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Structural Studies of Retroviruses: Characterization of Oligomeric Complexes of Murine and Feline Leukemia Virus Envelope and Core Components Formed upon Cross-Linking

ABRAHAM PINTER* AND ERWIN FLEISSNER

Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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To examine the protein proximity and subunit organization of type C retroviruses, preparations of AKR murine leukemia virus were treated with bifunctional cross-linking reagents and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The cross-linked components obtained were characterized by immunoprecipitation with monospecific antisera against purified viral proteins, followed by SDS-PAGE analysis both before and after cleavage of the cross-links. With these procedures, complexes of both viral envelope and core components were identified. The major envelope subunit obtained was a large (apparent molecular weight of 450,000 to 500,000), glycosylated complex, composed of four to six gp70-p15(E) subunits. This complex was detected over a 100-fold range of cross-linker concentration and thus seems to represent a particularly stable viral substructure. The cross-linked complexes of the core proteins consisted of oligomers of p30 dimers, suggesting that the p30 dimer is a basic structural unit of the viral core. When virion preparations, which had previously been disrupted with the nonionic detergent Nonidet P-40, were cross-linked, the envelope complex was still observed, indicating that this structure is stable in the presence of Nonidet P-40. A similar envelope structure was observed for feline leukemia virus, suggesting that such a complex may be a conserved feature of oncornavirus structure.

The type C oncornaviruses possess distinct core and envelope morphological structures which have been visualized in the electron microscope. The presence of surface "knobs" approximately 8.0 to 10.0 nm in diameter covering the envelope of intact virions has been demonstrated after negative staining of murine leukemia virus (MuLV) preparations with uranyl acetate or by a combination of phosphotungstic acid staining followed by freeze-drying (5, 22). Regularly arranged subunits of viral cores, which possess a diameter of approximately 6.0 nm, have also been visualized after freeze-drying and shadowing of viral cores released after various treatments (22). The MuLV envelope has been shown to contain two major protein components, a large glycosylated protein of approximately 70,000 daltons, gp70 (1), and a smaller, nonglycosylated hydrophobic protein, p15(E) (13); both are derived by proteolytic cleavage of a common precursor (8, 15, 26, 28). The two envelope components can, under appropriate conditions, form a disulfide-linked complex, referred to as [gp90] (23, 24), demonstrating an integral association between gp70 and p15(E). The major internal structural components of the virus consist of four proteins—p30, p15, p12, and p10—which are derived by proteolytic processing of the *"gag"* gene product (7). To better correlate viral proteins with the morphological substructures seen in virions, we have studied the protein proximities and spatial arrangements of proteins in viral substructures by treating virions with two chemical cross-linking reagents, dithiobis(succinimidyl proprionate) (DSP) and 1,5-difluoro-2,4-dinitrobenzene (DFDNB). Both reagents contain two functional groups which react readily at physiological conditions with lysine amino groups, thus allowing the cross-linking of neighboring proteins. DSP possesses an internal disulfide bond; thus cross-links formed with this reagent can be readily cleaved by reduction, facilitating the analysis of the cross-linked products. These studies have resulted in the identification of specific complexes of both viral envelope and viral core components; these complexes may be related to the morphological substructures which have been visualized in the electron microscope.

MATERIALS AND METHODS

Viruses. AKR MuLV was obtained from producer SC-1 cells, and feline leukemia virus (FeLV) AB was obtained from an infected feline lung fibroblast cell

line. Viruses were radiolabeled by culturing in the presence of either ^{14}C-labeled mixed amino acids or ^{3}H-labeled glucosamine precursors, at a concentration of 100 μCi/ml, for 18 h, and purified by banding directly on 15 to 60% sucrose gradients. Concentrated AKR MuLV, obtained from Electro-Nucleonics Laboratory Inc., was labeled with [^{3}H]$NaBH_4$ by the method of Gahmberg and Hakomori (10).

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 7 to 21% gradient slab gels by the system described by Laemmli (18). Gels were analyzed by fluorography, according to the procedure described by Bonner and Laskey (3). Samples analyzed under nonreducing conditions were boiled in the presence of 1% SDS for 1 min. Samples analyzed under reducing conditions were boiled in the presence of 1% SDS and 1% mercaptoethanol for 1 min.

Alkylation of virions with NEM. A stock solution of 10% *N*-ethylmaleimide (NEM) in acetonitrile was diluted 1:10 with phosphate-buffered saline (PBS). One volume of the PBS solution was then added to 9 volumes of the virus sample and incubated at room temperature for 15 min.

Cross-linking procedure. Stock solutions of the cross-linking reagents were prepared in Me_2SO at a concentration of 20 mg/ml. These were then diluted with PBS to a concentration fivefold greater than the desired final concentration, then further diluted 1:5 with the virus sample. DSP reactions were performed in the presence of TN buffer (0.01 M Tris–0.10 M NaCl); for DFDNB reactions, virus samples prepared in PBS were used. Reaction times were typically 15 min at room temperature. Occasionally, unreacted reagent was quenched by the addition of 0.1 M ammonium acetate before lysis of virions; however, similar results were obtained if this step was omitted. The concentration of radiolabeled virions used in these studies was less than 20 μg/ml. Similar patterns resulted upon cross-linking of virions at a concentration of 1 mg/ml.

Radioimmunoprecipitations. Virions were lysed by the addition of Nonidet P-40 (NP-40) and NaCl to final concentrations of 0.5% and 0.5 M, then incubated with antisera at a final concentration of 1:50 for 1 h at 37°C. Immune complexes were then pelleted by treatment with 50 μl of a 10% solution of staphylococcus A (Pansorbin, Calbiochem) prepared as described by Kessler (17). After pelleting at 3,000 rpm for 10 min, the staphylococcus A was washed with 10 ml of high salt (1 M NaCl, 0.5% NP-40, 0.02 M Tris) and 10 ml of low salt (0.004 M Tris, 0.5% NP-40) buffers, pelleted, and then extracted with 0.1 ml of SDS-containing buffer at 100°C for 1 min.

Reagents. Goat anti-Rauscher gp70 and anti-p30 sera were obtained from R. Wilsnack; rabbit anti-p15(E) was prepared as described (13). DSP and DFDNB were obtained from Pierce Chemical Co., SDS (specially pure) was obtained from BDH Chemicals Ltd., NEM was from Sigma Chemical Co., and radiochemicals were obtained from New England Nuclear Corp.

RESULTS

Cross-linking of AKR MuLV with DSP. We have previously shown that the majority of the viral envelope proteins, gp70 and p15(E), exist in native virions in the form of a noncovalently linked complex. Upon disruption of virions with NP-40, a covalent disulfide linkage can form spontaneously between the two proteins, resulting in the stable gp70-p15(E) complex referred to as [gp90]. In addition, activation of appropriate thiol residues of gp70 and p15(E) by treatment with the thiol-specific reagents NEM or dithiobis(*m*-nitropyridine) (DTNP) results in formation of [gp90] in intact virions (23, 24) (Fig. 1). Samples of ^{14}C-amino acid-labeled AKR virions analyzed by SDS-PAGE under nonreducing conditions contained gp70 and p15(E) (Fig. 1A); after treatment with NEM, the gp70 band is not observed, the p15(E) band diminishes in intensity, and the [gp90] band appears (Fig. 1B). To determine whether [gp90] was part of a larger envelope structure, virions pretreated with NEM were reacted with DSP and analyzed under nonreducing conditions. At a final DSP concentration of 10 μg/ml, new bands with apparent molecular weights of 60,000 (a) and 120,000 (b) were observed, along with a more diffuse band at a molecular weight of approximately 180,000 (c), and a band of higher molecular weight ("x") (Fig. 1C). When the concentration of cross-link-

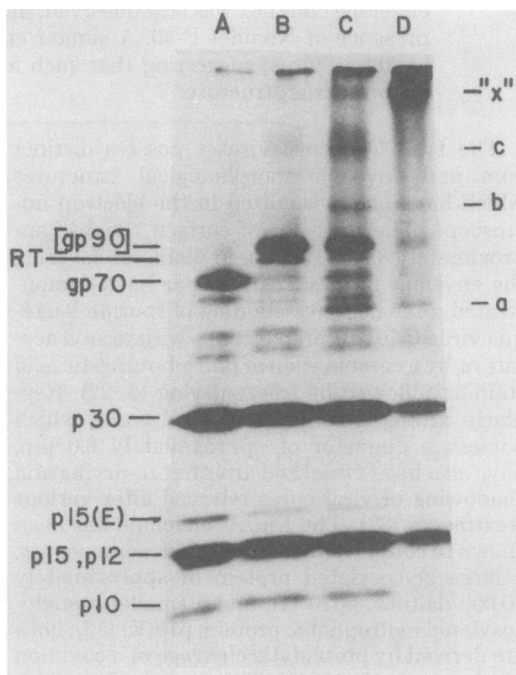

FIG. 1. *Cross-linking of ^{14}C-amino acid-labeled AKR virions with DSP. Virus samples were analyzed by SDS-PAGE under nonreducing conditions after the following treatments: (A) untreated virus; (B) +NEM; (C) +NEM, +DSP (10 μg/ml); (D) +NEM, +DSP (100 μg/ml). "x" represents the cross-linked envelope component.*

ing reagent was increased to 100 μg/ml, a significant decrease in intensity of the monomeric viral protein bands was observed, as well as of bands a, b, and c, and there was a large increase in material running at position "x" (Fig. 1D).

To ascertain which cross-linked bands contained gp70, the above experiments were repeated with virions in which the glycoprotein had been specifically labeled by galactose oxidase oxidation, followed by reduction with [^{3}H]$NaBH_4$ (10). Figure 2A shows the gel pattern obtained for NEM-treated virions under nonreducing conditions. The major band observed corresponds to [gp90], with a faint band of gp70 and only trace amounts of high-molecular-weight components. Lanes B, C, and D show the patterns obtained after treatment with 10, 100, and 1,000 μg of DSP per ml, respectively. At the lowest DSP concentration, a new component with the mobility of "x" appears along with some smaller, more diffuse material (Fig. 2B). At 100 μg of DSP per ml, essentially all of the [gp90] has been cross-linked, with a concomitant increase in band "x" (Fig. 2C). A significant percentage of the radioactivity is still present as component "x" after treatment with 1,000 μg of DSP per ml, although under these conditions, most of the protein has been cross-linked to still larger aggregates which can no longer enter the gel (Fig. 2D). These experiments indicate that band "x" contains a cross-linked complex of the viral glycoprotein; the observations that it is detected over a 100-fold concentration range of the cross-linking reagent and that well-characterized complexes of smaller size are not readily observed suggest that the large complex represents a particularly stable viral substructure.

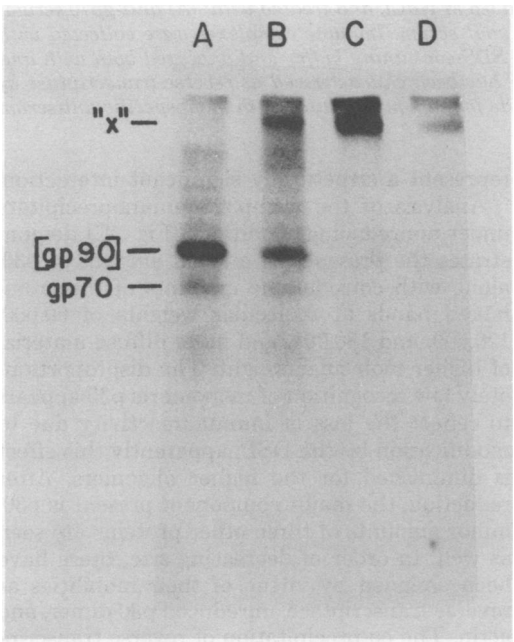

FIG. 2. *Cross-linking of AKR MuLV glycoproteins. Virions, for which gp70 had been labeled by [^{3}H]-$NaBH_4$ reduction after galactose oxidase oxidation (10), were analyzed by SDS-PAGE under nonreducing conditions after the following treatments: (A) +NEM; (B) +NEM, +DSP (10 μg/ml); (C) +NEM, +DSP (100 μg/ml); (D) +NEM, +DSP (1,000 μg/ml).*

The size of the glycosylated complex was estimated by comparison of its SDS-PAGE mobility with that of the LETS monomer and dimer purified from JLSV9 cells by immunoprecipitation with monospecific antiserum prepared against human cold insoluble globulin (25). The LETS protein monomer has a molecular weight of 220,000 and has been shown to exist as a disulfide-linked dimer (16). Such an analysis showed that the cross-linked complex has a slightly lower mobility than that of the purified LETS dimer (data not shown; see Fig. 5), and thus we have assigned it an apparent molecular weight of 450,000 to 500,000.

Characterization of DSP-cross-linked components by immunoprecipitation. To determine the composition of the cross-linked components obtained after DSP treatment, cross-linked virions were lysed with NP-40, and the cross-linked complexes were isolated by immunoprecipitation with monospecific antisera against individual viral proteins. This procedure has the advantage that it allows the analysis of individual complexes even in cases where there may be several different cross-linked complexes of similar size. The components of the complexes are thus identified by their antigenic reactivity as well as by their SDS-PAGE mobility after reduction.

Figure 3 shows the SDS-PAGE pattern obtained for ^{14}C-amino acid-labeled AKR virions before and after cross-linking with 100 μg of DSP per ml. In this experiment, the NEM treatment was omitted. The pattern obtained after cross-linking was similar to the one described earlier; almost all of the gp70 and most of the p15(E) have been cross-linked, and there is a significant reduction in the intensity of the p30 band. The 60,000- and 120,000-molecular-weight bands are readily apparent, and there is a considerable amount of high-molecular-weight material which is more diffuse. Upon reaction with anti-gp70 serum, very little radioactivity is precipitated; this suggests that the DSP treatment resulted in a considerable modification of the gp70 molecule with a consequent loss of immunoreactivity. A faint band is observed for the anti-gp70 precipitate (Fig. 3A). In the unreduced sample, it has the mobility of "x"; after reduction, the disulfide bonds of the cross-linking

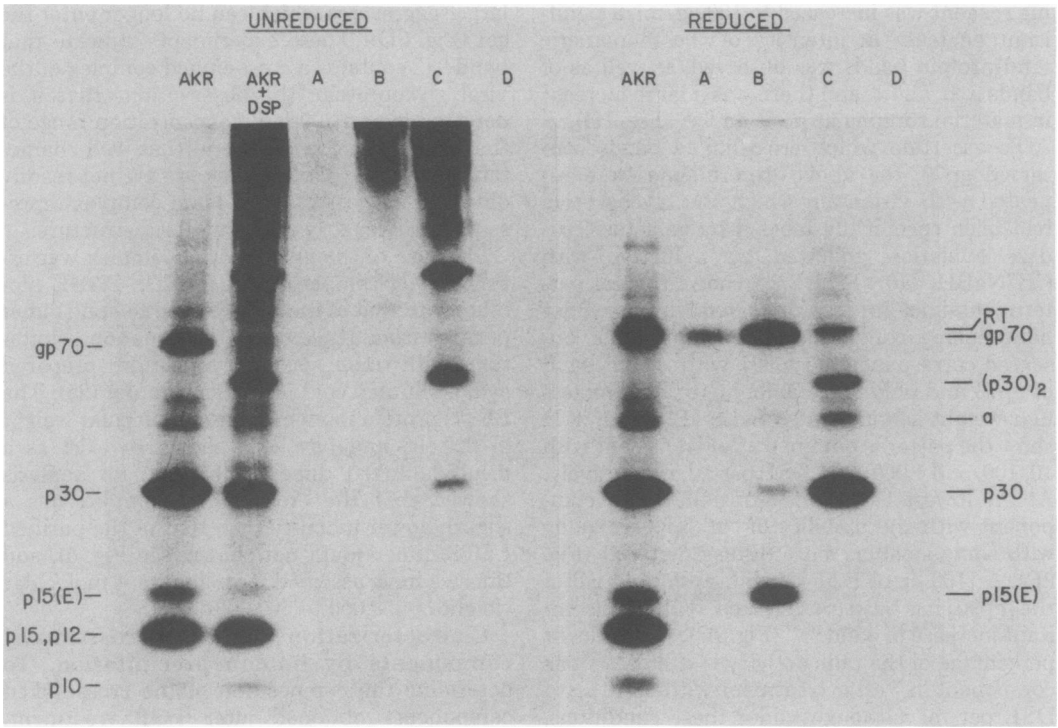

FIG. 3. *Immunoprecipitation of DSP cross-linked components. ^{14}C-amino acid-labeled virions were cross-linked with 100 μg/ml of DSP, lysed with 0.5% NP-40 and 0.5 M NaCl, and treated with: (A) anti-gp70 serum; (B) anti-p15(E) serum; (C) anti-p30 serum; (D) normal goat serum. Immune complexes were collected with staphylococcus A and, after washing, were dissolved in SDS-containing buffer and analyzed both with and without adding mercaptoethanol. The band labeled RT has been characterized as reverse transcriptase by virtue of its identical mobility with that of the polypeptide immunoprecipitated with monospecific antiserum to purified reverse transcriptase.*

reagent are cleaved, and a band with the mobility of free gp70 is observed. The sample is too dilute to detect any additional components present.

Reaction with antisera to p15(E) resulted in the immunoprecipitation of considerably more material (Fig. 3B); apparently the immunoreactivity of p15(E) is more resistant to modification. Upon analysis under nonreducing conditions, a large diffuse band, similar to that recognized by the anti-gp70 serum, is observed. After reduction, this band is shown to consist of only two major components, gp70 and p15(E), indicating that a large complex of gp70 and p15(E) is stabilized by DSP treatment. This complex does not contain any p15, p12, or p10; however a slight amount of p30 is observed after reduction (Fig. 3B). It is not clear whether this is due to some trapping of cross-linked p30 aggregates with the cross-linked envelope complexes or whether this reflects actual cross-linking between p30 and the envelope components. In any case, the relative amount of p30 observed in this sample is so low that this does not appear to represent a structurally significant interaction.

Analysis of the anti-p30 immunoprecipitate under nonreducing conditions (Fig. 3C) demonstrates the presence of a small amount of p30, along with considerable amounts of the cross-linked bands at molecular weights of 60,000, 120,000, and 180,000, and more diffuse material of higher molecular weight. The disproportionately low recognition of monomeric p30 appears to reflect the loss of immunoreactivity due to modification by the DSP; apparently this effect is diminished for the higher oligomers. After reduction, the major component present is p30; minor amounts of three other proteins are seen as well. In order of decreasing size, these have been assigned by virtue of their mobilities as reverse transcriptase, unreduced p30 dimer, and actin. The co-precipitation of reverse transcriptase with p30 has been previously observed (14), and thus is not an indication that the two components were cross-linked. Actin has also been observed to be a contaminant of viral immunoprecipitates, and thus it is not clear whether there is an actual p30-actin cross-linked complex

formed by DSP treatment.

Treatment with nonimmune serum did not result in the precipitation of any labeled components (Fig. 3D), indicating that nonspecific adsorption of the cross-linked components to staphylococcus A is not occurring.

DSP treatment of NP-40-solubilized virions. Recent studies have indicated that certain MuLV morphological structures can survive NP-40 treatment (9, 31). To study the MuLV substructures which exist in the presence of nonionic detergent, AKR virions were disrupted with 0.5% NP-40 and subsequently cross-linked with 100 μg of DSP per ml. Cross-linking of intact virions results in the pattern described earlier; gp70 and p15(E) are cross-linked into a large complex, and p30 oligomers are formed (Fig. 4B). DSP treatment of NP-40-lysed virions still results in the cross-linking of gp70 and p15(E); however the p30 oligomers do not form, thus allowing the clear resolution of the envelope complex (Fig. 4C). A similar effect is observed for virions pretreated with NEM (Fig. 4C'). These results indicate that the large gp70-p15(E)

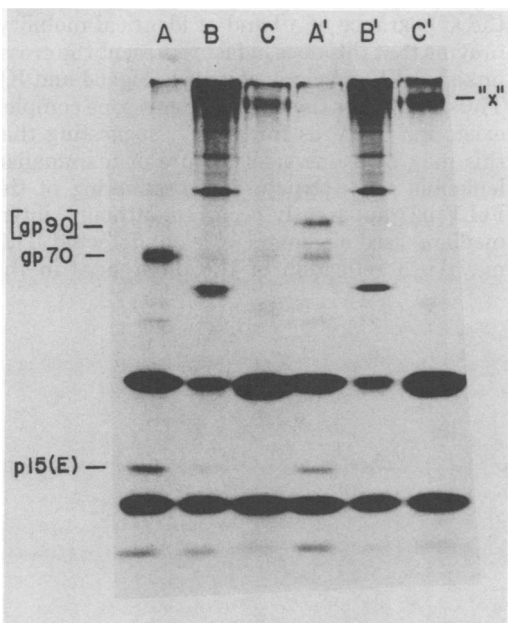

FIG. 4. *DSP cross-linking of AKR virions disrupted with NP-40. ^{14}C-amino acid-labeled virions analyzed by SDS-PAGE under nonreducing conditions after the following treatments: (A) uncross-linked virions; (B) cross-linked with 100 μg of DSP per ml; (C) disrupted with 0.5% NP-40 for 15 min at room temperature followed by cross-linking with 100 μg of DSP per ml. Samples A', B' and C' were pretreated with 0.1% NEM before the other manipulations to convert gp70 to the gp70-p15(E) disulfide-linked complex [gp90].*

complex is quite stable in the presence of nonionic detergent, whereas the p30 complexes are not. It is believed that nonionic detergents bind primarily to hydrophobic regions of proteins (12); thus the dissociation of the p30 complexes by NP-40 suggests that the p30 associations are due mainly to hydrophobic interactions.

Cross-linking of AKR virions with DFDNB. To further characterize the subunit composition of native oncornaviruses, intact preparations of AKR MuLV were treated with DFDNB, a bifunctional cross-linking reagent which reacts readily with protein amino groups, resulting in the formation of cross-links with a maximum span of 0.5 nm. Since this span is considerably shorter than that of DSP (approximately 1.1 nm), DFDNB is useful as a probe for protein associations of closer proximity than those detected by DSP. For this particular experiment, a virus preparation at a concentration of 1 mg/ml was used, and the protein pattern was determined by staining the polyacrylamide gels with Coomassie blue. The patterns observed upon cross-linking were independent of virus concentration, and similar results were obtained for more dilute, radioactively labeled preparations.

Treatment of intact virions with 100 μg of DFDNB per ml resulted in the loss of the gp70 band and the appearance of higher-molecular-weight material which possesses a similar mobility to that of the envelope complex formed with DSP (Fig. 5C). There does not appear to be a detectable decrease in the intensity of the p30 band, and bands corresponding to the p30 oligomers are not present. DFDNB treatment of virus which had been pretreated with NEM also resulted in the cross-linking of gp70 and the resolution of a large complex (Fig. 5D). This band appears slightly larger than the corresponding band observed in lane C; this may be an indication that the complex formed without NEM treatment contains less than the maximum number of gp70 or p15(E) constituents. For the NEM-treated virions, DFDNB treatment does result in a decrease in the intensity of the p30 band, indicating that cross-linking has occurred; however, the p30 intermediates detected after DSP treatment are not observed. This may indicate that NEM treatment induces a change in conformation of the p30 molecules, which allows the occurrence of large-scale cross-linking by DFDNB. Figure 5E shows the gel patterns obtained after DSP cross-linking. The p30 dimer, tetramer, and hexamer are detected, and a broad band, encompassing the mobilities of the cross-linked envelope complexes resolved in lanes C and D, is observed.

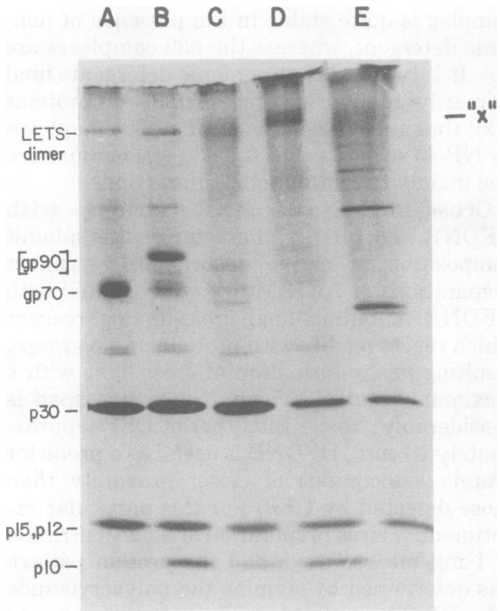

FIG. 5. *AKR virions cross-linked with DFDNB. Coomassie blue staining pattern of AKR virions analyzed by SDS-PAGE under nonreducing conditions, after the following treatments: (A) untreated virions; (B) virions treated with 0.1% NEM; (C) virions cross-linked with 100 μg of DFDNB per ml; (D) virions treated with 0.1% NEM followed by cross-linking with 100 μg of DFDNB per ml; (E) virions cross-linked with 100 μg of DSP per ml. (The band labeled "LETS dimer" is an impurity found in virions which comigrates with purified LETS protein analyzed under nonreducing conditions. Upon reduction, this band is not observed and a band which comigrates with monomeric LETS protein appears.)*

Cross-linking of FeLV. The studies described above have been performed with the AKR strain of MuLV; similar results have been obtained with other MuLV strains, including Rauscher and Moloney virions (data not shown). To determine whether these structures are present in retroviruses of other species as well, the DSP cross-linking experiments were repeated with a representative strain of FeLV. Upon SDS-PAGE analysis of ^{14}C-amino acid-labeled FeLV, the overall pattern obtained was similar to that observed with AKR MuLV, although the absolute mobilities of the various proteins were different. As was the case for MuLV, analysis under nonreducing conditions demonstrated the presence of free gp70 (Fig. 6B and C), whereas NEM treatment resulted in the formation of a disulfide-linked gp70-p15(E) complex (Fig. 6D). Upon treatment with increasing amounts of DSP, the intensity of the [gp90] band diminished, as did that of the core components. The only new component resolved was a large band with similar mobility to that of the cross-linked MuLV envelope complex (Fig. 6G). Cross-linking of [^{3}H]glucosamine-labeled FeLV resulted in the appearance of a band of identical mobility, proving that this does in fact represent the cross-linked FeLV envelope complex (Fig. 6J and K). Thus, it appears that a similar envelope complex exists for FeLV as for MuLV, suggesting that this may be a universal feature of mammalian leukemia virus structure. Cross-linking of the FeLV p27 apparently occurred, although intermediate-sized oligomers were not detected. This may be a reflection of the differences in the

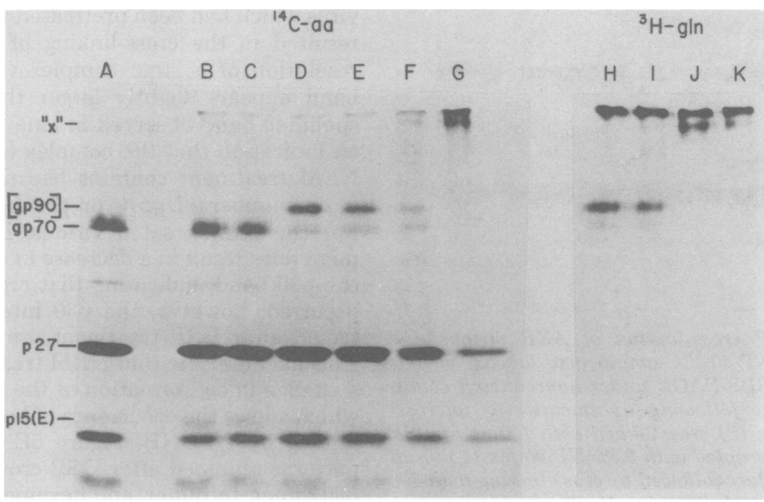

FIG. 6. *Cross-linking of FeLV proteins with DSP. ^{14}C-amino acid-labeled (A–G) and [^{3}H]glucosamine-labeled (H–K) FeLV analyzed by SDS-PAGE under reducing (A) and nonreducing (B–K) conditions after the following treatments: (A and B) untreated virus; (C) +1% iodoacetamide; (D) +0.1% NEM; (E) +0.1% NEM, +DSP (4 μg/ml); (F) +0.1% NEM, +DSP (20 μg/ml); (G) +0.1% NEM, +DSP (100 μg/ml); (H) +0.1% NEM; (I) +0.1% NEM, +DSP (10 μg/ml); (J) +0.1% NEM, +DSP (100 μg/ml); (K) +0.1% NEM, +DSP (1,000 μg/ml).*

primary structures of p27 and p30, or may actually indicate a difference between the structural arrangement of the major core proteins of the two viruses.

DISCUSSION

The cross-linking experiments described in this paper demonstrate the presence of distinct associations between leukemia virus envelope components and between viral core proteins. Treatment of MuLV with the reversible cross-linking reagent, DSP, results in the formation of a large complex (component "x") which is glycosylated (Fig. 2) and can be immunoprecipitated by both anti-gp70 and anti-p15(E) sera. SDS-PAGE of the immunoprecipitated complex after reduction demonstrates that the only components present in appreciable amounts are gp70 and p15(E) (Fig. 3). This complex is formed both with native virions and with virions which have been treated with NEM, and which therefore contain the gp70-p15(E) disulfide-linked complex. The acylation rate of proteins by DSP is very rapid, with a half-life of less than 1 min at neutral pH (19); thus it does not appear likely that the formation of "x" requires lateral diffusion of components in the viral membrane. The envelope complex is observed over a greater than 100-fold concentration range of DSP; in addition, an apparently identical complex is formed after treatment of virions with a second cross-linking reagent, DFDNB, which has a shorter span (0.5 nm compared with 1.1 nm for DSP) and different functional groups than DSP (Fig. 5). These results indicate that gp70 and p15(E) exist in the viral membrane in the form of a specific structural complex, which corresponds to the component "x" observed in gels after stabilization with the cross-linking agents. The fact that the envelope complex survives in the presence of NP-40 (Fig. 4) is a further demonstration of the stability of the complex.

The apparent size of the envelope complex as determined by SDS-PAGE, using the LETS dimer as a molecular-weight marker, is approximately 450,000 to 500,000 (Fig. 5). However, the cross-linked structure of the complex could result in an anomalous migration rate. It has recently been reported that cross-linked proteins with molecular weights larger than 50,000 migrate in SDS-Laemmli gels as if they were 17 to 25% larger than the molecular weights calculated from their polypeptide composition (21). By using the larger value as the limit of error, a minimum molecular weight of 360,000 can be calculated for the cross-linked complex, suggesting that it is composed of at least four gp70-p15(E) components; by calculating a molecular weight directly from the observed mobility of the complex, a maximum value of six subunit components is obtained. The demonstration that a similar complex is present on FeLV indicates that this structure is a conserved feature of retroviruses. Since the size of the complex is large enough to account for the 8- to 10-nm projections observed on the surface of MuLV in the electron microscope, it is attractive to hypothesize that this complex actually corresponds to the individual knobs of the viral envelope.

The major cross-linked core components detected after DSP treatment of intact MuLV consisted of p30 oligomers. The p30 dimer, tetramer, and hexamer were resolved and characterized by immunoprecipitation (Fig. 3) and by two-dimensional gel electrophoresis, in which the first dimension was run under nonreducing conditions and the second dimension was run after reduction (data not shown). Larger complexes were also formed, but these were not resolved in the gels and formed a broad band near the top of the gel. The formation of only the even-numbered oligomers indicates the stability of the p30 dimer and suggests that it is a basic subunit of the viral core. Our observation that the p30 complexes are not cross-linked in the presence of NP-40 suggests that hydrophobic forces play a role in the self-association of p30 molecules; this is consistent with the demonstrated presence of hydrophobic sites on p30 (20, 27). It is interesting to compare these results with those of Burnette et al. (4), who demonstrated that purified p30 of Moloney MuLV self-associates into multimeric forms at increasing concentrations. At the highest concentration tested, the observed molecular weight for p30 as determined by sedimentation velocity analysis was approximately 120,000, and a computer-simulated analysis of the data suggested a monomer-octomer equilibrium. These p30 complexes may be related to the 6-nm core structures which have been visualized in the electron microscope (22).

Cross-linked complexes of the other major components of the viral core, p15, p12, and p10, were not detected under conditions which resulted in extensive cross-linking of the envelope components and of p30. This was not due to a lack of accessible amino groups on these proteins, since we have observed that a radioactive analog of DSP, *N*-succinimidyl [2,3-^{3}H]propionate (Amersham Corp.), can effectively label all of the core proteins (data not shown). In addition, after treatment of virions with DSP at 100 μg/ml, p15 can no longer be immunoprecipitated with anti-15 sera, indicating that it is heavily substituted with DSP molecules. A lack of cross-linking is not conclusive evidence that molecular associations do not exist, since it may

merely indicate that the necessary functional groups are not arranged in the proper spatial orientation to be cross-linked by a particular reagent. Nonetheless, the fact that the smaller core components are not readily cross-linked may be an indication that they do not exist as specific multiprotein complexes in virions.

Structural studies of other enveloped viruses, performed with the same or related cross-linking reagents, have demonstrated the existence of heterocomplexes between virion core and envelope components. Treatment of Semliki Forest virus with dimethyl suberimidate resulted in the cross-linking of viral envelope components to cores (11). Cross-linking of vesicular stomatitis virus with DSP and related reagents resulted in the detection of complexes formed between the viral glycoprotein, G, and the membrane protein, M (6), and also between the G protein and the nucleocapsid protein (21). Such heterocomplexes between envelope and core components of retroviruses were not detected in our study. In particular it is of interest that cross-linked complexes of p15 and the envelope proteins were not obtained (Fig. 3B), since p15 has been shown to possess properties of a membrane-associated protein (2). Although failure to detect such interactions by this type of a study is not conclusive, it does indicate an increased probability that such interactions are not present. It has been demonstrated that the envelope and core components of MuLV are synthesized via separate classes of mRNA's (29); thus they must find their way to the viral assembly site, the plasma membrane, by independent routes. Since retroviral proteins are relatively minor components of infected cells, there must be an efficient and specific interaction between the viral envelope and core proteins which results in their association in the presence of a large excess of cellular material. Recently it has been shown that the assembly of budding virus particles can occur with uncleaved core precursor Pr70 (30). It is interesting to speculate that the association of viral core and envelope components at the plasma membrane requires the uncleaved core precursor and that, in extracellular particles which contain the cleaved core proteins, these interactions are not retained. A possible approach toward resolving this question would be to cross-link budding virus particles and to look for the presence of envelope-core complexes in either solubilized membranes or subsequently released virus particles.

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