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Myristyl amino-terminal acylation of murine retrovirus proteins: An unusual post-translational protein modification

(gas chromatography-mass spectrometry/membrane proteins/amino acid sequence)

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ABSTRACT **The primary structure of the NH_2-terminal region of the *gag* gene encoded internal membrane-associated protein p15 has been determined for both Rauscher and Moloney murine leukemia viruses. Peptides generated by endopeptidases and purified by HPLC were subjected to semi-automated Edman degradation. Dipeptides obtained with dipeptidyl carboxypeptidase were identified by gas chromatography-mass spectrometry. The amino acid sequence of the first 16-residue segment of Rauscher p15 is identical to the sequence of Moloney p15 except for a single amino acid substitution (Gly → Asp) at position 13. Both proteins were found to have an acylated NH_2 terminus. By mass spectroscopy, myristic acid [$CH_3(CH_2)_{12}COOH$] was found to be bound through an amide linkage to the NH_2-terminal glycyl residue in both p15s. The results of liquid chromatography show that the NH_2-terminal myristyl group greatly contributes to the strong binding of these modified proteins and peptides to hydrophobic surfaces. Because p15 is known to be derived from the NH_2-terminal region of a precursor polyprotein $Pr65^{gag}$ by proteolytic cleavage in the assembled virus, it is suggested that myristylation *in vivo* takes place during the biosynthesis of $Pr65^{gag}$. Preliminary data indicate that such modification of *gag* precursor polyproteins may be common to mammalian retroviruses. The role of NH_2-terminal myristyl acylation of $Pr65^{gag}$ in virus assembly and the possibility of similar NH_2-terminal modifications of *gag*-related fusion proteins of transforming viruses are discussed.**

Type C murine leukemia viruses (MuLV) are representatives of a morphologically and biochemically defined group of RNA viruses (family Retroviridae) which replicate via a DNA intermediate synthesized by the virus-encoded enzyme reverse transcriptase (for review see ref. 1). Viruses of the Retroviridae family have been associated with animal and human leukemia and other types of cancer (for review see refs. 2 and 3).

The type C MuLVs are composed of a RNA and a protein inner core structure encapsulated by a lipoprotein envelope. The inner core structural proteins of type C MuLVs are synthesized as a precursor polyprotein designated $Pr65^{gag}$. In the process of virus assembly, $Pr65^{gag}$ is associated with the inner plasma membrane where it also forms a specific complex with viral RNA. During subsequent viral budding and maturation, $Pr65^{gag}$ is proteolytically cleaved into four major fragments which constitute the inner core structural proteins of the virus. In MuLVs these fragments are designated p15, pp12, p30, and p10, in the order that they occur from the NH_2 to the COOH terminus of $Pr65^{gag}$ (for review see ref. 4).

The complete nucleotide structure of the proviral DNA has been reported for the Moloney (M) strain of MuLV and murine sarcoma virus (5, 6). The complete amino acid sequences of p10 (7) pp12 (8), and p30 (unpublished data) have been determined by protein microanalytical techniques. These data clearly define the coding region for most of $Pr65^{gag}$ in the viral genome. However, knowledge of the NH_2-terminal amino acid sequence of the viral p15 is required to define the complete coding sequence for $Pr65^{gag}$. Previous efforts to identify the NH_2-terminal residue of viral p15 by dansylation and by Edman degradation have failed. The protein was assumed to have a "blocked" NH_2 terminus (9) but the nature of the "blocking" group was not determined.

In this communication we describe the isolation and structural determination of peptides derived from the NH_2-terminal region of Rauscher (R) and M-MuLV p15s. By mass spectroscopy we identified the blocking group on the NH_2 terminus of both p15s to be a myristyl radical linked to the NH_2-terminal glycine by an amide bond. The implication of this *in vivo* protein modification in eukaryotic cells with respect to virus assembly and its possible significance to the structure and function of the *gag–onc* fusion proteins of transforming viruses will be discussed.

MATERIALS AND METHODS

Viruses. R-MuLV, grown in BALB/c mouse bone marrow JLS-V9 cells (10), and M-MuLV, grown in N/3T3 cells (11), were purified by sucrose density gradient centrifugation at the Viral Resources Laboratory, National Cancer Institute–Frederick Cancer Research Facility.

Chemicals. Endoproteinase Lys-C and thermolysin were obtained from Boehringer Mannheim. Acetonitrile was obtained from Burdick and Jackson (Muskegon, MI) and was used without further treatment. Trifluoroacetic acid (F_3CCOOH) was sequenal grade obtained from Pierce. All chemicals used in the spinning cup liquid Sequenator were as described (7).

Purification and Analysis of Proteins and Peptides. Viral proteins were purified by reversed-phase HPLC (RP-HPLC) as described (12, 13). Endoproteinase Lys-C digestions were performed in buffered (0.1 M NH_4CO_3, pH 8) solutions of 40% (vol/vol) acetonitrile containing 0.3–0.5 mg of viral p15 per ml and 6 μg of enzyme. Thermolysin digestions were done as above except the final solution was 20% in acetonitrile. All digestions were performed at room temperature (23°C). Peptides were purified by RP-HPLC as described (13). Amino acid analysis and semi-automated micro sequence determination were performed as described (7, 14, 15).

Dipeptidyl Carboxypeptidase Digestion. Dipeptidyl carboxypeptidase was prepared and used as described (16). Digestions were carried out in 20 μl of 50 mM *N*-methylmorpho-

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Abbreviations: MuLV, murine leukemia virus; M, Moloney; R, Rauscher; *gag*, group specific antigen; *onc*, oncogene; F_3CCOOH, trifluoroacetic acid; Me_3Si, trimethylsilyl; GC, gas chromatography; MS, mass spectrometry; RP-HPLC, reversed-phase HPLC.

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line·HCl (Pierce) at pH 7.5 and 37°C for 4 hr and were stopped by lyophilization. Dried samples were treated with 20 μl of 50% (vol/vol) bis(trimethylsilyl)-trifluoroacetamide (Supelco, Bellefonte, PA) in acetonitrile for 10 min at 140°C in sealed tubes. Aliquots were taken directly for gas chromatography/mass spectrometry (GC/MS).

Preparation of Acid-Hydrolyzed Samples for GC/MS. Acid-hydrolyzed samples (6 M HCl, 100°C) were vacuum dried at room temperature and extracted with benzene. The benzene extract was transferred to another tube and evaporated to dryness. Trimethylsilyl (Me_3Si) derivatives of benzene-soluble compounds were prepared as described above. Aliquots were taken directly for GC/MS.

Analysis by GC/MS. All Me_3Si-derivatized samples (see above) were introduced by GC into an LKB model 2091 mass spectrometer set in the EI mode and interfaced with a DEC PDP 11/05-computer. The GC column was a 0.3 mm × 25 m crosslinked SE-54 fused silica column (Hewlett–Packard), and a temperature rise of 10°C per min from 110°C to 270°C was used. Peaks from the GC column were detected by a total ion detector in the mass spectrometer, and spectra were scanned with a trap current of 50 μA and an accelerating voltage of 3.5 kV at 70 eV. A complete mass spectrum of each 2-sec window in the GC trace was taken and stored in the computer. After the GC/MS run was completed, the computer was first directed to present the accumulated data as a total ion plot (which is similar to the GC trace) and then directed to search the accumulated mass spectra for a selected ion to show the presence of a specific class of compounds that exhibit the selected ion in their mass spectrum. Next, the computer was directed to display the mass spectra of the material under particular peaks. The structures of Me_3Si-dipeptides were deduced from their mass spectra by using rules previously established for their identification via MS (16, 17). According to these rules, assignments are made from the presence of two ion masses in the spectrum. One ion mass determines the sequence of the dipeptide. Dipeptides containing leucine are distinguished from dipeptides containing isoleucine by a third ion mass. The identity of Me_3Si-myristate (Me_3Si-tetradecanoate) was deduced from characteristic peaks in its mass spectrum and verified by comparison to the published mass spectrum (18).

RESULTS

The complete amino acid sequences of M- and R-MuLV p15 were determined and will be described elsewhere. In this report we focus attention on the nature of the NH_2-terminal blocking group of MuLV p15 and its contribution to the hydrophobic properties of the protein. MuLV p15s were isolated from disrupted virus by RP-HPLC on μBondapak C_{18} as described (12, 13). The *gag* proteins of MuLVs were eluted from the support in the following order of increasing retention: p10, pp12, p30, $Pr65^{gag}$, $Pr27^{gag}$ (an incomplete cleavage product consisting of p15 and p12), and p15. The relative hydrophobicity of MuLV *gag* proteins is inferred from their order of elution from the hydrophobic support. MuLV p15 is the most hydrophobic *gag* protein and elutes from μBondapak C_{18} at about 44% acetonitrile; $Pr65^{gag}$ and $Pr27^{gag}$ elute at slightly lower concentrations of acetonitrile. This suggests that the major hydrophobic center of $Pr65^{gag}$ resides in the p15 portion of the structure.

M-MuLV p15 was digested with endoproteinase Lys-C for 18 hr, and the resulting peptide mixture was separated by RP-HPLC (data not shown). NH_2-terminal peptides were identified as having blocked NH_2-terminal residues. Incomplete digestion gave rise to more than one NH_2-terminal peptide; however, the total yield (nanomoles) of NH_2-terminal peptides was about 80% of the nanomoles of protein digested. The amino acid composition of the major NH_2-terminal peptide isolated from M-MuLV p15 (residues 1–30) is given in Table 1 (M 1–30). R-MuLV p15 was digested for 72 hr with endoproteinase Lys-C, and the NH_2-terminal peptide was isolated and identified as above. The amino acid composition of the R-MuLV NH_2-terminal peptide (residues 1–16) is also given in Table 1 (R 1–16).

For further structural studies, Lys-C peptides derived from the NH_2-terminal region of p15s were digested with thermolysin and these peptide mixtures were separated by RP-HPLC (Fig. 1). Amino acid analysis of the peptides in Fig. 1 gave the following results: peptide 1, $Thr_{2.0}Ser_{1.1}Pro_{0.9}Val_{1.0}Leu_{1.0}$; peptide 2, $Asx_{1.0}Thr_{1.0}Leu_{2.0}His_{1.0}Lys_{1.0}$; peptide 3, identical to peptide 2; peptide 4, $Thr_{1.0}Glx_{1.0}Gly_{1.0}$; peptide 5, $Thr_{3.3}Ser_{1.0}Glx_{1.4}Pro_{1.0}Gly_{1.4}Val_{1.0}Leu_{1.0}$; peptide 6, undigested R 1–16 (see Table 1). The amino acid sequences of peptides 1 and 2 were determined by semi-automated NH_2-terminal Edman degradation; the results are summarized in Fig. 2 (residues 4–9 and 10–16, respectively, in the R sequence). Peptides 4 and 5 had blocked NH_2-terminal residues. Composition data indicated that peptide 4 was a tripeptide (R 1–3, Fig. 2). Peptide 5 (R 1–9) was an incomplete cleavage product which helped to establish the order of the thermolytic peptides from R 1–16 as shown in Fig. 2.

Peptide M 1–30 (Table 1) from M-MuLV p15 was also digested with thermolysin and the resulting peptides were isolated by RP-HPLC (data not shown). Results of amino acid analysis of the thermolytic peptides (numbered according to the M sequence in Fig. 2) were as follows: M 1–3, $Thr_{1.0}Glx_{1.0}Gly_{1.0}$; M 4–7, $Thr_{1.9}Pro_{1.0}Val_{1.0}$; M 8–9, $Ser_{0.8}Leu_{1.0}$; M 10–11, $Thr_{0.9}Leu_{1.0}$; M 8–11, $Thr_{0.9}Ser_{1.0}Leu_{1.9}$; M 12–20, $Asx_{1.1}Glx_{1.2}Gly_{1.0}Val_{0.8}Leu_{1.0}His_{1.0}Lys_{1.0}Arg_{0.9}$; M 21–30, $Asx_{2.1}Ser_{1.1}Glx_{1.4}Ala_{1.0}Val_{1.3}Ile_{1.0}His_{1.0}Lys_{1.0}$. The amino acid sequences of M 4–7 and M 12–20 were determined by semi-automated Edman degradation as summarized in Fig. 2. These results were in complete agreement with the amino acid sequence for M-MuLV p15 predicted from the nucleotide sequence of M-MuLV proviral DNA (6). R-MuLV p15 has aspartic acid instead of glycine in position 13.

In the separation of the Lys-C and thermolytic peptides (Fig. 1), the NH_2-terminal fragments were eluted in 40–43% acetonitrile whereas all other peptide fragments derived from other

Table 1. Amino acid compositions of NH_2-terminal peptides from R- and M-MuLV p15s produced by endoproteinase Lys-C

Amino acid	Peptide*	
	M 1–30	R 1–16
Asp/Asn	2.8 (2+1)	1.1 (1+0)
Thr	3.9 (4)	3.8 (4)
Ser	1.9 (2)	1.1 (1)
Glu/Gln	3.0 (1+2)	1.1 (0+1)
Pro	1.3 (1)	1.0 (1)
Gly	1.9 (2)	1.0 (1)
Ala	1.1 (1)	0 (0)
Val	3.5 (4)	1.0 (1)
Ile	1.0 (1)	0 (0)
Leu	3.0 (3)	2.7 (3)
His	1.4 (2)	0.9 (1)
Lys	1.8 (2)	0.9 (1)
Arg	1.0 (1)	0 (0)
Trp	ND (1)	ND (1)

* Peptides are numbered according to the position of their first and last residue in the amino acid sequence of p15 (see Fig. 3). Values are expressed as moles of amino acid residues per mole of peptide. Numbers in parenthesis are from the sequence. ND, not determined. Values for Cys, Met, Phe, and Tyr were 0.

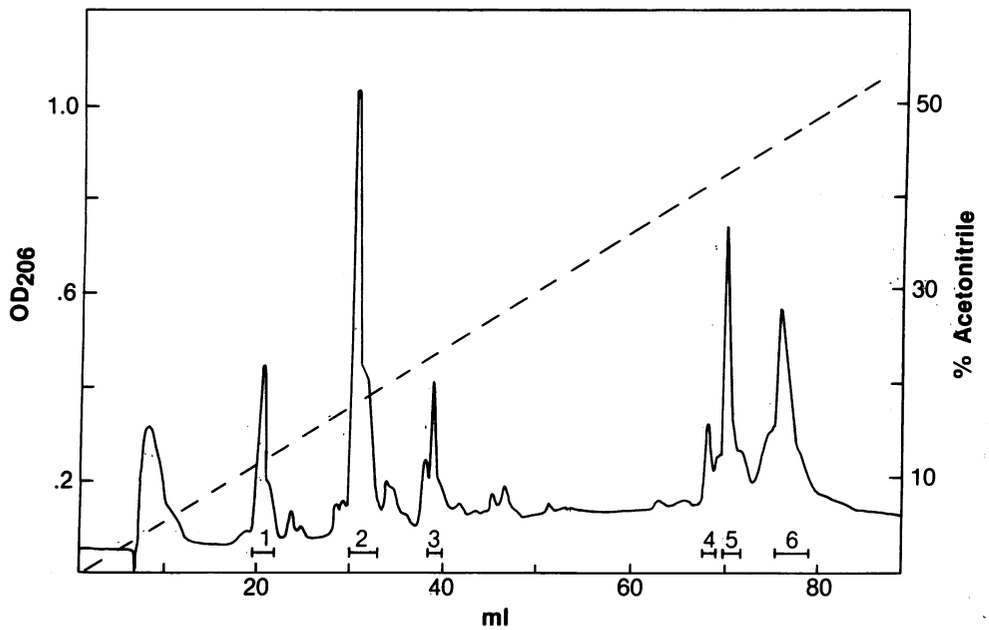

FIG. 1. Separation of thermolysin digest of 30 nmol of R-MuLV p15 NH_2-terminal Lys-C peptide (R 1–16 in Table 1) by RP-HPLC on μBondapak C_{18}. Gradient elution was accomplished at pH 2 with acetonitrile (----). Peptides were detected by UV absorption at 206 nm (——). Peptide fractions 1 through 6 are identified in the text.

parts of the proteins were eluted at lower concentrations of organic reagent. These observations and the fact that NH_2-terminal tripeptides of both p15s do not contain hydrophobic amino acid residues strongly suggested that a major hydrophobic center of p15 is located within the NH_2-terminal blocking group of the protein.

The nature of the NH_2-terminal blocking group on p15 was determined as follows. The thermolytic tripeptides R 1–3 and M 1–3 containing the unknown blocking group (X) and having the structure X-Gly-Gln-Thr (Fig. 2) were digested with dipeptidyl carboxypeptidase. The resulting mixtures were derivatized with Me_3Si and analyzed by GC/MS. The data for M 1–3 are shown in Fig. 3. Identical results were obtained for R 1–3. All components of the separated mixture were analyzed by MS as they eluted from the GC column (Fig. 3*A*). The mass 102 ion, $Me_3Si—NH—CH_2^+$, resulting from the loss of $R'CH{=}C{=}O$ from $R'CH_2CON(Me_3Si)CH_2^+$ is an expected ion in the mass spectrum of any $Me_3Si—N—$acylGly present in the mixture. As the selected ion trace (Fig. 3*B*) shows, compound I (Fig. 3*A*) is the only component of the separated mixture giving an ion with a mass of 102. The mass spectrum of compound I is shown in Fig. 3*C*. The molecular ion has a mass of 429 mass units, which is consistent with the acyl group being a myristyl moiety. All of the major ions and observed splitting patterns of compound I (Fig. 3*C*) are consistent with the structure $CH_3(CH_2)_{12}CONZCH_2CO_2Z$ in which Z indicates Me_3Si groups replacing reactive hydrogens on un-derivatized myristylglycine.

The mass spectrum of compound II (Fig. 3*A*) is shown in Fig. 3*D*. This spectrum is consistent with compound II being the Me_3Si derivative of the dipeptide Gln-Thr. The sequence-determining ion is at mass 245, and the molecular weight-determining ion is at mass 520. In this case, the molecular ion itself (mass 535) is also present in the spectrum. These data show that the blocked NH_2-terminal tripeptide derived from the either M- or R-MuLV p15 has the structure $CH_3(CH_2)_{12}$-CO—Gly—Gln—Thr—OH. In addition, peptides R 1–9 and R 1–16 were digested with dipeptidyl carboxypeptidase and analyzed by GC/MS. The results were in complete agreement with the amino acid sequence indicated in Fig. 2 and again showed the NH_2-terminal residue to be myristylglycine (data not shown). To verify further that MuLV p15s are myristylated at the NH_2 terminus, all R-MuLV p15 NH_2-terminal peptides (R 1–3, R 1–9, and R 1–16) were hydrolyzed with 6 M HCl and analyzed by GC/MS after trimethylsilylation. In all cases, myristic acid was identified as the only major fatty acid species. The mass spectrum obtained matched the mass spectrum of authentic Me_3Si-myristic acid (Me_3Si-tetradecanoic acid) (18).

DISCUSSION

In vivo chemical modification of proteins has been reviewed recently (19). Some bacterial outer membrane proteins were

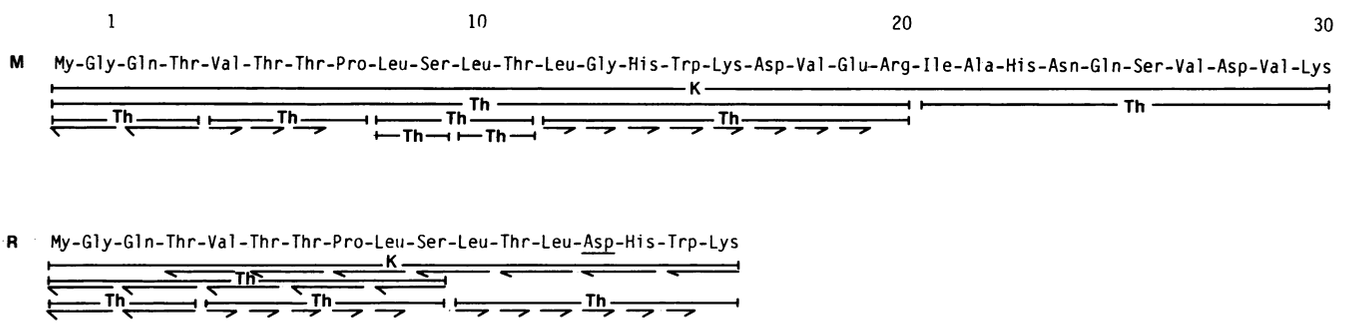

FIG. 2. Summary of NH_2-terminal amino acid sequence data for M- and R-MuLV p15. My, myristyl group covalently bound to the NH_2-terminal amino group by an amide bond; ——, peptides derived by enzymatic cleavage with endoproteinase Lys-C (K) or thermolysin (Th); →, results of Edman degradation of the peptide; ←, results of dipeptidyl carboxypeptidase digestion. The amino acid sequence of MuLV p15 is in agreement with that predicted by nucleic acid sequence (5, 6).

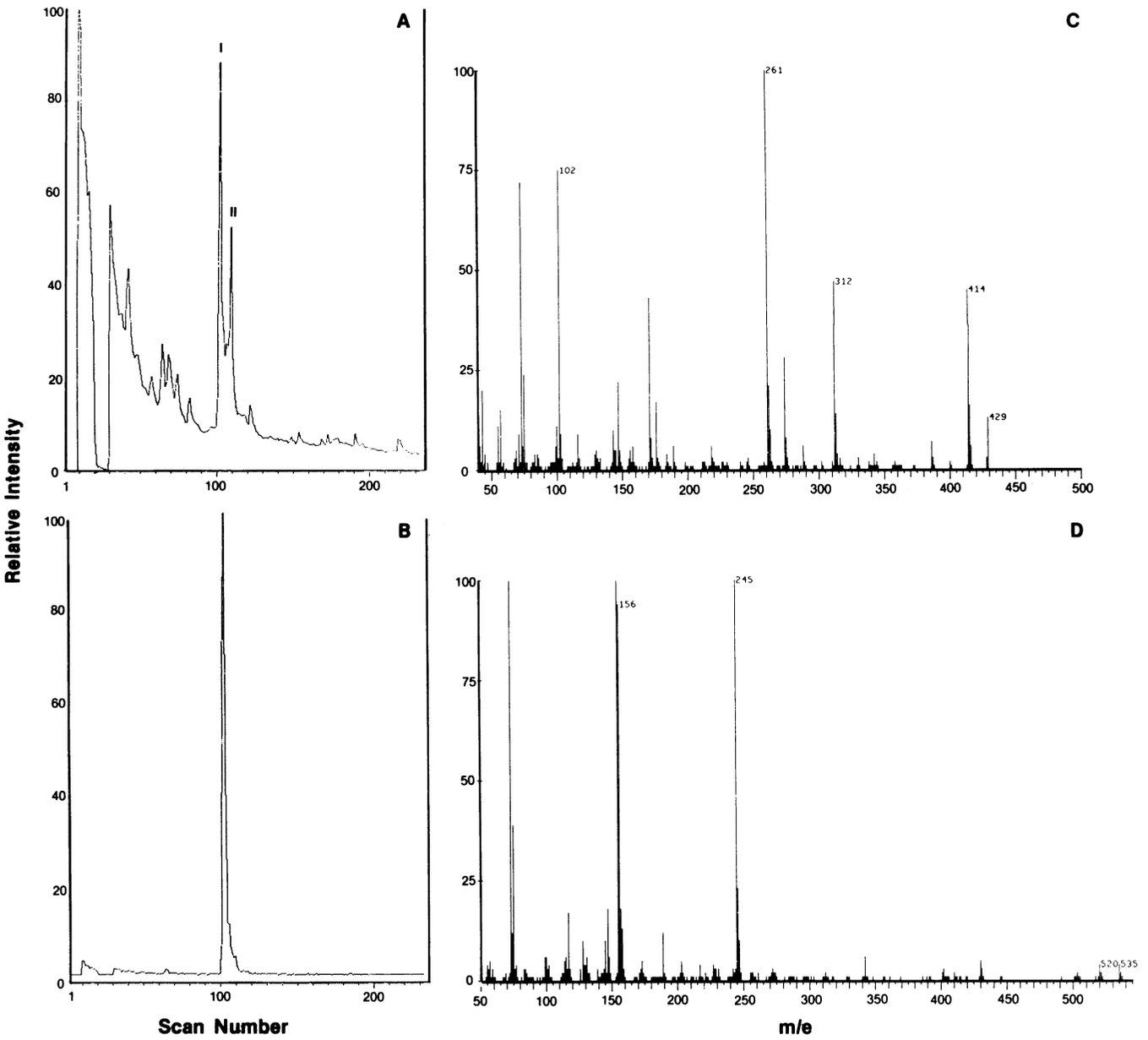

FIG. 3. GC/MS of compounds produced by dipeptidyl carboxypeptidase digestion of NH_2-terminal tripeptide M 1–3 from M-MuLV p15. (*A*) Total ion trace obtained for the separation of Me_3Si digestion products. (*B*) Selected ion trace for $Me_3Si—NH—CH_{2+}$ (m/e = 102). (*C*) Complete mass spectrum of compound I (*A*). (*D*) Complete mass spectrum of compound II (*A*).

shown to have palmityl-, palmitoleyl-, or *cis*-vaccenyl radicals linked to NH_2 termini (20). The data presented in this report show that the p15 structural proteins from M- and R-MuLVs are modified by myristyl acylation of their common NH_2-terminal glycine residues. MS clearly identified the structure of myristylglycine with the expected amide linkage, CH_3-$(CH_2)_{12}$—CO—NH—CH_2—COOH. The viral p15 represents the NH_2-terminal portion of the precursor polyprotein $Pr65^{gag}$. The results of Edman degradation indicated that $Pr65^{gag}$ and one of its incomplete cleavage products, $Pr27^{gag}$ (consisting of p15 and p12), are also blocked at their NH_2-terminal residues. The finding that p15 is blocked by NH_2-terminal myristyl acylation suggests that the NH_2-terminus of $Pr65^{gag}$ may also be myristylated. This has now been confirmed by the specific incorporation of [^{3}H]myristic acid into $Pr65^{gag}$ (unpublished data).

The biosynthetic mechanism for NH_2-terminal long-chain fatty acid acylation of viral proteins is unknown. The following model is proposed. Synthesis of $Pr65^{gag}$ probably initiates at the methionine codon immediately preceding the codon for the NH_2-terminal glycine (5, 6) of $Pr65^{gag}$ (Fig. 2). Removal of the initiator methionine, a cotranslational event, precedes NH_2-terminal fatty acid acylation which can occur co- or post-translationally. In the cases presented here, the acylation step is specific for the addition of a myristyl group. This specificity suggests an enzyme-catalyzed acylation step. It remains to be determined whether the putative myristyl *N*-acyl transferase is a cellular of virus-encoded enzyme. We assume that long-chain fatty acid NH_2-terminal acylation is not confined to retroviral proteins; there may exist cellular proteins modified similarly *in vivo*.

Covalent attachment of a long-chain fatty acid should greatly increase the hydrophobic character of acceptor proteins. This expectation is supported by the observations that myristylated $Pr65^{gag}$ and all NH_2-terminal peptide fragments derived from it, including $Pr27^{gag}$, p15, and NH_2-terminal peptides of p15, show similar strong hydrophobic binding to RP-HPLC supports

(see refs. 12 and 13; Fig. 1). These observations suggest that the myristyl group is the most dominant hydrophobic part of $Pr65^{gag}$ and may greatly enhance its capacity to bind to cellular and viral membranes.

During the early stages of virus assembly, intact $Pr65^{gag}$ is located on the cytoplasmic side of the cell membrane where it also complexes with viral RNA (21, 22). Covalently bound long-chain fatty acid can serve to anchor the $Pr65^{gag}$–RNA complex to the cell membrane. Thus, one of the functions of NH_2-terminal myristyl acylation of $Pr65^{gag}$ may be to assist in the early stages of virus assembly and budding. In the mature virus, in which $Pr65^{gag}$ has been cleaved to the viral structural proteins, the NH_2-terminal fatty acid modification of p15 may serve to stabilize the viral membrane. The hydrophobic character and membrane association of p15 have been documented (23, 24). If NH_2-terminal fatty acid acylation of $Pr65^{gag}$ does indeed play a role in MuLV assembly, then we expect that a similar modification occurs on the *gag* precursor polyproteins of other mammalian retroviruses. The following observations support this hypothesis. In addition to M- and R-MuLV, the natural cleavage products of *gag* precursors from many different viruses including Gross, Friend, and AKR MuLVs, rat and feline leukemia viruses, baboon endogenous virus (BaEV), and avian reticuloendotheliosis virus have been separated by RP-HPLC. In all cases the NH_2-terminal fragment of the *gag* precursor uniformly showed strong binding to the RP-HPLC support and was shown to be blocked against NH_2-terminal Edman degradation (unpublished data). In ongoing studies we have found that baboon endogenous virus NH_2-terminal *gag* protein also has myristylglycine as its NH_2-terminal residue.

Numerous genetic recombinants between retroviral *gag* gene and host cell DNA have been described (for review see ref. 25). Such recombinants are able to cause transformation of appropriate cells grown in tissue culture and cause rapid onset of disease in test animals. Many such recombinants were shown to code for a transformation-specific protein of cellular origin (*onc* gene product) fused to the COOH terminus of virus-encoded *gag* protein. Some examples of this class of transforming proteins are $P29^{gag\text{-}ras}$ from Rasheed rat sarcoma virus (26), $P120^{gag\text{-}abl}$ from Abelson MuLV (27), and $P85^{gag\text{-}fes}$ from Snyder–Theilen feline sarcoma virus (28, 29) (numerous other examples are listed in ref. 25). The data presented here suggest that the *gag–onc* gene products of the recombinant transforming viruses may also be NH_2-terminal fatty acid acylated. This suggests a mechanism for virus-mediated translocation of cellular gene products to the cell membrane. Such a translocation may be an important part of virus-mediated cellular transformation and oncogenesis.

Note Added in Proof. While this paper was in press a report by S. A. Carr *et al.* (30) showed that the NH_2-terminal glycine of the catalytic subunit of cyclic AMP-dependent protein kinase from bovine cardiac muscle is also myristylated.

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1. Varmus, H. & Swanstrom, R. (1982) in *Molecular Biology of Tumor Viruses: RNA Tumor Viruses*, eds. Weiss, R. A., Teich, N. M., Varmus, H. E. & Coffin, J. M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 369–512.
2. Teich, N., Wyke, J., Mak, T., Bernstein, A. & Hardy, W. (1982) in *Pathogenesis of Retrovirus Induced Disease*, eds. Weiss, R. A., Teich, N. M., Varmus, H. E. & Coffin, J. M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 785–998.
3. Gallo, R. B. & Wong-Staal, F. (1982) *Blood* **60**, 545–557.
4. Dickson, C., Eisenman, R., Fan, H., Hunter, E. & Teich, N. (1982) in *Molecular Biology of Tumor Viruses: RNA Tumor Viruses*, eds. Weiss, R. A., Teich, N. M., Varmus, H. E. & Coffin, J. M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 513–648.
5. Shinnick, T. M., Lerner, R. A. & Sutcliffe, J. G. (1981) *Nature (London)* **293**, 543–548.
6. Van Beveren, C., van Straaten, F., Galleshaw, J. A. & Verma, I. M. (1981) *Cell* **27**, 97–108.
7. Henderson, L. E., Copeland, T. D., Sowder, R. C., Smythers, G. W. & Oroszlan, S. (1981) *J. Biol. Chem.* **256**, 8400–8406.
8. Versteegen, R. J., Copeland, T. D. & Oroszlan, S. (1982) *J. Biol. Chem.* **257**, 3007–3013.
9. Oroszlan, S., Henderson, L. E., Stephenson, J. R., Copeland, T. D., Long, C. W., Ihle, J. N. & Gilden, R. V. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1404–1408.
10. Wright, B. S., O'Brien, P. A., Shibley, G. P., Mayyasi, S. A. & Lasfargues, J. C. (1967) *Cancer Res.* **27**, 1672–1677.
11. Enjuanes, L., Lee, J. C. & Ihle, J. N. (1979) *J. Immunol.* **122**, 665–674.
12. Henderson, L. E., Sowder, R. & Oroszlan, S. (1981) in *Chemical Synthesis and Sequencing of Peptides and Proteins*, eds. Liu, D. T., Schechter, A. N., Heinrikson, R. & Condliffe, P. G. (Elsevier/North-Holland, Amsterdam), pp. 251–160.
13. Henderson, L. E., Sowder, R. & Oroszlan, S. (1981) in *Methods in Protein Sequence Analysis*, ed. Elzinga, M. (Humana, Clifton, NJ), pp. 409–416.
14. Copeland, T. D., Grandgenett, D. P. & Oroszlan, S. (1980) *J. Virol.* **36**, 115–119.
15. Henderson, L. E., Copeland, T. D. & Oroszlan, S. (1980) *Anal. Biochem.* **102**, 1–7.
16. Krutzsch, H. C. (1980) *Biochemistry* **19**, 5290–5296.
17. Krutzsch, H. C. & Pisano, J. J. (1978) *Biochemistry* **17**, 2791–2797.
18. Heller, S. R. & Milne, G. W. A. (1978) *EPA/NIH Mass Spectral Data Base* (Department of Commerce/National Bureau of Standards, Washington, DC), p. 2237.
19. Wold, F. (1981) *Annu. Rev. Biochem.* **50**, 783–814.
20. Braun, V. (1975) *Biochim. Biophys. Acta* **415**, 335–377.
21. Bolognesi, D. P., Montelaro, R. C., Frank, H. & Schafer, W. (1978) *Science* **199**, 183–186.
22. Yeger, H., Kalnins, V. I. & Stephenson, J. R. (1978) *Virology* **89**, 34–44.
23. Barbacid, M. & Aaronson, S. A. (1978) *J. Biol. Chem.* **253**, 1408–1414.
24. Pepinsky, B. B. & Vogt, V. M. (1979) *J. Mol. Biol.* **131**, 819–837.
25. Coffin, J. M., Varmus, H. E., Bishop, J. M., Essex, M., Hardy, W. D., Jr., Martin, G. S., Rosenberg, N. E., Scolnick, E. M., Weinberg, R. A. & Vogt, P. K. (1981) *J. Virol.* **40**, 953–957.
26. Young, H. A., Shih, T. Y., Scolnick, E. M., Rasheed, S. & Gardner, M. B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3523–3527.
27. Witte, O. N., Rosenberg, N. E., Paskind, M., Shields, A. & Baltimore, D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2488–2492.
28. Stephenson, J. R., Kahn, A. S., Sliski, A. H. & Essex, M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5608–5612.
29. Sherr, C. J., Fedele, L. A., Oskarsson, M., Maizel, J. & Vande Woude, G. (1980) *J. Virol.* **34**, 200–212.
30. Carr, S. A., Biemann, K., Shoji, S., Parmalee, D. C. & Titani, K. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6128–6131.