Palmitoylation of the Intracytoplasmic R Peptide of the Transmembrane Envelope Protein in Moloney Murine Leukemia Virus

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Previously it was reported that the 16-amino-acid (aa) C-terminal cytoplasmic tail of Moloney murine leukemia virus (MoMLV) transmembrane protein Pr15E is cleaved off during virus synthesis, yielding the mature, fusion active transmembrane protein p15E and the 16-aa peptide (R peptide or p2E). It remains to be elucidated how the R peptide impairs fusion activity of the uncleaved Pr15E. The R peptide from MoMLV was analyzed by Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunostained with antibody against the synthetic 16-aa R peptide. The R peptide resolved with an apparent molecular mass of 7 kDa and not the 4 kDa seen with the corresponding synthetic peptide. The 7-kDa R peptide was found to be membrane bound in MoMLV-infected NIH 3T3 cells, showing that cleavage of the 7-kDa R-peptide tail must occur before or during budding of progeny virions, in which only small amounts of the 7-kDa R peptide were found. The 7-kDa R peptide was palmitoylated since it could be labeled with $[^{3}H]$palmitic acid, which explains its membrane association, slower migration on gels, and high sensitivity in immunoblotting. The present results are in contrast to previous findings showing equimolar amounts of R peptide and p15E in virions. The discrepancy, however, can be explained by the presence of nonpalmitoylated R peptide in virions, which were poorly detected by immunoblotting. A mechanistic model is proposed. The uncleaved R peptide can, due to its lipid modification, control the conformation of the ectodomain of the transmembrane protein and thereby govern membrane fusion.

The envelope proteins of retroviruses are important for the viral entry and subsequent delivery of viral RNA into the host cells. In the ecotropic Moloney murine leukemia virus (MoMLV), the envelope precursor protein gpr80 is proteolytically cleaved into two subunits, surface protein (SU) and transmembrane protein (TM), by a cellular protease (45). The SU is involved in receptor recognition and binding (3), whereas the TM is responsible for the fusion between viral and cellular membranes (16). In MoMLV, SU is a 70-kDa glycoprotein (gp70) and TM is a 17-kDa polypeptide, Pr15E. Further processing of the Pr15E by a viral protease, at the moment of budding or in virions, reveals a 15-kDa protein, p15E (or p12E), and a 16-amino-acid (aa) oligopeptide, the R peptide (or p2E) (12, 36), which in virions ends up in a 1:1 ratio to p15E (14).

Truncation of the full R peptide renders the Env complex highly fusogenic, resulting in massive syncytium formation in NIH 3T3 cells (28, 29). The R peptide thus appears to act as a safety catch preventing premature fusion, but it is not known how it acts.

Lipid modification by palmitic acid (S-acylation being prevalent) has been reported for a number of viral and cellular membrane proteins (7, 31, 43). Palmitoylation of proteins has been shown to play a considerable role, especially in protein-protein interactions such as signal transduction between G-proteins and G protein-coupled receptors and G proteins in eukaryotic cells (23). Reversible palmitoylation due to the unstable esterification of cysteine thiol groups by palmitic acid may be regulated, unlike N-myristylation, which is a “permanent” modification (30). The covalent fatty acid binding to carbon chains facilitates plasma membrane localization (6). Additionally, it has a significant function in assembly and release of virus particles (18). A recent study has shown that palmitoylation of human thyrotropin receptor enhances the rate of intracellular trafficking of the receptor (39). Finally, the issue of whether fatty acid modification is important in catalyzing the fusogenic abilities of influenza A virus hemagglutinin (HA) has been discussed (24, 27).

Lately, it has been noted that palmitoylation of viral envelope proteins (retroviruses, adenoviruses, togaviruses, and paramyxoviruses) usually takes place at cysteine residues located within the transmembrane domain or in the cytoplasmic tail close to this domain (13, 15, 34, 41, 48). The thioester linkage of fatty acids to a number of viral membrane glycoproteins (vesicular stomatitis virus G, Sindbis virus E1, and influenza A virus HA) is a posttranslational event that takes place in the cis or medial Golgi after exit from the endoplasmic reticulum (ER) and after oligomerization but prior to acquisition of endo H (endo-β-N-acetylglucosaminidase H) resistance (5, 42). Studies have verified that a number of envelope proteins from retroviruses are palmitoylated, e.g., Pr15E of Friend murine leukemia virus, gp41 of human immunodeficiency virus type 1 (HIV-1), gp65 of spleen focus-forming virus and gp35 of Rous sarcoma virus (11, 38, 46, 48).

In the present study, we have resolved the R peptide by tricine-sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (Tricine-SDS-PAGE) and visualized it by immunostaining with an antibody against the synthetic 16-aa R peptide. The R peptide was observed in the host cell, where it was membrane bound and palmitoylated.

MATERIALS AND METHODS

Cells and viruses. NIH 3T3 cells, obtained as a generous gift from B. M. Willumsen, University of Copenhagen, were grown in Dulbecco modified Eagle...
medium (DMEM) supplemented with 10% fetal calf serum, nonessential amino acids, penicillin, and streptomycin. MoMLV (an ecotropic MLV) was obtained from Research Resources, National Cancer Institute (NCI), Bethesda, Md. MoMLV-containing medium was collected for the fractionation of infected NIH 3T3 cells with a titration corresponding to ca. 10^6 PFU per ml.

Membrane preparations from infected NIH 3T3 cells. Membrane fragments from infected NIH 3T3 cells were made according to the method of Maeda et al. (Method Three [20]). Briefly, cells were homogenized in hypotonic buffer, and membranes were collected on 41% sucrose by centrifugation at 45,000 rpm for 30 min at 4°C in a Beckman SW60 swinging-bucket rotor. The protein concentration of the membrane preparations dissolved in phosphate-buffered saline (PBS) was determined by use of the Micro BCA protein assay reagent (Pierce, Rockford, Ill.).

Chemical cross-linking. The thiol-cleavable cross-linking reagent, DSP (di-thiobis(succinimidylpropionate)) was obtained from Pierce. Cross-linking was performed in 25-μl aliquots, containing membranes. DSP was used at 0.05 to 1 mM and incubated 1 h on ice and 15 min at room temperature (RT). Tris base was added to give a 50 mM final concentration and, after an additional 15 min at RT, a gel loading buffer (50 mM Tris-HCl, pH 6.8; 2.5 mM EDTA; 2% SDS; 5% glycerol; 20 mM dithiothreitol [DTT]) was added. The samples were then boiled for 5 min at 100°C. Nonreduced samples were neither boiled nor supplied with DTT.

Detection of viral proteins. Virus samples were concentrated from 1.5 ml of supernatant obtained from subconfluent infected NIH 3T3 cells grown for 2Y in 8.8-cm^2 dishes (Nunc/Life Technologies, Copenhagen, Denmark). The supernatant was centrifuged through a 10% sucrose cushion in a microcentrifuge for 1 h at 4°C, 30,000 × g. The pellet was then lyzed in gel loading buffer. Cell lysates were prepared in the same way as described above, adding 200 μl of PBS to 1 ml of cell lysate (250 mM NaCl; 25 mM Tris-HCl, pH 7.5; 5 mM EDTA; 1% NP-40; 1% SDS; 100 μM of phenylmethylsulfonyl fluoride per ml). Lysates (40 μl) were mixed with gel loading buffer and boiled as described above.

Protein separation was done by Tricine-SDS-PAGE. The gels were essentially made according to the method of Schägger and von Jagow (32). Briefly, proteins were denatured in 50 mM Tris-HCl, pH 6.8; 2.5 mM EDTA; 2% SDS; 5% glycerol; 20 mM dithiothreitol; 10 mM DTT. Running conditions were 15 min at 400 V and then 3.5 h at 750 V, with 45 mM NaOH as the lower buffer. The gels were stained with Coomassie Blue R-250 and visualized with DTT. Protein bands were visualized on Hybond ECL nitrocellulose membranes (Amersham, Little Chalfont, Buckinghamshire, United Kingdom).

RESULTS

Detection of the R peptide. In Fig. 1 (lanes 3, 9, and 10) it can be seen that the antiserum raised against the synthetic R peptide is specific, since it recognizes Pr15E but not p15E. With 5 μg of synthetic R peptide a weak, broad band at an apparent size of 4 kDa was observed (Fig. 1, lane 4), which is designated as the synR peptide. This band was the detection limit for synR peptide (data not shown).

The differences in the observed and expected migration lengths of the synthetic peptide can be explained by the Tricine gel system, where Tricine is used as a trailing ion. In the gel system separates proteins of less than 20 kDa from the bulk SDS and thereby causes the small peptides to resolve in accordance with their own composition, i.e., by their charge and not by the size and charge of the peptide-SDS micelle (32).

It is surprising that the antiserum recognized an intense band with an apparent size of 7 kDa in infected cells (and not in uninfected cells) (see Fig. 1, lanes 1, 2, 7, and 8). This band was not p15E, as seen from the lanes stained with a mixture of anti-R and anti-p15E. The band is designated the palmR peptide.

Membrane fragments from infected NIH 3T3 cells were made according to the method of Maeda et al. (Method Three [20]). Briefly, cells were homogenized in hypotonic buffer, and membranes were collected on 41% sucrose by centrifugation at 45,000 rpm for 30 min at 4°C and at 37°C for 30 min prior to loading.

2D-gel PAGE. Isoelectric focusing gels were prepared in glass capillaries with dimensions of 7.3 by 0.1 cm. Gel mixtures were essentially prepared as described by Laemmli (32). The gels were stained with Coomassie Blue R-250 and visualized with DTT. Protein bands were visualized on Hybond ECL nitrocellulose membranes (Amersham, Little Chalfont, Buckinghamshire, United Kingdom).

Radioactive labeling with palmitic acid. Virus samples were concentrated from 1.5 ml of supernatant obtained from subconfluent infected NIH 3T3 cells grown for 2Y in 8.8-cm^2 dishes (Nunc/Life Technologies, Copenhagen, Denmark). The supernatant was centrifuged through a 10% sucrose cushion in a microcentrifuge for 1 h at 4°C, 30,000 × g. The pellet was then lyzed in gel loading buffer. Cell lysates were prepared in the same way as described above, adding 200 μl of PBS to 1 ml of cell lysate (250 mM NaCl; 25 mM Tris-HCl, pH 7.5; 5 mM EDTA; 1% NP-40; 1% SDS; 100 μM of phenylmethylsulfonyl fluoride per ml). Lysates (40 μl) were mixed with gel loading buffer and boiled as described above.

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The R peptide was detected by using a 1:500 dilution of rabbit polyclonal antipeptide serum raised against a synthetic R peptide (NH2-L-T-Q-Q-Y-Q-L-K-P-I-E-Y-E-P-COOH; 1.896 kDa). The gp70, as well as Pr15E and p15E, was detected with antiserum kindly donated by B. A. Neus and Alan Rein, respectively. The secondary antibody was horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins (Dako, Glostrup, Denmark) and used at dilutions of 1:1,500. Positive bands were visualized with the enhanced chemiluminescence (ECL) detection system (Amersham) and Cronex X-ray films (DuPont-NEC, Boston, Mass.).

Analysis of fatty acids by TLC. Infected NIH 3T3 cells were labeled with [3H]palmitic acid, immunoprecipitated, and run on 2D gels as described above. The R peptide was localized by fluorography, and the spots were then excised and washed with water to remove the scintillator. The spots were hydrolyzed in 6 N HCl at 110°C overnight. The lipid portion was extracted with hexane and then dried by a gentle N2 stream. Dried samples were redissolved in 40 μl of hexane and applied onto an RP-18 thin-layer chromatography (TLC) plate (Merck, Mannheim, Germany), with acetonitrile-glacial acetic acid (1:1) as the solvent system. Fractions were scraped off the plate, and β-emissions were counted in a scintillation counter. [14C]palmitic acid and [14C]myristic acid (Du-Pont-NEC) were used as the standards.

Analysis of hydroxylamine stability of palmitic acid-labeled proteins. Gels were sliced, and the slices were swelled in water. They were washed overnight at RT in 500 μl of 1 M Tris-HCl (pH 7.5) and subsequently washed overnight in 500 μl of 1 M NH4OH-HCl at pH 7.5. For each slice, the radioactivity remaining in the gel and in the hydroxylamine wash was determined with a beta-counter.
MoMLV was found by high-pressure liquid chromatography (HPLC) analysis to be hydrophilic (14). It should be noted that the broad palmR-peptide band consisted of two close bands, seen more clearly in Fig. 2B (a magnification of the palmR-peptide bands in Fig. 2A, lane 3). This doublet is striking since the R peptide has been found in virions in two variants (p2E and p2E*) by Henderson et al. (14), who suggested these to be genetic variants.

Cross-linking studies on membrane fragments, isolated from infected NIH 3T3 cells, were performed with the lipid soluble homobifunctional chemical cross-linker DSP (Fig. 3). At concentrations of >0.5 mM, nearly complete cross-linking of the palmR-peptide band and Pr15E to complexes of high molecular mass was observed. Since DSP does not act on soluble proteins (40), the cross-linkage of the palmR peptide indicates that it is located in or close to the membrane, a finding in agreement with its membrane copurification. The 50-kDa band seen at 0.05 mM DSP is possibly the Pr15E trimer.

From the sequence, the R peptide has an estimated pI of 5.35, whereas the pKₐ for palmitic acid is 4.9 (22). Labeled samples were separated by 2D-gel electrophoresis. As shown in Fig. 4B, 2D gels of palmitic acid-labeled infected cells resulted in two closely localized spots at the expected pI of between 5 and 6 and an apparent molecular mass of 7 kDa, which demonstrated that the R peptide was labeled by [³H]palmitic acid. In Fig. 4C, the results from a similar labeling experiment illustrate, in a more pronounced manner, that the left spot ran slower in the second dimension than the right one, correspond-

FIG. 2. Subcellular fractionation of infected NIH 3T3 cells. Sucrose gradient fractionation was done on cell homogenates, followed by Tricine-SDS-PAGE and immunoblotting with anti-R peptide by using the ECL detection system. (A) Lanes: 1, NIH membrane; 2, NIH cytoplasm; 3, infected NIH membrane; 4, infected NIH cytoplasm. Equalized cytoplasm and membrane amounts were added. (B) Magnification of the R peptide resolved in Fig. 2A, lane 3.

The palmR peptide is palmitoylated. Labeling of the MoMLV envelope protein with [³H]palmitic acid resulted in bands corresponding to the envelope precursor gPr80” and Pr15E (Fig. 4A). A band most likely corresponding to the palmR peptide was also seen, but it was unfortunately partly covered by free palmitic acid or lipids containing palmitic acid (Fig. 4A, compare lanes 1 and 2). Further separation was necessary.

In Fig. 4C, the results from a similar labeling experiment illustrate, in a more pronounced manner, that the left spot ran slower in the second dimension than the right one, correspond-

FIG. 1. Identification of the R peptide in cell lysates and virus pellets. Proteins in cell lysates and virus pellets were analyzed by Tricine-SDS-PAGE and immunoblotted as described in Materials and Methods with anti-R peptide, anti-gp70, and anti-p15E by using the ECL detection system. Lanes: 1, NIH lysate; 2, infected NIH lysate; 3, MoMLV; 4, synthetic R peptide (5 µg); 5, infected NIH lysate; 6, MoMLV; 7, NIH lysate; 8, infected NIH lysate; 9 and 10, MoMLV obtained from the NCI (10 µg).
ing to the doublet seen in Fig. 2B. Pr15E and gPr80 envelope were not detectable in Fig. 4B, presumably because the detection limit is higher in the 2D gel than in the 1D gel (Fig. 4A).

Identification of the label incorporated into palmR peptide as palmitic acid. Identification of the fatty acid incorporation into proteins is important, since [3H]palmitic acid can be converted into other fatty acid species of different chain lengths or saturations before it is attached to the acyl protein (32). We used a reversed-phase TLC assay. The palmR peptide from Fig. 4B was analyzed. In Fig. 5, the radioactivity from the hydrolyzed peptide is shown. Standards of palmitic and myristic acid peaked at \( R_f \) values of 0.38 and 0.47, respectively. This result thus shows that the majority of the label was incorporated into the palmR peptide as a palmitoyl group. (The small peak at \( R_f 0.1 \) might represent large lipids, e.g., polyisoprenoids known to carry sugars for the membrane-associated synthesis of glycoproteins.) When the two palmR-peptide spots from Fig. 4B were analyzed individually, the same results were obtained (data not shown). The finding that palmitic acid could be recovered shows that it was added by acylation and not subjected to interconversion to other fatty acids or to amino acids.

Palmitoylation of Pr15E and gPr80 envelope observed in reducing gels is not bound by thioacylation. The R peptide does not contain cysteine but does contain lysine, threonine, and tyrosine, to which palmitic acid could be coupled by an amide or

![FIG. 3. Cross-linking with DSP of membrane fragments from infected NIH 3T3 cells. Membrane fragments were isolated, followed by cross-linking with DSP in the noted concentrations. Samples treated with DTT cleaving the disulphide bond in the cross-linker are indicated (+). The samples were electrophoresed and immunoblotted with anti-R peptide by using the ECL detection system.](image)

![FIG. 4. Labeling of the R peptide by [3H]palmitic acid. (A) Labeled cell lysates immunoprecipitated with anti-R peptide subjected to gel loading buffer containing DTT prior to Tricine-SDS-PAGE. Lanes: 1, NIH lysate; 2, infected NIH lysate. (B) Labeled infected NIH 3T3 cell lysates immunoprecipitated with anti-R peptide before isoelectric focusing and subsequent Tricine-SDS-PAGE. The preparation of the isoelectrical focusing gel is described in Materials and Methods. (C) Similar experiment to that described for panel B (only the lower left quadrant is shown).](image)

![FIG. 5. TLC analysis of the [3H]palmitic acid-labeled R peptide of MoMLV. As described in Materials and Methods, fatty acids from hydrolyzed labeled R peptide from infected NIH 3T3 cells were analyzed on an RP-18 TLC plate. Fractions were scraped off the plate, and each fraction was counted. The disintegrations per minute (dpm) were plotted against the \( R_f \) which was defined as the distance from the origin to the fractions relative to the corresponding distance of the front (16 cm). The arrows indicate the relative migration of the two standards: [3H]palmitic acid (p) and [3H]myristic acid (m).](image)
oxy-ester bond. If palmitic acid is bound to the R peptide before cleavage, then Pr15E and possibly also gPr80^{env} are labeled on the R-peptide tail. Palmitic acid is, however, also known to be bound to a cysteine residue in the membrane-spanning domain of p15E (48). Cysteine-bound palmitic acid was excluded in the experiment shown in Fig. 4, since DTT was used to reduce the samples. Furthermore, thio-ester bonds are labile, whereas amide and oxy-ester bonds are stable in hydroxylamine at neutral pH (44). The gPr80^{env} and Pr15E bands from Fig. 4A were cut out and treated with hydroxylamine. The remaining radioactivity levels in gPr80^{env} and Pr15E were 451 and 661 dpm, respectively, compared to 218 and 92 dpm in the corresponding controls at the same locations of labeled uninfected cells. Fewer than 20 dpm were observed in the hydroxylamine washes. Thus, more than 95% of the radioactivity in the two proteins was stable in hydroxylamine, which shows that the radioactivity observed in Fig. 4A was not thio-ester bound. It should be noted that the observed label could be added through metabolic conversions, though this is unlikely since the label on the R peptide is bound by acylation.

**DISCUSSION**

Altogether, the results show that the palmR-peptide band is a derivative of the R peptide. It is detected by R-specific antiserum (recognizing Pr15E but not p15E), and it is present in infected cells. In virions it is also present, but only in small amounts. It resolves into two very close bands which have also been observed by HPLC analysis of MoMLV R peptide (designated p2E and p2E*, with the latter containing an extra alanine) (14). The palmR peptide must be a derivative, since it runs more slowly in gels than does the synthetic R peptide. It is palmitoylated, which explains its membrane association and its slower gel migration.

The different sensitivities in immunoblotting of the palmitoylated and synthetic R peptides can be explained by the different binding properties to nitrocellulose. In contrast to Pr15E, the R peptide is hydrophilic, as seen by its weak binding to hydrophobic (reversed-phase C_{18}) columns (14). Binding of proteins to nitrocellulose occurs mainly by hydrophobic interactions and can be weak, especially for small peptides (25). Binding of the native hydrophilic R peptide is therefore expected to be weak. Palmitoylation of the hydrophilic R peptide will presumably create a strong hydrophobic binding and give a higher sensitivity.

Since we did not observe native R peptide in virions, immunoblotting onto polyvinylidene difluoride (PVDF) membranes (generally used for peptide mapping) was compared to that of nitrocellulose. In virus preparations, a band below the palmR peptide band was observed on the PVDF membrane (results not shown).

Which amino acid(s) in the R peptide the palmitic acid is linked to remains to be elucidated. In the absence of cysteines the fatty acid must either be bound by an ester linkage to threonine or tyrosine or by an amide linkage to lysine (35) (or if it is added after cleavage possibly to the terminal amino group). The palmitoylation of lysine has been proposed for the early region 1B 176R protein of human adenovirus type 5 (21). A general consensus signal, specifying the site of palmitoylation, is not known. For proteins belonging to the Ras family, it has been suggested that a cysteine followed by two aliphatic residues could function as the signal for palmitoylation (37). In Pr15E Friend murine leukemia virus, palmitoylation was demonstrated to occur on Cys-606 (corresponding to cysteine 630 in MoMLV), located close to the transmembrane domain (48), which actually is followed by isoleucine and leucine. Aliphatic amino acids in the R peptide are neighboring both the lysine and threonine, which together with a bound fatty acid will make up a strong hydrophobic domain. It should be mentioned that the shown non-S acylation on Pr15E and gPr80^{env} does not exclude the possibility that thio-esterification also occurs on Pr15E (48).

The present results give some insights into the formation and fate of the palmitoylated R peptide. The finding that Pr15E and gPr80^{env} also were palmitoylated on other atoms than sulfur suggests that the R-peptide palmitoylation occurs before gPr80^{env} and Pr15E cleavage. Based on cell fractionation of Semliki Forest virus-infected BHK cells, Berger and Schmidt (4) have proposed that the fatty acyltransferase is located in the ER. Since gPr80^{env} is cleaved in the cis compartment of the Golgi (10), the finding of labeled gPr80^{env} suggests a lipid modification in the ER or Golgi. More data is needed, however, in order to show whether all R-peptide palmitoylation occurs before cleavage and whether all Pr15E and gPr80^{env} molecules are palmitoylated on their R peptides.

Pr15E has been shown by pulse-labeling to have a turnover time in cells of ca. 1 h (12), with which the present intensities of Pr15E in cells and virions agree (Fig. 1 and calculations not shown). It has previously been suggested that the R cleavage occurs after budding (14); however, the present results show that the R peptide at least in the palmitoylated form is cleaved before budding.

Since both p15E and R peptide (irrespective of its form) are created by a cleavage of Pr15E, their total amounts must equal (assuming no degradation), which has been shown to be true in virions (14). However, this does not appear to be the case in cells, where only minute amounts of p15E are present (Fig. 1 and reference 12). In the present study, we looked at the situation after 20 h of virus production. The cellular Pr15E amount was lower than the viral Pr15E amount, which again only comprised approximately one-tenth of the viral p15E amount (Fig. 1 and reference 12). The cellular (palmitoylated) R peptide has an intensity approximately equal to that of the cellular Pr15E (Fig. 1). According to the relative amounts discussed above, the cellular R peptide apparently only comprises a small percentage of the viral p15E and thus viral (unpalmitoylated) R peptide.

It is interesting that the cellular p15E has a much lower intensity than the cellular palmitoylated R peptide (Fig. 1). This difference shows that p15E after the cleavage in cells is rapidly moved to the virions, whereas the palmitoylated R peptide remains in cells, a finding which indicates that it possibly has a function there. However, the results do not show whether (i) only a fraction of the Pr15E is cleaved in the cell (represented by the cellular palmitoylated R peptide) or (ii) all Pr15E is cleaved in the cell. In the first case, the palmitoylated R peptide is accumulated (dead end) in cells, whereas in the second case it slowly would move to the virions with approximately the same turnover time as that for the cellular Pr15E. In the latter case, a depalmitoylation of R peptide during entry into virions must occur.

A transient existence of the palmitoylated form was suggested by others (32a). MoMLV was propagated in SC-1 cells. MoMLV from these cells showed considerable amounts of the 7-kDa R peptide; these amounts decreased after prolonged incubation.

The membrane association of the palmitoylated R peptide is presumably important for its function. Since the palmitoylated R peptide was seen in cells but basically not in virions, it apparently operates before or during budding. Palmitoylated R peptide may thus function as a transport signal or in the control of conformational changes or the budding process.
Transport signal. The apparent palmitoylation at an early point after Env synthesis fits with the transport signal model, thus bringing the Env complex to the plasma membrane. If so, the R peptide is not needed after virus budding. Late, the sorting in polarized epithelial cells of HIV-1 and MoMLV was investigated (19). To confer polarized basolateral budding in Madin-Darby canine kidney cells, at least one crucial membrane-proximal intracytoplasmic tyrosine residue, Tyr-622 (residue 6 in the R peptide), in MoMLV was needed. If this tyrosine is palmitoylated then lipid modification appears to have a role in compartmentalization.

Control of conformational changes. A palmitoylated R tail of Pr15E will most likely attach to the membrane as the free palmitoylated peptide does. It might thus tilt the Pr15E molecule in the membrane, whereby it consequently can control the conformation of the Pr15E trimer (9) on the outside of the membrane. After cleavage, p15E can erect into its fusogenic form. As such it can act as a safety catch, which is in agreement with previous results (28, 29) showing that R-truncated Pr15E is fusogenic. The model for this is shown in Fig. 6A and B.

In order to tilt Pr15E in the membrane, the transmembrane and cytoplasmic domains of p15E need to be rigid, which fits with the suggestions that the domains are α-helical (47). The cytoplasmic α-helix is amphiphatic, with hydrophobic faces (47), which can bind the p15E molecules together and thus erect them. Chimeras in which the cytoplasmic tail of the simian immunodeficiency virus (SIV) transmembrane protein was replaced by the MoMLV cytoplasmic tail in the entire form or the R-truncated form were made (47). The R-peptide tail was then able to inhibit SIV propagation in HeLa T4 cells. This ability could be due to its palmitoylation (Fig. 6A and B), which would be independent of the virus (e.g., SIV), but not of the cell type used for virus propagation.

Control in the budding process. The palmitoylated R peptide can possibly control budding by creating membrane curvature. Seen from the cytoplasm, budding needs an outward membrane curvature at the brim of the site of budding. As an amphiphilic molecule the palmitoylated R peptide has a high head group area (17) compared to the hydrophobic tail (the palmitic acid), presumably generating outward curvature of the amphiphilic molecule the palmitoylated R peptide has a high head group area (17) compared to the hydrophobic tail (the palmitic acid), presumably generating outward curvature of the membrane. Later, during budding, the membrane curvature reverses, where a high head group area is a disadvantage, thus explaining a depalmitoylation of the R peptide. This model is shown in Fig. 6C.

REFERENCES


