 4, protein labeling also occurred when bloodstream *T. brucei* was grown with [^{3}H]MVL and simvastatin. Bloodstream *T. brucei* proteins were labeled significantly better with [^{3}H]MVL/simvastatin than were proteins in procyclic cells (compare Fig. 4 to Fig. 1 of Ref [15]). One possible explanation for this differential labeling is that the endogenous pool of mevalonic acid is greater in the procyclic form than in the bloodstream form, which would explain why the latter are more sensitive to simvastatin. If this is true, then the specific radioactivity of ^{3}H-mevalonic acid in the procyclic form would be less than in bloodstream parasites, which would lead to less labeling of procyclic cell proteins. The concentration of simvastatin used in radiolabeling experiments was 40 μM, which is 4-fold below the IC_{50} for growth inhibition of *T. brucei* procyclic form, and is 20-fold higher than the IC_{50} seen with the bloodstream form (Table 2).

Next, we tested the effect of PFT inhibitor farnesyl-O-NH-PA ester on radiolabeling of *T. brucei* bloodstream proteins. As shown in Fig. 4, the presence of 25 μM farnesyl-O-NH-PA ester, which was sufficient to completely block parasite growth, led to significant suppression of the labeling of a subset of prenylated proteins (those with molecular weights of 45, 60, 90, and 110 kDa). The levels of prenylation of several proteins were not detectably affected

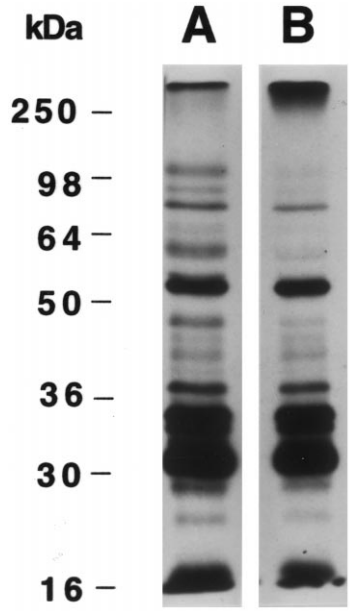


Fig. 4. Inhibition of in vivo protein prenylation by farnesyl-O-NH-PA ester in bloodstream form *T. brucei*. Bloodstream form *T. brucei* was labeled for 24 h with 6.7 μM (100 μCi) [^{3}H]MVL and 40 μM simvastatin in the absence (lane A) or presence (lane B) of 25 μM farnesyl-O-NH-PA. Radiolabeled proteins were analyzed by SDS-PAGE on a 12.5% gel followed by fluorography. Film exposure time was 5 days.

by farnesyl-O-NH-PA ester. Most notable were proteins in the molecular weight range of 25 to 35 kDa. At least some of these proteins may be *T. brucei* homologs of mammalian Rab GTPases. Such proteins have been recently identified in *T. brucei* by molecular cloning [24–26]. Rab proteins contain a pair of C-terminal cysteines and are substrates for mammalian protein geranylgeranyltransferase-II [11,36]. Rab proteins may be geranylgeranylated by the *T. brucei* homolog of this enzyme. This enzyme activity has been recently detected in *T. brucei* cytosol using mammalian Rab6 as a substrate (Yokoyama and Gelb, unpublished), and the radiolabeling data in Fig. 4 would suggest that farnesyl-O-NH-PA does not inhibit this enzyme. Some of the radiolabeled proteins may be mono-geranylgeranylated by a protein geranylgeranyltransferase-I [36], but the existence of this enzyme is not certain because only very low levels have been detected in *T. brucei* cytosol [15]. Together, the results in Fig. 4 and Table 2 are consistent with the idea that the inhibition of *T. brucei* growth by PFT inhibitors is due to inhibition of parasite protein farnesylation, but other effects of PFT inhibitors cannot be ruled out.

3.4. Protein prenylation in T. cruzi and L. mexicana and effect of PFT inhibitors

To examine whether protein prenylation also occurs in *T. cruzi* and *L. mexicana*, insect forms of *T. cruzi* (epimastigote) and *L. mexicana* (promastigote) were incubated with [^{3}H]MVL in the presence of simvastatin. As shown in Fig. 5, a number of radiolabeled proteins from both protozoans are seen on fluorographs of SDS-PAGE gels. Proteins in insect forms of *T. cruzi* and *L. mexicana* seem to radiolabel about as well with [^{3}H]MVL/simvastatin, based on cpm incorporated, as did proteins in *T. brucei* insect form labeled in the same way [15]. As with *T. brucei*, prominent labeled proteins of MW 30 and 50 kDa are seen.

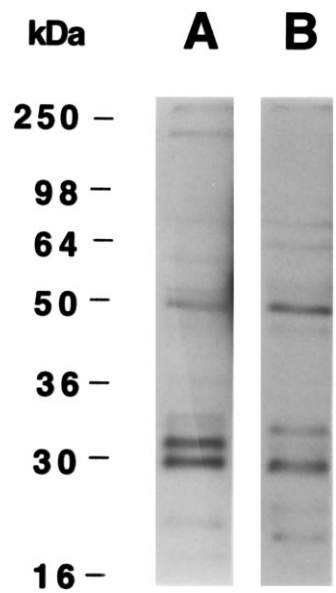


Fig. 5. Prenylation of proteins in *T. cruzi* and *L. mexicana*. *T. cruzi* epimastigotes (lane A) or *L. mexicana* promastigotes (lane B) were labeled for 24 h with 6.7 μM (100 μCi) [^{3}H]MVL in the presence of 40μM simvastatin. Radiolabeled proteins were analyzed by SDS-PAGE on a 12.5% gel followed by fluorography. Exposure time was 4 days.

The mammalian intracellular form of *T. cruzi* (amastigote) grown in mouse 3T3 host cells, and insect forms of *T. cruzi (*epimastigote) and *L. mexicana* (promastigote) were tested for growth inhibition by PFT inhibitors (Table 2). Growth of *T. cruzi* amastigotes was inhibited by both CaaX and FPP analogs with IC_{50} values ranging from 10 to 50 μM, while none of the PFT inhibitors affected the growth of 3T3 host cells when tested at concentrations up to 100 μM. In contrast, *T. cruzi* epimastigotes were resistant to 50–100 μM PFT inhibitors. The only PFT inhibitor to block the growth of *L. mexicana* promastigotes was rCrVFM, which displayed an IC_{50} of 30 μM. Further studies are needed to explore the sensitivities of *T. cruzi* and *L. mexicana* PFTs to PFT inhibitors. It also remains to be seen if these PFT inhibitors are toxic to *L. mexicana* amastigotes.

4. Closing remarks

The fact that growth of *T. brucei*, *T. cruzi*, and *L. mexicana* is blocked by PFT inhibitors suggests that the trypanosomatid PFT is a good target for the development of drugs for treating sleeping sickness, Chagas' disease, and leishmaniasis. It should be possible to obtain parasite-selective CaaX mimetic PFT inhibitors, since *T. brucei* PFT displays very different specificity for the CaaX substrate compared to the mammalian enzyme [15]. The fact that mammalian PFT is 18-fold more sensitive to rCrVFM than *T. brucei* PFT (Table 1), is another indication that the CaaX binding regions of these enzymes are distinct. A perfectly parasite-selective PFT inhibitor may, however, not be needed as a lead compound for drug development. This is based on the fact that 3T3 cell growth is not affected by PFT inhibitors (Table 2). In fact, the growth of mammalian cells is generally not affected by PFT inhibitors, but protein geranylgeranyltransferase-I inhibitors tend to be cytostatic [37]. For some mammalian cell proteins that are normally farnesylated such as K-Ras, it has been shown that treatment of cells with PFT inhibitors leads to the geranylgeranylation of these proteins presumably by protein geranylgeranyltransferase-I, which is not inhibited by these PFT inhibitors [38,39]. As noted above, *T. brucei* may lack protein geranylgeranyltransferase-I, and this may contribute to the sensitivity of this protozoan to PFT inhibitors. Finally, it should be noted that there is tremendous effort devoted toward the development of mammalian PFT inhibitors because such compounds are showing great promise for the treatment of human malignancies [40]. Thus, it should be possible to make use of the wealth of information on mammalian PFT inhibitors for the development of inhibitors of trypanosomatid PFTs once these latter enzymes have been purified and characterized.

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