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Amino Acid Sequence of p15 from Avian Myeloblastosis Virus Complex†

Robert T. Sauer,* David W. Allen, and Hugh D. Niall

ABSTRACT: The complete amino acid sequence of the p15 *gag* protein from avian myeloblastosis virus (AMV) complex has been determined by sequential Edman degradation of the intact molecule and of peptide fragments generated by limited tryptic cleavage, cleavage with staphylococcal protease, and cyanogen bromide cleavage. AMV p15 is a single-chain protein containing 124 amino acids. The charged amino acids tend to be clustered in the primary structure. p15 contains a single cysteine at position 113 which may be essential for the p15 associated proteolytic activity. However, p15 shows no appreciable sequence homology with papain or other classical thiol proteases.

Retroviruses of the avian leukosis-sarcoma group are enveloped ribonucleic acid (RNA)[1] tumor viruses displaying the characteristic morphology of C-type particles (Tooze, 1973). The internal structural proteins of these viruses are encoded by the viral *gag* gene and are designated p27, p19, p15, and p12 (August et al., 1974) since their monomer molecular weights are approximately 27 000, 19 000, 15 000, and 12 000, respectively (Fleissner, 1971). *gag* proteins from avian myeloblastosis virus (AMV) complex, the Prague strain of Rous sarcoma virus, avian myelocytamostosis virus (MC-29), and avian sarcoma virus B77 have been isolated and partially characterized (Niall et al., 1970; Fleissner, 1971; Herman et al., 1975; Fletcher et al., 1975; Hunter et al., 1978; Reynolds et al., 1978; Palmiter et al., 1978; Wiesemann et al., 1978). The homologous *gag* proteins from these different species of avian retroviruses are very similar in amino acid composition, molecular weight, and, where studied, amino acid sequence. The *gag* proteins are synthesized as a precursor polyprotein (Pr76) of 76 000 molecular weight which is subsequently cleaved to generate the four known *gag* proteins (Vogt & Eisenman, 1973; Vogt et al., 1975).

The structural locations of the *gag* proteins within the virion and the general morphology of the retroviruses have been recently reviewed [see Montelaro & Bolognesi (1978) and references cited therein]. p12 is a phosphoprotein which interacts with the viral RNA to form the centrally located, electron-dense ribonucleoprotein complex of the virus. p27 molecules form a core shell surrounding the ribonucleoprotein, and p19 molecules form an ill-defined structure between the core shell and the viral membrane. A small number of p19 molecules are also found associated with the viral genome (Sen & Todaro, 1977) and may be involved in control of intracellular processing of the RNA (Leis et al., 1978). The structural role of the p15 molecule in the avian retroviruses is poorly understood. p15 is found with p27 in core structures, but its recovery is low and variable when compared with that of p27 (Bolognesi et al., 1973) and it has been termed "core associated".

In contrast to its uncertain structural role, recent evidence suggests an important regulatory role for p15 in the viral life cycle. Highly purified preparations of p15 contain an associated protease activity which cleaves the Pr76 polyprotein precursor (von der Helm, 1977; Dittmar & Moelling, 1978; Vogt et al., 1979). At least two of the mature *gag* proteins can be generated by p15-mediated cleavage of Pr76, and the proteolytic activity appears to be moderately specific for Pr76 although certain other proteins also act as substrates when denatured (Vogt et al., 1979; Dittmar & Moelling, 1978). The idea that p15 may be an essential protease is supported by the analysis of a temperature-sensitive mutant (LA 3342) of avian sarcoma virus (Hunter et al., 1976). This conditional mutant apparently codes for an altered p15 protein (Rohrschneider et al., 1976), and at the nonpermissive temperature (41 °C) produces aberrant, noninfectious virions containing reduced amounts of certain *gag* proteins and new proteins that appear to be intermediates in the cleavage of Pr76. Cleavage of Pr76 in LA 3342 infected cells is slower at 41 °C than at the permissive temperature (Hunter et al., 1976). It is unlikely that the LA 3342 defect defines a cis-acting site where proteolysis occurs since LA 3342 can be complemented at the nonpermissive temperature by a nondefective avian leukosis virus (Hunter et al., 1976). Moreover, certain defective retroviruses such as MC-29 and avian erythroblastosis virus (AEV) produce *gag*-related fusion proteins lacking p15 (Bister et al., 1977; Hayman et al., 1979). In nonproducer cell lines, these fusion proteins are not cleaved as would be expected if functional p15 were necessary for such processing. The *gag*-related fusion proteins from AEV and MC-29 can be

† From the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 (R.T.S.), the Hematology Section, Veterans Administration Medical Center, Minneapolis, Minnesota 55417 (D.W.A.), and the Howard Florey Institute, University of Melbourne, Parkville, Australia 3052 (H.D.N.). *Received October 17, 1979; revised manuscript received October 3, 1980.* This work was supported in part by grants form the American Cancer Society (NP-27J), the National Institutes of Health (AI-15706), and the Athwin Foundation and by a contract from the National Cancer Institute (NIH-NCI-E-73-3265).

[1] Abbreviations used: RNA, ribonucleic acid; AMV, avian myeloblastosis virus; $NaDodSO_4$, sodium dodecyl sulfate; LC, liquid chromatography; TPCK, tosylphenylalanine chloromethyl ketone; CM, carboxymethyl.

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cleaved by purified p15 in vitro (Vogt et al., 1979), but in vivo cleavage of these fusion proteins following superinfection by a nondefective virus producing p15 has not been observed.

In this paper, we report the complete amino acid sequence of the p15 protein purified from avian myeloblastosis virus complex. Previous chemical characterization of this protein by our group was reported by using the nomenclature "group specific antigen b (gs-b)" (Allen et al., 1970; Niall et al., 1970).

Materials and Methods

Purification. Avian myeloblastosis virus complex was obtained from Dr. J. W. Beard, Life Sciences Inc., St. Petersburg, FL. p15 was isolated from Tween 80 and ether-disrupted virus by centrifugation, gel filtration on Sephadex G-100, and ion-exchange chromatography on carboxymethylcellulose as described (Allen et al., 1970). One batch of p15 was the generous gift of Drs. T. Vanaman and M. Veigl, Duke University, Durham, NC.

Many preparations of p15 are contaminated with small quantities of other viral *gag* proteins. Samples of *S*-carboxymethylated p15 used for amino acid analysis were therefore further purified by $NaDodSO_4$–polyacrylamide gel electrophoresis using 13.5% Laemmli gels. The p15 was detected by staining guide strips of the gel with Coomassie Blue and was electroeluted from the gel slice. The eluted protein was then desalted on a Sephadex G-50 (fine) column equilibrated in 0.2 M NH_4HCO_3 and 0.2% $NaDodSO_4$. The protein was then dialyzed against 0.2 M NH_4HCO_3 to remove the $NaDodSO_4$ and was finally frozen and lyophilized prior to hydrolysis.

Edman Degradation. Automated degradations were performed in a Beckman Model 890 sequencer by using the single-coupling, double-cleavage program of Edman & Begg (1967) with minor modifications (Sauer et al., 1974). The automated degradations on peptides CNBr-44 and BNPS were performed in a Beckman Model 890C sequencer by using the 0.1 M Quadrol program described by Brauer et al. (1975) with added polybrene (6 mg) as carrier. Sequencer reagents and solvents were obtained from Beckman Instruments (Spinco Division, Palo Alto, CA) and were used without further purification. 1,4-Butanedithiol (1:20 000) was added to the 1-chlorobutane to protect the labile phenylthiohydantoin (PTH) amino acid derivatives of serine and threonine (Hermodson et al., 1970). Manual degradations were performed by a modification of the three-stage procedure of Edman (Edman, 1960; Sauer et al., 1974). The 2-anilinothiazolinone amino acid derivatives were converted to PTH derivatives at 80 °C for 10 min with 1 N HCl (Ilse & Edman, 1963).

Identification of PTH Derivatives. Most PTH derivatives were identified and quantitated by gas–liquid chromatography (GLC) (Pisano & Bronzert, 1969; Niall, 1973), and where the amount of material permitted, identifications were confirmed by thin-layer chromatography (TLC) in the Edman H system (Edman, 1970) using 250-μm silica gel TLC plates (Analtech, Newark, DE). The PTH derivative of ϵ-maleyllysine was identified solely by TLC in the Edman H system (Sauer et al., 1974). PTH-Arg was routinely identified by the phenanthrenequinone reaction (Yamada & Itano, 1966), and PTH-His was identified by the Pauly reaction (Easley, 1965). In two experiments, PTH amino acid derivatives were identified by high-pressure LC using a Waters C_{18} μBondapak column (Zimmerman et al., 1977).

PTH-Ala, Ser, Gly, Thr, Val, and Pro were identified by GLC using isothermal elution at 170 °C on a 10% DC-560 support. PTH-Leu and Ile were distinguished by isothermal elution at 135 °C on a 1.5% AN-600 support. The PTH derivatives of Asp, Met, Glu, and Phe were identified as their trimethylsilyl derivatives on the 10% DC-560 support with isothermal elution at 210 °C. The PTH derivatives of Asn, Gln, Lys, Tyr, and Trp were identified by elution at 250 °C on a 2% OV-25 support. All gas chromatography was performed with a Beckman GC-45 gas chromatograph equipped with hydrogen flame ionization. Unknown PTH derivatives were quantitated by comparison of peak areas with those of known amounts of crystalline PTH amino acid standards obtained from Pierce Chemical Co. (Rockford, IL).

Residue Assignments. In most degradations, residue assignments were made on the basis of a clear predominance by yield of a single PTH amino acid derivative. Where this was not possible (i.e., in the latter stages of a degradation or where labile derivatives such as serine were involved), assignments were based on a minimum 3-fold rise of a single PTH amino acid above background levels with a subsequent fall to those levels within two cycles of degradation.

Amino Acid Analysis. Samples for analysis were hydrolyzed in an evacuated desiccator at 110 °C for 24, 48, or 72 h with 5.7 N HCl to which 2-mercaptoethanol (1:2000) had been added for protection of methionine residues (Keutmann & Potts, 1969). Analyses were performed on a Beckman Model 121 amino acid analyzer. Peak integrations were performed with an Infotronics Model CRS 12 AB integrator.

Lysine and Arginine Blockade. Lysine residues were modified with maleic anhydride. A total of 3.5 mg of p15 was added to 1.0 mL of 0.2 N $Na_2B_4O_7$ (pH 9.0). The insoluble protein was dispersed with a glass rod to form a fine suspension. This suspension was maintained by constant magnetic stirring at room temperature. At 5-min intervals, 5-μL aliquots of maleic anhydride (200 mg/mL in 1,4-dioxane) were added. The pH was maintained between 8.5 and 9.0 by addition of 0.1 N NaOH. Following the addition of 15 mg of maleic anhydride, the reaction mixture was stirred overnight, centrifuged to remove insoluble material, and desalted on a 1.2 × 65 cm Bio-Gel P-2 column equilibrated with 0.2 M NH_4HCO_3. The maleylated p15 emerged at the void volume of the column and was lyophilized.

In one case, lysine residues were blocked by using succinic anhydride. The procedure was the same as for maleylation except that 0.15 mg of p15 was used and excess reagent was removed by dialysis against 0.2 M NH_4HCO_3, followed by lyophilization.

Arginine residues were reacted with 1,2-cyclohexanedione by using the method described by Toi et al. (1967). Twice recrystallized 1,2-cyclohexanedione (Eastman) was dissolved in 0.5 mL of 0.2 N NaOH, and 4.1 mg of p15 was added. The protein did not dissolve and was dispersed into a fine suspension with a glass rod. The reaction mixture was stirred at room temperature for 18 h at which time the protein had dissolved. The reaction mixture was then titrated to pH 8.6 with 0.2 N HCl and was desalted as described above.

Tryptic Digestion of Modified p15. p15 modified either by maleylation or by reaction with 1,2-cyclohexanedione was digested with TPCK-trypsin (Worthington Biochemical Corp.) in 0.5 mL of 0.2 M triethylamine–acetate buffer (pH 8.2), at 37 °C for 4 h, with an enzyme/substrate ratio of 1:100 (mol/mol). Digests were frozen and lyophilized prior to peptide fractionation.

Digestion with Staphylococcal Protease. The staphylococcal protease used in these studies was kindly provided by Dr. G. R. Drapeau, University of Montreal, Montreal, Quebec. Digestion conditions were chosen to restrict cleavage to peptide bonds following glutamyl residues (Houmard & Drapeau,

Table I: Amino Terminal Degradation of p15

cycle	residue (PTH)	yield (nM)	cycle	residue (PTH)	yield (nM)
1	Leu	245.0	20	Thr	24.3
2	Ala	223.2	21	Gly	62.5
3	Met	135.7	22	Ser	36.4
4	Thr	203.1	23	His	*a*
5	Met	119.5	24	Pro	15.8
6	Glu	143.0	25	Val	24.5
7	His	*a*	26	Lys	16.4
8	Lys	67.6	27	Gln	18.8
9	Asp	116.4	28	Arg	*b*
10	Arg	*b*	29	Ser	17.4
11	Pro	39.3	30	Val	21.4
12	Leu	92.6	31	Tyr	16.9
13	Val	82.5	32	Ile	20.0
14	Arg	*b*	33	Thr	13.1
15	Val	75.2	34	Ala	15.3
16	Ile	59.3	35	Leu	12.3
17	Leu	79.0	36	Leu	8.1
18	Thr	61.7	37	Asp	5.6
19	Asn	35.6	38	Ser	4.2

[a] PTH-His was identified by the qualitative Pauly reaction. [b] PTH-Arg was identified by phenanthrenequinone reaction.

1972). A total of 3.3 mg of p15 was incubated with the protease in 0.5 mL of 0.2 M ammonium acetate buffer (pH 4.0) for 18 h at 37 °C, with an enzyme/substrate ratio of 1:20 (mol/mol). The sample was frozen and lyophilized prior to peptide isolation.

Cleavage with Cyanogen Bromide. A total of 3.0 mg of p15 was incubated with a 100-fold molar excess of cyanogen bromide (Eastman) in 0.5 mL of 70% formic acid, for 24 h at room temperature in the dark. The sample was then diluted 10-fold with distilled water, frozen, and lyophilized. A second cyanogen bromide digest was performed on 4 mg of p15 that had been reduced and alkylated with iodoacetic acid (Eastman) as described (Konigsberg, 1972). Otherwise, reaction conditions were identical with the first digestion.

Cleavage with BNPS-skatole. Approximately 10 nM of dried, succinylated p15 was dissolved in 0.25 mL of 70% formic acid. A 50-fold molar excess of solid BNPS-skatole (Pierce Chemical Co.) was added, and the reaction mixture was sealed under nitrogen and incubated at room temperature for 24 h in the dark. The reaction mixture was constantly stirred during this time. The reaction mixture was then dried with a stream of nitrogen, redissolved in 0.5 mL of distilled water, and extracted 5 times with ethyl ether. The aqueous phase was then frozen, lyophilized, and used directly for automated sequencing.

Peptide Isolation and Detection. Most peptides used in this study were isolated by gel filtration alone. Column effluents from gel filtration were monitored by absorbance at 280 nm with a Beckman DG spectrophotometer. Peptide digests containing maleyl groups were additionally monitored at 250 nm. Fluorescamine protein determinations (Udenfriend et al., 1972) were performed by dissolving the sample in 1.5 mL of 0.2 M $Na_2B_4O_7$ (pH 8.6) and adding 0.5 mL of a fluorescamine solution (5 mg/mL in acetone). Fluorescence was monitored in an American Instrument Co. fluoromicrophotometer. Samples containing either ammonium acetate or ammonium bicarbonate buffers were lyophilized thoroughly before reaction with fluorescamine. In one experiment, a cyanogen bromide digest of reduced and alkylated p15 was fractionated on a 0.9 × 23 cm column on SP-Sephadex (C25). The column was developed with a linear gradient (200 mL total) from 6.6 M urea, 0.1 N HOAc, and 0.01 M NH_2OH to 6.6 M urea, 0.1 N HOAc, 0.01 M NH_2OH, and 0.25 M NaCl. The effluent from this column was monitored by absorbance at 200 nm with a Beckman Model 25 spectrophotometer, following desalting of the fractions into 50 mM phosphoric acid.

Results

Amino-Terminal Sequence. The sequence of the amino-terminal 38 residues of p15 was established on the basis of automated Edman degradation on 4.2 mg of purified protein. The yields of the PTH amino acid derivatives identified during this degradation are presented in Table I.

Fractionation and Sequencing of Maleylated Tryptic Peptides of p15. The tryptic digest of maleylated p15 was fractionated on a Bio-Gel P-4 column (Figure 1). The four peaks designated MT-1–4 were pooled and lyophilized. Aliquots of each pool were then sequenced by using the manual Edman procedure. The results of these degradations are shown in Table II.

The degradation on ~40 nM MT-1 proceeded through cycle 25. No PTH amino acid was identified at cycle 22. The sequence of MT-1 overlaps the amino-terminal sequence of p15 from residue 29 of the native sequence onward. The native sequence can thus be extended to residue 53.

Peptide MT-2 was sequenced for five cycles before breakage of the reaction tube terminated the degradation. The degradation of pool MT-3 revealed a mixture of two peptides in approximately equimolar amounts. The sequence designated MT-3a in Table II corresponds to the sequence of residues 15–26 of native p15. The second sequence (MT-3b) was deduced by difference.

Table II: Maleylated Tryptic Peptides[a]

cycle no.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
MT-1	29 Ser- 30	30 Val- 39	31 Tyr- 29	32 Ile- 35	33 Thr- 34	34 Ala- 32	35 Leu- 25	36 Leu- 28	37 Asp- 15	38 Ser- 15	39 Gly- 23	40 Ala- 20	41 Asp- 12	42 Ile- 19	43 Thr- 15	44 Ile- 19	45 Ile- 17	46 Ser- 6	47 Glu- 12	48 Glu- 12	49 Asp- 6	50 · · ·	51 Pro- 4	52 Ala- 3	53 Asp- 2 · · ·
MT-2	88 Asp- 12	89 Gly- 20	90 Ser- 25	91 Leu- 36	92 Glu 15 · · ·																				
MT-3a	15 Val- 10	16 Ile- 16	17 Leu- 14	18 Thr- 7	19 Asn- 6	20 Thr- 8	21 Gly- 8	22 Ser- 3	23 His- b	24 Pro- 4	25 Val 4 · · ·	} degraded as mixture													
MT-3b	78 Asp- 10	79 Met- 17	80 Ile- 15	81 Glu- 10	82 Val- 9	83 Gly- 7	84 Val- 6	85 Ile- 8	86 Asn 6 · · ·																
MT-4a	121 Leu- 39	122 Thr- 25	123 Asn- 17	124 Leu 28	} degraded as mixture																				
MT-4b	106 Gly- 21	107 Ser- 26	108 Ile 12 · · ·	} degraded as mixture																					

[a] Numbers under residues are yields in nanomoles of the PTH amino acid derivative. Numbers above residues represent residue position in final sequence (Figure 4). [b] PTH-His identified by Pauly reaction.

Table III: Staphylococcal Protease Peptides[a]

cycle no.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
SPC-4	Val-	Gly-	Val-	Ile-	Asn-	Arg-	Asp-	Gly-	Ser-	Leu-	Glu-	Arg-	Pro-	Leu-	Leu-	Leu-	Phe-	Pro-	Ala-	Val-	Ala-	Met-	Val-	Arg-	Gly-	Ser-	Ile ···
	34	32	37	37	29	c	8	14	5	27	15	c	17	14	16	12	8	9	9	7	6	2	6	c	7	3	5
	1	2	3	4																							
SPC-5	Leu-	Ala-	Met-	Thr ···																							
	6	7	3	4																							
	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78							
SPC-6	Ala-	Ala-	Asn-	Pro-	Gln-	Ile-	His-	Gly-	Ile-	Gly-	Gly-	Gly-	Ile-	Pro-	Met-	···	Lys-	Ser-	···	Asp ···							
	50	46	20	37	21	24	b	30	23	28	22	18	13	5	4		3	2		4							
	69	70	71	72	73																						
SPC-7	Gly-	Gly-	Ile-	Pro-	Met ···																						
	13	11	10	7	6																						
	59	60	61	62																							
SPC-8	Ala-	Ala-	Asn-	Pro ···																							
	14	15	10	8																							
	49	50	51	52	53	54	55	56	57	58																	
SPC-9	Asp-	Trp-	Pro-	Ala-	Asp-	Arg-	Pro-	Val-	Met-	Glu-COOH																	
	35	28	17	24	21	c	10	16	14	15																	

[a] Numbers under residues are yields of PTH amino acid derivative in nanomoles. Numbers above residues represent residue positions in final sequence (Figure 4). [b] PTH-His identified by Pauly reaction. [c] PTH-Arg identified by phenanthrenequinone reaction.

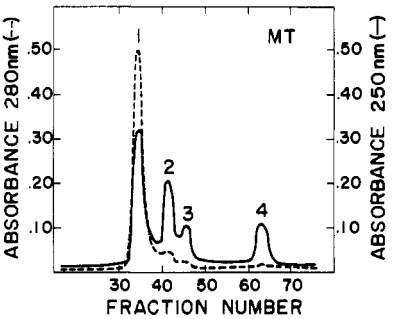

FIGURE 1: Column chromatography of a tryptic digest of maleylated AMV p15 on Bio-Gel P-4 (1.2 × 140 cm) equilibrated in 0.2 M NH_4HCO_3 (pH 8.2). Fractions were 1.5 mL each, and the column flow rate was 8 mL/h.

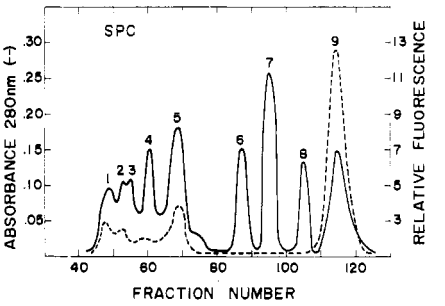

FIGURE 2: Fraction of peptides generated by staphylococcal protease digestion of AMV p15 on a 1.2 × 150 cm column of Sephadex G-50 (superfine) equilibrated in 0.05 M ammonium acetate (pH 4.1). Fractions were 1.2 mL each, and the column flow rate was 10 mL/h.

Pool MT-4 also proved to be a mixture of two peptides. However, neither peptide originated from a known sequence region. For resolution of the mixture, an aliquot was spotted on a cellulose thin-layer plate (Eastman 6060), and the plate was developed with pyridine–butanol–acetic acid–water (150:100:30:120). The plate was sprayed lightly with ninhydrin, and a fast-running spot (R_f = 0.76) was recovered in sufficient yield after elution with 50% acetic acid for hydrolysis and amino acid analysis. The composition of this peptide was $Asp_{1.16}$, $Thr_{0.97}$, and $Leu_{1.85}$. This composition, in combination with the results of the mixture digestion, allows deduction of the sequence NH_2-Leu-Thr-Asn-Leu-COOH, designated MT-4a in Table II. Since this maleylated tryptic peptide contained no arginine, it was assigned as the carboxy-terminal peptide of p15. This assignment is consistent with the release of leucine following carboxypeptidase A digestion of native p15 (Niall et al., 1970).

Fractionation and Sequencing of Staphylococcal Protease Peptides of p15. The staphylococcal protease digest of p15 was fractionated on Sephadex G-50 (superfine), and nine peaks were detected either by absorbance at 280 nm or by fluorescamine protein determination (Figure 2). These were pooled, lyophilized, and subjected to manual Edman degradation. The pools designated SPC-1–3 were found to contain nonstoichiometric mixtures of peptides, and extended degradations were not attempted. The results of degradations on the remaining pools are presented in Table III.

SPC-4 (40 nM) was sequenced for 27 cycles before clear identifications became impossible. The sequence determined for SPC-4 overlaps the sequence of MT-3b from residue 5 of MT-3b onward and includes the MT-2 sequence as residues 7–11 of SPC-4. This establishes the order and contiguity of these two maleylated tryptic peptides. In addition, residues 25–27 of SPC-4 are identical with the MT-4b sequence. This establishes the order MT-3b–MT-2–MT-4b. The sequence determined for SPC-5 represents the amino-terminal sequence of p15.

The peptide SPC-6 was sequenced through residue 20 with no residue being identified as position 19. As shown in Table III, the sequence of SPC-8 is identical with the amino-terminal sequence of SPC-6, and the SPC-7 sequence corresponds to residues 11–15 of SPC-6. The simplest interpretation of these data is that a minor cleavage has occurred between the residues corresponding to positions 11 and 12 of SPC-6. However, since glycine, not glutamic acid, was observed at cycle 10 of the SPC-6 degradation, the nature of the putative minor cleavage is unclear. Either there has been cleavage of the Gly^{10}–Gly^{11} bond by staphylococcal protease or by a contaminating protease (perhaps p15 itself) or p15 is heterogeneous at this position, containing both glycine and glutamic acid. Unfortunately, peptide SPC-8 was not recovered in sufficient yield to allow determination of its carboxy-terminal residue. We have, however, observed that digestion of porcine parathyroid hormone with staphylococcal protease results in cleavage of Gly–Ala bond (unpublished experiments), and thus we favor the former possibility.

The decapeptide designated SPC-9 was sequenced completely, and glutamic acid was identified as its carboxy-terminal residue by amino acid analysis (without hydrolysis) following the ninth cycle of Edman degradation. The sequence of SPC-9 overlaps the sequence of MT-1 from residue 21 of MT-1 onward. The gap at position 22 of MT-1 is thus occupied by a tryptophan residue.

Peptides Generated by Tryptic Cleavage at Lysines of p15. Peptides generated by tryptic cleavage of cyclohexanedione-

Table IV: CHD-tryptic and BNPS-skatole Degradations[a]

cycle no.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
CHD-1	Gln-	· · ·	Ser-	Val-	Tyr-	Ile-	Thr-	Ala-	Leu-	Leu-	Asp-	Ser-	Gly-	Ala	· · ·
	7		19	11	9	23	11	7	12	13	4	6	7	6	
	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
CHD-2	Ser-	· · ·	Asp-	Met-	Ile-	Glu-	Val-	Gly-	Val-	Ile-	Asn-	· · ·	Asp-	Gly-	Ser · · ·
	36		15	30	66	13	36	23	17	16	4		5	15	10
	9	10	11	12	13	14	15	16	17						
CHD-3	Asp-	· · ·	Pro-	Leu-	Val-	· · ·	Val-	Ile-	Leu · · ·						
	10		17	18	15		16	13	9						
	1	2	3	4	5	6	7								
CHD-4	Leu-	Ala-	Met-	Thr-	Met-	Glu-	His · · ·								
	25	21	7	14	9	12	[b]								
	51	52	53	54	55	56	57	58	59	60	61	62	63	64	
BNPS	Pro-	Ala-	Asp-	Arg-	Pro-	Val-	Met-	Glu-	Ala-	Ala-	Asn-	Pro-	Gln-	Ile · · ·	
	4	4	[c]	2	3	3	1	2	2	3	2	1	1	2	

[a] Numbers under residues are yields of the PTH amino acid derivative in nanomoles. Numbers above residues represent residue position in final sequence (Figure 4). [b] PTH-His identified by Pauly reaction. [c] 0.5 nmol of PTH-Arg identified by high-pressure liquid chromatography.

Table V: CNBr Peptides[a]

cycle no.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	1	2	3	4	5	6															
CNBr-1	Leu-	Ala-	· · ·	Thr-	· · ·	Glu-	· · ·														
	27	17		10		5															
	6	7	8	9	10	11	12														
CNBr-2	Glu-	His-	Lys-	Asp-	Arg-	Pro-	Leu-	· · ·													
	17	[b]	15	17	[c]	13	16														
	80	81	82	83	84	85	86														
CNBr-4	Ile-	Glu-	Val-	Gly-	Val-	Ile-	Asn · · ·														
	30	19	34	13	15	9	4														
	58	59	60	61	62	63	64														
CNBr-6	Glu-	Ala-	Ala-	Asn-	Pro-	Gln-	Ile · · ·														
	14	21	14	9	5	6	3														
	1	2																			
CNBr-8	Leu-	Ala · · ·																			
	39	27																			
	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124
CNBr-44	Val-	Arg-	Gly-	Ser-	Ile-	Leu-	Gly-	Arg-	Asp-	Cys-	Leu-	Gln-	Gly-	Leu-	Gly-	Leu-	Arg-	Leu-	Thr-	Asn-	Leu-COOH
	2.7	0.8	1.7	1.7	3.0	2.5	1.5	0.8	1.2	[d]	1.7	1.1	1.1	1.6	1.0	1.5	0.4	0.9	0.3	0.4	0.6

[a] Numbers under residues are yields of PTH amino acid derivatives in nanomoles. Numbers above residues represent residue position in final sequence (Figure 4). In degradation CNBr-44, PTH amino acid derivatives were identified by high-pressure LC and GLC. [b] PTH-His identified by Pauly reaction. [c] PTH-Arg identified by phenanthrenequinone reaction. [d] 0.7 nmol of PTH-*S*-CM-Cys found.

blocked p15 were fractionated on a 1.2 × 160 cm column of Bio-Gel P-6 equilibrated in 0.2 N NH_4HCO_3 (pH 8.2) (data not shown). Four peaks were detected, pooled, and sequenced manually. The results of these degradations are shown in Table IV.

The sequence CHD-1 corresponds to residues 27–40 of the native p15 sequence. The gap at residue 2 of CHD-1 can be seen to be an arginine residue by alignment with the native sequence. The CHD-3 and CHD-4 sequences also represent known areas of the p15 sequence. CHD-4 is an amino-terminal peptide while CHD-3 corresponds to residues 9–17 of p15. In both degradations, no PTH amino acid was detected at positions known to contain arginyl residues. This is consistent with the fact that CHD-Arg is unstable and unable to react with phenanthrenequinone (Patthy & Smith, 1975).

The CHD-2 sequence allows the sequences of peptides SPC-6 and SPC-4 to be linked. The gaps at positions 2 and 12 in the CHD-2 degradation can be assigned as arginines, the latter by alignment with known sequence regions and the former by the specificity of the limited tryptic cleavage producng peptide MT-3b.

Isolation and Sequence of p15 Cyanogen Bromide Peptides. The initial CNBr digest of p15 was fractionated on Sephadex G-50 (superfine), and eight peaks were detected (data not shown). The pools designated CNBr-1, -2, -4, -6, and -8 yielded interpretable sequence data following manual Edman degradation (see Table V). Degradations on the remaining pools yielded either no PTH amino acids or complex mixtures of several peptides.

CNBr-1 and CNBr-8 represent the amino-terminal sequence of p15. CNBr-2 and CNBr-4 also correspond to known sequence regions. The CNBr-2 sequence corresponds to residues 6–12 of p15 while the CNBr-4 sequence corresponds to residues 3–9 of MT-3b.

The sequence designated CNBr-6 overlaps the sequence of SPC-6 from residue 2 of CNBr-6 onward. The placement of a methionyl residue prior to the amino-terminal glutamic acid residue of CNBr-6, on the basis of CNBr specificity, further allows the overlapping of SPC-9 and CNBr-6. This is a rather weak overlap since it consists of only two residues which are not unique. However, the other known Met-Glu sequence occurs at positions 5 and 6 of the native p15 sequence and is thus well accounted for in a known sequence region.

No CNBr peptide corresponding to cleavage of the Met–Val bond at positions 21–22 of SPC-4 was recovered in the experiments described above. A second cyanogen bromide digest on reduced and alkylated p15 was therefore performed. The digest was fractioned by ion-exchange chromatography on SP-Sephadex (see Materials and Methods). Peptides detected by absorbance at 200 nm were desalted on Bio-Gel P-2 columns equilibrated in 0.1 N acetic acid and were lyophilized. End group analysis revealed a single peptide (eluting near 200 mM NaCl) with amino-terminal valine. This peptide was subjected to automated Edman degradation for 20 cycles using the 0.1 N Quadrol program of Brauer et al. (1975) and polybrene as carrier. The results of this degradation (CNBr-44) are presented in Table V. The CNBr-44 degradation data allows the sequences designated SPC-4 and MT-4a to be

Table VI: Peptide Compositions

	SPC-5	MT-1	CNBR-6	SPC-6	SPC-4	CNBR-44
CM-Cys	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (1)	0.87 (1)
Asx	6.04 (6)	4.65 (5)	1.13 (1)	2.02 (2)	3.79 (4)	1.91 (2)
Thr	4.91 (5)	2.00 (2)	0.09 (0)	0.06 (0)	1.08 (1)	0.85 (1)
Ser	3.89 (4)	2.89 (3)	0.12 (0)	0.84 (1)	1.87 (2)	0.94 (1)
Glx	5.27 (5)	4.21 (4)	1.96 (2)	1.90 (2)	2.12 (2)	1.12 (1)
Pro	3.68 (4)	3.83 (4)	1.84 (2)	1.97 (2)	1.64 (2)	0.08 (0)
Gly	1.99 (2)	4.96 (5)	4.23 (4)	3.86 (4)	5.75 (6)	3.84 (4)
Ala	4.15 (4)	4.76 (5)	1.96 (2)	2.08 (2)	1.77 (2)	0.06 (0)
Val	4.80 (5)	2.02 (2)	0.08 (0)	0.12 (0)	3.98 (4)	0.86 (1)
Met	2.87 (3)	1.83 (2)	0.00 (1)	1.83 (2)	0.83 (1)	0.00 (0)
Ile	4.22 (5)	5.35 (7)	2.65 (3)	3.51 (4)	1.81 (2)	1.03 (1)
Leu	5.16 (5)	2.13 (2)	0.11 (0)	0.16 (0)	10.41 (10)	5.94 (6)
Tyr	0.89 (1)	0.98 (1)	0.00 (0)	0.00 (0)	0.02 (0)	0.01 (0)
Phe	0.00 (0)	0.07 (0)	0.01 (0)	0.00 (0)	0.93 (1)	0.00 (0)
His	2.14 (2)	1.05 (1)	0.88 (1)	0.97 (1)	0.02 (0)	0.02 (0)
Lys	1.82 (2)	0.12 (0)	0.03 (0)	1.14 (1)	0.00 (0)	0.07 (0)
Arg	3.74 (4)	1.89 (2)	0.05 (0)	2.12 (2)	4.76 (5)	3.20 (3)
Trp	ND[a] (1)	ND[a] (1)	ND[a] (0)	ND[a] (0)	ND[a] (0)	ND[a] (0)
sequence position	1–58	29–74	58–73	59–81	82–124	104–124

[a] ND, not determined.

Table VII: p15 Composition[a]

	24	48	72	composite	sequence
CM-Cys	0.78	0.72	0.69	0.73	1
Asx	12.34	12.23	12.26	12.27	12
Thr	5.86	5.69	5.52	6.02	6
Ser	6.43	6.26	5.80	6.70	7
Glx	8.91	8.94	8.93	8.92	9
Pro	8.27	8.31	8.22	8.26	8
Gly	11.99	11.74	11.81	11.84	12
Ala	7.93	7.85	7.83	7.87	8
Val	7.82	8.20	8.46	8.46	9
Met	5.67	5.59	5.52	5.59	6
Ile	8.12	9.27	9.89	9.89	11
Leu	15.19	15.26	15.30	15.25	15
Tyr	0.84	0.87	0.86	0.85	1
Phe	1.02	0.98	1.05	1.01	1
His	2.69	2.92	2.81	2.80	3
Lys	3.15	3.11	3.09	3.11	3
Arg	10.87	10.76	10.94	10.85	11
Trp	ND[b]	ND[b]	ND[b]	ND[b]	1

[a] Composite values were determined by averaging 24-, 48-, and 72-h values, except for Ser and Thr which were extrapolated to zero time and Ile and Val for which the 72-h values were used. The Ile and Val values may be low because of slow hydrolysis of the Ile–Ile and Val–Ile bonds at positions 44–45, 15–16, and 84–85 of p15 (Figure 4). [b] ND, not determined.

linked. Moreover, the assignment of MT-4a as the carboxy-terminal peptide of p15 is supported since free leucine was detected following 20 cycles of Edman degradation on CN-Br-44.

Amino Acid Sequence and Confirming Data. The sequence data presented in Tables I–V define a continuous sequence of 124 amino acid residues for the p15 protein. This sequence is shown in Figure 4. The positions of the peptide overlaps used to generate this sequence are indicated schematically in Figure 3. The overlap of sequences determined for peptides SPC-9 and CNBr-6 is based, however, only on a two-residue overlap. To confirm this peptide order, 10 nM intact, succinnylated p15 was cleaved at the single tryptophan residue with BNPS-skatole (Omenn et al., 1970) and was subjected to automated Edman degradation. The results of this degradation are presented in Table IV. Since the α-amino group of p15 was succinnylated, only the amino acid sequence following the tryptophan residue at position 50 was revealed in this degradation. The results of this degradation completely confirm the overlapping of the SPC-9 and CNBr-6 sequences.

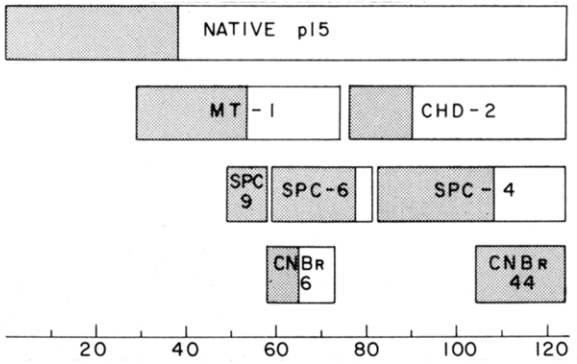

FIGURE 3: Schematic representation of peptides used to establish p15 amino acid sequence. The shaded regions indicate extent of sequential Edman degradations.

NH_2-Leu-Ala-Met-Thr-Met-Glu-His-Lys-Asp-Arg-Pro-Leu-Val-Arg-Val-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Ile-Leu-Thr-Asn-Thr-Gly-Ser-His-Pro-Val-Lys-Gln-Arg-Ser-Val-
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Tyr-Ile-Thr-Ala-Leu-Leu-Asp-Ser-Gly-Ala-Asp-Ile-Thr-Ile-Ile-
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

Ser-Glu-Glu-Asp-Trp-Pro-Ala-Asp-Arg-Pro-Val-Met-Glu-Ala-Ala-
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Asn-Pro-Gln-Ile-His-Gly-Ile-Gly-Gly-Gly-Ile-Pro-Met-Arg-Lys-
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75

Ser-Arg-Asp-Met-Ile-Glu-Val-Gly-Val-Ile-Asn-Arg-Asp-Gly-Ser-
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90

Leu-Glu-Arg-Pro-Leu-Leu-Leu-Phe-Pro-Ala-Val-Ala-Met-Val-Arg-
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105

Gly-Ser-Ile-Leu-Gly-Arg-Asp-Cys-Leu-Gln-Gly-Leu-Gly-Leu-Arg-
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120

Leu-Thr-Asn-Leu-COOH
121 122 123 124

FIGURE 4: Amino acid sequence of AMV p15.

The amino acid sequence determined here for p15 is supported by the amino composition determined for p15 (Tables VII) and by the amino acid compositions determined for several purified peptides of p15 (Table VI). Moreover, the

monomer molecular weight of 13 538 calculated for p15 from its sequence is also in good agreement with the molecular weight of 14 000–15 000 determined by gel filtration in the presence of 6 M guanidine hydrochloride (Fleissner, 1971; Vogt et al., 1975) and the molecular weight of 12 000 determined by $NaDodSO_4$–polyacrylamide gel electrophoresis (Fleissner, 1971).

Discussion

Avian myeloblastois virus, the etiologic agent in AMV complex, is itself replication defective, and recent studies have shown that it lacks p15 coding sequences (M. Hayman and T. Graf, unpublished experiments). The p15 purified from AMV complex is presumably encoded by the nondefective helper virus of the complex, but this helper virus has not been well characterized. Historically, the p15 from AMV complex has been called "AMV p15", but it is now clear that this nomenclature is potentially misleading. However, in the absence of a more desciptive alternative, we propose that the AMV p15 designation be retained with the understanding that the source of the protein is AMV complex.

The p15 sequence presented here differs at two positions from the partial sequence determined previously by our group (Niall et al., 1970). We originally reported residue 6 to be heterogeneous, containing both glutamic acid and threonine, and position 19 to be serine. In the present study we have found only glutamic acid at residue 6 and only asparagine at residue 19. We cannot rule out that the preparations of AMV complex used for these experiments were slightly different, but we feel it more likely that our original report, based on a single degradation, was in error.

p15 does not show extensive homology with any currently known protein sequences as determined by the computer SEARCH program of the National Biomedical Research Foundation [see Dayhoff (1976)]. At present, there are only partial sequences known for the other avian retrovirus *gag* proteins. Residues 1–8 of p19 (Palmiter et al., 1978) showed limited homology with residues 57–64 of p15 (four of eight residues are identical), but lack of further sequence information for p19 prevents a critical appraisal of this possible homology.

There are several interesting aspects of the AMV p15 sequence. Many of the charged residues occur in small clusters. This is best illustrated by the sequences Glu^{6}-His^{7}-Lys^{8}-Asp^{9}-Arg^{10}, Glu^{47}-Glu^{48}-Asp^{49}, and Arg^{74}-Lys^{75}-Ser^{76}-Arg^{77}-Asp^{78}. An association of basic and acidic residues seems to be especially favored. Six of the eleven arginine residues are either preceded or followed by an acidic residue. Furthermore, in each case where the acidic residue precedes the arginine, a proline residue follows, giving rise to the identical Asp-Arg-Pro sequences at positions 9–11 and 53–55 and the Glu-Arg-Pro sequence at 92–94. The sequences which follow these acidic-basic-prolyl clusters can be generally categorized as hydrophobic. In each case, the 14 residues following the cluster contain no more than two charged amino acids.

The single cysteine residue at position 113 of p15 merits special attention since the proteolytic activity associated with AMV p15 is inhibited by reagents such as *N*-ethylmaleimide, *p*-(chloromercuri)benzoic acid, and iodoacetate which react with free SH groups (Dittmar & Moelling, 1978; Vogt et al., 1979). p15 therefore superficially resembles active "thiol" proteases such as papain. There are, however, several problems with assigning Cys^{113} as an active-site residue or in considering p15 to be a thiol protease. First, the above-mentioned inactivating reagents will react with functional groups other than cysteine, and it has not been demonstrated that the single cysteine in native p15 is the sole site or even a site of modification by these reagents. Second, the active site SH group in papain and the related thiol proteases is superreactive toward reagents such as the α-halo acids and can also be alkylated by phenylmethanesulfonyl fluoride and TLCK [see Glazer & Smith (1971) and references cited therein]. In contrast, p15 reacts sluggishly or not at all with these reagents (Dittmar & Moelling, 1978). Finally, there is no extended amino acid sequence homology between the active-site sequences of papain, ficin, bromelain, chymopapain, and streptococcal protease and the sequence surrounding Cys^{113} of p15. This lack of homology is manifested in more than the primary structure. The thiol proteases named above all contain a glycine residue penultimate to the active-site cysteine residue. This glycine appears to be essential since a bulky side chain at this position in the tertiary structure would block access of substrate to the active site (Drenth et al., 1971). p15 contains the bulky Arg^{111} at the equivalent position, and thus it seems highly unlikely that the peptide backbone of p15 is folded in a manner similar to the active-site regions of the thiol proteases. In the absence of further studies, it would seem unwise to refer to p15 as a thiol protease.

The complete nucleotide sequence of genomic RNA from the Prague C strain of Rous sarcoma virus (Pr-RSV) has recently been determined (D. Schwartz, R. Tizard, and W. Gilbert, unpublished experiments). The p15 protein encoded by Pr-RSV and the p15 protein from AMV complex are highly homologous. These two species of p15 differ at no more than five amino acids positions. This high degree of amino acid conservation is not surprising, since the relatively small p15 protein probably faces the dual constraint of retaining proteolytic as well as structural function.

The RNA sequence of the *gag* region of Pr-RSV in combination with the p15 protein sequence and the partial protein sequences of p12, p19, and p27 (Wiesemann et al., 1978; Palmiter et al., 1978; Niall et al., 1970; R. Asbury, D. Allen, H. Niall, and R. Sauer, unpublished experiments) suggests that the coding order of the *gag* proteins in the genomic RNA is N-p19–p27–p12–p15-C. This order is consistent with the order predicted from pactamycin mapping studies (Shealy & Rueckert, 1978). Other studies, however, have reversed the p27–p12 positions (Vogt et al., 1975, 1979; Rettenmier et al., 1979). These latter results presumably reflect the experimental difficulty in correctly ordering p12 because of the relative paucity and difficulty of recovering marker peptides in this *gag* protein [see Vogt et al. (1979) for discussion].

In the Pr-RSV RNA sequence, the codon for the amino-terminal leucine of p15 directly follows the codon for the carboxy-terminal serine of p12, and an amber termination codon directly follows the codon for the carboxy-terminal residue of p15. Since p12 and p15 are contiguous in Pr76, and if the amber termination codon is present and utilized during translation in vivo, then a single proteolytic cleavage of the Ser–Leu peptide bond joining p12 and p15 should be sufficient to generate intact p15. However, some modification of this simple model may be required, since a precursor intermediate slightly larger and related to p15 has been observed (Vogt et al., 1975). At present, the source of the additional material in this precursor is unknown.

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