Interstrain Variation of the Major Internal Structural Component (p30^{gag}) of Two Murine Oncornaviruses: Comparative Immunochemical, Biochemical, and Biophysical Analysis

W. NEAL BURNETTE† AND WILLIAM M. MITCHELL*

*Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461, and Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232**

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The major internal structural protein (p30^{gag}) of the Moloney leukemia virus and the endogenous Y-1 murine oncornavirus was examined for biochemical and biophysical manifestations of interstrain antigenic variation. Although the two viral proteins share murine group-specific antigenic determinants, the Y-1 virus p30 appeared to have both a lower relative number of such determinants and a decreased affinity at the cross-reactive sites for Moloney virus p30 monospecific antibodies. Further, immunological analysis indicated the presence of unique antigenic sites on the Moloney virus p30 not shared by the analogous Y-1 virus molecule. The two polypeptides copurified and had similar isoelectric points (pH 6.2 to 6.3) and sedimentation coefficients (2.47S). However, equilibrium sedimentation yielded a significant mass difference between the two proteins, 28,300 ± 600 and 31,000 ± 300 daltons for the Moloney and Y-1 virus molecules, respectively. Amino acid analysis indicated a concomitant increase in total residues for the Y-1 virus p30, although a number of residues appeared to have been conserved between the two viral proteins. Conformational studies and hydrodynamic calculations demonstrated marked secondary and tertiary structural differences; with the Y-1 virus p30 being an asymmetric prolate ellipsoid containing 27 to 28% α -helix and Moloney virus p30 being somewhat more spherical and possessing an α -helical content of 50 to 55%. Two-dimensional mapping of ¹²⁵I-labeled tryptic peptides of each p30 suggested that considerable sequence heterogeneity is responsible for many of the biophysical, biochemical, and immunochemical differences in these two analogous structural proteins.

The provirus genome of mammalian oncornaviruses code for a number of structural polypeptides (2) whose primary and secondary structures immunologically define the interspecies (13, 14, 41), species-specific (15, 18, 28, 30-33, 36, 42), and type-specific (17, 35, 39, 42, 44) antigenic reactivities of various virus strains in general, and analogous proteins shared among strains in particular. The major internal structural protein of the murine oncornaviruses is the approximately 30,000-dalton polypeptide, p30^{gag} (2, 8, 9, 18, 26, 30, 36). The p30^{gag} and the polypeptides p15^{gag}, p12^{gag}, and p10^{gag} are cleavage products of a 65,000-dalton polypeptide precursor (1, 38, 39), Pr65^{gag}, which is itself the product of the *gag* (group-specific antigen) gene constituent (4, 5) of the integrated provirus genome (45). The p30 has been termed the group-specific antigen (15) because it possessed murine-specific cross-

reactive determinants in the greatest relative concentration. It does, however, also possess demonstrable type-specific reactivity, and p30 molecules from various strains of murine virus exhibit unique antigenic determinants (42). Unlike the hemagglutinating and interference properties of gp69/71^{env} (6, 20), the RNA-dependent DNA polymerase activity of the virion reverse transcriptase enzyme (3, 46), and the nucleic acid-binding ability of p12 (37) and p10 (12; M. Schülein, W. N. Burnette, and J. T. August, *J. Virol.*, in press), the p30 has as yet no definitive function, although its involvement in the process of host range restriction has been suggested (19), and, because of its proclivity for self-association, it may play a role in viral core assembly at the cell membrane (8).

We have previously described a number of biophysical and biochemical properties of p30 from the laboratory strain of Moloney murine leukemia virus (8). In this report we compare

† Present address: Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

many of those properties with the p30 of the Y-1 N-ecotropic endogenous murine oncornavirus (9) to elucidate the primary and secondary structural characteristics responsible for the antigenic variation exhibited by this evolutionarily conserved polypeptide.

MATERIALS AND METHODS

Cells and viruses. Moloney leukemia virus was propagated in high-passage-level Swiss mouse embryo cells maintained in Eagle minimal essential medium with 10% fetal calf serum. Murine adrenal carcinoma cells (clone Y-1, ATCC no. CCL-79) producing the N-tropic Y-1 virus were grown in Ham F-10 medium containing 15% fetal calf serum and 2.5% horse serum. Virus was harvested and purified as previously described (9).

Antisera. Heterospecific goat anti-Moloney leukemia virus (1S-166) and goat anti-AKR virus (2S-296) sera were obtained through the courtesy of R. Wilsnack, Huntingdon Laboratories, Md. Monospecific antisera against p30 were obtained by hyperimmunization of rabbits with the purified protein (see below) in complete Freund adjuvant followed by two fortnightly boosters in incomplete Freund adjuvant. Goat anti-rabbit immunoglobulin G was purchased from Calbiochem (La Jolla, Calif.).

p30 Purification. p30 was purified under non-denaturing conditions from core preparations of virus by phosphocellulose ion exchange and Sephadex G-100 (Pharmacia) gel filtration chromatography as described by Strand and August (43). Column effluents were monitored for relative fluorescence at 287 nm/348 nm.

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in cylindrical 10% polyacrylamide gels as previously described (8).

Radioiodination. Purified p30 was radioiodinated for radioimmunoassay by the chloramine T method of Hunter (21) and chromatographed on Sephadex G-25 superfine to remove unbound radioiodine.

Immunodiffusion and radioimmunoassay. Immunodiffusion was performed in 1% Difco agar containing 100 mM NaCl, 1 mM EDTA, and 10 mM Tris-hydrochloride (pH 7.2) for 24 to 72 h at room temperature and then for 24 h at 4°C. The gels were washed extensively in phosphate-buffered saline and stained with Coomassie brilliant blue R-250. Competition radioimmunoassay was performed as described by Strand and August (42); unlabeled p30 competed against radioiodinated p30 for antibodies in a monospecific antiserum dilution sufficient to bind approximately 50% of the trichloroacetic acid-precipitable input radioiodinated protein. Precipitation was effected by the addition of goat anti-rabbit immunoglobulin G, and the precipitate was washed extensively to remove nonspecific radioactivity. Radioactivity in the precipitate was determined by gamma counting.

Peptide analysis. Y-1 and Moloney leukemia virus p30 bands, each containing approximately 5 µg of protein, were cut from dried Coomassie brilliant blue-stained sodium dodecyl sulfate-polyacrylamide gels;

the slices were minced; and the proteins were eluted overnight at 37°C in a buffer containing 50 mM NH_4HCO_3 (pH 8.3), 0.05% sodium dodecyl sulfate, and 1 mM phenylmethylsulfonyl fluoride. Protein recovery was routinely 80 to 90% by this procedure. The eluants were centrifuged briefly to remove gel debris, KCl was added to the supernatants to a final concentration of 0.2 M, and, after 15 min on ice, the precipitates were collected by centrifugation (7). The precipitates were washed once with ice-cold acetone containing 0.1 N HCl, washed again with acetone alone, and dried. They were suspended in 20 µl of 0.5 M sodium phosphate (pH 7.5) containing 0.1% sodium dodecyl sulfate, heated for 2 min at 100°C, and allowed to cool. The protein preparations were then radioiodinated by the chloramine T method (21), and free radioiodine was removed from the quenched reaction mixtures by passage over columns (0.8 by 14.5 cm) of Sephadex G-25 superfine equilibrated in a buffer of 1.0 M Tris-hydrochloride (pH 8.6) with 0.1% sodium dodecyl sulfate. Reduction and S-aminoethylation were performed by modifications of previously published procedures (10, 11). Briefly, the radioiodinated proteins from the G-25-excluded volumes (1 ml each) were reduced by adding dithiothreitol to a final concentration of 0.1 M, flushing the reaction vessels with N_2 , capping them, and incubating them for 3 h at 37°C. The reduced protein preparations were then S-aminoethylated by the sequential addition of one 10-µl portion and two 5-µl portions of ethyleneimine (ICN Pharmaceuticals, Inc.) at 10-min intervals under a N_2 atmosphere. A 50-µg quantity of carrier bovine immunoglobulin was added to each preparation (16), and the mixtures were precipitated at 4°C with 10% trichloroacetic acid. The precipitates were collected by centrifugation and washed twice with ice-cold acetone. The dried precipitates were resuspended in 50 µl of 50 mM NH_4HCO_3 (pH 8.3), 1 µg of tosylsulfonyl phenylalanyl chloromethyl ketone-treated trypsin (Worthington Biochemicals) was added to each suspension, and the preparations were incubated for 3 to 4 h at 37°C. An additional 1 µg of trypsin was added to each preparation, and incubation was continued overnight. Digestion was routinely found to be more than 90% complete as measured by inclusion on Sephadex G-25. After brief centrifugation to remove insoluble material, the samples were spotted at one corner of Polygram Cel 300 plastic-backed cellulose sheets (Brinkman Instruments). The sheets were wetted with a pH 3.5 buffer of pyridine-acetic acid-water (1:10:89) and electrophoresed under Varsol (Savant Instruments, Inc.) for approximately 30 min at 1,000 V with acid fuchsin as a migration marker. The sheets were dried and chromatographed at ascending right angles to the direction of electrophoresis in an atmosphere-equilibrated tank containing a pH 5.3 buffer of *n*-butanol-acetic acid-water-pyridine (15:3:12:12). The sheets were again dried, and autoradiography was performed by exposure to Kodak no-screen medical X-ray film.

Other biochemical and biophysical analyses. Extinction coefficient determinations, liquid column and polyacrylamide gel isoelectric focusing, sedimentation equilibrium and velocity analysis, amino acid analysis, and far-UV circular dichroism were performed as previously described (8).

RESULTS

Purity of the protein preparations. Fractionation of the solubilized core proteins from the Moloney and Y-1 viruses on phosphocellulose with a continuous gradient of 0 to 1.0 M KCl revealed a major peak for both viruses at about 0.2 M (Fig. 1). When these peaks were subsequently rechromatographed by gel filtration, a single homogeneous peak was observed for each preparation in the 30,000-molecular-weight region of the column effluent (Fig. 2). Electrophoresis of these peak fractions in the presence of sodium dodecyl sulfate demonstrated a single band for each p30 preparation at about 30,000 daltons (Fig. 3), although it is apparent that the Moloney virus p30 migrates slightly faster, and thus has a lower molecular mass, than the corresponding Y-1 virus protein. Isoelectric focusing in a sucrose-ampholyte column (Fig. 4) yielded an isoelectric point of approximately pH 6.3 for both viral proteins. The minor peak seen as a shoulder at about pH 7 may be analogous to the heterogeneously charged species of p30 observed by Oroszlan et al. (33) in complement fixation assays of isoelectric-focusing column effluents. High-resolution

FIG. 1. Ion-exchange chromatographic fractionation of the proteins of Moloney and Y-1 murine oncornaviruses. Moloney leukemia virus and Y-1 virus were disrupted in Triton X-100, the soluble proteins were applied to parallel columns of Whatman P-11 phosphocellulose, and p30 was eluted with a linear gradient of 0 to 1.0 M KCl in 10 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 6.5), and 1 mM EDTA. Moloney leukemia virus proteins (Δ); Y-1 virus proteins ($\circ$).

FIG. 2. Purification of the major structural protein of the Moloney and Y-1 murine oncornaviruses by molecular sieve chromatography. The peak fractions eluting from phosphocellulose at approximately 0.2 M KCl were concentrated by precipitation with $(\text{NH}_4)_2\text{SO}_4$ at 75% saturation and chromatographed in parallel columns of Sephadex G-100 in a buffer of 1.0 M NaCl, 1 mM EDTA, and 10 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 6.5). Moloney leukemia virus protein (Δ); Y-1 virus protein ($\bullet$).

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the major structural protein of the Moloney and Y-1 murine oncornaviruses. Electrophoresis was performed in 10% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate and 3 M urea as described in the text. (1) and (6), molecular weight markers; (2) whole disrupted Moloney leukemia virus; (3) purified Moloney virus p30; (4) purified Y-1 virus p30; (5) whole disrupted Y-1 virus. Protein bands were stained with Coomassie brilliant blue R-250.

FIG. 4. Column isoelectric focusing of the purified major structural protein of the Moloney and Y-1 murine oncornaviruses. The viral proteins were focused in a 1.2% ampholyte pH gradient of 3.5 to 10 at 4°C. The pH gradient was stabilized with a 0 to 40% sucrose gradient to prevent convection, and focusing was at 500 V for the time indicated. Moloney leukemia virus protein, 68 h (○); Y-1 virus protein, 94 h (●); pH (▲).

focusing in a polyacrylamide-ampholyte matrix (not shown) demonstrated a difference of about 0.1 pH unit between the isoelectric points of the two proteins, with the Y-1 virus polypeptide being the slightly more basic component. No evidence of minor species in these gels was observed. Thus, the minor charge heterogeneity obtained with column isoelectric focusing may be secondary to self-association (8) at the higher concentrations required for analysis.

Immunological analysis. In immunodiffusion (Fig. 5), the Y-1 and the Moloney virus p30 polypeptides extensively cross-reacted with both anti-Moloney virus and anti-AKR virus hetero-specific sera. The presence of a small spur in the precipitin band pointing toward the Y-1 p30 well with the anti-Moloney virus serum and the apparent absence of spurs in the cross-reaction with the antiserum directed against AKR virus, a virus very similar to Y-1 (9), indicated the presence of determinants on the Moloney virus p30 not shared by the Y-1 virus protein. In competition radioimmunoassay with monospecific anti-Moloney virus p30 serum, the differences between the two proteins became more obvious (Fig. 6). Although Y-1 p30 could compete with Moloney p30, indicative of the group-specific and type-specific cross-reactivity observed in immunodiffusion, the displacement of the Y-1 competition curve, the decreased slope relative to the homologous Moloney virus protein competition, and the failure of the Y-1 polypeptide to compete fully for all the antibody species in the anti-Moloney p30 serum popula-

FIG. 5. Immunodiffusion analysis of the murine-specific cross-reactivity of the Moloney and Y-1 virus p30 proteins. Immunodiffusion was performed as described in the text with the purified viral components. IS-166, goat anti-Moloney virus serum; 2S-296, goat anti-AKR virus serum. Gels were stained with Coomassie brilliant blue R-250. (A) Immunodiffusion plate; (B) illustration of immunodiffusion plate to highlight spur.

FIG. 6. Analysis of the antigenic determinants of the p30 of Moloney and Y-1 murine oncornaviruses. Competition radioimmunoassay was performed with a final dilution of monospecific anti-Moloney virus p30 serum sufficient to precipitate approximately 50% of the radioiodinated Moloney p30 in the assay. The amount of competing protein is as indicated. Moloney virus p30 (▲); Y-1 virus p30 (●).

tion clearly demonstrated unique determinants present on the Moloney virus p30 that were not exposed on the analogous Y-1 protein.

Amino acid composition. The amino acid compositions of the Moloney virus and the Y-1 virus p30 polypeptides are presented in Table 1. The best integral fit of the recovery data was obtained with three residues of histidine for the Y-1 and four residues for the Moloney virus p30. Because of its greater apparent mass, the Y-1 virus polypeptide was found to contain more residues than the Moloney protein. However, the total number of lysine and alanine residues appeared to have been conserved, and histidine and methionine were actually in excess in Moloney virus p30. Two to three half-cystine residues were assigned to both polypeptides since in each case the calculated value was found to be nonintegral.

Sedimentation analysis. Although p30 exhibits a marked proclivity for concentration-dependent self-association (8), sedimentation velocity and molecular weight analysis can be per-

formed in the analytical ultracentrifuge, with initial loading concentrations and ionic strength favoring the monomer. Under these conditions the $s_{20,w}^0$ for both the Y-1 and Moloney virus p30 proteins was found to be 2.46 to 2.47S (data not shown). However, high-speed, meniscus-depletion equilibrium sedimentation revealed a significant difference in the mass of the two proteins (Fig. 7), confirming the initial observation in sodium dodecyl sulfate-polyacrylamide gels. Using partial specific volumes of 0.719 ml/g for Moloney virus p30 and 0.722 ml/g for Y-1 p30 (determined from amino acid analysis) for the calculation of weight average molecular weights and extrapolating to zero concentration, we obtained molecular weights of $28,300 \pm 600$ for the Moloney virus protein and $31,000 \pm 300$ for the Y-1 p30.

On the basis of the mass and velocity parameters, a frictional ratio relative to a perfectly spherical ellipsoid could be calculated. A ratio of 1.38 for Moloney virus p30 and 1.45 for Y-1 p30 corresponded to axial ratios of about 5 and 6,

TABLE 1. *Amino acid composition of the major internal structural protein of the Y-1 and Moloney oncornavirus*

Amino acid	Apparent residues per mol of protein ^a				Observed (Y-1) minus Moloney
	Y-1 virus protein		Moloney virus protein ^b		
	Avg or extrapo- lated value	Assumed compo- sition	Avg or extrapo- lated value	Assumed compo- sition	
Lysine	16.7	17	17.3	17	0
Histidine	3.0	3	4.0	4	−1
Arginine	31.2	31	28.6	29	+2
Tryptophan ^c	3.8	4	3.0	3	+1
Aspartic Acid	29.1	29	26.9	27	+2
Threonine	18.0	18	14.5	15	+3
Serine	17.0	17	15.8	16	+1
Glutamic Acid	51.2	51	44.5	45	+6
Proline	20.1	20	16.3	16	+4
Glycine	18.3	18	16.0	16	+2
Alanine	14.4	14	13.6	14	0
Half-cystine ^d	2.6	3 (2)	2.3	2 (3)	(0−1)
Valine	9.2	9	7.5	8	+1
Methionine ^d	2.0	2	3.2	3	−1
Isoleucine	6.6	7	4.8	5	+2
Leucine	35.0	35	27.8	28	+7
Tyrosine	6.3	6	5.3	5	+1
Phenylalanine	5.6	6	5.9	6	0
Calculated mol wt		33,400		30,000	

^a Calculated with histidine as the integral residue. Analyses were performed in triplicate on samples dialyzed extensively against distilled water, lyophilized, and hydrolyzed at 110°C for 20, 40, and 72 h, respectively, in 6 N HCl containing 0.01% (vol/vol) phenol to preserve tyrosine residues. The recovery values of threonine and serine were obtained by extrapolation of the recoveries from the various hydrolysis times to zero time. Valine, leucine, and isoleucine values were taken as their maximum recoveries from the timed HCl hydrolyses. The remaining residue recovery values were obtained from an average of the timed HCl hydrolyses.

^b Data for Moloney virus p30 taken from Burnette et al. (8).

^c Tryptophan analyzed after hydrolysis at 115°C for 22 h in 4 N methanesulfonic acid.

^d Half-cystine determined as cysteic acid and methionine as the sulfone by performic acid oxidation followed by 20 h of HCl hydrolysis.

respectively, assuming a hydration value of 0.3 g of water per g of protein. These ratios indicate that, although both are apparently asymmetric ellipsoids, the Y-1 p30 is a more elongated globular molecule.

Conformational analysis. Figure 8 illustrates the major conformational differences between the analogous Moloney and Y-1 virus p30 proteins as determined by far-UV circular dichroism. We have utilized two independent methods for estimating the type and amount of

ordered secondary structure as reported previously for the Moloney virus p30 alone (8), namely, a resolution of the patterns into a minimum number of gaussian curves to achieve a best fit of the data and a least-squares fit of ellipticity to a linear combination of the possible structural contributions. A tabulation of the rotational strengths of the resolved gaussian bands by the former method is presented in Table 2, although both procedures gave closely comparable results. While both polypeptides have

FIG. 7. Plots of $1/\text{molecular weight}$ versus initial loading concentration in analytical ultracentrifuge cell for the major internal structural protein of the Moloney and Y-1 murine oncornaviruses. High-speed sedimentation equilibrium was performed as previously described (8), and the molecular weights at various initial protein concentrations were extrapolated to zero concentration by the method of least squares. (A) Moloney leukemia virus protein; data taken from Burnette et al. (8). (B) Y-1 virus protein.

FIG. 8. Far-UV circular dichroic spectra of the major internal structural protein of the Moloney and Y-1 murine oncornaviruses. Circular dichroism was performed in 0.5-mm path length fused-silica cells from 250 nm to 200 nm in 0.2 M borate (pH 8.0) and 0.15 M potassium fluoride. (A) Moloney virus protein, 360 $\mu\text{g}/\text{ml}$. (B) Y-1 virus protein, 350 $\mu\text{g}/\text{ml}$. $[\Theta]$, Reduced mean residue ellipticity.

TABLE 2. Mean residue rotational strengths of the far-UV circular dichroic bands of the major internal structural protein of Moloney leukemia virus and Y-1 virus

Virus protein and λ_0^a	$[\theta]_{\text{max}}^b$	R^c	Transition	Conformation	% Conformation ^d
Y-1					
223 nm	-10,500	-5.81	n- π^*	α -helix	28
215 nm	-3,500	-0.83	n- π^*	β -structure	11
Moloney leukemia					
224 nm	-17,000	-10.88	n- π^*	α -helix	52
216 nm	-3,000	-0.80	n- π^*	β -structure	10
192 nm	+41,000	+17.25	π - π^*	α -helix	50

^a λ_0 , Wavelength of circular dichroic transition.

^b $[\theta]_{\text{max}}$, Maximum mean residue ellipticity.

^c R , Rotational strength in cgs units, $\times 10^{-40}$, of each resolved gaussian band determined as previously described (8).

^d Based on a comparison of the resolved gaussian band rotational strengths calculated as previously described (8).

about 10 to 14% β -structure, Moloney p30 exhibits an exceptional 50 to 55% α -helicity (8), whereas the Y-1 p30 is predicted to possess a significantly lower 27 to 28% α -helix. The remainder of the structure is presumably apolar.

Tryptic peptide analysis. Two-dimensional peptide mapping (Fig. 9) demonstrates that the Y-1 and Moloney virus p30 proteins share two major radioiodinated tryptic peptides. However, the Y-1 virus p30 appears to possess five unique major iodinated peptides, and the Moloney leukemia virus p30, three. In view of this observed sequence heterogeneity, it is not surprising that the two proteins exhibit significant type-specific antigenic variation and secondary structural disparity.

Assuming that each radioiodinated tyrosine residue is located on a separate peptide, the number of labeled major peptides corresponds rather well to the amino acid analyses. The presence of minor peptides that amount to less than 10% of the total radioactivity may be the result of a number of secondary reactive processes, such as diiodination of tyrosine and formation of moniodohistidine (22), incomplete S-aminoethylation of cysteinyl residues (23, 24) and other chemical modifications (27), arginyl and lysyl residues that are conformationally

hindered, and proline-adjacent arginyl and lysyl bonds that are not hydrolyzed by trypsin.

DISCUSSION

The principal physical and chemical characteristics of Moloney leukemia virus p30 and the endogenous Y-1 virus p30 are summarized in Table 3. For analogous proteins from two strains of murine oncornavirus possessing group-specific cross-reactivity, they display a surprising dissimilarity. Although they share common isoelectric points and sedimentation coefficients, distinct differences exist in molecular weight, amino acid composition, tryptic peptides, and conformation. The conformational disparity observed by circular dichroic analysis is, in conjunction with the peptide maps, the most significant interstrain variation detected as an explanation of the observed antigenic heterogeneity. The helical differences are most likely due to

TABLE 3. *Summary of the physical and chemical properties of the major internal structural protein of Moloney leukemia virus and Y-1 virus*

Property	Major structural protein	
	Moloney leukemia virus ^a	Y-1 virus
Extinction coefficient ($E_{1\text{cm},280}^{1\%}$)	13.7 ^b	9.5 ^c
Absorption maximum (nm)	280-281	279
Isoelectric point ^d	6.2	6.3
Molecular weight ^e	28,300 $\pm$ 600	31,000 $\pm$ 300
Partial specific volume (ml/g) ^f	0.719	0.722
Sedimentation coefficient ($s_{20,w}^g$)	2.47	2.47
Diffusion coefficient ($D_{20,w}^h$) (cm ² /s)	7.33×10^{-7}	6.69×10^{-7}
Frictional ratio (f/f_0)	1.38	1.45
Amino acid residues ⁱ	259	290
α -Helicity (%)	50	27
β -Structure (%)	10-13	11-14
Major radioiodinated tryptic peptides (shared/unique)	2/3	2/5

^a Data on Moloney virus p30 taken from Burnette et al. (8).

^b Determined both by dry weight measurement and synthetic boundary formation.

^c Determined by dry weight measurement only.

^d Difference estimated on the basis of focusing in polyacrylamide gels.

^e Determined by high-speed sedimentation equilibrium.

^f Calculated from amino acid composition.

^g Calculated from sedimentation equilibrium and velocity data.

^h Based on timed hydrolysis and amino acid recovery data.

FIG. 9. Two-dimensional tryptic peptide analysis of the Y-1 and Moloney leukemia virus p30 proteins. The purified proteins were iodinated, S-aminoethylated, digested with trypsin, and mapped on thin-layer sheets as described in the text. Electrophoresis was in the horizontal plane and chromatography was vertical (ascending). Symbols: ○, shared major peptides; ◐, shared minor peptides; ●, unique major Moloney virus peptides; ◑, unique major Y-1 virus p30 peptides.

the presence of amino acid sequences and particular residues (e.g., proline) that act as helix-breakers at critical regions in the Y-1 virus p30. These sequentially different and/or additional residues in the Y-1 polypeptide hence interfere with the formation of α -helix as observed in the Moloney virus molecule. How the conformational differences affect the tertiary structure of these proteins is not known, although the hydrodynamic studies indicate some minor ratio differences. Steric hindrance or other factors due to regional conformational differences about the immunologically cross-reactive sites may contribute to the observed decrease in antibody affinity for the Y-1 virus p30 molecule.

On a biological level, the Moloney leukemia virus and the Y-1 endogenous virus also differ greatly. The Moloney virus is highly oncogenic in vivo and displays high titer infectivity with NB-tropic host range in vitro (C. Riggin, W. N. Burnette, and W. Mitchell, unpublished data). Since the laboratory strain of Moloney leukemia virus arose from an endogenous murine virus (24, 25) as a result of the application of evolutionary pressures designed to select for enhanced infectivity and oncogenicity, it is reasonable to expect these pressures to be reflected in both the structure and functional nature of its genomic products. The nature of the variations that give rise to the immunochemical differences, for example, can be ascribed to the chemical and physical disparities noted in this study. Conversely, the conservation of the intrinsic charge and general shape between the p30 molecules argues for an important functional role, such as molecular association (8) and subviral core self-assembly.

Our data suggest considerable sequence heterogeneity between the major structural proteins of these two viruses, although Oroszlan et al. (29) have described only a single amino acid difference in the first 24 N-terminal residues of the p30 proteins from six different murine oncornaviruses. It is possible that the N-terminus is the most highly conserved portion of the molecule and may be the portion responsible for the species-specific immunological cross-reactivity. Nevertheless, the mechanism by which the heterogeneity arises is not currently understood. On a structural level, unequal crossing over within the p30 gene during recombinational events could have led to the exclusion of genetic information from the endogenous virus that is not essential, and perhaps inimical, to exogenous viral infectivity and oncogenicity functions. This is particularly intriguing with respect to recent observations of mass variation in p30 molecules in host range revertants of Moloney leukemia virus (19) and host range clones of Friend leu-

kemia virus (W. N. Burnette, S. Duttagupta, and R. Soeiro, manuscript in preparation). Such intragenic exclusion could account for the approximately 9% difference in molecular weight seen between the N-tropic Y-1 virus p30 and the p30 from the NB-tropic Moloney virus used in our studies. Intramolecular recombination has been previously described in oncornaviruses by Wyke et al. (47) for transformation-dependent, temperature-sensitive mutants of the Rous sarcoma virus genome.

The sequence heterogeneity observed in these investigations is also compatible with point mutations. Such point mutations would give rise to helix-forming amino acid residues and sequences and thus to the increased helicity of the Moloney virus p30, since the 9% mass exclusion probably cannot fully account for the 22 to 28% difference in helicity between the proteins from the endogenous and exogenous viruses. Further, point mutations could also be the source of the mass difference by leading to altered processing and cleavage of the p30 from the *gag* gene polyprotein precursor. If altered processing were the case, one might expect a concomitant mass change in those polypeptides (e.g., p10^{gag}) that are contiguous with the p30^{gag} in the polyprotein. Alternate cleavage of the precursor for Rauscher leukemia virus envelope glycoproteins is thought to be the source of the two components of the gp69/71^{env} complex (W. N. Burnette and J. T. August, Fed. Proc. 35:1736).

A functional approach to the effect of evolutionary pressure on the major internal structural protein component of mammalian oncornaviruses has not yet been possible, since the functions of this polypeptide have yet to be established. However, investigations to elucidate the functions of viral p30, particularly in relation to its possible role in virion self-assembly and host range restriction, are currently in progress.

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