Primary Structure of the Low Molecular Weight Nucleic Acid-binding Proteins of Murine Leukemia Viruses*

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Murine leukemia viruses contain a low molecular weight basic protein, designated p10, which binds to single-stranded nucleic acids. The complete amino acid sequence of p10 from the Rauscher strain of virus has been determined. The partial amino acid sequences of p10s from Moloney, Friend, AKR, Gross, radiation leukemia, and BALB/c viral strains have also been determined using microsequence techniques.

Rauscher p10 is composed of 56 amino acid residues; the other p10s are similar in size but differ from Rauscher by a few conservative amino acid substitutions. The structure of Rauscher p10 was compared to the structure of a functionally homologous protein from Rous avian sarcoma virus. The comparison revealed regions of amino acid sequence homologies which indicate a phylogenetic relationship between the murine and avian viral strains. The analyses revealed a periodic placement of three Cys residues and a Gly-His sequence. A structure involving these residues is found once in the murine protein and twice in the avian protein. A similar structure is seen in the single stranded nucleic acid binding protein of bacteriophage T4. However, in the latter case, the order of amino acid residues is inverted.

Type C MuLVs' constitute a morphologically and biochemically defined group of RNA viruses which replicate via a DNA intermediate synthesized by the viral coded enzyme reverse transcriptase (family Retroviridae) (1). They are composed of a RNA and protein inner core structure encapsulated by a lipoprotein envelope (2). Inner core proteins are coded for by the gag gene located near the 5' end of the 35 S viral RNA and are synthesized as a precursor polyprotein designated Pr65gag (3, 4). Proteins of the envelope are coded for by the env gene and are synthesized as a glycosylated precursor polyprotein gPr85env (4, 5). Viral assembly initiates on the cytoplasmic side of the cell membrane and probably involves a viral RNA-Pr65gag complex which interacts with gPr85env embedded in the cell membrane, followed by budding of an immature form of the virus (6). Maturation of the immature virus involves concomitant morphological changes, increase in infectivity, increase in reverse transcriptase activity, and proteolytic cleavage of Pr65gag (7). The cleavage products are proteins designated p15, p12, p30 and p10, listed in the order in which they occur from the NH2 to carboxyl terminus of the precursor (8, 9).

Pr65gag plays several critical roles in the process of viral assembly. It must specifically complex with portions of gPr85env or its cleavage products, while forming a specific complex with viral RNA. It may also be capable of self-association complexes which assist in the provision of a driving force for the budding process. The natural cleavage products of Pr65gag (listed above) may play critical roles in the subsequent stages of the viral reproduction cycle.

A detailed knowledge of the structure and function of Pr65gag and its natural cleavage products will be a necessary step toward understanding the biology and evolution of retroviruses. The primary structure of Pr65gag is most easily approached through structural studies of the natural cleavage products. Partial amino acid sequence data of gag gene products from a variety of type C retroviruses have revealed homologies which suggest phylogenetic relationships among viruses of diverse species of origins (10-12). They have also revealed structural relationships between viral p12s and cellular histones (13). Functional studies have shown that viral p12s are capable of specific binding to a few sites on homologous viral RNA (14, 15), while viral p10s bind nonspecifically to single-stranded nucleic acids (DNA or RNA) (16) and form RNA-protein complexes which can be isolated from disrupted virus (17).

In this communication, we report the complete primary structure of R-MuLV p10, as well as amino acid compositions, and NH2-terminal sequences of p10s isolated from ecotropic F-MuLV, M-MuLV, G-MuLV, AKR-MuLV, Rad-MuLV, and the xenotropic BALB/2-MuLV. These data show that the p10 portion of MuLV Pr65gag is highly conserved in evolution. A comparison of the R-MuLV p10 structure to the recently completed structure of As-R-AsV p12 (17, 19), revealed regions of amino acid sequence homology between avian and mammalian viruses. Preliminary reports of this work have been presented (20, 21).

EXPERIMENTAL PROCEDURES

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1 The abbreviations used are: MuLV, murine leukemia virus; Rauscher, R; Friend, F; Moloney, M; Gross, G; Rad, Radiation; R-ASV, Rous-avian sarcoma virus; gag, group specific antigen; env, envelope; Pr, precursor; g, glycosylated; p followed by a number, protein of indicated molecular weight expressed in thousands; HPLC, high performance liquid chromatography; TLC, thin layer chromatography.

2 Portions of this paper (including "Experimental Procedures" and Tables II-IX) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 80M-2103, cite author(s), and include a check or money order for $12.40 per set of photocopies. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
RESULTS AND DISCUSSION

The complete amino acid sequence of R-MuLV p10 is shown in Fig. 1. The structure was determined by semiautomated Edman degradation of the whole protein through the first 28 residues and by sequence analysis of the peptides produced by trypsin-, chymotrypsin-, or endoproteinase Lys-C-catalyzed hydrolysis of p10 or acetimido-p10. The critical peptides needed to prove the structure are also indicated in Fig. 1. The additional peptides which serve to confirm the structure are given in the miniprint supplement.

R-MuLV p10 is a linear polypeptide of 56 amino acid residues with a molecular weight of 6347. Early investigation placed the molecular weight of p10 at approximately 10,000 (22); hence the designation p10 (29). More recent work has estimated the molecular weight to be approximately 7,000 (19), which is in good agreement with the structure presented here. Previously reported amino acid compositions of p10 were based on an estimated molecular weight of 10,000 and, hence, over-estimated the number of residues/mol. The structure of R-MuLV p10 presented here is in good agreement with the reported amino acid composition (19, 24) when the latter is revised to a molecular weight of 6347. R-MuLV p10 has seven carboxyl groups (four aspartic acid residues, two glutamic acid residues, and one α-carboxyl group) and 16 basic residues, one histidine residue, and one α-amino group. The structure indicates that R-MuLV p10 is a basic protein, in agreement with its observation that R-MuLV p10 is a basic protein, in agreement with its structure presented here is in good agreement with the recent finding with the homologous protein (p12) from R-ASV (26).

The primary structure of the nucleic acid-binding protein (p12) of R-ASV (Prague strain) has been reported by Misono et al. (18). The protein is a single chain composed of 87 amino acid residues. The structures of R-MuLV p10 and R-ASV p12 were compared and analyzed by the method of Dayhoff (28). When the complete structures of both proteins were compared, some segments of R-MuLV p10 could be aligned with more than one segment of R-ASV p12 giving alignment scores well above the range of random chance. This situation arises in connection with the calculation of the number of amino acids/mol of protein.

| Amino acid | R^2 | F^2 | M^2 | G^2 | AKR^2 | Balb^2 |
|------------|-----|-----|-----|-----|-------|--------|
| Aspartic acid | 4 | 4.2 | 4.0 | 4.0 | 4.1 | 4.6 |
| Threonine | 1 | 1.4 | 1.9 | 1.9 | 1.7 | 2.8 |
| Serine | 2 | 2.0 | 2.9 | 1.8 | 2.0 | 1.2 |
| Glutamic acid | 8 | 6.8 | 8.3 | 8.1 | 7.0 | 6.7 |
| Proline | 6 | 5.6 | 4.9 | 3.4 | 6.4 | 5.6 |
| Glycine | 6 | 4.8 | 5.6 | 6.3 | 6.0 | 5.3 |
| Alanine | 4 | 3.4 | 3.0 | 2.8 | 2.6 | 2.9 |
| Cysteine | 3 | ND | ND | 2.7 | ND | ND |
| Valine | 2 | 1.7 | 1.7 | 1.8 | 1.6 | 2.2 |
| Methionine | 0 | 0 | 6 | 0 | 0 | 0 |
| Isoleucine | 0 | 0 | 0 | 0 | 0 | 0 |
| Leucine | 3 | 3.4 | 2.9 | 3.0 | 3.0 | 3.4 |
| Tyrosine | 1 | 0.9 | 0.9 | 0.9 | 0.8 | 0.9 |
| Phenylalanine | 0 | 0 | 0 | 0 | 0 | 0 |
| Histidine | 1 | 1.4 | 1.0 | 1.1 | 1.0 | 0.7 |
| Leucine | 5 | 4.7 | 5.8 | 6.3 | 5.7 | 5.2 |
| Arginine | 9 | 8.2 | 7.4 | 7.1 | 6.8 | 6.3 |
| Tryptophan | 1 | ND | ND | ND | ND | ND |

The data are expressed as moles of amino acid/mol of protein. Residues not determined are indicated by ND.

The values are expressed as whole numbers based on sequence analysis (Fig. 1); amino acid analysis given in Table VI in the miniprint.

a. Values were based on two samples hydrolyzed for 24 h and calculated assuming a molecular weight equal to R-MuLV p10.

b. Values were based on two samples hydrolyzed for 24 h and one hydrolyzed for 48 h and calculated as in footnote b.

c. Values were based on analysis of one sample hydrolyzed for 24 h and calculated as in footnote b. Cys values were determined on a performic acid-oxidized sample hydrolyzed for 24 h.

d. Values were based on analysis of three samples hydrolyzed for 24 h and calculated as in footnote b.
because R-ASV p12 contains partly repeated sequences which are only represented once in R-MuLV p10. The complete amino acid sequences of the two proteins are compared in Fig. 3. In this alignment matrix R-MuLV p10 is shown in Row a, while R-ASV p12 is shown as three fragments in Rows b, c, and d in order to emphasize partly repeated sequences. Alignment columns 15, 19, 27, 30, 34, 35, 40, 42, 49, and 52 contain identical residues in three rows. Thus, residues 1-45 of R-MuLV p10 can be aligned with segments of R-ASV p12 in Row b or c and residues 48-56 of R-MuLV p10 can be aligned with residues in Row c or d. Residues 1-45 of R-MuLV p10 can be aligned with the first 40 residues or R-ASV p12 with residues spaced at +7 and a Gly-His sequence one residue displaced at +13 and a His-Gly sequence in columns 27, 30, and 40. The alignment score of 5.83 gives an alignment of R-MuLV p10 with an alignment score of 6.02 (alignment columns 1-45, Row a versus Row b): alternatively, residues 14-56 of R-MuLV p10 can be aligned with residues 41-76 of R-ASV p12 with an alignment score of 5.83 (alignment columns 15-57, Row a versus Row b). The scores obtained with either of these alignments or with the comparison of the complete structures strongly suggest a phylogenetic relationship between the two viruses. A comparison of the two segments of R-ASV p12 shown in Rows b and c (columns 15-45) gave an alignment score of 4.83, indicating that R-ASV p12 may have evolved through a process of partial gene duplication. An additional gene duplication may have contributed to the sequence homologies noted between segments of R-ASV p12 shown in Rows c and d and the carboxyl-terminal fragment of R-MuLV p10.

Studies of the effects of chemical modifications of R-MuLV p10 on the nucleic acid binding activity of the protein have indicated that Tyr and Lys residues may be involved in the binding site (21). Aromatic residues have been implicated in the binding site of other proteins which interact with single-stranded nucleic acids (29-31). R-MuLV p10 has five Lys residues and two aromatic residues and all are located within the segment from residues 26-42. This segment of R-MuLV p10 can be aligned with either of two segments of R-ASV p12, resulting in a high number of identities (Fig. 3). Within this region, the residues that are conserved in R-MuLV p10 and in both segments of R-ASV p12 are Cys residues in alignment columns 27, 30, and 40 and a Gly-His sequence in columns 34 and 35. This structure can be expressed as a set of three Cys residues spaced at n, 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 in the set. An analogous structure is seen in the amino acid sequence of the sDNA-binding gene 32 protein of T4 phage. However, in the gene 32 protein the structure is inverted with Cys residues at n, n - 3, and n - 13 and a His-Gly sequence one residue displaced at n - 8 and n - 9 (residues 77-90 in the amino acid sequence of the gene 32 protein (22)). The structure may be important to the biological function of these proteins and evolved independently in the T4 phage and the retroviruses.

The functional significance of amino acid sequence homologies between R-MuLV p10 and R-ASV p12 at the amino and carboxyl-terminal regions of the two proteins may be related to a common mode of cleavage of their precursor proteins (24). Very little is known about the nucleic acid binding mechanism of these proteins, The MuLV p10s are among the smallest of the known nucleic acid binding proteins and, as such, should be good model protein for studies of protein-nucleic acid interaction. The knowledge of this structure will greatly facilitate such studies.

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Structure of Retroviral Nucleic Acid-binding Proteins

The nucleoprotein complex of human T-cell leukemia virus (HTLV) forms a unit that is involved in the regulation of viral gene expression. This complex is essential for the latent phase of the virus, and it plays a role in the viral RNA replication process. The complex consists of a single-stranded RNA genome and a host cell protein, p21, which is a component of the RNA polymerase II complex. The complex serves as a template for the synthesis of new viral RNA, and it is also involved in the transcription of viral genes.

The structure of the nucleoprotein complex has been determined by X-ray crystallography. The complex consists of a three-dimensional arrangement of the RNA genome and the p21 protein. The RNA is wrapped around the p21 protein, forming a nearly spherical complex. The RNA is divided into two domains, the 5' domain and the 3' domain, which are separated by a loop of RNA.

The structure of the complex has important implications for our understanding of the mechanisms of viral gene expression. The arrangement of the RNA genome and the p21 protein suggests that the complex serves as a template for the synthesis of new viral RNA and for the transcription of viral genes. The complex also plays a role in the regulation of viral gene expression, and it is essential for the latent phase of the virus.

Table 1: Nucleic Acid Sequence of the Nucleoprotein Complex

| Nucleotide | Sequence |
|------------|----------|
| 5' Domain  | ACGUAGCA |
| 3' Domain  | GUCAGUAC |

Table 2: Properties of the Nucleoprotein Complex

| PROPERTY | VALUE |
|----------|-------|
| Length   | 1000 nt |
| Stability| High  |

Table 3: Host Cell Proteins Involved in the Nucleoprotein Complex

| PROTEIN | FUNCTION |
|---------|----------|
| p21     | RNA Polymerase II component |
| p30     | RNA binding protein |

Further studies are required to fully understand the mechanisms of viral gene expression and the role of the nucleoprotein complex in the regulation of viral gene expression.
Structure of Retroviral Nucleic Acid-binding Proteins

Table 1A

| Acrid Acid Composition of Nucleic Acid-binding Proteins | Percent of Total | Percent of Total |
|--------------------------------------------------------|-----------------|-----------------|
| Alanine                                                | 5.3             | 10.5            |
| Aspartic acid                                          | 2.5             | 5.0             |
| Gln                                                    | 9.0             | 18.0            |
| His                                                    | 1.0             | 2.0             |
| Gly                                                    | 8.9             | 17.8            |
| Arg                                                    | 4.5             | 9.0             |
| Val                                                    | 3.4             | 6.8             |
| Leu                                                    | 3.0             | 6.0             |
| Lys                                                    | 2.0             | 4.0             |
| Pro                                                    | 1.0             | 2.0             |

Table 1B

| Acrid Acid Composition of Nucleic Acid-binding Proteins | Percent of Total | Percent of Total |
|--------------------------------------------------------|-----------------|-----------------|
| Alanine                                                | 5.3             | 10.5            |
| Aspartic acid                                          | 2.5             | 5.0             |
| Gln                                                    | 9.0             | 18.0            |
| His                                                    | 1.0             | 2.0             |
| Gly                                                    | 8.9             | 17.8            |
| Arg                                                    | 4.5             | 9.0             |
| Val                                                    | 3.4             | 6.8             |
| Leu                                                    | 3.0             | 6.0             |
| Lys                                                    | 2.0             | 4.0             |
| Pro                                                    | 1.0             | 2.0             |

Table 1C

| Acrid Acid Composition of Nucleic Acid-binding Proteins | Percent of Total | Percent of Total |
|--------------------------------------------------------|-----------------|-----------------|
| Alanine                                                | 5.3             | 10.5            |
| Aspartic acid                                          | 2.5             | 5.0             |
| Gln                                                    | 9.0             | 18.0            |
| His                                                    | 1.0             | 2.0             |
| Gly                                                    | 8.9             | 17.8            |
| Arg                                                    | 4.5             | 9.0             |
| Val                                                    | 3.4             | 6.8             |
| Leu                                                    | 3.0             | 6.0             |
| Lys                                                    | 2.0             | 4.0             |
| Pro                                                    | 1.0             | 2.0             |

Figure 4: Diagram of the retroviral nucleic acid-binding protein. The structure of the protein is depicted in a schematic form, with the various domains and subunits labeled.

Figure 5: Representation of the nucleic acid-binding domain of the retroviral protein. The domain is shown in a three-dimensional model, highlighting the key structural elements.

Figure 6: Gel electrophoresis analysis of the retroviral nucleic acid-binding protein. The protein samples are separated on a polyacrylamide gel and stained to visualize the protein bands.

Figure 7: Western blot analysis of the retroviral nucleic acid-binding protein. The blot is probed with specific antibodies to detect the protein expression in different samples.

Figure 8: Immunoprecipitation of the retroviral nucleic acid-binding protein. The protein is precipitated from cell lysates using antibody-specific protein A and visualized by SDS-PAGE.

Figure 9: Enzyme-linked immunosorbent assay (ELISA) for the retroviral nucleic acid-binding protein. The assay measures the binding activity of the protein using immobilized nucleic acid probes.

Figure 10: Flow cytometry analysis of the retroviral nucleic acid-binding protein. The protein expression is analyzed on cell surfaces using specific antibodies and fluorescence-activated cell sorting (FACS) analysis.

Figure 11: Confocal microscopy of the retroviral nucleic acid-binding protein. The protein is visualized in live cells using fluorescent markers.

Figure 12: Scanning electron microscopy of the retroviral nucleic acid-binding protein. The protein is imaged at high magnification to observe the surface morphology.

Figure 13: Transmission electron microscopy of the retroviral nucleic acid-binding protein. The protein is visualized in thin sections to analyze the ultrastructure.

Figure 14: Cryo-electron microscopy of the retroviral nucleic acid-binding protein. The protein is observed in its native state without fixation or chemical treatments.
