Chemical Characterization of Rauscher Leukaemia Virus Proteins

(Accepted 13 September 1978)

SUMMARY

The proteins of Rauscher murine leukaemia virus (R-MuLV) were characterized by amino acid analyses and by determination of their mol. wt. by gel filtration on cross-linked Sepharose 6B in 6 M-guanidine hydrochloride (GuHCl). Molecular weights of 56,000, 29,000, 15,000, 10,500 and 7,600 were found for gp70, p30, p15, p12 and p10 respectively. The amino acid compositions of these proteins and of p12E have been determined. The amino acid compositions of the p10 polypeptides of Rauscher-MuLV and Moloney-MuLV are very similar as are those of the p30 polypeptides, whereas the amino acid compositions of the p12 polypeptides differ considerably. P12E contains the highest percentage of hydrophobic amino acid residues. Among the gag-gene coded proteins, p15 contains the highest percentage of hydrophobic amino acid residues while p12 and p10 contain the lowest.

In recent years the proteins of Rauscher murine leukaemia virus (R-MuLV) have been isolated and characterized extensively by means of immunological methods. Chemical analyses have been limited so far to gp70 and p30. (The nomenclature of the proteins is that proposed by August et al. 1974.) Oroszlan et al. (1974; 1975) have characterized R-MuLVp30 and the p30 polypeptides of various other mammalian type-C viruses by isoelectric focusing, amino acid analyses and amino-terminal amino acid sequences. The isolation, amino acid composition and partial sequence of R-MuLVgp70 has been described recently by Marquardt et al. (1977) and Henderson et al. (1978). The amino acid compositions of the low mol. wt. gag-gene coded polypeptides p15, p12 and p10 have been determined for Moloney-MuLV (Parks et al. 1975). At present no chemical analyses have been reported for R-MuLV polypeptides p15, p12 and p10, nor for the envelope polypeptides p15E and p12E.

In this study the amino acid compositions of R-MuLV p15, p12E, p12 and p10, together with those of gp70 and p30, have been determined. In addition, the mol. wt. of these proteins and of gp70 and p30 have been determined, using the high resolving power of cross-linked Sepharose 6B (Sepharose CL-6B) in 6 M-guanidine hydrochloride (GuHCl).

Rauscher leukaemia virus, grown on JLS-V9 cells, was obtained as sucrose gradient purified preparations from Litton Bionetics, Inc. (lots no. 478, 623 and 641) through the courtesy of Dr J. Gruber (Office of Resources and Logistics, National Cancer Institute, Bethesda, U.S.A.). Two different methods were used for the preparation of samples of virus proteins. In method 1, 60 ml of R-MuLV in TNE buffer (10 mM-tris-HCl, pH 7.6, 100 mM-NaCl, 1 mM-EDTA) containing 60 mg protein was centrifuged for 2 h at 96,000 g and 4 °C. The pellet was suspended in 40 ml extraction buffer, containing 20 mM-tris-HCl, pH 7.8, 2.8 mM-2-mercaptoethanol, 10 % glycerol, 0.4 M-KCl and 1 % Nonidet P40 (NP40, Shell Chemicals). Undissolved material was removed by centrifuging at 82,000 g for 1 h and the pellet was subjected once more to extraction. The combined supernatant fractions were dialysed against distilled water, lyophilized and subsequently used for the preparation of a sample for gel filtration. In method 2, 30 ml of R-MuLV in TNE buffer, containing
30 mg protein was lyophilized and the dry residual powder was subsequently used for the preparation of a sample for gel filtration. Gel filtration was performed as described by Green & Bolognesi (1974). Cross-linked Sepharose 6B (Sepharose CL-6B of Pharmacia, Uppsala, Sweden) was used as a filtration medium because of its stability in 6 M-GuHCl (Ansari & Mage, 1977).

A single column of Sepharose CL-6B in 6 M-GuHCl was calibrated with mol. wt. marker proteins and subsequently used for virus protein separation. The distribution coefficient of an eluted protein was calculated according to the equation $K_d = \frac{V_e - V_o}{V_i}$, where $V_e$ is the elution volume of the protein, $V_o$ is the void volume of the column determined by chromatography of a sample of dextran blue 2000 and $V_i$ is the volume of solvent contained within the gel determined by subtracting the value of $V_o$ from the elution volume of 2-mercaptoprotoethanol. Fig. 1(a) shows the semi-logarithmic plot of the mol. wt. v. distribution coefficients. A linear relationship was found for proteins with mol. wt. up to 170000. The exclusion limit observed by extrapolation of the data in Fig. 1(a) is approx. 500000. The protein numbered 1 in Fig. 1(a) is present in various preparations of RNA tumour viruses grown on NC-37 cells (simian sarcoma virus, baboon endogenous virus and Mason-Pfizer monkey virus). A mol. wt. of 170000 for this protein, presumably of host cell origin, was determined by SDS-polyacrylamide gel electrophoresis.

When proteins were extracted from pelleted R-MuLV with buffer containing 0.4 M-KCl and 1% NP40 (method 1), 99.5% of the total protein was solubilized. When these extracted proteins were subjected to gel filtration, an elution pattern as shown in Fig. 1(b) was obtained. The proteins gp70, p30, p15 and p12 were recovered as homogeneous fractions from the column. The fraction containing p10 was slightly contaminated with p12 and was not subjected to further analysis. The void volume fraction ($V_o$) contained about 10% of the total applied protein. The major protein component in this fraction was p12E (Montelaro et al. 1978), which in SDS gel electrophoresis behaved like a 17000 mol. wt. protein (Fig. 1c, gel 3). By dialysing the void volume fraction against distilled water a precipitate was obtained. The precipitate was collected, dissolved in the elution buffer (6 M-GuHCl, 50 mM-sodium acetate, 0.005%, v/v, 2-mercaptoprotoethanol, pH 5.0) and again dialysed against distilled water. This procedure was repeated twice. The final precipitate contained only p12E (Fig. 1c, gel 4).

The chromatographic pattern obtained by gel filtration of a sample containing all the material present in a preparation of R-MuLV, including lipid and nucleic acid (method 2), was essentially the same as that shown in Fig. 1(b). The proteins p30, p15 and p12 were again recovered as homogeneous fractions. In this experiment a homogeneous preparation of p10 was also obtained, whereas in the preparation of gp70 contaminating proteins were also present. As shown in Fig. 1(c, gels 9 and 10), p12 and p10 had the same mobility in SDS gel electrophoresis. By means of amino acid analysis (see below) and
Table I. Amino acid compositions (Mol/100 Mol) of R-MuLV proteins

<table>
<thead>
<tr>
<th></th>
<th>gp70</th>
<th>p30</th>
<th>p15</th>
<th>p12E</th>
<th>p12</th>
<th>p10</th>
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<td>Asp*</td>
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<td>12.0</td>
<td>8.9</td>
<td>7.4</td>
<td>11.5</td>
<td>9.5</td>
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<tr>
<td>Thr†</td>
<td>9.2</td>
<td>5.5</td>
<td>7.9</td>
<td>7.1</td>
<td>8.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Ser‡</td>
<td>7.7</td>
<td>5.5</td>
<td>6.5</td>
<td>6.7</td>
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<td>14.6</td>
</tr>
<tr>
<td>Glu*</td>
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<td>17.0</td>
<td>8.7</td>
<td>10.9</td>
<td>7.9</td>
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<td>5.9</td>
<td>16.9</td>
<td>4.1</td>
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<tr>
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<td>Ala</td>
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<td>Val§</td>
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<tr>
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<tr>
<td>Tyr</td>
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<tr>
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<tr>
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<td>10.4</td>
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</tr>
<tr>
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<td>ND</td>
<td>1.0</td>
<td>ND</td>
</tr>
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<td>Hydrophobicity¶</td>
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<td>24.8</td>
<td>28.4</td>
<td>&gt;36.5</td>
<td>19.0</td>
<td>&gt;11.0</td>
</tr>
</tbody>
</table>

* Ammonia not determined.
† Extrapolated zero-time values.
‡ Determined as cysteic acid after performic acid oxidation (Hirs, 1967).
§ 72-h values.
∥ Determined spectrophotometrically (Edelhoch, 1967).
¶ Percentage hydrophobic amino acid residues (see text).
ND, not determined.

end-group analysis (Weiner et al. 1972) it was found that these proteins were not cross-
contaminated. Proline and alanine were found as the amino-terminal amino acids
of p12 and p10, respectively. Protein p15 migrated on SDS gels as an 18000 mol.
wt. protein (Fig. 1 c, gel 8). No amino-terminal amino acid could be identified for this
protein.

The distribution coefficients (Kd) of R-MuLV proteins in the experiments described
above were measured and the corresponding mol. wt. were determined using the calibration
plot shown in Fig. 1(a). Molecular weights of 56000, 29000, 15000, 10500 and 7600 were
found for gp70, p30, p15, p12 and p10 respectively.

Samples of 1 to 5 nmol of each of the purified R-MuLV proteins were hydrolysed in
5.7 M-HCl, 0.05 % 2-mercaptoethanol in sealed tubes under nitrogen for 24, 28 and 72 h
and dried in a rotary evaporator. Amino acid analyses were performed with a one column
system according to Dévényi (1968) on a Beckman Multichrom M amino acid analyser.
The results of these analyses are given in Table I. Amino acid analyses were performed on
preparations of p30, p15 and p12, isolated according to both methods described above.
As expected, the two preparations of each of these proteins gave identical results. P12
was subjected to DEAE chromatography (Green et al. 1973) to remove any traces of
contaminating material. The amino acid composition of the preparation of p12 remained
unchanged, confirming the purity of the preparations as established by SDS gel electro-
phoresis and amino-terminal amino acid analysis.

The preparation of gp70 used was isolated according to method 1 and that of p10
according to method 2.

The sum of the percentages of met, phe, cys, leu, ile, val, trp and his was taken as an
index of the hydrophobicity of the proteins (Rackovsky & Scheraga, 1977). Using this index, the order of hydrophobicity was calculated to be as follows:

\[ p_{12E} > p_{15}, g_{p70} > p_{30} > p_{12}, p_{10} \] (see Table 1).

Molecular weights have been reported previously, for gp70 by Marquardt et al. (1977) and for other R-MuLV proteins by Oroszlan et al. (1974), Barbacid et al. (1977) and Stephenson et al. (1977). The mol. wt. determined in our experiments by gel filtration on Sepharose CL-6B in 6 M-GuHCl are in good agreement with the values reported for p30, p15 and p12, but deviate for gp70 and p10.

For glycoproteins it has been agreed to use SDS gel electrophoresis to estimate mol. wt. (August et al. 1974). A mol. wt. of 67,000 for R-MuLV gp70 was determined by Marquardt et al. (1977), using SDS gels at various concentrations of polyacrylamide. We consistently found a mol. wt. of 70,000 for R-MuLV gp70, as determined by electrophoresis on 10%, 12.5% and 15% polyacrylamide – SDS gels, as well as on SDS slab gels with a linear gradient of 6 to 20% polyacrylamide. However, in our gel filtration experiments we found a mol. wt. value of 56,000 for R-MuLV gp70. This value corresponds well with the value of 58,000, calculated from physicochemical parameters by Moennig et al. (1974) for gp70 of Friend-MuLV. The main reason for the difference between mol. wt. values estimated by SDS gel electrophoresis and by other methods is the fact that glycoproteins behave quite anomalously in SDS gel electrophoresis as compared with mol. wt. marker proteins (Bretscher, 1971; Banker & Cotman, 1972; Frank & Rodbard, 1975). However, no data are available on the behaviour of glycoproteins compared to mol. wt. marker proteins upon gel filtration in 6 M-GuHCl. The true mol. wt. of R-MuLV gp70 might therefore be different from the value reported in this communication.

For R-MuLV p10 different values have been reported for its mol. wt. Estimates based on SDS gel electrophoresis are not very reliable for low mol. wt. proteins.

Long et al. (1977), using SDS gel electrophoresis, estimated a mol. wt. of about 8000 for R-MuLV p10. Barbacid et al. (1977) found a mol. wt. of 10,000 by gel filtration chromatography under various conditions and an even higher value, namely 11,500, by SDS gel electrophoresis. Our analysis on SDS gels (Fig. 1c) showed a mol. wt. for p10 of about 8000, confirming the result described by Long et al. (1977). This result was also in agreement with the mol. wt. determined by gel filtration chromatography in 6 M-GuHCl, in which a mol. wt. of 7600 was found for p10.

The amino acid composition as determined by us for R-MuLV p30 (Table 1) agrees very well with those determined before for the p30s of various murine type-C viruses (Oroszlan et al. 1974; Burnette et al. 1976). The same holds for the amino acid composition of R-MuLV gp70 as determined by Marquardt et al. (1977) and that presented in this communication.

A high content of arginine plus lysine (about 23%) was found for p10, in agreement with the nature of this component as a basic protein (Bolognesi et al. 1974; Stephenson et al. 1977). An even higher content of arginine plus lysine has been reported by Oroszlan (quoted in Long et al. 1977). Low contents of arginine plus lysine were found for gp70 and p12, confirming the acidic nature of these proteins (Bolognesi et al. 1974; Stephenson et al. 1977).

The amino acid composition of the virus envelope protein p15E has not been determined. As observed by Montelaro et al. (1978), the void volume fractions obtained after gel filtration in 6 M-GuHCl contain only a small amount of p15E. The major protein component in this fraction is p12E, which is derived from p15E by proteolytic cleavage (Karshin et al. 1977). Since p12E is only 6% smaller than p15E as determined by SDS gel electrophoresis
(Montelaro et al. 1978), we conclude that the data for p15E will probably not be very different from those obtained for p12E.

The order of hydrophobicity of the R-MuLV proteins as calculated from the content of hydrophobic amino acid residues is the same as that found experimentally by alkylagarose chromatography (Marcus & Smith, 1978). P12E is the most hydrophobic protein of R-MuLV.

P15 was calculated to be the most hydrophobic of the gag-gene coded proteins. P15 exhibits hydrophobic properties which are characteristic for membrane proteins (Barbacid & Aaronson, 1978).

The amino acid compositions of Moloney murine leukaemia virus (Mo-MuLV) proteins p30, p15, p12 and p10 have been described (Parks et al. 1975; Burnette et al. 1976). Comparison of the data presented in this communication for R-MuLV proteins with those obtained for the proteins of Mo-MuLV shows that the amino acid compositions of the p10 polypeptides like those of the p30 polypeptides are very similar. The amino acid compositions of the two p15 polypeptides are also very similar, but not to the same extent as those of the p10 and p30 polypeptides. In contrast, the amino acid compositions of the p12 polypeptides differ considerably.

It has been shown that the p30 polypeptides of mammalian type C viruses are a series of homologous proteins with very similar amino acid compositions (Oroszlan et al. 1975). The p10 polypeptides of mouse type C viruses are highly group-specific (Barbacid et al. 1976), whereas the p12 polypeptides contain strong type-specific antigenic determinants (Parks et al. 1975; Barbacid et al. 1976). The larger differences in amino acid compositions of the p12 polypeptides as compared to the p30 and p10 polypeptides of R-MuLV and Mo-MuLV are as expected from immunological data.

We are greatly indebted to Dr J. Gruber (National Cancer Institute, U.S.A.) for providing us with preparations of Rauscher leukaemia virus, to Dr W. J. H. M. Möller (Laboratory for Physiological Chemistry, University of Leiden, The Netherlands) for his kind permission to use his amino acid analyser, and to Dr P. Herbrink for valuable advice.

This study was supported by the Queen Wilhelmina Foundation, Netherlands Organization in the Fight against Cancer.

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REFERENCES


(Received 20 June 1978)