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Posttranslationally processed structure of the human platelet protein smg p21B: Evidence for geranylgeranylation and carboxyl methylation of the C-terminal cysteine

(ras p21/small GTP-binding protein/mevalonate/prenylation)

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ABSTRACT **smg p21A and -B are small GTP-binding proteins that share putative effector and consensus C-terminal sequences with ras p21 proteins. In the present report, we showed that human platelet smg p21B became labeled when intact platelets were incubated with exogenous [^{3}H]mevalonolactone and when a purified preparation of smg p21B was incubated with bovine brain membranes and *S*-adenosyl-L-[*methyl*-^{3}H]methionine. In addition, we demonstrated by gas chromatography/mass spectrometry that treatment of smg p21B with Raney nickel released a geranylgeranyl moiety in a molar ratio of about 1:1. In contrast, treatment of smg p21B with NH_2OH or KOH yielded no evidence for the presence of a palmitoyl thioester. Extensive digestion of smg p21B with *Achromobacter* protease I yielded two C-terminal tripeptides that contained serine and cysteine in a molar ratio of 2:1. Both peptides were modified by a thioether-linked geranylgeranyl group. One of the peptides comigrated with a ^{3}H-labeled proteolytic product of methylated smg p21B on reverse-phase HPLC and this peptide appeared at the same retention time as that of the other peptide after being treated with KOH. Since the cDNA-predicted C-terminal sequence of smg p21B contains a unique Ser-Ser-Cys peptide within its C-terminal domain, -Lys-Lys-Ser-Ser-Cys-Gln-Leu-Leu^{184}, these results indicate that smg p21B is posttranslationally modified by geranylgeranylation of Cys-181 and suggest that further modifications cause proteolytic removal of the three predicted C-terminal amino acids followed by partial methylation of the cysteinyl carboxyl group.**

smg p21A and -B, also referred to as the rap1A or Krev-1 and rap1B proteins, respectively, belong to a superfamily of ras p21/ras p21-like small GTP-binding proteins (G proteins) (refs. 1–10; M.K., Y. Kawahara, M. Sunako, S. Araki, T. Tsuda, H. Fukuzaki, and Y.T., unpublished data). They are found in most mammalian tissues, though their tissue and subcellular distributions differ somewhat from those of ras p21 proteins (11). smg p21 proteins have consensus amino acid sequences for GDP/GTP-binding and GTPase activities and exhibit these activities (3–10). The GTP-bound form of smg p21 proteins is active whereas the GDP-bound form is inactive, and the two forms can be interconverted by a specific GDP/GTP exchange protein, named GDP dissociation stimulator (12), and by a specific GTPase-activating protein (13, 14).

A striking feature of smg p21 proteins is that they share a putative effector domain with ras p21 proteins (1–6, 10) which interacts with ras p21 GTPase-activating protein (for review, see ref. 15). This suggests that smg p21 proteins may exert actions similar or antagonistic to those of ras p21 proteins in addition to their own specific actions (1–6, 10). Indeed, Krev-1 has been shown to suppress the transforming activity of activated Ki-ras in NIH 3T3 cells (6). Moreover, smg p21B and the rap1A protein inhibit ras p21 GTPase-activating protein activity (16, 17). smg p21 proteins also share a cDNA-predicted consensus C-terminal amino acid sequence with ras p21 proteins (1–6, 10). This sequence, Cys-Ali-Ali-Xaa, where Ali is an aliphatic amino acid and Xaa is any amino acid, is predicted for many other proteins including yeast a factor, lamin B, the γ subunits of heterotrimeric G proteins, and a small G protein referred to as G25K (18–20). Three kinds of posttranslational modifications can affect the cysteine in this type of sequence: prenylation (21–30), removal of the three terminal amino acids (31, 32), and carboxyl methylation (22, 23, 28, 33, 34). In some cases palmitoylation of upstream cysteines may also occur (25). The C-terminal cysteines of ras p21 proteins are farnesylated, and this is essential both for their binding to membranes and for their transforming activity (25–27). Furthermore, yeast a factor and lamin B are also farnesylated, and this may also be essential for their membrane-binding activity (23, 24). The C-terminal cysteines of the γ subunits of brain heterotrimeric G proteins and G25K are geranylgeranylated (28–30), but the structure that determines farnesylation or geranylgeranylation remains to be identified.

We show here that human platelet smg p21B also contains a C-terminal cysteine residue that is geranylgeranylated and carboxyl methylated. This finding provides insight concerning structural elements of the C terminus that may direct the prenylation of proteins.

MATERIALS AND METHODS

Materials and Chemicals. Human platelets were prepared as described (8). smg p21B was purified to near homogeneity from human platelets as described (8) except that smg p21B from the Mono Q column chromatography step was purified by YMC-pack AP-802 C_4 reverse-phase HPLC (3). The purified smg p21B was >95% pure as determined by SDS/PAGE. The C-terminal peptide of smg p21B was prepared by completely digesting smg p21B (2.5 nmol) with *Achromobacter* protease I (API) followed by Bakerbond WP-octyl C_8 reverse-phase HPLC (3) (*R,S*)-[^{3}H]mevalonolactone (MVA) (40 Ci/mmol; 1 Ci = 37 GBq) and *S*-adenosyl-L-[*methyl*-^{3}H]-methionine (AdoMet) (73.8 Ci/mmol) were obtained from DuPont/New England Nuclear. Plavastatin (a derivative of compactin) was provided by Sankyo.

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Abbreviations: G proteins, GTP-binding proteins; API, *Achromobacter* protease I; MVA, mevalonolactone; AdoMet, *S*-adenosyl-L-methionine; GC/MS, gas chromatography/mass spectrometry; t_R, retention time; Ali, aliphatic amino acid.

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Treatment of smg p21B with NH_2OH, KOH, and CH_3I. smg p21B (50 pmol) in 20 mM Tris·HCl (pH 7.5; 0.13 ml) containing 2 M urea and 1 mM dithiothreitol was incubated for 1 hr at 23°C with either 2 M NH_2OH (0.13 ml) or 0.2 M KOH (0.13 ml). smg p21B (100 pmol) in 20 mM Tris·HCl (pH 8.0; 0.5 ml) containing 1 mM EDTA, 1 mM dithiothreitol, 5 mM $MgCl_2$, and 0.6% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate was incubated for 24 hr in the dark at room temperature with CH_3I (0.1 ml) in the presence of 3% (vol/vol) formic acid (0.1 ml) containing 0.6% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. Then, CH_3I was removed under reduced pressure. After this treatment, each sample was subjected to YMC-pack C_4 reverse-phase HPLC. The protein peaks were detected by absorbance at 215 nm and confirmed by SDS/PAGE with silver staining.

Labeling of Human Platelets with [^{3}H]MVA. Human platelet-rich plasma was obtained from whole blood (50 ml) (35). After adding EGTA to a final concentration of 1 mM, the platelets were sedimented by centrifugation at 1200 × *g* for 15 min. The pellets were suspended in plasma and the number of platelets was counted and adjusted to 1.0×10^9 platelets per ml. The suspension (2 ml) was incubated with [^{3}H]MVA (0.4 mCi) in the presence of 0.5 mM plavastatin for 4 hr at 37°C. The labeled platelets were centrifuged at 1200 × *g* for 15 min and the pellets were washed 3 times with 20 mM sodium phosphate buffer (pH 6.5; 5 ml) containing 113 mM NaCl and 5.5 mM glucose. The pellets were sonicated twice for 10 sec in 20 mM Tris·HCl (pH 8.0; 0.2 ml) containing 1 mM EDTA, 1 mM dithiothreitol, 1 μM (4-amidinophenyl)-methanesulfonyl fluoride, and 0.25 M sucrose at 4°C. The homogenate was centrifuged at 100,000 × *g* for 1 hr at 4°C. Samples of the pellet (membrane fraction) and the supernatant (soluble fraction) were subjected to SDS/PAGE. Samples of the pellet were also analyzed by two-dimensional gel electrophoresis (36). Fluorography was performed with Amplify (Amersham) as a reagent. Western blot analysis was performed with an anti-smg p21 polyclonal antibody (11).

Gas Chromatography/Mass Spectrometry (GC/MS). The isoprenoid released from smg p21B or its proteolytic peptides was quantified by GC/MS in the selected ion monitoring mode (28). To obtain full ionization spectra of the released material for determination of double-bond configuration and positive identification against standards, smg p21B (1.0 nmol) was dried in a siliconized glass tube with a Speed Vac concentrator, resolubilized in formic acid/ethanol [1:4 (vol/vol); 0.4 ml], and extracted with three 1-ml volumes of pentane that had been equilibrated with the formic acid/ethanol. Raney nickel-activated catalyst (50 mg) and pentane (1 ml) were then added to the screw-capped tube and the sample was incubated for 15 hr at 100°C. The sample was then chilled for 1 hr at −20°C, whereupon water (0.4 ml) was added and the sample was vortex-mixed. After centrifugation at 1500 × *g* for 1 min, the pentane phase was removed. The sample was reextracted with pentane (0.5 ml) and the extracts were pooled. Half of the extract was concentrated for immediate analysis, and the remainder was hydrogenated for 3 hr over Adams' catalyst prior to analysis, as described (24) except that the temperature program started with the column at 80°C and increased to 250°C at 4°C/min.

Methylation of smg p21B. smg p21B (62 pmol) was carboxyl methylated using stripped bovine brain membranes (50 μg of protein) and [^{3}H]AdoMet (5.5 μCi) (34). The methylated smg p21B was subjected to SDS/PAGE. The gels were stained with Coomassie brilliant blue followed by fluorography.

Amino Acid Analysis. API-digested peptides that had been separated by C_8 reverse-phase HPLC were dried, dissolved in 0.2 ml of chilled performic acid, and incubated for 2 hr at 4°C. The performic acid was prepared by mixing 30% (vol/vol) H_2O_2 and 88% (vol/vol) formic acid [1:9 (vol/vol)] and allowing the mixture to stand for 2 hr at room temperature. The peptide solutions were dried under reduced pressure. After being hydrolyzed with 6 M HCl at 110°C for 20 hr, the samples were derivatized with phenyl isothiocyanate and subjected to Pico-Tag amino acid analysis according to the manufacturer's manual (Millipore).

Determination of Protein Concentrations. Protein concentrations were determined by the method of Bradford with bovine serum albumin as a standard protein (37). Where specified, protein concentrations were alternatively determined by densitometric tracing of protein bands stained with Coomassie brilliant blue on an SDS/polyacrylamide gel (3).

RESULTS

Treatment of smg p21B with NH_2OH, KOH, or CH_3I. When smg p21B was analyzed by C_4 reverse-phase HPLC, it appeared as a single peak with a retention time (t_R) of 24.5 min. After being treated with NH_2OH or KOH to remove a possible thioester-linked palmitoyl moiety, it appeared at the same t_R as the control (data not shown). However, after being treated with CH_3I to remove a possible thioether-linked moiety, it appeared at a t_R of 21.5 min (data not shown). These results seemed consistent with earlier observations that smg p21B shares a cDNA-predicted C-terminal amino acid sequence, Cys-Ali-Ali-Xaa, with ras p21 proteins (1, 5, 10) and that ras p21 proteins are modified by a cysteinyl thioether-linked isoprenoid group at their C termini (25–27).

Labeling of smg p21B with [^{3}H]MVA in Intact Human Platelets. To investigate whether smg p21B is also modified with an isoprenoid group, we incubated intact human platelets with [^{3}H]MVA, then disrupted them by sonication, and prepared the membrane and soluble fractions by centrifugation. Upon analyzing the membrane fraction by SDS/PAGE followed by fluorography, we detected four proteins with molecular weights of 22,000, 24,000, 24,500, and 25,000 (Fig. 1*A*, lane 2). In contrast, we were unable to detect any labeled proteins in the soluble fraction (Fig. 1*A*, lane 3). The M_r 22,000 protein migrated at the same position as that of smg p21B (Fig. 1*A*, lanes 1 and 2) and was furthermore recognized by an anti-smg p21 polyclonal antibody (Fig. 1*A*, lane 5). When the radiolabeled proteins were analyzed by two-dimensional gel electrophoresis, at least six spots were observed (Fig. 1*B*). Among them, two spots corresponded to smg p21B (Fig. 1 *B* and *C*). These results provided additional evidence that smg p21B might be modified by an isoprenoid group.

GC/MS Analysis of Raney Nickel-Treated smg p21B. To obtain definitive evidence concerning the isoprenoid modification of smg p21B, we treated it with Raney nickel to cleave a potential thioether linkage, extracted the treated material with pentane, and then analyzed the pentane extract by GC/MS. This approach identified a peak that had a t_R corresponding to that of authentic all-*trans*-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene (24). Furthermore, the material in the peak yielded a fragmentation pattern that was nearly identical to that of this hydrocarbon standard (Fig. 2 *A* and *B*). When the pentane extract was first hydrogenated and then analyzed by GC/MS, it yielded a peak with a t_R and spectrum nearly identical to those of phytane (Fig. 2 *C* and *D*). Analysis by GC/MS in the selected ion monitoring mode showed that about 0.9 mol of the C_{20} isoprenoid was recovered per mol of smg p21B and that no C_{15}, C_{25}, or C_{30} isoprenoids were present (Fig. 3*B*). These results indicated that smg p21B is modified by a single thioether-linked all-*trans*-geranylgeranyl moiety.

Methylation of smg p21B. Proteins that are modified by thioether-linked isoprenoid groups are often also α-carboxyl methylated (refs. 22, 23, and 28; for review, see ref. 38). To determine whether smg p21B could be α-carboxyl methylated, we incubated it with stripped bovine brain membranes, a known source of α-carboxyl methyl transferase activity (34), and [^{3}H]AdoMet, as a substrate. Upon analyzing the

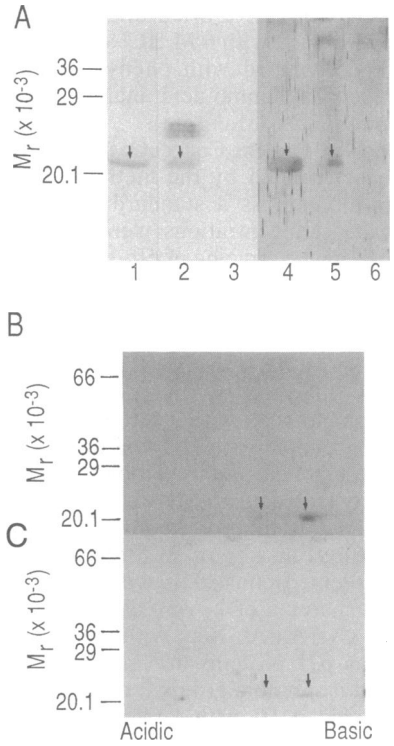

FIG. 1. Labeling of smg p21B with [^{3}H]MVA in intact human platelets. Human platelets were incubated with [^{3}H]MVA (0.4 mCi) in the presence of 0.5 mM plavastatin. (*A*) The purified unlabeled smg p21B (50 pmol), the labeled platelet membrane fraction (100 μg of protein), and the labeled soluble fraction (100 μg of protein) were analyzed by SDS/PAGE followed by Coomassie brilliant blue staining, fluorography, and Western blotting. Lanes: 1, Coomassie brilliant blue staining; 2 and 3, fluorographs; 4–6, Western blots; 1 and 4, smg p21B; 2 and 5, the membrane fraction; 3 and 6, the soluble fraction. Arrows indicate smg p21B and M_r 22,000 protein. (*B* and *C*) Two-dimensional gel electrophoresis of the purified unlabeled smg p21B (0.5 nmol) and the labeled membrane fraction (200 μg of protein). (*B*) Coomassie brilliant blue staining of smg p21B. (*C*) Fluorography of the membrane fraction. Arrows indicate smg p21B and M_r 22,000 protein.

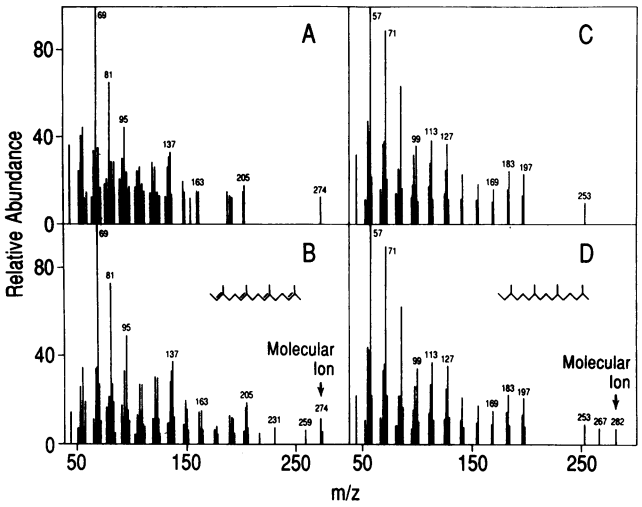

FIG. 2. Enhanced electron ionization spectra of C_{20} isoprenoids released from smg p21B after Raney nickel treatment. smg p21B was treated with Raney nickel and the released pentane-extractable material was analyzed by GC/MS before or after hydrogenation in the scan mode of data acquisition. The t_R and spectrum were then compared to those of an authentic standard, all-*trans*-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene, and the corresponding saturated compound phytane. (*A*) Spectrum of a selected GC peak [t_R = 14:22 (min:sec)] from the nonhydrogenated smg p21B. (*B*) Spectrum of all-*trans*-2,6,10,14-tetramethyl-2,6,10,14-hexadecatetraene (t_R = 14:23). (*C*) Spectrum of a selected GC peak (t_R = 11:43) from the hydrogenated smg p21B. (*D*) Spectrum of phytane (t_R = 11:43). The spectra shown have been enhanced by a standard method to highlight ions of low abundance (24). The absence of several low-abundance ion species from the spectra of the smg p21B-derived isoprenoids probably reflected the limited amount of protein available for analysis.

radiolabeled products by SDS/PAGE followed by fluorography, we detected a single radioactive band at the same position as that of smg p21B (Fig. 4, lanes 1 and 4). No radioactivity was incorporated into this protein in the absence of membranes or smg p21B (Fig. 4, lanes 2 and 3) or in the presence of membranes or smg p21B that had previously been denatured by boiling (data not shown). Furthermore, the methylation of smg p21B was completely blocked in the presence of *S*-adenosyl-L-homocysteine (Fig. 4, lane 5). These results demonstrated that smg p21B can be methylated by the brain enzyme preparation.

Identification of the Site of Geranylgeranylation of smg p21B. To locate the site of geranylgeranylation on smg p21B, we first completely digested it with API and then separated the API digests by C_8 reverse-phase HPLC. Many peptide peaks appeared (Fig. 5*A*), and the amino acid composition of several of the peptides was analyzed. Two of the peptides emerged from the column with a t_R of 63.5 min (peptide 1) and 67 min (peptide 2) in yields of 7.8% and 16%, respectively. When these peptides were separately treated with performic acid for 2 hr at 4°C and subjected to amino acid analysis, serine and cysteic acid in a molar ratio of about 2:1 were detected in each one. This amino acid composition corresponds uniquely to that of the Ser-Ser-Cys^{181} sequence in -Lys-Lys-Ser-Ser-Cys-Gln-Leu-Leu^{184}, at the cDNA-predicted C terminus of smg p21B (5, 10). These results provided evidence that smg p21B lacks the C-terminal Gln-Leu-Leu that is predicted from its cDNA.

When the two peptides were separately treated with Raney nickel and the released pentane extractable material was hydrogenated and analyzed by GC/MS selected ion monitoring, a peak with a t_R and ion ratios identical to those of phytane was observed in each case (Fig. 3*C*). In contrast, no phytane was found in a peak adjacent to peptides 1 and 2 that was analyzed as a control (Fig. 3*D*). These results indicated that Cys-181 of smg p21B is the site of geranylgeranylation.

Identification of the Site of Methylation of smg p21B. When smg p21B was methylated by [^{3}H]AdoMet as described above, then completely digested with API, and subjected to C_8 reverse-phase HPLC, a single major radioactive peak and three minor peaks appeared (Fig. 5*B*). This major peak comigrated with peptide 2. When peptide 2, which had been isolated from the API-digested smg p21B by HPLC, was treated with KOH and rechromatographed, it appeared at the same position as that of peptide 1 (Fig. 5*C*). The t_R of peptide 1 was not affected by treatment with KOH (data not shown). These results indicated that Cys-181 of smg p21B is the site of methylation and that peptide 2 is methylesterified, whereas peptide 1 is not.

DISCUSSION

We have shown herein that human platelet smg p21B is geranylgeranylated at Cys-181 with a molar ratio of about 1:1. Moreover, we have presented evidence that this cysteine is C-terminal and that it is at least partly carboxyl methylated. The cDNA-predicted sequence of smg p21B ends with -Lys-Lys-Cys-Gln-Leu-Leu^{184} (5, 10). By analogy with the proposed posttranslational processing of ras p21 proteins (25–27), it is likely that Cys-181 of smg p21B is geranylgeranylated

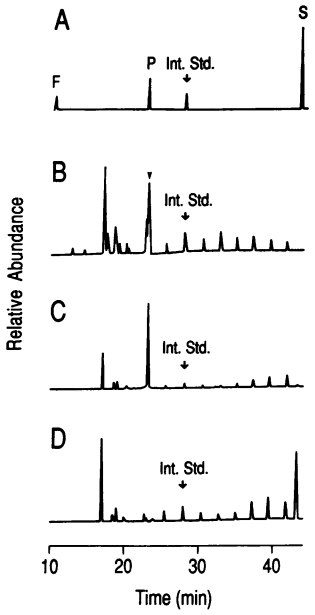

FIG. 3. Selected ion current chromatograms for m/z 183 of Raney nickel-released material from smg p21B and its proteolytic fragments. After digestion of smg p21B with API, the resulting peptides were purified by C_8 reverse-phase HPLC. Selected peptides and the intact smg p21B were separately hydrogenated and then treated with Raney nickel. The released pentane extractable material was analyzed by GC/MS in the selected ion monitoring mode. (*A*) Chromatogram of the saturated isoprenoid standards. F, farnesane (t_R = 10:47); P, phytane (t_R = 22:52); S, squalane (t_R = 43:15); Int. Std., an internal standard, eicosane (t_R = 27:54). (*B*) Chromatogram of the Raney nickel-released material from smg p21B. The t_R of the major peak shown by an arrowhead was 22:53. (*C*) Chromatogram of the Raney nickel-released material from C-terminal peptide 2 (see Fig. 5*A*). The t_R of the major peak was 22:53. A similar chromatogram was obtained for peptide 1 (data not shown). (*D*) Chromatogram of Raney nickel-released material from a peptide adjacent to peptides 1 and 2 shown by an arrowhead in Fig. 5*A*. Signals in each chromatogram were normalized to the most intense peak.

followed by removal of the three terminal amino acids and partial methylation of the liberated cysteinyl carboxyl group.

The structural features that determine whether proteins are geranylgeranylated or farnesylated remain to be fully defined, but a farnesyl transferase that can modify ras p21 proteins has recently been purified from rat brain cytosol and characterized (39). This enzyme catalyzes the transfer of a farnesyl group from farnesyl pyrophosphate to unmodified ras p21 proteins that have been synthesized in *Escherichia*

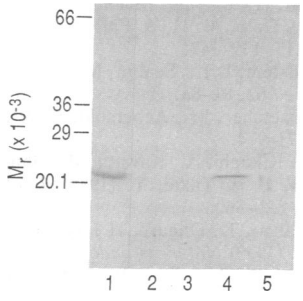

FIG. 4. Labeling of smg p21B with [^{3}H]AdoMet by bovine brain membranes. smg p21B was incubated with stripped bovine brain membranes and [^{3}H]AdoMet in the absence or presence of 25 μM *S*-adenosyl-L-homocysteine and subjected to SDS/PAGE followed by fluorography. Lanes: 1, Coomassie brilliant blue staining of the control smg p21B; 2–5, fluorographs; 2, smg p21B alone; 3, brain membranes alone; 4, smg p21B plus brain membranes; 5, smg p21B plus brain membranes plus *S*-adenosyl-L-homocysteine.

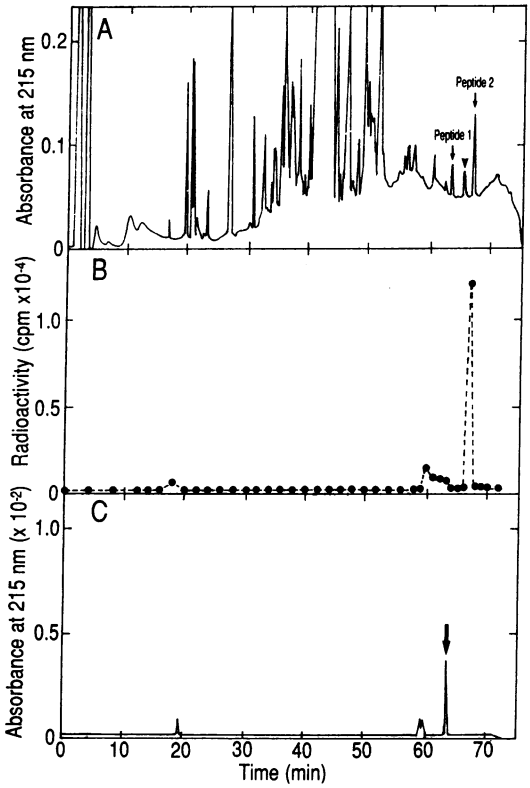

FIG. 5. C_8 reverse-phase HPLC of the API digests of smg p21B, ^{3}H-labeled methylated smg p21B, and the KOH-treated C-terminal peptide of smg p21B. (*A*) smg p21B (2.5 nmol) was completely digested with API and subjected to C_8 reverse-phase HPLC. Two peaks (peptides 1 and 2) were determined to contain Ser-Ser-Cys as described in the text. (*B*) ^{3}H-labeled methylated smg p21B (0.25 nmol) was digested with API and subjected to the same HPLC. Fractions (1 ml) were pooled and dried, and the radioactivity in each tube was determined. (*C*) Peptide 2 (0.4 nmol) obtained from the experiment in *A* was treated with 0.1 M KOH for 1 hr at 23°C in the presence of 4 M urea and 0.6% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate and then rechromatographed. The absorbance of the control reaction mixture was subtracted from that of the sample.

coli (39). The enzyme appears to recognize the Cys-Ali-Ali-Xaa structure of proteins such as yeast a factor, lamin B, c-Ki-ras p21, and the γ subunit of transducin, which have cDNA-predicted sequences that end with -Cys-Val-Ile-Ala, -Cys-Ala-Ile-Met, -Cys-Val-Ile-Met, and -Cys-Val-Ile-Ser, respectively (1, 18, 19, 42). It is possible that the enzyme that geranylgeranylates smg p21B, the γ subunits of brain heterotrimeric G proteins, and G25K may also recognize the Cys-Ali-Ali-Xaa structure of its substrates (28–30). The cDNA-predicted C-terminal sequence of one of the γ subunits ends with Cys-Ala-Ile-Leu, whereas the corresponding sequence of human brain G25K is Cys-Cys-Ile-Phe (40, 43). Therefore, on the basis of currently available information, it is possible that specific C-terminal amino acids may selectively direct the farnesylation and geranylgeranylation of proteins, as suggested below.

$$\text{-Cys-Ali-}\left[\ \right]\text{-}\begin{bmatrix}\text{Ala}\\ \text{Ser}\\ \text{Met}\end{bmatrix} = \text{farnesylation}$$

and

$$\text{-Cys-Ali-}\left[\ \right]\text{-}\begin{bmatrix}\text{Phe}\\ \\ \text{Leu}\end{bmatrix} = \text{geranylgeranylation}$$

Our results demonstrate that treatment of smg p21B with API yields two geranylgeranylated C-terminal tripeptides that have the same amino acid composition. One of these peptides is carboxyl methylated whereas the other is not. It is unknown whether this heterogeneity is physiological or due to the removal of methyl ester from smg p21B during purification procedures. However, we have shown (36) that phosphorylated smg p21B can be resolved into two spots by two-dimensional gel electrophoresis and have shown herein that smg p21B that is labeled with [^{3}H]MVA can be similarly resolved (Fig. 1*C*). This heterogeneity may partly reflect the methylation state of smg p21B *in vivo*.

The geranylgeranylation and methylation of smg p21B may be important because we have found that C-terminal modification by lipids is required for the binding of smg p21B to membranes (M. Hiroyoshi, K. Kaibuchi, S. Kawamura, Y. Hata, and Y.T., unpublished data). Furthermore, we have reported (36, 41) that smg p21B is phosphorylated by cAMP-dependent protein kinase, have identified Ser-179 as the site of phosphorylation, and have shown that the phosphorylation enhances the GDP/GTP exchange reaction stimulated by the GDP dissociation stimulator (Y. Hata, K. Kaibuchi, S. Kawamura, M. Hiroyoshi, and Y.T., unpublished data). Therefore, at least four kinds of posttranslational modification appear to affect the C-terminal region of smg p21B. A major challenge for the future will be to determine how these posttranslational modifications affect the function of smg p21B in intact cells.

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