Structural and Antigenic Analysis of the Nucleic Acid-Binding Proteins of Bovine and Feline Leukemia Viruses

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The nucleic acid-binding proteins of bovine leukemia virus (BLV) and feline leukemia virus (FeLV) were isolated in a high state of purity with chloroform-methanol extraction followed by reversed-phase liquid chromatography. Selective solubilization and purity of BLV p12 and FeLV p10 was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The compositions and molecular weights were determined by amino acid analysis. An abundance of lysine and arginine residues along with their size identifies both BLV p12 and FeLV p10 as small basic proteins similar to well-defined type C viral nucleoproteins. NH₂-terminal degradation by the semiautomated Edman method provided the sequence of the first 40 amino acids for both proteins. The putative nucleic acid binding site found in several type C viral nucleoproteins was contained within this sequence, with the most homology centered around an eight-amino acid region involving seven identical residues and one substitution. Antisera were developed in rabbits, and specificity and titers were determined by electroblotting and immunautoradiography. By this technique, an immunological cross-reaction was found between BLV p12 and FeLV p10. The shared antigenic determinant most likely exists in the highly conserved eight-amino acid region. Although this sequence is also highly conserved in the nucleic acid-binding proteins of murine leukemia viruses, the shared antigenic determinant is not found in these or any other type C viruses tested. It is suggested that substitution of arginine (BLV p12/FeLV p10) to lysine (murine leukemia virus p10) is sufficient to elicit a change in antibody specificity.

Bovine leukemia virus (BLV) and feline leukemia virus (FeLV) are non-genetically transmitted exogenous retroviruses shown to be associated with disease in their respective hosts (4, 5).

BLV is regarded as the causative agent of enzootic bovine leukosis, a lymphosarcoma, found in domesticated cattle widespread in both North America and Europe. It infects 20% of all dairy cattle and is found in 60% of all herds in the United States. It can be transmitted horizontally through cell-to-cell contact or insect vectors and vertically by congenital means (6). Recently infectious BLV was demonstrated in the milk of dairy cows (7). It is also infectious in other species including sheep, goat, pig (18), and chimpanzee (32). The *gag* gene products are the internal structural proteins; p24, the major structural protein (8); p15, probably a major phosphorylated component (31); p12, the nucleic acid-binding protein (NBP) (17); and p10, which is less well characterized. The precursor to *gag* gene products is a ~65,000-dalton protein (Pr65) which was shown to contain p24, p15, p12, and

p10 by tryptic peptide mapping (9). BLV has not been readily classified into one of the well-defined groups of retroviruses. For example, antiserum to BLV p24 failed to detect cross-reactivity with the internal protein of FeLV, Rauscher murine leukemia virus (R-MuLV), foamy-like bovine syncytia virus, Mason-Pfizer monkey virus (8, 20), or avian oncornaviruses (19).

FeLV is also a naturally occurring infectious retrovirus which causes leukemia and lymphosarcoma in cats and is responsible for one-third of all cancer deaths. The primary mode of virus transmission is horizontal through saliva, infecting most free-roaming pet cats. Pathogenic forms of leukemia can be induced by inoculation of cats with FeLV (5). FeLV can also grow in human embryonic lung cells, dog kidney cells, and pig embryo cells (15) and can produce lymphosarcoma in dogs (28). FeLV strains can be divided into subgroups A, B, and C based on their patterns of attachment to receptors at the host cell membrane. The *gag* gene-encoded internal proteins are p15, p12, p27, and p10 (15),

which are similar in properties to the proteins of MuLV. Based on protein sequence homology and immunological relatedness with MuLV, FeLV is classified as type C, subgroup I (23).

The only BLV protein partially sequenced is p24, which is found to contain sequence homology with type C virus p30 structural proteins, especially with FeLV p27, suggesting that BLV p24 and FeLV p27 are evolutionarily related proteins (22). BLV p24 has recently been found (25) to have structural homology with the analogous 24,000-molecular-weight protein from a virus associated with human adult T-cell leukemia-lymphoma and designated HTLV (26). Several lines of evidence indicate that the NBPs of the virion may be among the most conserved *gag* gene products of retroviruses. As the RNA-associated internal core proteins of the virus, they may have an important function in replication and assembly and they may be under high evolutionary constraint. As a continuation of our studies on the BLV-FeLV relationship, we sequenced and immunologically characterized the NBPs of these viruses. The primary structure data of BLV p12 and FeLV p10 show the presence of a highly conserved segment in the putative nucleic acid binding site region. This and the results of immunological analysis which indicate cross-reaction permit the delineation of the shared antigenic determinant.

MATERIALS AND METHODS

Virus. BLV was grown in fetal lamb kidney cells (33), and the Rickard strain (A, B) of FeLV was grown in feline lymphoblasts (27). Viruses were purified by sucrose density gradient centrifugation according to standard procedures and obtained from the Viral Resources Laboratory of the National Cancer Institute-Frederick Cancer Research Facility, Frederick, Md.

Chloroform-methanol extraction. NBPs of BLV and FeLV were partially purified by chloroform-methanol extraction (21). Chloroform-methanol (2:1, vol/vol) was added to the virus suspension in the presence of low-ionic-strength buffer, 0.01 M Tris-hydrochloride, pH 7.4–0.001 M EDTA–0.05 M NaCl. After vigorous agitation for 2 min, the phases were separated by centrifugation at 2,000 rpm for 20 min at 4°C. The phosphoprotein and RNA partitioned to the aqueous phase, the viral lipids partitioned to the organic phase, and the remaining viral proteins were contained in the interphase. To solubilize BLV p12 and FeLV p10, the interphase was resuspended in high-ionic-strength buffer, 0.01 M Tris-hydrochloride (pH 7.4)–0.001 M EDTA–1.0 M NaCl, and the extraction was repeated with the NBP contained in the high-ionic-strength aqueous phase.

RPLC. Further purification was performed by reversed-phase liquid chromatography (RPLC) on a Waters μ Bondapak C₁₈ column (13). The NBP contained in the aqueous phase from high-ionic-strength chloroform-methanol extraction was lyophilized and then resuspended in 6 M guanidine-HCl and adjusted to a pH of 2.0 with 10% trifluoroacetic acid. A 0 to 30%

gradient of acetonitrile in 0.05% trifluoroacetic acid at pH 2.0 was used to elute the NBP from the column by reduction of the hydrophobic interactions. Proteins were recovered by lyophilization.

Reduction and carboxamidomethylation. Protein was reduced in the presence of 6 M guanidine-HCl–0.1 M NaHCO₃ (pH 8.5) buffer with 0.17 M dithiothreitol. Samples were flushed with N₂ and incubated at room temperature for 3 h. Reduced proteins were carboxamidomethylated with 0.4 M iodoacetamide (room temperature; 13 to 14 h).

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to the formulation of Laemmli (17), was used to determine purity of the NBPs and approximate molecular weights.

Amino acid analysis. The composition and molecular weight of the NBPs were determined by amino acid analysis on a Durrum D500 amino acid analyzer as previously described (11). The raw data were analyzed by a program run on the National Institutes of Health DEC 10 computer which selects a molecular weight for the protein based in such a way as to minimize the departure from integral values for all residues (3).

NH₂-terminal sequence analysis. NH₂-terminal sequence analysis by semiautomated Edman degradation was performed with the spinning-cup liquid phase system (24) on a Beckman 890C sequencer in the presence of Polybrene (30). RPLC on a Waters phenyl-alkyl column was used to identify and quantitate the PTH derivatives of amino acids (10).

Antisera. Antisera to the purified NBPs were developed in rabbits. The initial injection was made intradermally in the back footpads and subcutaneously in four sites on the hind of the rabbit with 150 μ g of NBP in phosphate-buffered saline mixed with an equal volume of Freund complete adjuvant. The rabbits were boosted subcutaneously approximately every 10 days with 50 μ g of purified protein each time in phosphate-buffered saline mixed with an equal volume of Freund incomplete adjuvant. Ten days after the second boost, the first test bleed of 10 ml from the ear vein was obtained. The rabbits were repeatedly boosted and then bled according to this schedule. Animals immunized with BLV p12 and FeLV p10 were bled out by heart puncture after 130 and 182 days, respectively. Anti-BLV p24 guinea pig serum and anti-FeLV p27 rabbit serum were available from previous studies (8).

Electroblotting and immunautoradiography. The specificities of the antisera were determined by electroblotting and immunautoradiography (29). BLV and FeLV were electrophoresed on 7.5 to 20% SDS-polyacrylamide slab gels. After electrophoresis the gel was equilibrated in transfer buffer (0.04 M sodium phosphate, pH 6.5). Aminobenzoyloxymethyl paper was converted to the active diazobenzoyloxymethyl form (DBM paper) by treatment with freshly prepared 4 mM NaNO₂ in 1.17 M HCl for 30 min on ice and then washed with water and equilibrated with transfer buffer. Virus was transferred from the gel to DBM paper electrophoretically for 2 h at 25 V. Remaining active groups on the paper were blocked after incubation at room temperature for 2 h with 1% bovine serum albumin in transfer buffer. The paper was rinsed in water and cut vertically into 0.5-cm-wide strips (representing a lane of separated viral proteins from SDS-PAGE) which were rotated at 37°C in rabbit antiserum

FIG. 1. RPLC of (A) BLV p12, (B) FeLV p10, and (C) reduced and carboxamidomethylated BLV p12.

diluted in 0.05 M Tris-hydrochloride (pH 7.4)–0.005 M EDTA–0.15 M NaCl–0.25% gelatin–0.05% Nonidet P-40 (TENG-N) for 15 to 16 h. The strips were rinsed in water and incubated first at 37°C for 2 h in four changes of TENG-N on a shaking water bath and then for 2 h in ^{125}I -labeled protein A at 40,000 to 50,000 cpm per ml of TENG-N. Label was removed and the strips were washed with four changes of 0.05 M Tris-hydrochloride (pH 7.4)–0.005 M EDTA–1.0 M NaCl–0.25% gelatin–0.4% Sarkosyl (TENG-S) for 2 h at 37°C. The strips were rinsed in water, air dried, and exposed to flashed X-ray film with an intensifying screen for 15 to 16 h.

DOT tests. Antibody titers were determined by directly spotting DBM paper strips with 1 μg of purified NBP in 0.04 M sodium phosphate buffer, pH 6.5. With this method an exact amount of protein was bound to DBM paper. Reaction with serial dilutions of antisera, ^{125}I -labeled protein A, and autoradiography were carried out as described above.

RESULTS

Purification of BLV and FeLV NBPs. The NBPs of BLV and FeLV were purified by chloroform-methanol extraction and RPLC. The proteins partitioned to the high-ionic-strength aqueous phase during extraction with chloroform-methanol. An approximate purity of >95%

after chloroform-methanol extraction was determined by SDS-PAGE. RPLC was used to remove possible contaminating protein components and RNA and allow for desalting and rapid concentration of protein by lyophilization from a volatile solvent. RPLC profiles of the extracted proteins from BLV, FeLV, and reduced and carboxamidomethylated protein from BLV are shown in Fig. 1A, B, and C, respectively. Both nucleoproteins eluted at 25% acetonitrile as the major component of the extraction. The elution patterns of BLV and FeLV NBPs indicated multiple protein peaks (three) eluting within a narrow range of acetonitrile gradient. When BLV p12 was reduced and carboxamidomethylated the elution pattern showed a single major peak, suggesting that the apparent chromatographic heterogeneity may have been due to the formation of inter- or intramolecular S–S bonds.

The purity of the proteins and approximate molecular weights were determined by SDS-PAGE. Purified NBP from BLV is demonstrated in Fig. 2A. In our system it has an M_r of $\sim 8,000$ compared with standard proteins. Previous nomenclature has identified this protein as BLV p12 (4). We will use the same designation. Figure 2B confirms the purity of FeLV p10, the

FIG. 2. SDS-PAGE of BLV virus and purified BLV p12 (A) and of FeLV virus and purified FeLV p10 (B). Standard proteins (molecular weights in parentheses) are phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (45,000), carbonic anhydrase (29,000), soybean trypsin inhibitor (21,000), lysozyme (14,300), and aprotinin (6,400).

NBP from FeLV, which has a molecular weight of ~6,000.

Amino acid composition. The amino acid compositional data of BLV p12 and FeLV p10, based on 24-, 48-, and 72-h hydrolysis, are given in Table 1. The total number of amino acids

TABLE 1. Amino acid composition of BLV p12 and FeLV p10

Amino acid	BLV p12		FeLV p10	
	Residues per protein	Nearest integer	Residues per protein	Nearest integer
Asp	3.72	4	6.09	6
Thr	2.90	3	1.81	2
Ser	2.33	2	1.93	2
Glu	(2.1)	2	5.29	5
Pro	14.83	15	3.70	4
Gly	5.82	6	3.23	3
Ala	3.08	3	3.25	3
Val	1.26	1	1.99	2
Met	0.71	1	0.00	0
Ile	1.03	1	0.94	1
Leu	2.04	2	3.59	4
Tyr	0.82	1	1.02	1
Phe	0.00	0	0.00	0
His	2.18	2	0.99	1
Lys	7.36	7	8.78	9
Arg	4.82	5	5.76	6
Cys (not determined)				
Trp (not determined)				
Mol wt	6,034		5,652	

minus cysteine and tryptophan is 55 for BLV p12 compared with 49 for FeLV p10. Note the abundance of basic amino acids, lysine and arginine, in both proteins. The number of residues of serine, alanine, isoleucine, and tyrosine are identical for both proteins as is the absence of phenylalanine. BLV p12 has a high proline content not shared by FeLV p10. The computer-assisted molecular weights from the composition (without cysteine and tryptophan) are 6,034 for BLV p12 and 5,652 for FeLV p10.

NH₂-terminal sequence. The NH₂-terminal sequence as determined by semiautomated Edman degradation of BLV p12 is shown in Fig. 3A. Assignments were based on the occurrence of a newly arising peak at each cycle compared with standard PTH amino acids. In a single microsequence analysis of 4 nmol of BLV p12, unambiguous assignments were made for residues 1 to 40 except cysteines in positions 24, 27, and 37, leucine residue 28 (which was lost), and threonine 39. Cysteine residues cannot be identified after Edman degradation of the unmodified protein due to their instability. The cysteines were determined by degrading purified peptides derived by endoproteinase Lys-C cleavage of reduced and carboxamidomethylated BLV p12. These two peptides, one representing the fragment of residues 18 to 29 and the other representing that of residues 30 to 40, were available from other studies. Sequence analysis of the peptides confirmed the initial results of Edman

BLV p12																					
		1				5				10				15				20			
a		Val-His-Thr-Pro-Gly-Pro-Lys-Met-Pro-Gly-Pro-Arg-Gln-Pro-Ala-Pro-Lys-Arg-Pro-Pro																			
	b	2.9	1.5	3.6	3.2	1.9	2.0	3.1	1.4	2.7	1.8	1.3	3.0	1.6	1.7	1.5	1.3	2.1	2.1	0.9	1.9
																		4.3 4.7 5.3			
		21				25				30				35				40			
a		Pro-Gly-Pro-Cys-Tyr-Arg-Cys-Leu-Lys-Glu-Gly-His-Trp-Ala-Arg-Asp-Cys-Pro-Thr-Lys																			
	b	6.8	6.2	4.5	2.9	7.5	6.2	2.3	4.1	1.1	17	14	17	8.9	19	17	16	6.2	3.1	2.9	2.5
FeLV p10																					
		1				5				10				15				20			
a		Ala-Thr-Val-Val-Ala-Gln-Asn-Arg-Asp-Lys-Asp-Arg-Glu-Glu-Lys-Lys-Leu-Gly-Asp-Gln																			
	b	6.8	4.7	9.0	5.6	5.7	3.4	3.0	3.4	3.3	4.8	2.9	2.4	3.7	3.3	3.2	5.1	2.1	1.3	2.1	2.0
		21				25				30				35				40			
a		Arg-Lys-Ile-Pro-Leu-Gly-Lys-Asp-Gln-Cys-Ala-Tyr-Cys-Lys-Glu-Lys-Gly-His-Trp-Val																			
	b	2.2	4.1	1.6	1.9	1.0	1.3	1.1	1.1	1.0	0.7	1.5	0.7	1.3	0.5	0.2	0.2	0.5			
						25 10 16 9.8 8.9				8.6 11 15 4.2 4.4											

FIG. 3. NH₂-terminal amino acid sequence of BLV p12 and FeLV p10. Numbers below residues are yields in nanomoles of PTH amino acid derivatives as obtained by degrading the unmodified proteins (rows a) and fragments (rows b). In the initial analysis of BLV p12 the assignments for residues 21 to 30 were based on qualitative data due to instrument malfunction which did not allow accurate quantitation. Inputs for Edman degradation were as follows: BLV p12—intact protein, 4 nmol; fragment 18 to 29, 21 nmol; fragment 30 to 40, 24 nmol; FeLV p10—intact protein, 12 nmol; fragment 23 to 27, 33 nmol; fragment 28 to 34, 18 nmol.

degradation and also allowed assignments to be made at positions 28 and 39 as shown.

Figure 3B shows the sequence for FeLV p10. The first 40 amino acid residues, except cysteine residues 30 and 33 and lysine 27, were determined by a single microsequencing analysis of 12 nmol of protein. Assignments of the cysteines and lysine residue 27 were made as previously discussed for BLV p12, using Lys-C cleavage

fragments (T. D. Copeland, M. A. Morgan, and S. Oroszlan, manuscript in preparation). BLV p12 and FeLV p10 NH₂-terminal sequences are compared for homology with each other and the amino acid sequence of the NBP from R-MuLV (12) in Fig. 4.

Immunological characterization of BLV p12 and FeLV p10. Antisera specific for the NBPs were developed in rabbits. The immunization

	5	10	15	20	25	30	35	40																																					
FeLV p10	<u>A</u>	<u>T</u>	<u>V</u>	<u>V</u>	<u>A</u>	<u>Q</u>	<u>N</u>	<u>R</u>	<u>D</u>	<u>K</u>	<u>D</u>	<u>R</u>	<u>E</u>	<u>E</u>	<u>K</u>	<u>K</u>	<u>L</u>	<u>G</u>	<u>D</u>	<u>Q</u>	<u>R</u>	<u>K</u>	<u>I</u>	<u>P</u>	<u>L</u>	<u>G</u>	<u>K</u>	<u>D</u>	<u>Q</u>	<u>C</u>	<u>A</u>	<u>Y</u>	<u>C</u>	<u>K</u>	<u>E</u>	<u>K</u>	<u>G</u>	<u>H</u>	<u>W</u>	<u>V</u>	<u>R</u>	<u>D</u>	<u>C</u>	<u>P</u>	<u>K</u>
BLV p12	<u>V</u>	<u>H</u>	<u>T</u>	<u>P</u>	<u>G</u>	<u>P</u>	<u>K</u>	<u>M</u>	<u>P</u>	<u>G</u>	<u>P</u>	<u>R</u>	<u>Q</u>	<u>P</u>	<u>A</u>	<u>P</u>	<u>*</u>	<u>*</u>	<u>*</u>	<u>K</u>	<u>R</u>	<u>P</u>	<u>P</u>	<u>P</u>	<u>G</u>	<u>P</u>	<u>*</u>	<u>*</u>	<u>*</u>	<u>C</u>	<u>Y</u>	<u>R</u>	<u>C</u>	<u>L</u>	<u>K</u>	<u>E</u>	<u>G</u>	<u>H</u>	<u>W</u>	<u>A</u>	<u>R</u>	<u>D</u>	<u>C</u>	<u>P</u>	<u>T</u>
R-MuLV p10	<u>A</u>	<u>T</u>	<u>V</u>	<u>V</u>	<u>S</u>	<u>G</u>	<u>Q</u>	<u>R</u>	<u>Q</u>	<u>*</u>	<u>D</u>	<u>R</u>	<u>Q</u>	<u>G</u>	<u>G</u>	<u>E</u>	<u>*</u>	<u>*</u>	<u>*</u>	<u>R</u>	<u>R</u>	<u>R</u>	<u>P</u>	<u>Q</u>	<u>L</u>	<u>D</u>	<u>R</u>	<u>D</u>	<u>Q</u>	<u>C</u>	<u>A</u>	<u>Y</u>	<u>C</u>	<u>K</u>	<u>E</u>	<u>K</u>	<u>G</u>	<u>H</u>	<u>W</u>	<u>A</u>	<u>K</u>	<u>D</u>	<u>C</u>	<u>P</u>	<u>K</u>

FIG. 4. NH₂-terminal sequences of BLV p12, FeLV p10, and R-MuLV p10 are compared in single-letter amino acid code: A, alanine; T, threonine; V, valine; Q, glutamine; N, asparagine; R, arginine; D, aspartic acid; K, lysine; E, glutamic acid; L, leucine; G, glycine; I, isoleucine; P, proline; Y, tyrosine; H, histidine; W, tryptophan; M, methionine; S, serine; C, cysteine. Any residue identical with a residue from either of the other proteins is underlined. Gaps are indicated by an asterisk. The sequence of FeLV p10 beyond residue 40 is taken from other studies (Copeland et al., unpublished data). The sequence of R-MuLV p10 is taken from Henderson et al. (12).

FIG. 5. Immunization schedules and titers of anti-BLV p12 rabbit serum and anti-FeLV p10 rabbit serum. Symbols: $\circ$, test bleed; $\downarrow$, injection.

schedules with the resulting antibody titers for BLV p12 and FeLV p10 are shown in Fig. 5. Arrows indicate days when injections (a total of nine for each rabbit) were made, and circles indicate the titers of test bleed and bleed-out sera. Each serum was titrated by immunoautoradiography, using the DOT test. The highest titers obtained for anti-BLV p12 serum, and FeLV p10 antiserum were 16,200 and 5,800. A total of 550 μ g of each purified protein was used for each rabbit. Immunization with BLV p12 was completed in 130 days and that with FeLV p10 was completed in 182 days. In spite of the relatively large doses of antigens and long period of immunization, the resulting antisera were highly spe-

cific. This reflects on the high state of antigenic purity of the RPLC-purified proteins.

Figure 6 shows an actual titration pattern of anti-BLV p12 and anti-FeLV p10 rabbit sera as obtained by the DOT test. All test bleeds and bleed-out sera were titrated by this procedure, but only results of two bleeds (one early and one later) from each rabbit immunized with the NBPs are shown. Test bleed 1 of anti-BLV p12 rabbit serum titrated at 1,800 with an increase to 16,200 at test bleed 5, which did not react when tested against BLV p24. Test bleed 2 of anti-FeLV p10 rabbit serum titrated at 1,800 with an increase to 5,400 in test bleed 7, which was negative when spotting FeLV p27. Prebleeds

FIG. 6. Titration of anti-BLV p12 rabbit serum and anti-FeLV p10 rabbit serum by NBP spotting of DBM paper. TB, Test bleed.

taken before immunization of either rabbit were negative.

Specificity of antisera to BLV p12 and FeLV p10 was further studied by immunautoradiography involving the electrotransfer of SDS-PAGE-separated viral proteins to DBM paper (Fig. 7). In lanes 1 to 3 FeLV proteins were transferred and stained with appropriate antisera. Lane 1 shows the reaction with anti-FeLV p27 rabbit serum. Lane 2 was stained with anti-FeLV p10 rabbit serum which reacted with p10 only. The same protein was also recognized by antiserum to BLV p12 as shown in lane 3. BLV proteins were transferred in lanes 4 to 6 and stained with appropriate antisera. Lane 4 shows the reaction of BLV with anti-BLV p24 guinea pig serum. Lane 5 was stained with anti-BLV p12 rabbit serum. Rabbit antiserum to FeLV p10 also cross-reacted with BLV p12. Both antisera to BLV and FeLV NBPs reacted specifically with BLV, recognizing p12 only. Various other retroviruses were tested with anti-BLV p12 and anti-FeLV p10 rabbit sera after virus proteins were transferred to DBM paper. Table 2 summarizes these results. As expected, anti-FeLV p10 serum reacted in an interspecies fashion with other mammalian type C virus NBPs but not with Rous avian sarcoma virus p12. The anti-BLV p12 serum, however, did not recognize any type C virus tested except FeLV. Homologous and heterologous titers (numbers in parentheses) for both sera are also given in Table 2.

DISCUSSION

Chloroform-methanol extraction and RPLC in combination are effective methods in which to purify to a high state of purity the NBPs of BLV

and FeLV. The purity of BLV p12 and FeLV p10 was confirmed by SDS-PAGE. In spite of the apparent homogeneity in this system, RPLC patterns indicated multiple protein peaks eluting within a narrow range of an acetonitrile gradient. This heterogeneity of chloroform-methanol-extracted BLV p12 was shown to be due to disulfide cross-links between cysteine residues. The reduced and carboxamidomethylated protein eluted as a single peak. It is assumed that similar intra- or intermolecular cysteine interactions may be responsible for the chromatographic heterogeneity of FeLV p10. It is clear from the partial sequence data that both proteins have more than one cysteine.

The amino acid composition data indicate that both NBPs contain an abundance of basic amino acids. No amino acid appeared with higher residue number than lysine plus arginine in either protein except the 15 residues of proline found in BLV p12. This unusually high figure for proline in BLV p12 is not shared in the compositions of FeLV p10 and of other known NBPs of type C viruses. Recently, the primary structure of the NBP (p10) of R-MuLV was reported (12). R-MuLV p10 is a basic protein containing 5 lysine, 9 arginine, and 2 histidine residues, a total of 16 basic amino acid residues of 56 in the complete protein. Gel filtration and electrophoretic analysis had originally estimated the molecular weight to be between 7,000 and 10,000. The exact molecular weight is reported as 6,347. The molecular weights calculated from composition (without cysteine and tryptophan) for BLV p12 (6,034) and for FeLV p10 (5,652) are also smaller than molecular weights determined by gel electrophoresis. Results indicate that BLV p12 and FeLV p10 are similar in size and composition,

FIG. 7. Specificity of anti-BLV p12 and anti-FeLV p10 rabbit sera by immunodot radiography. FeLV was transferred to DBM paper (lanes 1 to 3) and stained with anti-FeLV p27 rabbit serum at a 1:6,000 dilution (lane 1), anti-FeLV p10 rabbit serum at a 1:6,000 dilution (lane 2), and anti-BLV p12 rabbit serum at a 1:100 dilution (lane 3). BLV was transferred to DBM paper (lanes 4 to 6) and stained with anti-BLV p24 guinea pig serum at a 1:2,000 dilution (lane 4), anti-BLV p12 rabbit serum at a 1:10,000 dilution (lane 5), and anti-FeLV p10 rabbit serum at a 1:100 dilution (lane 6). Arrows indicate position of protein after SDS-PAGE.

except for proline in BLV p12 (see above), to the structurally defined MuLV viral NBP.

The alignments of FeLV p10, BLV p12, and R-MuLV p10 are shown in Fig. 4. BLV p12 and FeLV p10 NH₂-terminal sequences do not contain much homology in the first 40 residues of the proteins. The most homology between the proteins centers around a common region also contained in R-MuLV p10. This conserved region has been expressed as a set of three Cys residues spaced at $N + 3$, and $N + 13$ and a Gly-His sequence at $N + 7$ and $N + 8$, where N is the position in the amino acid sequence of the first Cys residue of the set (cysteine in position 30 in the alignment). Involvement of tyrosine and lysine residues in nucleic acid binding activity of proteins has been indicated with studies on the chemical modification of R-MuLV p10 (L. E. Henderson, C. W. Long, and S. Oroszlan, Fed. Proc. 39:1606, 1980) and the DNA-binding protein coded for by gene 5 of bacteriophage fd (2). Tyrosine has also been implicated in the ability of gene 32 protein from bacteriophage T4 to bind to single-stranded DNA (1). The conserved region of FeLV p10 contains both tyrosine and lysine residues and a Gly-His sequence at the appropriate positions. The cys-

teines and the Gly-His sequence of BLV p12 in positions 31 and 32 align with that in FeLV p10 and R-MuLV p10. The homologous region of BLV p12 also contains tyrosine and lysine. Due to their sequence homology with a well-identified NBP in a region considered to be important for nucleic acid binding, BLV p12 and FeLV p10 are determined to be the NBPs from the respective viruses.

The results from immunological studies indicate an authentic cross-reaction between BLV p12 and FeLV p10. Therefore, BLV p12 and FeLV p10 must share a common antigenic site within their structure. Antisera to BLV p12 and FeLV p10 were tested in immunodot radiography with other retroviruses. Anti-FeLV p10 serum reacted with the NBPs of all mammalian but not the avian type C viruses tested (Table 2). Anti-BLV p12 serum does not react and thus does not share antigenic determinants with any of the other viruses tested except FeLV. These results also show that antigenic determinants shared between feline p10 and murine p10 are not present in BLV p12. The comparison of NH₂-terminal sequences (first 40 residues) of these proteins (see Fig. 4) does not demonstrate a homologous region between FeLV p10 and BLV p12 not shared by R-MuLV p10, which could readily account for the FeLV/BLV-specific antigenic cross-reaction (see above).

The amino acid sequence of BLV p12 and FeLV p10 shows a highly homologous region beginning with Gly-His, positions 37 and 38 in the alignment of Fig. 6. Within this eight-residue-long homologous region there is only a

TABLE 2. Reaction of anti-FeLV p10 and anti-BLV p12 rabbit sera with the NBPs of various retroviruses after transfer of separated viral proteins from SDS-PAGE to DBM paper

Virus ^a	Reaction with antiserum to:	
	FeLV p10 ^b	BLV p12 ^c
BLV	+ (400) ^d	+ (16,200)
FeLV	+ (5,400)	+ (1,600)
M-MuLV	+ (1,600)	—
RD-114	+	—
R-ASV	—	—

^a Besides BLV and FeLV, the following viruses were tested: Moloney-MuLV, cat endogenous type C virus (RD-114), and Rous avian sarcoma virus (R-ASV).

^b Antiserum was used at 1:6,000 dilution in the homologous system and 1:100 in the heterologous systems.

^c Antiserum dilutions were 1:10,000 in the homologous system and 1:100 in the heterologous systems.

^d The numbers in parentheses represent the titers of bleed-out sera as determined by the DOT test.

single amino acid difference between FeLV and BLV. Alanine of BLV p12 is substituted for valine in FeLV p10. In this position R-MuLV p10 has alanine, like BLV p12. C-terminal to this residue, both bovine p12 and feline p10 have arginine whereas the murine p10 has lysine. Since the complete amino acid sequence of BLV p12 (Copeland et al., manuscript in preparation) and the nearly complete sequence of FeLV p10 (T. D. Copeland et al., unpublished data) do not show a higher degree of homology in the other parts of the molecules, the above eight-residue segment is the most likely candidate to represent the shared antigenic site responsible for BLV p12/FeLV p10 cross-reaction readily detectable on denatured proteins. The Ala-Val exchange may not be considered significant enough to alter the immunogenic response and subsequent antigen-antibody complexing. Although arginine and lysine are both basic amino acids, they are sterically different. This difference could explain why anti-BLV p12 serum reacts with FeLV p10 but not with the murine p10. Chemical modification of arginine and lysine residues and the use of synthetic peptides will aid the accurate definition of the immunogenic determinant shared by BLV and FeLV.

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