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Murine Leukemia Virus Proteins Expressed on the Surface of Infected Cells in Culture

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Infection of JLS-V9 cells in culture with Rauscher murine leukemia virus induced the appearance on the cell surface of two classes of viral proteins: Rauscher murine leukemia virus gp70, and glycoproteins related to the viral core (*gag*) proteins with apparent molecular weights in sodium dodecyl sulfate polyacrylamide gels of 80×10^3 and 95×10^3. The latter proteins were identified by lactoperoxidase-catalyzed iodination of the cell surface and by metabolic labeling with [^{3}H]mannose followed by immunoprecipitation with an antiserum directed against the major viral core protein, p30. Tryptic peptide maps of chloramine T-iodinated proteins indicated that 80×10^3- and 95×10^3-molecular-weight proteins were closely related. The 95×10^3-molecular-weight protein from Rauscher murine leukemia virus-infected cells had a tyrosine fingerprint which was identical to that of the 95×10^3-molecular-weight *gag* surface polyprotein of endogenous virus-producing AKR-A cells, suggesting that expression on the cell surface of glycosylated forms of *gag* precursor polyproteins may not be an exclusive property of leukemic thymocytes, but a more general phenomenon in murine leukemia virus infection. Tryptic fingerprint analysis of iodinated viral and cell-bound gp70's before and after desialylation indicated a lower level of glycosylation in the cell-bound gp70 population than in virions. Analysis of only surface-iodinated gp70 showed a simple pattern of exposed tryptic peptides which was very similar in Rauscher murine leukemia virus-infected cells and in AKR-A cells.

Cells infected with oncornaviruses contain virus-specific structural proteins which are synthesized from the viral gene *gag*, coding for the internal (core) proteins, *pol*, coding for the RNA-dependent DNA polymerase, and *env*, coding for the envelope proteins. Their biosynthesis occurs through the processing of their respective precursor proteins, as demonstrated first for the *gag* genes of avian (38, 39) and murine (24, 36) viruses and reviewed by Eisenman and Vogt (6).

In cells infected with murine leukemia viruses (MuLV), the viral envelope glycoprotein gp70 was shown to be present on the cell surface by immunological and biochemical methods (3, 12, 13, 20). This finding can be explained by the membrane location of the final virus assembly, which occurs by budding (27) and is thought to require gp70 (2, 40). However, endogenous glycoproteins closely related to viral gp70 are also found on certain mouse cells without concomitant virus production (8, 21, 30, 31), and in the form of G_{IX} antigen they participate in the normal phenotypic differentiation of thymocytes of low-leukemia strains of mice (3, 29, 33, 34). MuLV envelope glycoprotein has recently been implicated in the process of leukemogenesis (7, 32).

In addition to gp70, a class of *gag*-related surface proteins with molecular weights of 95×10^3 (95 k) and 85k has been described on AKR leukemic cells (20, 35) and identified as the "Gross cell surface antigen" (18, 28). They represent glycosylated forms of *gag* precursor polyproteins that are not incorporated into virus particles (19). Their role is obscure at present, but their localization on the cell surface indicates a possibility for immunological recognition. Since these *gag* molecular species have been identified currently only on thymocytes, we wished to investigate whether they represent a general phenomenon in MuLV infection. In this paper we studied *gag*- and gp70-related proteins on the surface of cells in culture that had been infected with the exogenous Rauscher MuLV (R-MuLV). This investigation was prompted by a finding by P. Nobis (personal communication) that complement-mediated cytotoxicity against the same R-MuLV-infected cells could be achieved with an antiserum directed against the R-MuLV major *gag* protein, p30. The present analysis by surface iodination and immunoprecipitation identified on the surface of R-MuLV-infected JLS-V9 cells two *gag* polyproteins of approximately 80 and 95 kilodaltons. Tryptic fingerprinting of the iodinated proteins revealed that they are related to each other and identical,

in this type of analysis, to endogenous (AKR) virus-specified polyproteins. A similar study of the envelope glycoprotein gp70 showed that cell-bound gp70 was related to that of purified R-MuLV, with some differences due to sialylation.

MATERIALS AND METHODS

Cells. JLS-V9 cells (44), a line derived from bone marrow of a BALB/c mouse, were obtained from H. Fan (Salk Institute, San Diego, Calif.) through P. Nobis (presently at H. Pette Institute, Hamburg, West Germany). AKR-A cells (43) were obtained from R. B. Herberman (National Institutes of Health, Bethesda, Md.) through J.-C. Cerottini (Ludwig Institute, Epalinges, Switzerland). They were grown in Dulbecco-modified Eagle medium containing 5% heat-inactivated (56°C, 45 min) fetal calf serum in 10-cm plastic petri dishes (Corning Glass Works).

Virus. R-MuLV (cell passage 9 in JLS-V9 cells) was obtained from G. P. Shibley (Frederick Cancer Research Center, Frederick, Md.) and was propagated in the same cells. Tissue culture supernatants (kept at −70°C) were used for newly infecting JLS-V9 cells when seeded. Infected cells were used for experiments between passages 2 and 7 after infection. Virus was purified from tissue culture medium by sedimentation and equilibrium centrifugation in 15 to 60% sucrose gradients in 0.1 M NaCl–0.05 M Tris-hydrochloride (pH 7.5)–1 mM EDTA.

Antisera. Goat antisera prepared against purified proteins of R-MuLV were obtained from the National Cancer Institute, Bethesda, Md., and kindly provided by R. Wilsnack: anti-p30, lot 2S-658; anti-gp 70, lot 5S-129. Their specificity was tested by immunoprecipitation of [^{35}S]methionine-labeled R-MuLV and sodium dodecyl sulfate (SDS)-gel electrophoresis.

Cell surface labeling. JLS-V9 cell monolayers (about 80% confluent) were dispersed by incubation in phosphate-buffered saline containing 10 mM EDTA for 10 to 15 min at 37°C. In early experiments (see Fig. 1 and 2) they were trypsinized briefly and allowed to recover in suspension in medium containing 10% fetal calf serum for 3 to 5 h at 37°C. AKR-A cells and dispersed JLS-V9 cells were washed repeatedly and radioiodinated by the method of Vitetta et al. (37) with some modifications. To about 5×10^7 to 7×10^7 cells in 1 ml of Earle saline, 100 μg of lactoperoxidase (Sigma Chemical Co.) and 1 to 2 mCi of ^{125}I were added, followed by three additions, at 4-min intervals, of 25 μl of 0.06% H_2O_2; the tube was kept in gentle rotation at 20°C. Labeling was stopped by dilution with phosphate-buffered saline containing 10 mM potassium iodide and 0.1 mg of phenylmethylsulfonyl fluoride (Sigma) per ml. The cells were washed several times with the same buffer and then lysed in 20 mM Tris-hydrochloride (pH 7.5)–50 mM NaCl containing 0.5% Nonidet P-40, 0.5% deoxycholate, and 0.1 mg of phenylmethylsulfonyl fluoride per ml for 30 min on ice. After blending in a Vortex mixer for 30 s, nuclei were removed by centrifugation at 13,000 × *g* for 10 min, and the supernatant was further clarified at 100,000 × *g* for 45 min and then used immediately for immunoprecipitation.

Intracellular labeling. R-MuLV-infected JLS-V9 cells were labeled with 200 μCi of [^{3}H]mannose per plate in culture medium for 23 h, washed with cold phosphate-buffered saline, and scraped in lysis buffer. The lysate was centrifuged at 13,000 × *g* for 10 min, and the supernatant was used for immunoprecipitation.

Immunoprecipitation. The lysate (1 ml) was preadsorbed with 50 μl of fixed *Staphylococcus aureus* (10% suspension; prepared by the method of Kessler [15]). In some experiments, 0.1% SDS was added to reduce nonspecific binding during immunoprecipitation. About 5 μl of antiserum per plate equivalent was added; incubation was for 1 h at 37°C. Immune complexes were precipitated with a 10-fold excess of *S. aureus* suspension in 0.15 M NaCl–5 mM EDTA–50 mM Tris-hydrochloride (pH 7.5) (NET) plus 0.05% Nonidet NP-40, washed three times in the same buffer and once in NET without Nonidet P-40, and finally eluted with gel sample buffer (0.1 M Tris-hydrochloride [pH 7]–20% glycerol–4% SDS–0.02% bromophenol blue) for 15 min at room temperature.

Polyacrylamide gel electrophoresis. Samples were solubilized by heating at 100°C for 2 to 3 min in sample buffer containing 10% 2-mercaptoethanol and subjected to electrophoresis in 10% discontinuous SDS-polyacrylamide slab gels (17), cross-linked with diallyltartardiamide (1). Electrophoresis at 30 mA was stopped when the bromophenol blue marker reached the bottom of the slab. Gels were briefly fixed and stained with Coomassie brilliant blue in 50% trichloroacetic acid and destained in 7% acetic acid–30% methanol. After soaking for 1 to 2 h in water, individual lanes of the slab were cut out and sliced (two 1-mm slices per fraction) for counting. ^{125}I-labeled proteins were counted directly in a gamma counter. ^{3}H-labeled proteins were eluted from gel slices for 24 h at room temperature in 0.2 M ammonium carbonate (pH 8.5) containing 0.1% SDS, diluted with water-solubilizing scintillation fluid (Aquasol; New England Nuclear Corp.), and counted in a liquid scintillation counter.

Iodination and tryptic peptide analysis of proteins. Chloramine T iodination of proteins in gel slices was performed by the method of Elder et al. (9). The removal of terminal sialic acid residues from glycoproteins (4, 16) was performed before iodination by incubating the gel slices in 2 ml of 0.05 N H_2SO_4 at 80°C for 90 min. After iodination and extensive washing, the gel slices were dissolved in 2% periodic acid; the proteins were precipitated with 25% trichloroacetic acid and 100 μg of bovine serum albumin as a carrier and washed as described by Gibson (11). The protein preparations were then oxidized with 0.5 ml of 0.1 M H_2O_2 for 2 h at room temperature, lyophilized, and digested with trypsin as reported previously (39). The separation of tryptic peptides was obtained by chromatography at 50°C on a column of Chromobead P (Technicon Corp., Inc.) cation-exchange resin which was eluted with a quadratic gradient of acetic acid-pyridine between pH 2.5 and 4.5 as described previously (16, 39). Fractions (3 ml each) were counted directly in a Packard gamma counter. After each run, the column was washed with the pH 4.5 column buffer containing 10 mg of potassium iodide per ml to remove any residual free iodine. Tritium label was counted with Aquasol (New England Nuclear Corp.) scintilla-

tion fluid after evaporating the column buffer and dissolving the samples in 0.01 N HCl. Values were corrected for ^{125}I overlap in the tritium channel.

RESULTS

Virus-related proteins on the surface of R-MuLV-infected cells. R-MuLV-infected JLS-V9 cells were surface labeled with ^{125}I and lactoperoxidase and then lysed with Nonidet P-40 and deoxycholate. Labeled proteins were immunoprecipitated with antisera directed against R-MuLV protein p30 or gp70 and analyzed by electrophoresis in acrylamide gels (Fig. 1). Anti-gp70 serum precipitated one protein species of approximately 70 kilodaltons (Fig. 1B). Anti-p30 serum (Fig. 1A) precipitated two main species, one of approximately 80 kilodaltons and a more heterogeneous one of approximately 90 to 95 kilodaltons. The smaller peak at about 45 kilodaltons was probably cellular actin that adhered to the immune complexes. The relative amount of label incorporated into the 80 and 95k proteins was always 10 to 25 times less than that incorporated into the 70k species. All of these proteins were glycosylated; Fig. 1 also shows the superimposed pattern (obtained in a separate experiment) of 80 and 95k proteins metabolically labeled with [^{3}H]mannose. When surface-labeled uninfected and R-MuLV-infected JLS-V9 cells were compared (Fig. 2), both possessed a 70k protein in comparable amounts, whereas 80 and 95k proteins were detectable only in infected cells. Since the 70k protein of infected cells was precipitated by anti-gp70 serum and comigrated on gels with viral gp70, we considered it to be viral envelope glycoprotein gp70 expressed on the cell surface. Uninfected-cell gp70 is probably due to the expression of an endogenous gp70-related gene (8, 21). Since the 80 and 95k proteins were precipitated by an antiserum directed against the major *gag* protein p30, we tentatively called them "*gag* surface polyproteins" by analogy with those described by others (20, 35) on the surface of leukemia cells, although their migration in gels with respect to protein markers was slightly different.

Tryptic peptide mapping of *gag* surface polyproteins from R-MuLV-infected cells. Both polyproteins were recognized by anti-p30 serum and contained, therefore, p30 determinants. Their high molecular weight suggests that they contained more amino acid sequences, possibly of other *gag* proteins. To determine whether these sequences were the same, we compared their tyrosine tryptic fingerprints. 80 and 95k *gag* proteins were re-iodinated in vitro with chloramine T, oxidized, and digested with trypsin, and the resulting peptides were separated on a cation-exchange chromatography column.

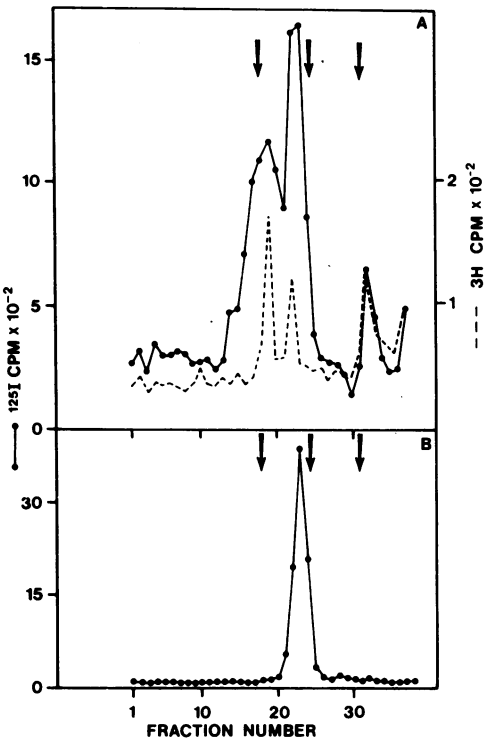

FIG. 1. *SDS-polyacrylamide slab gel electrophoresis of immunoprecipitates from surface-iodinated, R-MuLV-infected JLS-V9 cells. (A) Nine-tenths of the preparation immunoprecipitated with anti-p30 serum (continuous line). Superimposed is the pattern of a [^{3}H]mannose-labeled anti-p30 immunoprecipitate (broken line) obtained in a separate experiment. (B) One-tenth of the preparation precipitated with anti-gp70 serum. Arrows indicate the position of unlabeled molecular weight markers electrophoresed in neighboring slots. Left to right: phosphorylase a (97 kilodaltons), bovine serum albumin (68 kilodaltons), and immunoglobulin G, heavy chain (50 kilodaltons).*

Figure 3A and B shows that the two proteins had essentially identical fingerprints: all eight clearly identifiable peptides in one protein were present in the other, and no major peptide difference between them could be found. When tryptic digests of 80 and 95k proteins were mixed in equal amounts and chromatographed together, the same number of peaks and the same overall pattern of the single components were obtained (data not shown). The fingerprint of these *gag* proteins was different from that of surface-labeled, re-iodinated gp70 shown below (see Fig. 5A). This confirmed that the polyproteins precipitated by anti-p30 antiserum were not contaminated by gp70, although this protein was much more abundant on the cell surface.

The difference in apparent molecular weight on gels between 80 and 95k might have been due

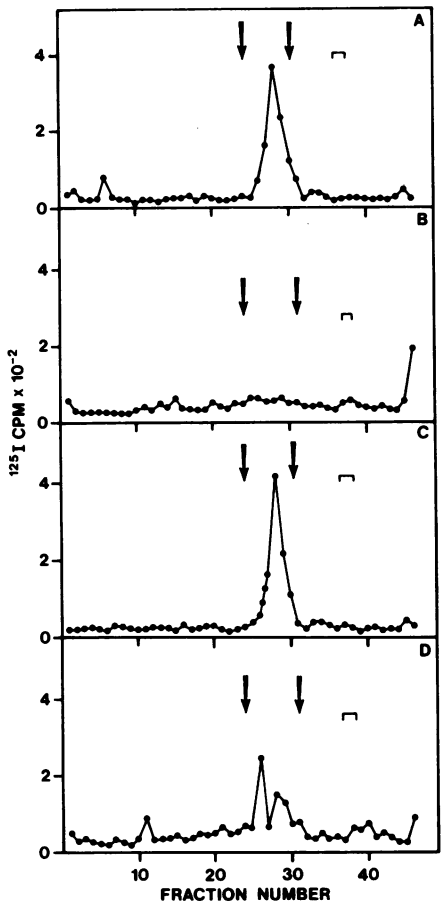

FIG. 2. *SDS-polyacrylamide slab gel electrophoresis of immunoprecipitates from surface-iodinated uninfected (A and B) and R-MuLV-infected (C and D) JLS-V9 cells. (A) and (C) show anti-gp70 precipitates (1/10 of the preparation); (B) and (D) show anti-p30 precipitates (9/10 of the preparation). Molecular weight markers as in Fig. 1.*

to peptides not containing tyrosine or to glycosylation, which could also have interfered with in vitro labeling, digestion, or peptide separation. The second hypothesis was tested by performing the same analysis on 80 and 95k proteins that had been treated with sulfuric acid to remove terminal sialic acid residues (4, 16). This treatment did not cause peptide breakage when tested on proteins with a known fingerprint (unpublished data). A comparison of the fingerprints showed that acid treatment did not affect the position of the peptides, except for one: peak g in Fig. 3A was absent in acid-treated 95k, whereas a new peak, g′ (Fig. 4A), appeared at about five fractions to the right, which was possibly the same peptide eluting now at a slightly more basic pH.

Relationship of *gag* surface proteins from R-MuLV-infected cells to endogenous virus *gag* surface polyproteins. Since the 80 and 95k proteins appeared on the cell surface after R-MuLV infection, they could carry some R-type specificity, or they could be the expression of endogenous viral information. In any case, we were interested in knowing to what extent these proteins were related to those present on the surface of leukemia cells expressing endogenous virus and previously identified serologically as Gross cell surface antigen. We therefore compared iodinated tryptic fingerprints of 95k *gag* proteins from R-MuLV-infected JLS-V9 cells and endogenous virus-producing AKR-A cells that had been surface labeled and immunoprecipitated with anti-p30 antiserum. (In AKR cells, only one prominent band comigrating with the phosphorylase marker was present in the immune precipitates, whereas no distinct peak was seen at the position of 80 kilodaltons.) Figure 4 shows the two chromatographic elution patterns of the acid-treated 95k proteins. They include an internal tritiated marker of a protein with a known fingerprint that was mixed with the iodinated sample before analysis; this allowed us to compare the exact position of peaks independently of experimental variations in the pH gradient. We concluded that the two fingerprints were identical, not only in the eight major tryptic peptides (identified with the same letters as in Fig. 3), but also in the minor features of the pattern. Two independent preparations of each type of cells gave identical results, and a mixture (in approximately equal amounts of radioactivity) of iodinated 95k from R-MuLV-infected cells and AKR cells produced a fingerprint with the same pattern as either of the individual components. These results show that the *gag* surface proteins of R-MuLV-infected JLS-V9 cells and of AKR-A cells are highly related and possibly identical, since no difference between them could be detected by iodinated tryptic peptide analyses.

Viral glycoprotein gp70. As a control to the data obtained for *gag* proteins, we wished to verify to which viral type the glycoprotein gp70 belonged. A similar type of analysis was therefore performed on the viral glycoproteins immunoprecipitated with anti-gp70 antiserum and re-iodinated with chloramine T. gp70 from R-MuLV-infected JLS-V9 cells had a tryptic fingerprint (Fig. 5A) that was clearly different from that of gp70 of AKR-A cells (Fig. 5C) and closely resembled that of R-MuLV gp70 (see below). Figure 5A and C shows the patterns of "native," untreated gp70's. A comparison with the pattern obtained after the removal of terminal sialic acid residues (4, 16) gives some clues about the state

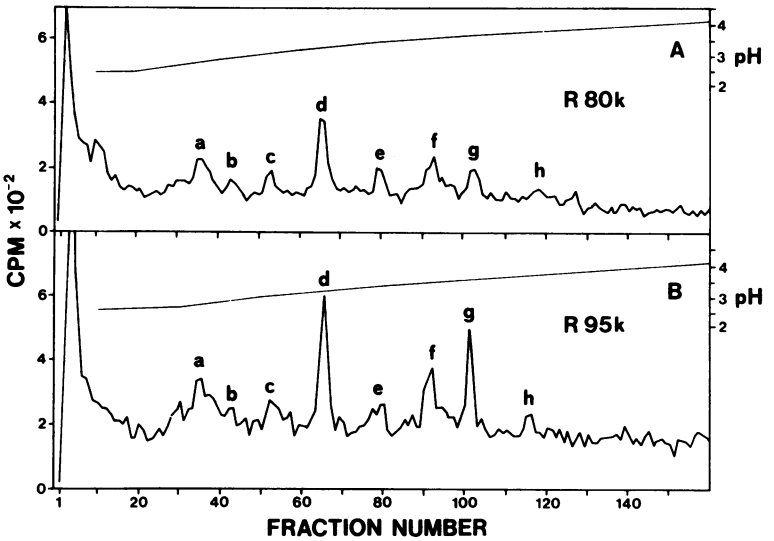

FIG. 3. *Chloramine T-iodinated tryptic peptides of 80k (A) and 95k (B) proteins. R-MuLV-infected JLS-V9 cells were surface iodinated and lysed, and the labeled polyproteins in an anti-p30 immunoprecipitate were isolated by electrophoresis on a gel that was sliced and counted. The polyproteins were re-iodinated in the gel slice and then isolated by trichloroacetic acid precipitation after dissolving the gel as described in the text. The H_2O_2-oxidized proteins were digested with trypsin, and peptides were separated by chromatography on a Chromobead P cation-exchange column. Corresponding peptides are indicated by lowercase letters.*

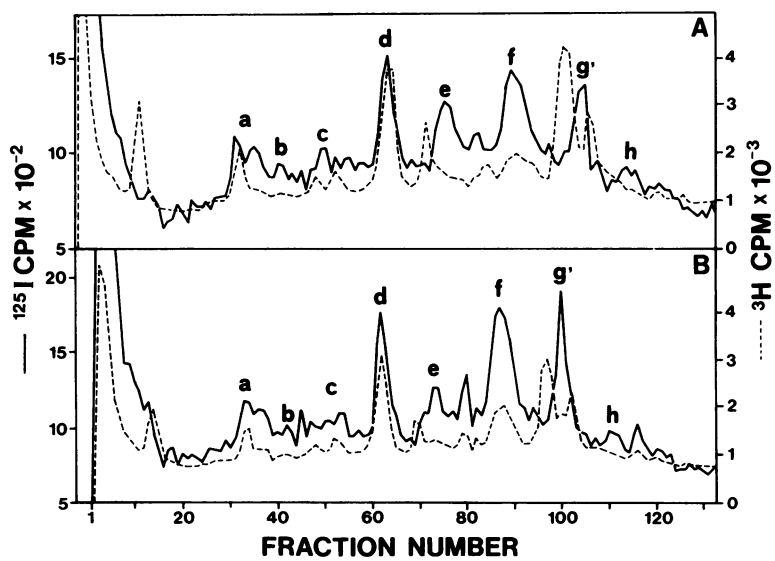

FIG. 4. *Comparison of tryptic fingerprints of 95k proteins from R-MuLV-infected JLS-V9 cells (A) and AKR-A cells (B). Surface-iodinated proteins were precipitated from the cell lysate with anti-p30 serum and isolated by SDS-gel electrophoresis. 95k proteins were acid treated (to remove terminal sialic acid residues), re-iodinated with chloramine T, trichloroacetic acid precipitated after dissolving the gel slice, oxidized, and digested with trypsin as described in the text. For the cation-exchange chromatography, iodinated tryptic digests* (——) *were mixed with tryptic digests of [³H]lysine-labeled vp19 from avian myeloblastosis virus as an internal marker* (---).

of glycosylation of the different proteins. In the case of R-MuLV-infected cells, treatment with acid to remove terminal sialic acid caused only a slight change in the fingerprint: peptide i disappeared and a new peptide (q) appeared, whereas the other 13 major peptides (b, c, d, e,

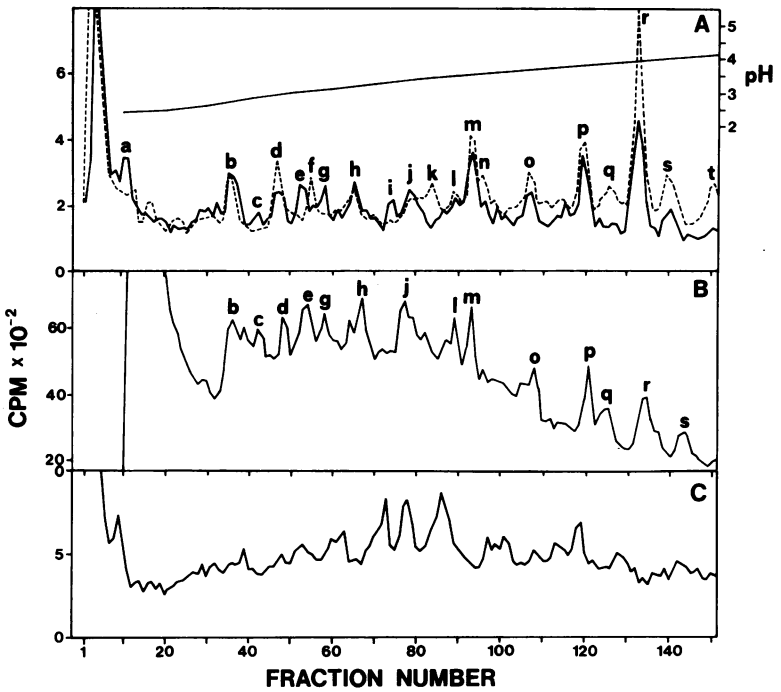

FIG. 5. *Comparison of tryptic fingerprints of chloramine T-iodinated cell-bound gp70's: native (A)* (——) *and acid-treated (B) R-MuLV-infected JLS-V9 cells and AKR-A cells (C). gp70 was immunoprecipitated with anti-gp70 serum from surface-iodinated cells, isolated by SDS-gel electrophoresis, relabeled with chloramine T, and processed for tryptic fingerprinting as described in the text. (A)* (---) *also shows the tryptic fingerprint of acid-treated gp70 from purified R-MuLV, isolated by SDS-gel electrophoresis, iodinated, and processed as the cellular samples. It was obtained in a separate run on the same column, and the pH gradients measured on fractions of each profile were superimposable.*

g, h, j, l, m, o, p, r, and s) were identical before and after acid treatment (Fig. 5B). The broken line in Fig. 5A is the fingerprint of acid-treated gp70 from R-MuLV grown in the same cells. Eleven peptides (b, d, h, j, l, m, o, p, q, r, and s) were in common with cellular glycoprotein; four cellular peptides (a, c, e, and g) were absent in virus, whereas four new ones were found (f, k, n, and t). These results suggest that the bulk of cell-bound gp70 molecules had a different level of glycosylation than did the gp70 in mature R-MuLV particles (see below). Interestingly, for AKR cells, the comparison of native and acid-treated gp70's also showed specific differences in the fingerprints, suggesting the occurrence of sialylated peptides in AKR gp70, as well.

Finally, we tried to gather information about the conformation of gp70 when it is inserted in the cellular membrane by investigating its availability to surface labeling. Fingerprint analysis of immunoprecipitated gp70 from surface-iodinated cells was performed without previous reiodination in vitro. The result shows (Fig. 6) that only a few (3 to 4) tryptic peptides were labeled out of the 14 major ones found in the uniformly iodinated protein (Fig. 5). One of them eluted in the acidic part of the gradient near the flow through of the column. Two highly labeled and a minor one eluted in the position around peptides d to g in Fig. 5A; the exact identification is at present still open. In AKR cells, a similar fingerprint was obtained (although with a somewhat higher background) despite the fact that the uniformly labeled AKR gp70 had a fingerprint different from that of R-MuLV gp70, as shown in Fig. 5.

DISCUSSION

Glycosylated forms of MuLV *gag* polyproteins on the cell surface have been described in AKR mouse thymocytes (20, 35) and Gross virus-induced leukemia cells (18, 28). We wished to determine whether this phenomenon was exclusive to leukemic thymocytes or rather a general result of MuLV infection. In this report we described the presence on the cell surface of R-MuLV-infected JLS-V9 cells of two related forms of glycosylated *gag* proteins with apparent molecular weights of 80 and 95k. We also showed that uninfected cells did not have detectable *gag*-related proteins on their surface, whereas they possessed a gp70-related protein that was

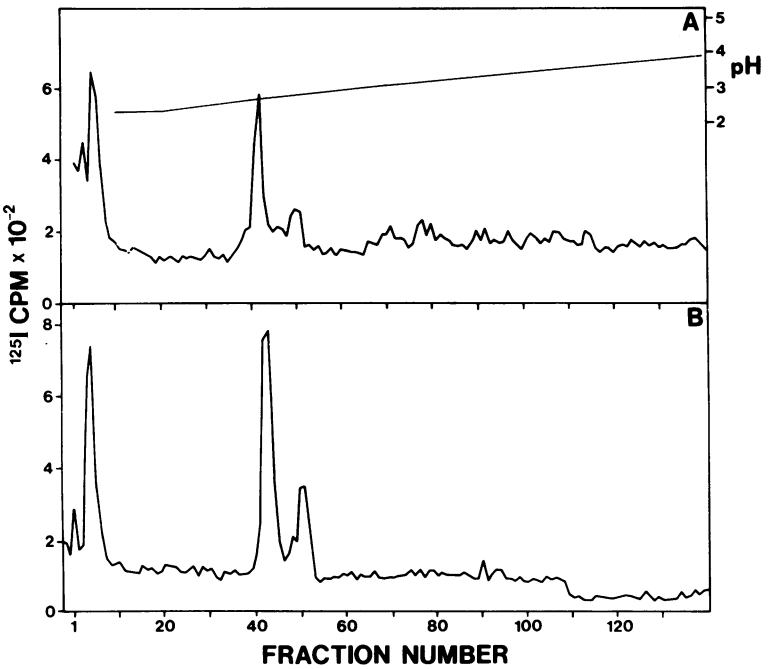

FIG. 6. *Tryptic fingerprints of cell-bound gp70's iodinated with lactoperoxidase on the surface of AKR-A (A) and R-MuLV-infected JLS-V9 (B) cells. Anti-gp70 immunoprecipitates from surface-labeled cells were analyzed by SDS-gel electrophoresis. Gel slices containing the radioactive gp70 were dissolved, and the protein was oxidized and trypsinized for chromatographic analysis as described in the text.*

not analyzed further. The expression of gp70 independently of virus production has been documented (21, 30, 31).

After this study was completed, the existence of glycosylated *gag*-related proteins was shown by metabolic labeling techniques in R-MuLV-infected JLS-V9 cells by Schultz et al. (26) and in Moloney MuLV-infected cells by Edwards and Fan (5), similar to findings of Evans et al. (10) in Friend MuLV-infected cells. In these studies an incompletely glycosylated precursor to $gP94^{gag}$, $gPr80^{gag}$, was described as opposed to $gP85^{gag}$ which would derive from $gP94^{gag}$ (19). The authors conclude from their data that all of these molecules differ only in their glycosylation level and have the same protein moiety; they are discriminated mainly by their different kinetics of intracellular labeling. Therefore, our analysis does not allow a definitive conclusion as to whether the 80k surface glycoprotein in this study represents $gPr80^{gag}$ or a derivative similar to $gP85^{gag}$.

The two molecular forms of *gag* polyproteins (80 and 95k) found on R-MuLV-infected JLS-V9 cells had very similar maps of tyrosine-containing tryptic peptides (Fig. 3); analogous observations have been reported for the Gross leukemia gag polyproteins of 85 and 95k molecular weight (19), using different labeled amino acids. Variations in the glycosylation level could be invoked to account for the different migration in SDS gels. Although we did not observe marked differences in the tryptic peptide map of 95k protein after the chemical removal of terminal sialic acid residues (4, 16), differences in internal sugar residues, in peptides not resolved by the column and eluting in the flow through, or in peptides not containing tyrosine are still possible.

Tryptic fingerprinting of the iodinated proteins also showed that the 95k *gag* proteins on the surface of R-MuLV-infected cells and AKR leukemic cells were indistinguishable (Fig. 4), whereas their cell surface gp70's could be clearly distinguished (Fig. 5). One could assume that a low level of endogenous virus production is induced by the exogenous infection and could account for the presence of *gag* polyproteins on the cell surface. However, the ratio of radioactive ^{125}I incorporation into gp70 versus *gag* polyproteins is approximately the same in R-MuLV-infected cells and in AKR cells (20; E. Buetti, unpublished data); if there were coordinate expression of glycoproteins and polyproteins of the endogenous virus, one would expect to see a significant contribution by the endoge-

nous gp70 to the fingerprint of R-MuLV-infected cell glycoprotein. Such an overlap was not observed (Fig. 5), suggesting that the presence of *gag* surface polyproteins in R-MuLV-infected cells was probably not due to the concomitant production of an endogenous virus. Our results can be compared with those obtained by O'Donnell and Stockert (25) by using a different experimental approach, namely, testing the ability of cells, when used for absorption, to reduce cytotoxic titers of G_{IX} and Gross cell surface antigen antisera. Cultured fibroblasts infected by exogenous MuLV gave negative absorption of G_{IX} antiserum (which detects an endogenous form of gp70), whereas they gave partial absorption of anti-Gross cell surface antigen reactivity (directed against *gag* polyproteins).

The 95k *gag* polyproteins contain several *gag* proteins with type specificity (19), which would be different in AKR and R-MuLV-infected cells. The identity of 95k proteins in our type of analysis may mean that type-specific peptides do not contain tyrosine or that they are not resolved by the column used or even that some are absent in the 95k polyproteins (19). It is also possible that the *gag* precursor is identical in different viruses and that differences in the fingerprints of the mature *gag* proteins could be the consequence of different processing.

In summary, we have shown that the infection of JLS-V9 cells in culture with R-MuLV induced the appearance on the cell surface, in addition to larger amounts of R-MuLV gp70 glycoprotein, of glycosylated forms of *gag* polyproteins. The latter are therefore not an exclusive feature of leukemic thymocytes (20, 35); their function in the viral infective cycle, if any, must still be elucidated.

Our chloramine T tryptic peptide analyses of cell-bound gp70 glycoproteins showed an extensive dissimilarity between R-MuLV-infected cells and AKR cells, in agreement with published data on the viral gp70's (8). However, the fingerprints of the same proteins, when they had only been iodinated in situ with lactoperoxidase, were intriguingly similar (Fig. 6). Although the chromatographic method used was not sufficient to prove the identity of the peptides, the result suggests a similarity at least in the charge, if not in the sequence, of the portion of protein exposed on the cell surface. A more detailed analysis would be needed to clarify this point.

The treatment of glycoproteins with sulfuric acid had been shown to be effective in removing their terminal sialic acid residues (4, 16). In the case of the avian glycoprotein gp85, this method had helped to clarify a complex fingerprint that was possibly due to heterogeneous sialylation (16). When we applied this treatment to viral and cellular gp70's (Fig. 5), we found specific differences in individual peptides that can provide some information on the maturation process of the virus. A recent report (23) analyzes the sources of the heterogeneity of MuLV gp70 as being both genetic and due to variability in the number of sialic acid residues attached to oligosaccharides (22). When we compared the fingerprints of native and desialylated cell-bound gp70, we found relatively little difference (one peptide displaced). This suggests a relatively low level of sialylation on the whole cellular gp70 population, which resides mainly in the cell membrane (14, 42). On the contrary, acid treatment of gp70 from purified virus resulted in the displacement of four additional peptides, suggesting a higher level of sialylation in virions. This may also explain our inability to obtain interpretable fingerprints of native gp70 from purified virus (data not shown).

In summary, our data for R-MuLV-infected cells are consistent with the existence of a large pool of cell surface-bound gp70 molecules that are incompletely glycosylated and a relatively small number that is further sialylated and participates in the budding of virus particles. Kinetic (41, 42) and immunoelectron microscopic (14) evidence supports this interpretation.

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