The Posttranslationally Modified C-terminal Structure of Bovine Aortic Smooth Muscle rhoA p21*

Masaya Katayama, Masahito Kawata, Yasuhisa Yoshida, Hisanori Horiiuchi, Takeshi Yamamoto, Yoshiharu Matsuura, and Yoshimi Takai

From the Department of Biochemistry, Kobe University School of Medicine, Kobe 650, and the Department of Veterinary Science, National Institute of Health, Tokyo 141, Japan

rhoA p21, a ras p21-like small GTP-binding protein, has the same C-terminal consensus motif of Cys-A-A-X (A is an aliphatic amino acid and X is any amino acid) as ras p21s, which is posttranslationally processed. We here determine the posttranslationally processed C-terminal structure of the rhoA p21 purified from bovine aortic smooth muscle. Incubation of rhoA p21-expressing insect cells with exogenous [3H]mevalonolactone caused the labeling of rhoA p21, suggesting that rhoA p21 is prenylated. Constantly, Raney nickel treatment of rhoA p21 released a geranyleranyl moiety as estimated by gas chromatography/mass spectrometry. No lipid moiety was released by KOH or NH4OH treatment. Extensive digestion of rhoA p21 with Achromobacter protease I yielded a C-terminal peptide, Ser-Gly-CysXn, that lacked the three C-terminal amino acids predicted from the cDNA but was geranyleranylated and carboxyl methylated at the cysteine residue. Bovine brain cytosol geranyleranylated the bacterial rhoA p21 having the three C-terminal amino acids predicted from the cDNA but not the protein lacking the three C-terminal amino acids. Bovine brain membranes methylated the synthetic C-terminal peptide with 10 amino acids of rhoA p21 which was geranyleranylated at its C-terminal cysteine residue but not the peptide which was not geranyleranylated. These results suggest that rhoA p21 is first geranyleranylated followed by removal of the three C-terminal amino acids and the subsequent carboxyl methylation of the exposed cysteine residue.

The rho protein family, composed of three homologous members, A, B, and C, belongs to the superfamily of ras p21/ras p21-like small G proteins (1–3, for reviews, see Refs. 4 and 5). They are present in various species from yeast to human as described for ras p21s (1–3, 6). All the rho proteins show M*, values of about 22,000, which are calculated from their cDNAs, and have consensus amino acid sequences for GDP/GTP-binding and GTPase activities (1–3, 6). The rho proteins purified from mammalian tissues exhibit GDP/GTP-binding and GTPase activities (7–11).

The definitive function of the rho proteins has not been clarified, but it has been shown that the rho proteins are ADP-ribosylated by botulinum ADP-ribosyltransferase C3 (8–13). It has been suggested, from the fact that this ADPribosyltransferase induces the morphological changes of several types of cells, that the rho proteins are involved in cytoskeletal control (13, 14). It has also been suggested that the rho proteins are involved in the regulation of cell proliferation and differentiation, on the basis of the observations that disruption of the yeast RHO1 gene results in lethality, that the human rhoA p21 is tumorigenic when the rhoA p21 cDNA-transfected cells are injected into nude mice and that the ADP-ribosylation of the rhoA proteins induces growth inhibition and neurite outgrowth in PC12 cells (6, 15, 16). The subcellular distribution analysis has shown that the rhoA proteins are found in both the membrane and soluble fractions depending on cell types (7–11, 17).

The rho proteins have two interconvertible forms: GDP-bound inactive and GTP-bound active forms. The GDP-bound form is converted to the GTP-bound form by a GDI/GTP exchange reaction and is regulated by two types of GDP/GTP exchange proteins named GDS and GDI (18–20). GDS stimulates the GDP/GTP exchange reaction by stimulating the dissociation of GDP from and the subsequent binding of GTP to the rho proteins. GDI inhibits the GTP/GDP exchange reaction by inhibiting the dissociation of GDP from and the subsequent binding of GTP to them. The GTP-bound form is converted to the GDP-bound form by a GTPase reaction catalyzed by the rho proteins, and this reaction is stimulated by GTPase activating protein, GAP (21, 22). Thus, the rho protein activity is regulated at least by these three regulatory proteins.

The rho proteins have a unique C-terminal amino acid sequence of Cys-X-A-X (A is an aliphatic amino acid and X is any amino acid) (1–3). It is well known that ras p21s have the same C-terminal amino acid sequence and that they undergo three kinds of posttranslational modifications in the C-terminal region: prenylation, most presumably farnesylation, of the cysteine residue through a thioether bond (23–26), removal of the three C-terminal amino acids (27, 28), and carboxyl methylation of the exposed cysteine residue (29, 30). In the case of Ha- and N-ras p21s, the cysteine residue a short distance upstream of the prenylated cysteine residue is furthermore palmitoylated through a thioester bond (24). Ki-ras...
p21 lacks this second cysteine residue and is not palmitoylated but has a polybasic region just upstream of the prenylated cysteine residue (24, 31). Among these modifications of ras p21s, the farnesoyl moiety but not the palmitoyl moiety is essential for their transforming activity, and both the isoprenoid and palmitoyl moieties of Ha- and N-ras p21s and both the isoprenoid moiety and the polybasic region of Ki-ras p21 are required for their membrane-binding activity (24, 31). We have recently found that both the GDP- and GTP-bound forms of the rhoB p21 purified from bovine brain bind to any type of membranes in a cell-free system, even after the membranes are digested with trypsin or boi (32). This membrane-binding activity is controlled by rho p21 GDP: GDPI GDP binds a complex with the GDP-bound form of rhoB p21 to membranes and moreover induces the dissociation of the prebound protein from the membranes (32). It is most likely that these results together with the unique C-terminal structure of the rho proteins that the carboxyl terminus is also posttranslationally processed as described for ras p21s.

In the present study, we have determined the posttranslationally processed structure of the rhoA p21 purified to homogeneity from bovine aortic smooth muscle. We report here that rhoA p21 is first geranylgeranylated followed by removal of the three C-terminal amino acids and the subsequent carboxyl methylation of the exposed cysteine residue.

**EXPERIMENTAL PROCEDURES**

**Materials and Chemicals**—Native rhoA p21 was purified to near homogeneity from bovine aortic smooth muscle as described (11). The purified rhoA p21 was more than 90% pure as determined by SDS-PAGE. The purity of rhoA p21 was kindly provided by T. Madaule (CNRS Laboratoire, Gif sur Yvette, France). rhoA p21s having the three C-terminal amino acids of Leu-Val-Leu and lacking the three C-terminal amino acids were produced by overexpressing Escherichia coli and purified to near homogeneity as described (33).

**YMC-pack** AP-802 C, and Bakerbond WP-octyl C reversed-phase HPLC columns were obtained from Yamamura Chemical Lab. Co. (Kyoto, Japan) and T. Baker Chemical Co., respectively. (RS)-[3H]MVA (1.5 TBq/mmol) was purchased from Du Pont-New England Nuclear. [3H]Geranylglycerol pyrophosphate (990 GBq/mmol) and [3H]AdoMet (3.2 TBq/mmol) were obtained from Amersham Corp. Methyl violonate, a competitive inhibitor of the 2-hydroxy-3-methylglutaryl-coenzyme A reductase, was provided by Merck Sharp & Dohme Research Laboratories. The peptides, Ala-Ar-Ar-Gly-Lys-Lys-Lys-Ser-Gly-all-trans-geranylglyceranyl-Cys and Ala-Ar-Ar-Gly-Lys-Lys-Lys-Ser-Gly-Cys, were synthesized (34, 35) and kindly supplied by Eisai Co. Ltd. (Tokyo, Japan). The structures of these peptides were confirmed by fast atom bombardment mass spectrometry and nuclear magnetic resonance spectroscopy as described (35, 36).

**Treatment of rhoA p21 with NH₂OH, KOH, or CH₃OH** —The purified rhoA p21 (95 pmol) dissolved in 20 mM Tris-HCl at pH 8.0 (0.4 ml) containing 1 mM EDTA, 1 mM DTT, 5 mM MgCl₂, 0.1 M NaCl, 0.6% CHAPS, and 2 ml urea was incubated for 1 h at 23 °C with either 2 M NH₂OH (0.4 ml) or 0.2 M KOH (0.4 ml). The purified rhoA p21 (240 pmol) dissolved in 20 mM Tris-HCl at pH 8.0 (0.5 ml) containing 1 mM EDTA, 1 mM DTT, 5 mM MgCl₂, 0.1 M NaCl, and 0.6% CHAPS was incubated for 2 h in the dark at room temperature with CH₃OH (0.1 ml) in the presence of 3% formic acid (0.1 ml) containing 0.6% CHAPS. Then, CH₃OH was removed under reduced pressure. After this treatment, each sample was subjected to C, reversed-phase HPLC. The protein peaks were detected by absorbance at 215 nm and confirmed by SDS-PAGE followed by staining with silver.

**Expression of rhoA p21 in SF9 Cells** — rhoA p21 was expressed using the insect/baculovirus system. The cDNA of rhoA p21 was inserted into a pACYC181 Autographa californica baculovirus transfer vector to express the cDNA under the control of the polyhedrin promoter in SF9 cells (37–39). Monolayers of SF9 cells were infected with the recombinant virus and cultured for 72 h. Details of the expression of rhoA p21 in SF9 cells will be described elsewhere.

**Labeling of rhoA p21 with DPMVA**—rhoA p21s were cultured in 24-well culture plates and used as confluent as described (31). The cells were incubated for 0.5 h at medium supplemented with 10% dialyzed fetal calf serum and 50 μM mevinolin. The medium was then replaced with 0.2 ml of fresh medium supplemented with 25 μM mevinolin, 10% dialyzed fetal calf serum, and 12.5 μM [3H]MVA (3.7 MBq), and the cells were further incubated for 19 h. The cells were washed twice with phosphate-buffered saline (pH 7.4) and centrifuged at 800 × g for 5 min. The pellets were sonicated twice for 30 s at 4 °C in 10 mM Tris- HCl at pH 7.5 (0.3 ml) containing 1 mM DTT, 10 mM MgCl₂, 50 mM NaCl, and 10 μM (p-amidinophenyl)methanesulfonil fluoride. The homogenate was centrifuged at 100,000 × g for 1 h. Aliquots were mixed with 1 ml of 100 mM Al(OH)₃ and the membrane fraction, and the soluble fraction were subjected to SDS-PAGE. Samples for two-dimensional gel electrophoresis were prepared by the method of Garrels (41). Two-dimensional gel electrophoresis was performed as described by OFarrell (42). Fluorography was performed with Amplify (Amer sham Corp.) as a reagent.

**GC/MS Analysis** —To obtain full ionization spectra of the isoprenoid released from the purified rhoA p21, it (1.0 nmol) was dried in a siliconized glass tube with a Speed Vac concentrator, resolubilized in water:formic acidethanol (1:940, v/v, 0.4 ml), and the contaminated lipids were extracted with three aliquots of pentane (1 ml) that had been equilibrated with detergent-containing water:formic acidethanol (1:940). Raney nickel-activated catalyst (100 mg) and pentane (1 ml) were then added to the screw-capped tube and the sample was incubated for 15 h at 100 °C. The sample was then chilled for 1 h at −20 °C, whereupon water (0.4 ml) was added and the sample was mixed by vortexing. After centrifugation at 1,500 × g for 1 min, the pentane phase was pooled. The aqueous phase was reextracted with pentane (0.5 ml) and the extracts were pooled. The extract was concentrated and immediately analyzed as described (43, 44), except that the temperature program started with the column at 80 °C and increased to 250 °C at 4 °C/min. For hydrogenation, the extract from the Raney nickel-activated catalyst was dried in vacuo and dissolved in 0.1 M KHO for 1 h at 23 °C in the presence of 2 μl tetraethylammonium hydroxide. The solution was hydrogenated over platinum (Adams’ catalyst) prior to analysis as described previously (40, 41). The isoprenoid released from rhoA p21 was quantified by GC/MS in the selected ion monitoring mode (35, 44).

**Peptide Map and Amino Acid Sequence Analyses** —The purified rhoA p21 (5.7 nmol) was subjected to C, reversed-phase HPLC to remove CHAPS, which was included in the buffer of the purified preparation, and was then completely digested with API as described (45). The digested peptides were separated by C, reversed-phase HPLC, dried, dissolved in 50% trifluoroacetic acid, and subjected to an automated gas-phase sequencer (Applied Biosystem model 477A) (40, 41). The isoprenoid released from rhoA p21 was quantified by GC/MS in the selected ion monitoring mode (35, 44).

**Geranylgeranylation of Bacterial rhoA p21s by Bovine Brain Cytosol** —The cytosol of bovine brain was prepared as described (46). The bacterial rhoA p21s having the three C-terminal amino acids of Leu-Val-Leu and lacking them (95 pmol each) were incubated for 1 h at 37 °C in a reaction mixture (25 μl) containing 50 mM Tris-HCl at pH 7.5, 50 μM ZnCl₂, 20 mM KCl, 1 mM DTT, 0.48 μM [3H]geranylgeranyl pyrophosphate (about 2,300 cpm/pmol), and bovine brain cytosol (11 μg of protein). After the incubation, the reaction was stopped by the addition of 0.5 ml of 4% SDS and then 0.5 ml of 30% trichloroacetic acid. The tubes were vortexed and left on ice for 45 min, after which 3 ml of 6% trichloroacetic acid and 2% SDS was added. The mixture was filtered on a 2.5-cm glass fiber filter (Wh atman GF/B), and the filter was washed three times with 3 ml of 6% trichloroacetic acid and 2% SDS, four times with 3 ml of 6% trichloroacetic acid, and once with 5 ml of 6% trichloroacetic acid, dried, and counted in a scintillation mixture (toluene, 0.6% 2,5-diphenyloxazole, 6.0 μl) by a scintillation counter.

**Methylation of the Synthetic C-terminal Peptides by Bovine Brain Cytosol** —The stripped peptides of bovine brain were prepared as described (47). Synthetic peptides, Ala-Ar-Ar-Gly-Lys-Lys-Lys-Ser-Gly-all-trans-geranylglyceranyl-Cys and Ala-Ar-Ar-Gly-Lys-Lys-Lys-Ser-Gly-Cys (0.77 nmol each), were incubated for 3 h at 37 °C in a reaction mixture (0.1 ml) containing 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid at pH 7.0, 7.0 mM MgCl₂, 100 μM [3H]AdoMet (0.37 MBq), and stripped membranes (100 μg of protein). The reaction was stopped with an SDS-stopping solution (50 mM containing 200 mM Tris-HCl at pH 6.7, 9% SDS, 0.2% mercaptoeth-
RESULTS

Treatments of rhoA p21 with NH$_2$OH, KOH, or CH$_3$I — When the purified rhoA p21 was analyzed by C$_4$ reversed-phase HPLC, it appeared as a single peak with a $t_{R}$ of 29.30 (Fig. 1A). After being treated with NH$_2$OH or KOH to remove a possible thioether-linked moiety, it appeared at the same $t_{R}$ as the control (Fig. 1, B and C). The KOH-treated rhoA p21 appeared as a broad peak, but the reason for this result is unknown. However, after being treated with CH$_3$I to remove a possible thioether-linked moiety, it appeared at $t_{R}$s of 27.30 and 27.50 (Fig. 1D). The reason for the appearance of two peaks is not known, but these results seem consistent with earlier observations that rhoA p21 shares a cDNA-predicted C-terminal amino acid sequence, Cys-A-A-X, with ras p21s, and that ras p21s are modified by a thioether-linked isoprenoid group at their C-terminal cysteines (23-26).

Labeling of rhoA p21 with $[^{3}H]$MVA in Sf9 Cells — To investigate whether rhoA p21 is modified by an isoprenoid group, the control and rhoA p21-expressing Sf9 cells were incubated with $[^{3}H]$MVA, disrupted by sonication, and centrifuged to separate the membrane and soluble fractions. Upon analyzing the homogenate by SDS-PAGE followed by fluorography, many radioactive bands were detected for the control cells (Fig. 2, lane 1), but one major band with two minor bands was observed for the rhoA p21-expressing cells (Fig. 2, lane 2). The migration position of this major radioactive band was the same as that of the purified smooth muscle rhoA p21 (Fig. 2, lanes 2 and 5). These results suggest that the major radioactive protein in the rhoA p21-expressing cells is rhoA p21. The reason why many bands detected in lane 1 disappeared in lane 2 is unknown, but it is possible that the radioactivity incorporated into other proteins decreased due to the presence of highly labeled rhoA p21 in the rhoA p21-expressing cells. Since labeled rhoA p21 was recovered in the membrane fraction (Fig. 2, lanes 3 and 4), this fraction was subjected to two-dimensional gel electrophoresis and the gel was stained with Coomassie Brilliant Blue. Many minor protein spots were observed (Fig. 3A). When the same sample together with the purified, unlabeled rhoA p21 was subjected to the same gel electrophoresis and the gel was stained with Coomassie Brilliant Blue, one major, one intermediate, and many minor protein spots were observed (Fig. 3B). Since many minor protein spots observed in Fig. 3B were identical with those seen in Fig. 3A, one major and one intermediate protein spots were identified as rhoA p21 (Fig. 3, A and B). The reason why rhoA p21 appeared in two spots is not known, but this result is consistent with our earlier observation (11). When the same gel was subjected to fluo-

![Fig. 1. Treatment of the purified rhoA p21 with NH$_2$OH, KOH, or CH$_3$I.](image)

![Fig. 2. SDS-PAGE analysis of the rhoA p21 labeled with $[^{3}H]$MVA in Sf9 cells.](image)
FIG. 3. Two-dimensional gel electrophoresis analysis of the rhoA p21 labeled with [3H]MVA in SF9 cells. The membrane fraction (30 μg of protein) of the [3H]MVA-labeled rhoA p21-expressing SF9 cells and the purified, unlabeled rhoA p21 (0.5 nmol) were analyzed by two-dimensional gel electrophoresis followed by Coomassie Brilliant Blue staining and fluorography. A, Coomassie Brilliant Blue staining of the membrane fraction of the rhoA p21-expressing SF9 cells; B, Coomassie Brilliant Blue staining of the membrane fraction of the rhoA p21-expressing SF9 cells plus the purified, unlabeled rhoA p21; C, a fluorograph of the membrane fraction of the rhoA p21-expressing SF9 cells plus the purified, unlabeled rhoA p21. Arrows indicate the positions of rhoA p21. The protein markers used were the same as those of Fig. 2.

rography, one major and one minor radioactive spots were observed and corresponded to the two protein spots of rhoA p21 (Fig. 3, B and C). These results suggest that rhoA p21 is modified by an isoprenoid group in intact cells.

GC/MS Analysis of Raney Nickel-treated rhoA p21—To obtain definitive evidence concerning the isoprenoid modification of rhoA p21, the purified sample was first treated with Raney nickel to cleave a potential thioether linkage. The treated material was then extracted with pentane, and the pentane extract was analyzed by GC/MS. This approach identified a peak that had a tR corresponding to that of authentic all-trans-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene. Furthermore, the material in the peak yielded a fragmentation pattern which was identical with that of this hydrocarbon standard (Fig. 4, A and B). When the pentane extract was first hydrogenated and then analyzed by GC/MS, it yielded a peak with a tR and spectrum identical with those of phytane (Fig. 4, C and D). Analysis by GC/MS in the selected ion monitoring mode showed that about 0.6 mol of the C20 isoprenoid was recovered per mol of rhoA p21, that no C15s, C15a, or C15b isoprenoid was present, and that the amounts of other unidentified peaks seemed less than 0.3 mol per mol of rhoA p21 (Fig. 5, A and B). These results indicate that rhoA p21 is modified by a single thioether-linked all-trans-geranylgeranyl moiety.

The Site of Geranylgeranylation—To locate the site of geranylgeranylation on rhoA p21, it was first completely digested with API, and the API digest was then separated by Cs reversed-phase HPLC. Many peptide peaks appeared (Fig. 6A), and the amino acid sequences of several peptides were analyzed. When a peptide that emerged from the column with a tR of 68:20 was subjected to amino acid sequence analysis, Ser-Gly was detected. This amino acid sequence corresponds to that of the unique Ser-Gly189 sequence that is located just upstream of the cDNA-predicted C-terminus (Leu193) of rhoA p21 (2). A C-terminal peptide, Ala-Arg-Arg-Gly-Lys-Lys-Lys-Gly-all-trans-geranylgeranyl-Cys, was synthesized and completely digested with API. When the digest was subjected to Cs reversed-phase HPLC, two peaks, major and minor, appeared at tR of 64:10 and 14:00, respectively (Fig. 6B). The major peak was identified as Ser-Gly by amino acid sequenc-
ing, but was not eluted at the same t_R as that of the C-terminal peptide obtained from the API-digested native rhoA p21. However, when the API-digested rhoA p21 was treated with KOH and subjected to the same HPLC, the peak at a t_R of 68:20 disappeared and a peak at a t_R of 64:10 appeared (Fig. 6C). These results indicate that rhoA p21 lacks the C-terminal three amino acids of Leu-Val-Leu^{36} which are predicted from its cDNA, and that the cysteine residue in the C-terminal region (Cys^{36}) is the site of geranylgeranylation.

Carboxyl Methylation of rhoA p21—We have previously shown that the smg p21B purified from human platelets was carboxyl methylated by a carboxyl methyltransferase contained in bovine brain membranes (44). It was next examined whether rhoA p21 is also carboxyl methylated. The purified rhoA p21 was first incubated with stripped bovine brain membranes and [3H]AdoMet and then was subjected to SDS-PAGE followed by fluorography. However, a radioactive band could not be detected at the same position as that of rhoA p21, suggesting that the purified rhoA p21 is already fully methylated. In fact, when the synthetic geranylgeranylated C-terminal peptide was completely digested with API and subjected to reversed-phase HPLC, a single radioactive peak appeared and the t_R of this peak was identical with that of the peptide determined in Fig. 6A (Fig. 6, A and D). These results indicate that the C-terminal cysteine residue is carboxyl methylated.

The Order of Posttranslational Modifications—In the last set of experiments, we analyzed the order of the three post-translational modifications of rhoA p21. A geranylgeranyltransferase contained in bovine brain cytosol transferred a geranylgeranyl moiety from geranylgeranyl pyrophosphate to the bacterial rhoA p21 having the three C-terminal amino acids of Leu-Val-Leu^{90} but not to the protein lacking the three C-terminal amino acids (Table I). This result suggests that the geranylgeranylation is followed by the proteolytic removal of the three amino acids.

A carboxyl methyltransferase contained in the stripped bovine brain membranes transferred a methyl moiety from AdoMet to the synthetic geranylgeranylated C-terminal peptide with 10 amino acids but not to the synthetic nongeranylgeranylated C-terminal peptide (Table II). Under the same conditions, the bacterial rhoA p21s having the three C-terminal amino acids and lacking the three C-terminal amino acids were not [3H]-methylated (data not shown). These results suggest that rhoA p21 is first geranylgeranylated followed by removal of the three C-terminal amino acids and the subsequent carboxyl methylation of the exposed cysteine residue.

**Discussion**

We have shown here that the rhoA p21 purified from bovine aortic smooth muscle is geranylgeranylated at the cysteine residue in the C-terminal region (Cys^{90}) at a molar ratio of about 1:1, is carboxyl methylated at this cysteine residue, and lacks the three C-terminal amino acids predicted from its cDNA. These results are consistent with the earlier observations made for other many proteins having the Cys-A-A-X motif, such as rhodotorunicine A, tremerogen a-13, tremerogen A-9291-1, yeast a-factor, ras p21s, lamin B, the y subunits of an spy-type of G proteins, and G25K, among which the y subunit of an a2y-type of G proteins, smg p21B, and G25K are geranylgeranylated, whereas other proteins are neither geranylgeranylated nor carboxyl methylated.

**Table I**

Geranylgeranylation of rhoA p21 having the three C-terminal amino acids but not of rhoA p21 lacking the three C-terminal amino acids

<table>
<thead>
<tr>
<th></th>
<th>CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>270</td>
</tr>
<tr>
<td>rhoA p21 with Leu-Val-Leu</td>
<td>4840</td>
</tr>
<tr>
<td>rhoA p21 without Leu-Val-Leu</td>
<td>260</td>
</tr>
</tbody>
</table>

**Table II**

Carboxyl methylation of a geranylgeranylated synthetic peptide but not of the nongeranylgeranylated peptide

<table>
<thead>
<tr>
<th></th>
<th>CPM</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>360</td>
</tr>
<tr>
<td>Geranylgeranylated peptide</td>
<td>2540</td>
</tr>
<tr>
<td>Nongeranylgeranylated peptide</td>
<td>350</td>
</tr>
</tbody>
</table>

*The radioactivity of a gel slice without any protein band was counted.*
farnesylated (23–26, 35, 36, 44, 50–57). The structural features that
determine whether proteins are geranylgeranylated or
farnesylated remain to be fully defined, but we have previously
proposed, on the basis of currently available information, that
specific C-terminal amino acids may selectively direct the
farnesylation and geranylgeranylation of proteins: if X of the
A-A-X portion is Leu or Phe, the protein is geranylgerany-
lated and if X is other amino acids, the protein is farnesylated
(44). This rule is also applied to the case of rhoA p21, since
rhoA p21 has the C-terminal predicted C-terminus, Cys-Leu-Val-
Leu (2).

Ha- and N-ras p21s have a cysteine residue a short distance
upstream of the prenylated cysteine residue, and this cysteine
residue is furthermore palmitoylated through a thioester bond
(24). Ki-ras p21 lacks this second cysteine residue and is not
palmitoylated but has a polybasic region just upstream of the
prenylated cysteine residue (24, 31). rhoA p21 lacks the second
cysteine residue a short distance upstream of the prenylated
cysteine residue, and our present analysis indicates that rhoA
p21 is not palmitoylated. Interestingly, rhoA p21 has a poly-

This result suggests that the enzyme recognizes the Cys-A-A-
structure but not the protein lacking the Cys-A-A-X structure.

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