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Identification and preliminary characterization of protein-cysteine farnesyltransferase

(*ras* oncogenes/p21 proteins/CAAX-box motif/farnesyl pyrophosphate)

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ABSTRACT **Ras proteins must be isoprenylated at a conserved cysteine residue near the carboxyl terminus (Cys-186 in mammalian Ras p21 proteins) in order to exert their biological activity. Previous studies indicate that an intermediate in the mevalonate pathway, most likely farnesyl pyrophosphate, is the donor of this isoprenyl group. Inhibition of mevalonate synthesis reverts the abnormal phenotypes induced by the mutant *RAS2*$^{Val-19}$ gene in *Saccharomyces cerevisiae* and blocks the maturation of *Xenopus* oocytes induced by an oncogenic Ras p21 protein of human origin. These results have raised the possibility of using inhibitors of the mevalonate pathway to block the transforming properties of *ras* oncogenes. Unfortunately, mevalonate is a precursor of various end products essential to mammalian cells, such as dolichols, ubiquinones, heme A, and cholesterol. In this study, we describe an enzymatic activity(ies) capable of catalyzing the farnesylation of unprocessed Ras p21 proteins *in vitro* at the correct (Cys-186) residue. This farnesylating activity is heat-labile, requires Mg^{2+} or Mn^{2+} ions, is linear with time and with enzyme concentration, and is present in all mammalian cell lines and tissues tested. Gel filtration analysis of a partially purified preparation of protein farnesyltransferase revealed two peaks of activity at 250–350 kDa and 80–130 kDa. Availability of an *in vitro* protein farnesyltransferase assay should be useful in screening for potential inhibitors of *ras* oncogene function that will not interfere with other aspects of the mevalonate pathway.**

ras genes code for a family of small (21-kDa) guanine nucleotide-binding proteins that interact with the inner side of cellular membranes (1). Genetic studies have indicated that such interaction requires a defined carboxyl-terminal structure (2–4). This structure, known as the CAAX box, consists of a conserved cysteine residue at position 186, two aliphatic amino acids, and a carboxyl-terminal amino acid residue. Mutations within the CAAX-box motif of oncogenic Ras p21 proteins abolished their transforming activity, presumably by impeding their interaction with the inner side of the plasma membrane (2–4). Such interaction requires a series of post-translational modifications within the CAAX box motif: (*i*) S-farnesylation of Cys-186, (*ii*) cleavage of the three carboxyl-terminal amino acid residues, and (*iii*) methylation of the carboxyl-terminal farnesylcysteine residue (5–9). The association of farnesylated Ras p21 proteins with cellular membranes is further strengthened by palmitoylation of neighboring upstream cysteine residues (7, 10–12).

The donor of the farnesyl moiety present in Ras p21 proteins is likely to be farnesyl pyrophosphate (FPP), a precursor of cholesterol biosynthesis (13). Genetic studies in *Saccharomyces cerevisiae* have shown that deprivation of mevalonate (a precursor of FPP) in mutants deficient in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme responsible for mevalonate synthesis, restores the normal phenotype to cells that either overexpress the wild-type *RAS2* gene or carry a mutated *RAS2*$^{Val-19}$ allele (14). Similarly, inhibitors of HMG-CoA reductase blocked the maturation of *Xenopus* oocytes induced by a transforming Ha-Ras^{Val-12} protein (14). These results have raised the possibility of using available inhibitors of cholesterol biosynthesis to block neoplastic transformation induced by *ras* oncogenes. However, FPP not only is a precursor in the biosynthesis of cholesterol but also participates in the synthesis of other essential compounds such as ubiquinones, dolichols, and heme A (13). Therefore, it is likely that attempts to block *ras*-induced transformation by inhibiting the synthesis of FPP will cause major disruptions in other cellular pathways.

We reasoned that a more logical approach would be to target a step subsequent to FPP biosynthesis, such as the putative farnesyltransferase that catalyzes farnesylation of Ras p21 proteins. In this study, we report the identification and preliminary characterization of a protein-cysteine farnesyltransferase activity that farnesylates mammalian Ha-Ras p21 proteins.

MATERIALS AND METHODS

Purification of Ha-Ras p21 Proteins. p21 proteins were partially purified (15) from bacterial carrying expression vectors with v-Ha-*ras* gene sequences (4, 16, 17). The various Ha-Ras p21 proteins used in this study (Table 1) were determined to be 20–40% pure by SDS/PAGE followed by Coomassie blue staining. Each of the protein preparations was tested by Western blot analysis with the anti-Ras monoclonal antibody Y13-259 and for [^{3}H]GDP binding activity as described (18).

Cell and Organ Extracts. Cell lines used in this study included murine NIH 3T3 cells transformed by either the human T24 Ha-*ras* oncogene (44-911 cell line; ref. 19) or an amplified Ha-*ras* protooncogene (115-611 cell line; ref. 20) and human medullary thyroid carcinoma cells (cell line TT) infected with the Harvey (Ha) strain of murine sarcoma virus (MSV) (21). Tissue culture cells were washed with ice-cold phosphate-buffered saline (PBS) and incubated at 4°C for 30 min in buffer C (10 mM Hepes, pH 7.4/1 mM $MgCl_2$/1 mM EGTA). Cells were sonicated and the lysate was centrifuged at 10,000 × *g* for 10 min. The supernatant (crude extract,

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Abbreviations: DTT, dithiothreitol; FPP, farnesyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MSV, murine sarcoma virus.

Table 1. Ha-Ras p21 proteins used in this study

Protein	Carboxyl-terminal sequence	GDP/GTP binding	Focus formation	Ref(s).
Wild type				
p21N	-Cys^{186}-Val^{187}-Leu^{188}-Ser^{189}	Yes	No	16
p21T	-Cys^{186}-Val^{187}-Leu^{188}-Ser^{189}	Yes	Yes	16
p21H	-Cys^{186}-Val^{187}-Leu^{188}-Ser^{189}	Yes	Yes	17
Mutant*				
pNW858	-Ser^{186}-Val^{187}-Leu^{188}-Ser^{189}	Yes	No	3, 4
pNW277	-Cys^{186}-Thr^{187}-Pro^{188}	Yes	No	3, 4
pNW754	-(Δ166–186)-Val^{187}-Leu^{188}-Ser^{189}	Yes	No	4
pNW739	-(Δ166–179)-. . .-Cys^{186}-Val^{187}-Leu^{188}-Ser^{189}	Yes	Yes	4

*Mutant Ha-Ras p21 proteins were isolated from *Escherichia coli* cells [strain C600(pRK248cIts)] transformed by plasmid pNW858, pNW277, pNW754, or pNW739. These bacterial expression plasmids were generated by replacing the 655-base-pair *Hin*dIII fragment of pJCL-E30 (17) with the corresponding sequences present in pBW858, pBW277, pBW754, and pBW739, respectively (3, 4).

S-10) was centrifuged further at 100,000 × *g* for 60 min at 4°C. The membranous pellet fraction (P-100) was resuspended in PBS and gently sonified to obtain uniform vesicles. The supernatant (S-100) was concentrated by ultrafiltration through an Amicon YM10 membrane.

Various organs were surgically removed from a pig, rinsed, and homogenized in a Waring blender with 4 volumes of buffer D [25 mM Tris·HCl, pH 7.4/1 mM dithiothreitol (DTT)/1 mM $MgCl_2$/1 mM EDTA/1 mM EGTA/1 mM phenylmethanesulfonyl fluoride]. The homogenate was centrifuged at 10,000 × *g* for 10 min, and the supernatant was passed through four layers of cheesecloth and centrifuged at 100,000 × *g* for 1 hr at 4°C. The amount of protein was determined by the method of Bradford (22) with bovine serum albumin as the standard.

Farnesyltransferase Assay. Reaction mixtures contained 1–2 μg of partially purified Ha-Ras p21 proteins and various amounts of cell or organ extract in 100 mM Hepes, pH 7.4/25 mM $MgCl_2$/10 mM DTT, in a total volume of 10 μl, unless otherwise stated. The reaction was initiated by the addition of 1 μl of 10 μM [^{3}H]FPP (20 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq). After incubation at 37°C for the indicated time, the reaction was terminated by the addition of 12 μl of 2× SDS/PAGE sample buffer (125 mM Tris·HCl, pH 6.8/20% glycerol/10% 2-mercaptoethanol/0.0025% bromophenol blue). Samples were boiled and analyzed by SDS/12.5% PAGE. Radioactivity in the Ha-Ras p21 proteins was determined by cutting the band from the gel, followed by solubilization and counting in a Tri-Carb model 2200CA (Packard) liquid scintillation counter.

RESULTS

***In Vitro* Farnesylation of Ha-Ras p21 Proteins.** Ras p21 proteins expressed in bacteria do not undergo posttranslational processing (10, 12). Therefore, we partially purified a series of Ha-*ras* p21 proteins expressed in *E. coli* cells (Table 1) that included p21T and p21H, the products of the v-Ha-*ras* oncogenes present in the BALB and Harvey strains of MSV, respectively (16, 17). In addition, we used a modified BALB-MSV Ha-Ras p21 protein in which the 12th residue, lysine, was converted to glycine (16). The resulting protein, Ha-Ras p21N, has an amino acid sequence identical to the products of the human and rat Ha-*ras* protooncogenes (except for Lys-143) and no longer possesses transforming properties (1).

Recent studies have indicated that farnesyl is the isoprenyl unit linked to the carboxyl terminus of Ras proteins (13). Therefore, the most likely donor of this farnesyl moiety is FPP. As a consequence, we based our assays to identify protein farnesyltransferase activity on the incorporation of radioactivity from [^{3}H]FPP into bacterially synthesized Ha-Ras p21 proteins in the presence of crude extracts obtained from various human and mouse cell lines. Fig. 1 depicts a representative experiment in which a partially purified preparation of Ha-Ras p21N was incubated with a crude extract of TT cells, a human medullary thyroid carcinoma cell line transformed in culture by v-Ha-*ras* (21). We selected Ha-*ras*-transformed cells because they should contain the necessary Ha-Ras p21-processing enzymes, including the putative farnesyltransferase. Addition of [^{3}H]FPP to the reaction mixture resulted in the labeling of a single protein of ≈23 kDa (Fig. 1*A*). Labeling of this protein was dependent upon the presence of both the bacterially expressed Ha-Ras p21N and the TT cell lysate (Fig. 1*A*). Fractionation of the crude extract

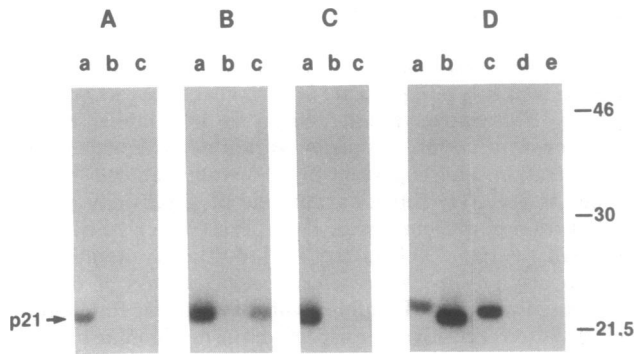

FIG. 1. *In vitro* farnesylation of Ras p21 proteins. Unless stated otherwise, reaction mixtures (10 μl) were incubated at 37°C for 1 hr and contained 1 μg of partially purified Ha-Ras p21N, 20 μg of protein (crude, P-100, or S-100) from human TT cells, 1 μM [^{3}H]FPP (0.2 μCi), 5 mM Mg^{2+}, and 5 mM DTT. (*A*) Incorporation of ^{3}H from [^{3}H]FPP into Ras p21 is dependent on the presence of *E. coli*-synthesized Ras p21 protein and an enzyme source. Lanes: a, complete reaction using a crude S-10 extract of human TT cells; b, same as lane a except p21N was omitted; c, same as lane a except the TT cell extract was omitted. (*B*) Subcellular localization of protein farnesyltransferase activity. Lanes: a, S-100 fraction; b, P-100 fraction; c, crude extract. (*C*) The activity is heat-labile and can be blocked with excess nonradioactive FPP. Lanes: a, Complete reaction using the S-100 fraction of TT cells; b, same as lane a except S-100 fraction was preincubated at 65°C for 30 min; c, same as lane a except a 100-fold excess of nonradioactive FPP was added to the reaction mixture. (*D*) Farnesylation of various *E. coli*-synthesized recombinant proteins by the S-100 fraction of human TT cells. Lanes: a, 1.2 μg of Ha-Ras p21N; b, 1 μg of Ha-Ras p21T; c, 1 μg of Ha-Ras p21H; d, 10 μg of interleukin 2; e, 7.5 μg of interferon γ. In all cases, reactions were terminated by the addition of SDS/PAGE sample buffer and analyzed by SDS/12.5% PAGE. After electrophoresis, gels were fixed, treated with Enlightning (New England Nuclear), and exposed at −70°C to Kodak X-Omat AR5 film in the presence of intensifier screens for 1–3 days. Coelectrophoresed molecular size markers included ovalbumin (46 kDa), carbonic anhydrase (30 kDa) and trypsin inhibitor (21.5 kDa). Position of the farnesylated Ha-Ras p21 proteins is indicated by an arrow.

derived from human TT cells into soluble (S-100) and membranous (P-100) fractions revealed that the Ha-Ras p21-farnesylating activity was almost entirely in the soluble, cytosolic fraction (Fig. 1*B*). Therefore, S-100 fractions were used in subsequent studies.

Preincubation of the S-100 fraction of TT cells at 65°C for 30 min completely abolished the incorporation of ^3H from [^3H]FPP into Ha-Ras p21N (Fig. 1*C*). These results suggested that the observed p21N farnesylation was catalyzed by a heat-labile enzyme. Incubation of the reaction mixture with an excess of nonradioactive FPP completely abolished the incorporation of ^3H from [^3H]FPP into p21N (Fig. 1*C*). The soluble S-100 TT cell extract also catalyzed the farnesylation of the transforming Ha-Ras isoforms p21T and p21H (Fig. 1*D*). These bacterially synthesized Ha-Ras p21 proteins were farnesylated with efficiencies comparable to that seen with p21N, indicating that this reaction is independent of the activated (oncogenic) state of Ha-Ras proteins. As controls, interleukin 2 and interferon γ, two proteins unrelated to the Ha-Ras proteins, were expressed in *E. coli* cells under the control of the same λP_L-derived vector used to express the Ha-Ras p21 proteins and were used as substrates in the farnesylation reaction. Neither interleukin 2, interferon γ, nor any of the *E. coli* proteins present in these reaction mixtures became farnesylated (Fig. 1*D*).

Preliminary Characterization of Protein Farnesyltransferase Activity. Based on the above results, S-100 extracts of V-Ha-*ras*-transformed human TT cells were used to characterize the farnesyltransferase activity. *In vitro* farnesylation of Ha-Ras p21N required the presence of Mg^{2+} or Mn^{2+}. In the case of Mn^{2+} the optimal concentration was 0.2 mM, whereas with Mg^{2+} optimal activity was observed at 25 mM (Fig. 2 *A* and *B*). In contrast, other divalent cations (Ca^{2+}, Zn^{2+}) did not support this *in vitro* farnesylating activity (data not shown). Addition of monovalent ions such as Na^+ (100 mM), K^+ (50 mM), or F^- (10 mM) had no significant effect on the reaction (data not shown). Farnesylation of *E. coli*-synthesized Ha-Ras p21 proteins took place at a relatively wide range of pH, with optimal activity between pH 6.8 and pH 7.8 (Fig. 2*C*) and required compounds, such as DTT, that are known to protect sulfhydryl groups (Fig. 2*D*).

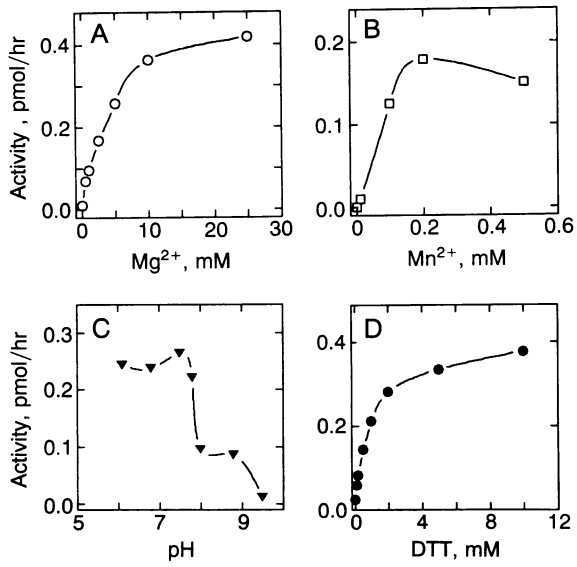

FIG. 2. Optimal conditions for protein farnesyltransferase activity. Effect of Mg^{2+} (*A*), Mn^{2+} (*B*), pH (*C*), and sulfhydryl-protecting compound. Reactions were carried out as for Fig. 1 and analyzed by SDS/PAGE. Radioactivity in Ha-Ras p21 proteins was determined by liquid scintillation counting of the gel slice containing the p21 band. Results are expressed as pmol of farnesyl groups incorporated per hr.

We also determined the kinetic properties of this crude farnesylating activity. The reaction was linear with time (up to 1 hr) and with the amount of enzyme (as S-100 extract) in the assay (Fig. 3 *A* and *B*). When the substrate saturation kinetics with Ha-Ras p21N and [^3H]FPP were investigated, the rate of the reaction was found to increase linearly with the concentration of substrate until it reached saturation (Fig. 3 *C* and *D*). The reaction did not require an external source of energy, since addition of ATP or GTP did not have any significant effect on the incorporation of ^3H from [^3H]FPP into p21N. Extensive dialysis of the TT S-100 cell extract did not result in significant loss of activity, suggesting that the putative farnesyltransferase does not require small (<10 kDa cofactors) for proper activity, unless they are tightly bound to the enzyme.

***In Vitro* Farnesylation of Ha-Ras p21 Proteins Occurs at Cys-186.** Farnesylation of Ha-Ras p21 proteins *in vivo* is known to take place at Cys-186, just three residues from the carboxyl terminus. To determine whether the *in vitro* farnesylation occurred at this specific residue, we used a mutated isoform of the transforming Ha-Ras p21H protein in which Cys-186 was replaced by serine (4). As a consequence of this mutation, the resulting protein, pNW858 (Table 1), does not interact with the inner side of the plasma membrane and lacks transforming activity (4). This mutant p21H$^{Ser-186}$ protein was not farnesylated in the *in vitro* assay (Fig. 4, lane b). These results confirm *in vivo* studies indicating that Cys-186 is required for farnesylation (7). A second p21H mutant, pNW277 (Table 1), in which the wild-type carboxyl-terminal sequence Val^{187}-Leu^{188}-Ser^{189} was replaced by Thr^{187}-Pro^{188} (4), did not serve as a substrate even though it carries the Cys-186 residue (Fig. 4, lane e). These results indicate that *in*

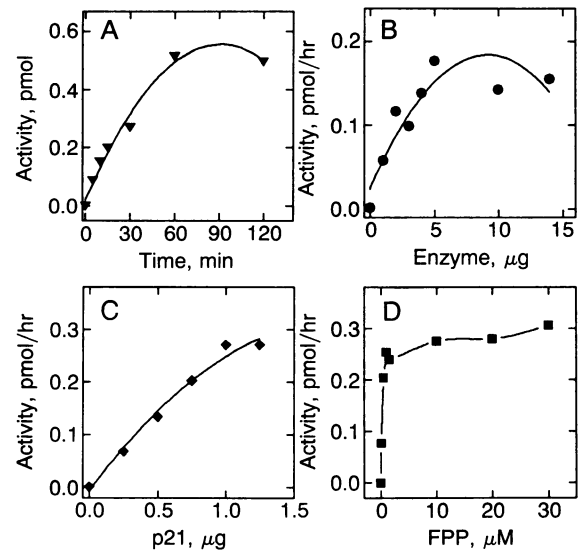

FIG. 3. Kinetics of farnesyltransferase activity. (*A*) Time course. A reaction mixture (80 μl) with 100 mM Hepes (pH 7.4), 5 mM DTT, 25 mM $MgCl_2$, 1 μM [^3H]FPP, 10 μg of p21N, and 165 μg of TT S-100 fraction was incubated at 37°C; aliquots (10 μl) were removed at the indicated times. (*B*) Enzyme concentration curve. Reaction mixtures (10 μl) with 1.25 μg of p21N and the indicated amount of TT S-100 cell extract were incubated for 1 hr. (*C*) Ha-Ras p21 substrate saturation kinetics. Activity was determined as in *B* except reactions contained 22 μg of TT S-100 extract and the indicated amount of p21N. (*D*) FPP substrate saturation kinetics. Activity was determined as in *B* in the presence of 1 μg of p21N protein, 11 μg of TT S-100 extract, and the indicated amount of [^3H]FPP. Activity was quantitated as for Fig. 2. In *A*, activity refers to the indicated time (not per hr).

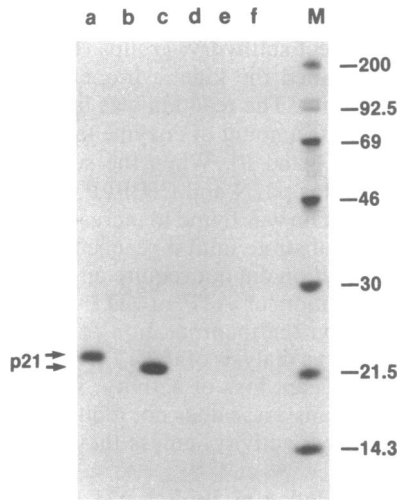

FIG. 4. *In vitro* farnesylation of Ha-Ras p21 proteins occurs at Cys-186 and requires an intact CAAX box. Farnesylation of p21H (lane a), pNW858 (lane b), pNW739 (lane c), pNW754 (lane d), and pNW277 (lane e) proteins (Table 1) was conducted for 1 hr, using 1 μg of each of these proteins and 20 μg of the TT S-100 cell extract. Another p21H reaction mixture (lane f) was the same as for lane a, but contained 1 μg of a tetradecapeptide (Tyr-Ser-Gly-Pro-Ser-Met-Ser-Ser-Lys-Cys-Val-Leu-Ser) whose sequence mimics the carboxyl terminus of p21H (1). Samples were analyzed by SDS/PAGE as in Fig. 1. Molecular size standards (lane M) were myosin heavy chain (200 kDa), phosphorylase *b* (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.3 kDa).

vitro farnesylation requires not only conserved Cys-186 residue but also the CAAX-box motif.

A partially purified mutant Ha-Ras p21H protein, pNW739, in which amino acid residues 166–179 were deleted (4) was efficiently farnesylated (Fig. 4, lane c). However, a similar mutant protein, pNW754, in which the deletion extended to Cys-186 (4) did not incorporate detectable radioactivity from [^{3}H]FPP (Fig. 4, lane d). These results indicate that modifications proximal to the carboxyl terminus of Ha-Ras p21 proteins do not affect their farnesylation, providing that they retain an intact CAAX motif. Finally, a synthetic tetradecapeptide whose sequence mimics the carboxyl terminus of Ha-Ras p21 proteins efficiently blocked *in vitro* farnesylation of p21N (Fig. 4, lane f). As a control, cysteine-containing peptides of unrelated sequences had no effect on Ha-Ras p21 farnesylation (data not shown).

Protein Farnesyltransferase Activity in Mammalian Cells and Tissues. Next, we searched for Ha-Ras p21-farnesylating activity in a variety of mammalian cell lines and tissues (Table 2). The activity was found in all mammalian cell and tissue extracts examined, albeit at different levels. For instance, porcine brain, kidney, and lung had the highest levels of activity (Table 2). In all cases, farnesylation of Ha-Ras p21 proteins *in vitro* was observed mostly in the soluble S-100 fraction, whereas the membrane-containing P-100 fractions did not exhibit significant activity. These findings indicate that the protein-cysteine farnesyltransferase identified in this study is a ubiquitous cytoplasmic enzyme.

To purify and characterize this enzymatic activity further, we submitted an S-100 fraction obtained from porcine kidney tissue to fractionation by ammonium sulfate precipitation followed by DEAE-cellulose (Whatman DE-52) chromatography. Most of the enzymatic activity was found in the 30–55% (% saturation) ammonium sulfate fraction and was from the DE-52 ion-exchange column as a single peak at ≈200 mM NaCl. This partially purified protein farnesyltransferase exhibited similar properties (divalent cation requirements, saturation kinetics, and time course) as the enzyme(s) present in the human S-100 extract (data not shown). When fractions eluted from the DE-52 column were concentrated and submitted to gel filtration chromatography using a Sephacryl S-200 (Pharmacia) column, two peaks of activity were identified (Fig. 5). Based on their elution with respect to previously chromatographed molecular size standards, we estimated that the molecular masses of these farnesyltransferase activities were 250–350 kDa and 80–130 kDa.

Table 2. Protein farnesyltransferase activity in various cells and tissues

Source of extract	Activity, pmol/hr per mg
Cells	
E. coli cells	0.0
Mouse 44-911 cell line	2.7
Mouse 115-611 cell line	6.5
Human TT cells	13.0
Ha-MSV-infected human TT cells	18.0
Tissues	
Human platelets	9.9
Porcine adrenal medulla	44.0
Porcine brain	62.0
Porcine heart	18.0
Porcine kidney	78.0
Porcine liver	14.0
Porcine lung	53.0

Partially purified Ha-Ras p21N (1.25 μg) was incubated at 37°C for 1 hr with 10–20 μg of the corresponding extracts as described in *Materials and Methods*. Activity is expressed per mg of extract protein.

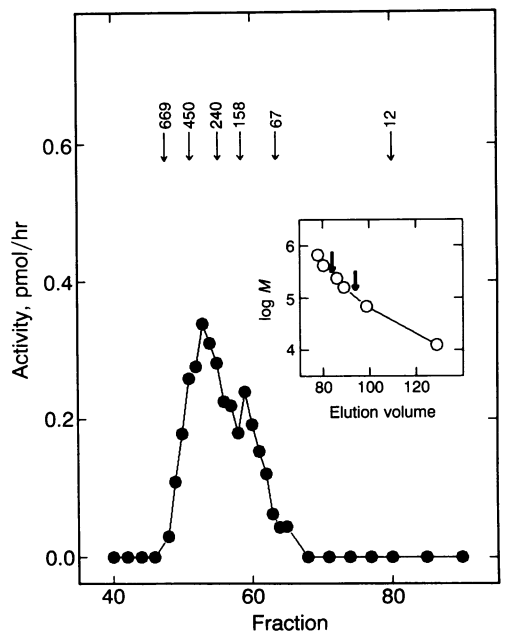

FIG. 5. Gel filtration of protein farnesyltransferase activity from porcine kidney. One milliliter (7 mg) of a partially purified preparation (see text) of farnesyltransferase was loaded onto a Sephacryl S-200 HR columns (98 cm × 1.6 cm) equilibrated with 50 mM sodium phosphate buffer, pH 7.0/200 mM NaCl/1 mM DTT/5% (vol/vol) glycerol. Elution was with the same buffer at 14 ml/hr, and 1.59-ml fractions were collected. Protein farnesyltransferase activity was assayed as for Fig. 2. Arrows indicate positions of molecular size markers chromatographed under similar conditions: thyroglobulin (669 kDa), ferritin (450 kDa), catalase (240 kDa), aldolase (158 kDa), bovine serum albumin (67 kDa), and cytochrome *c* (12.4 kDa). (*Inset*) Calibration plot used to determine the approximate molecular mass (*M*) of the farnesyltransferase. Bold arrows indicate the positions of the two peaks of activity.

DISCUSSION

Posttranslational modification of proteins by isoprenoid residues such as farnesyl and geranylgeranyl is emerging as a major mechanism by which cytosolic proteins interact with cellular membranes (23). Yeast mating factors, nuclear lamins, subunits of certain heterotrimeric guanine nucleotide-binding proteins, the α subunit of the cGMP phosphodiesterase, and the products of the *ras* gene family appear to be farnesylated at their carboxyl termini (13, 23). Molecular studies have indicated that the farnesylation of Ras proteins is the most critical step in their maturation process and is required for their biological activity (2–9). We have identified and partially characterized an enzymatic activity capable of transferring the farnesyl moiety of FPP to Cys-186 of mammalian Ha-Ras p21 proteins. This FPP:protein-cysteine farnesyltransferase activity is dependent upon the presence of [^{3}H]FPP, unprocessed Ha-Ras p21 proteins, and cell extracts of mammalian origin.

The enzyme(s) responsible for the farnesylation of Ha-Ras p21 proteins *in vitro* is located predominantly in the soluble cytosolic fraction and was detected readily in all cell lines and tissues tested. Most importantly, this *in vitro* farnesylation reaction exhibits the specificity expected from *in vivo* studies (2–9). This specificity involves targeting of the Cys-186 residue, as determined by the absence of farnesylation in the Ser-186 Ha-Ras p21 mutant, and requires an intact CAAX motif, since Ha-Ras p21 proteins carrying the carboxyl-terminal sequence-Cys^{186}-Thr^{187}-Pro^{188} did not serve as substrates. In addition, this protein farnesyltransferase activity recognized nontransforming (Gly-12) Ha-Ras p21 proteins as well as the oncogenic isoforms p21T (Lys-12) and p21H (Arg-12), the products of the Ha-*ras* oncogenes present in the BALB and Harvey strains of MSV, respectively (16, 17). These results indicate that the *in vitro* protein farnesyltransferase activity corresponds to the enzyme(s) responsible for the *in vivo* farnesylation of Ha-Ras p21 proteins. Gel filtration of the enzymatic activity present in porcine kidney gave two peaks of activity at ≈300 kDa and ≈100 kDa. Further purification is needed to determine whether these results reflect the presence of two different protein farnesyltransferases, partial proteolytic degradation of a large enzyme (≈300 kDa), or the existence of a complex containing a smaller enzyme (≈100 kDa).

The products of *ras* oncogenes have to be farnesylated to exert their neoplastic properties (7–9, 14). Schafer *et al.* (14) showed that deprivation of mevalonate in mutants deficient in HMG-CoA reductase activity restored the normal phenotype to cells that either overexpress the wild-type *RAS2* gene or carry a mutated *RAS2*$^{Val-19}$ allele. These observations suggested that inhibitors of cholesterol biosynthesis might be used to block the transforming activity of *ras* oncogenes in human tumors (13, 14). Unfortunately, the strict requirements of mammalian cells for cholesterol makes it likely that the concentrations of such inhibitors needed to block the action of *ras* oncogenes will have undesirable toxic effects. Availability of a fast, simple, and reproducible *in vitro* assay that detects farnesylation of Ras p21 proteins will make it possible to detect and/or design compounds that can block farnesylation of Ras proteins without interfering with any of the other vital cellular pathways that utilize FPP. The *in vitro* assay described in this study represents the first step in a rational approach toward the isolation of inhibitors that may block the neoplastic effect of *ras* oncogenes in human tumors.

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