The Isoelectric Point of the p30 Polypeptide as a Marker of Mouse Endogenous Viruses

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SUMMARY

The isoelectric point (pI) of the p30 polypeptide of members of the three known classes of mouse C-type endogenous viruses was determined both by column and by thin-layer gel isoelectric focusing. Each class was found to be characterized by a particular variant of p30 (isop30), with pI values of 6.1 for class I (ecotropic), 5.7 for class II (xenotropic), and 5.5 for class III (NZB, NIH, ATS124, also xenotropic). The 6.1-isop30 was found as a minor component of rat-grown NZB virus and of a number of laboratory strains of mouse C-type viruses.

The genetic information for multiple C-type RNA tumour viruses has been shown to be naturally integrated within the DNA of normal mouse cells (Aaronson, Hartley & Todaro, 1969; Huebner et al. 1970; Lowy et al. 1971; Gelb et al. 1973). In the inbred strain BALB/c, which has been intensively studied, there exists genetic information coding for polypeptides of at least three distinguishable endogenous viruses (Stephenson, Tronick & Aaronson, 1974). These three classes were found to exist in many other mouse strains (Stephenson et al. 1975). By using column isoelectric focusing, we have shown previously that the major polypeptide, p30, of murine C-type viruses can exist in several forms (Chuat et al. 1973), which, for the sake of brevity, might be designated as isop30s, an expression coined on the analogy of isoenzymes. Stocks of laboratory strains of murine leukaemia viruses (MuLV) contain a mixture of isop30s (Oroszlan et al. 1974; Chuat, Laprevotte & Boiron, 1975). The isop30 composition, and in particular the pI of the major isop30 appear to be characteristic of a given virus strain (Chuat et al. 1975).

The present work was undertaken to measure the pI of the major isop30 of mouse endogenous viruses, and to determine whether endogenous viruses, on the one hand, and some widely used laboratory strains on the other hand, had any isop30 in common. For this, in addition to the column method, we used thin-layer isoelectric focusing (TLIEF) in polyacrylamide gels, which permits direct visual comparison of electrofocused polypeptides.

Endogenous viruses were supplied by the Office of Program Resources and Logistics, Viral Oncology, Maryland, U.S.A. (NCI-VO). BALB: virus 1 was grown in the NIH-3T3 cell line and BALB: virus 2 [as a Kirsten sarcoma virus (Ki-MSV) pseudotype; Aaronson & Stephenson, 1973; Aaronson & Dunn, 1974] in Fischer rat cells; Ki-MSV (NZB:MuLV) and Ki-MSV (NIH:MuLV) (Levy & Pincus, 1970; Levy, 1973) in the A673 human cell line, and NIH:MuLV (ATS124) (Todaro et al. 1973) on RD human rhabdomyosarcoma cells. Laboratory strain viruses were obtained either from NCI-VO or from producer cell lines maintained in this department. All the virus lots were purified by two successive sucrose gradient runs. Virus disruption was accomplished by tween-ether treatment as described by Chuat et al. 1973. Column isoelectric focusing was carried out using 1% carrier ampholytes (LKB-Produkter, Bromma, Sweden) for 65 h at 5 °C, under a constant potential of 300 V. The pH measurements and complement-fixation tests were performed...
Fig. 1. Thin-layer isoelectric focusing (pH range: 3.5 to 10) of murine C-type viruses: (a) Without urea; (b) in 6 M-urea. 1, Carbonic anhydrase (marker); 2, NIH virus; 3, NZB virus; 4, BALB:virus 2; 5, BALB:virus 1; 6, Moloney; 7, BALB: virus 1 (non-gradient-purified); 8, Gross; 9, AKR; 10, Friend.

on the collected fractions as described by Chuat et al. 1973. Anti-p30 serum was obtained by immunization of WFU rats, and from J. T. August (Strand & August, 1973). The p30 polypeptide was separated from the other viral and cellular proteins by gel filtration on a Sephadex G-100 column. The fractions were analysed for the presence of gp 70, p30, p12, p10 and bovine serum albumin by use of the appropriate antisera. These assays established the monospecificity of all the anti-p30 sera utilized. The homogeneity of the isop30s separated by isoelectric focusing was checked by SDS-polyacrylamide gel electrophoresis according to the method of Weber & Osborn (1969), as modified by Ahmad-Zadeh, Piguet & Colli (1971) for molecular weight plotting. Protein content of the various solutions was estimated by the methods of Lowry et al. (1951) or Warburg & Christian (1941), and adjusted to 1 mg/ml after lyophilization.
Short communications

Table I. Isop3o composition of various strains of murine C-type viruses

<table>
<thead>
<tr>
<th>Major isop3os</th>
<th>Minor isop3os†</th>
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<tbody>
<tr>
<td><strong>Endogenous viruses</strong></td>
<td></td>
</tr>
<tr>
<td>Class I (BALB: virus 1)</td>
<td>6.1</td>
</tr>
<tr>
<td>Class II (BALB: virus 2)</td>
<td>5.7</td>
</tr>
<tr>
<td>Class III</td>
<td></td>
</tr>
<tr>
<td>NIH Swiss</td>
<td>5.5</td>
</tr>
<tr>
<td>ATS124</td>
<td>5.5</td>
</tr>
<tr>
<td>NZB</td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Laboratory strains‡</strong></td>
<td></td>
</tr>
<tr>
<td>Graffi</td>
<td>5.2</td>
</tr>
<tr>
<td>Moloney</td>
<td>5.9</td>
</tr>
<tr>
<td>Friend</td>
<td>6.5</td>
</tr>
<tr>
<td>Rauscher</td>
<td>6.5</td>
</tr>
<tr>
<td>Gross</td>
<td>6.6</td>
</tr>
<tr>
<td>AKR</td>
<td>6.6</td>
</tr>
<tr>
<td>Wild (1504E)</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* The pI values given have been chosen to provide a basis for a convenient nomenclature of the various isop3os. Statistical evaluations give slightly different values, e.g. with a confidence interval of 5%, m = 6.09 ± 0.045 for the 6.1-isop30 (data of 8 column isoelectric focusing runs).
† Only those isop3os which have been characterized by immunological tests on column isoelectric focusing fractions combined with alignment studies in TLIEF are shown. The actual number of components is higher in most cases.
‡ Most of the viruses studied were obtained from NCI-VO and have well-documented histories. Several lots of each were analysed. These and other viruses were obtained from colleagues either as growth fluids or concentrates: Graffi-MuLV (S. Gisselbrecht, Paris), Rauscher-MuLV (J. P. Lévy, Paris), Moloney-MuLV (line E81, R. Bassin, Bethesda, Md.), Gross (rat line ERTh, H. Ioachim, New York), wild type (line 1504E, obtained from S. Oroszlan, Rockville, Md.).

TLIEF was carried out in polyacrylamide gels (T = 5%, C = 3%) 0.2 mm thick, containing 12.5% (w/v) sucrose, 5% carrier ampholytes of pH range 3.5 to 10, with or without urea (6 M), maintained at 8°C. The samples were applied directly on the gel surface at 0.5 cm from the anode, by means of a plastic frame fastened into position by a holder (Chuat & Pilon, 1977). Various amounts of protein (from 10 to 40 μg) were tested. The power was raised manually without ever exceeding 20 W. The overall focusing time was about 2 h. The pH gradient was determined by measuring the pH every cm by means of a surface microelectrode and by comparison with reference markers. The gel was stained with Coomassie brilliant blue R250 at 60°C (Vesterberg, 1972).

The results are shown in Table 1 and illustrated in Fig. 1, which also include, for comparison, results obtained with laboratory strain viruses. Due to their masking effect on minor components in complement-fixation tests, the number of peaks specifically identified by anti-p30 immune serum in column isoelectric focusing was predictably less than the number of isop3os recognized by Coomassie staining. Only those isop3os that could be immunologically identified appear in the Table. It can be seen that the major isop3os of the endogenous viruses examined fall into three classes, with pI values of 6.1, 5.7 and 5.5, respectively. Early studies of endogenous viruses had partially distinguished BALB: virus 1 from BALB: virus 2, mainly on the basis of seroneutralization and host range tests, and of the manner in which the cell regulates their expression, BALB: virus 1 being eco-N-tropic, spontaneously released from cell cultures and activated by halogenated pyrimidines, while BALB: virus 2 is xenotropic and also inducible by protein synthesis inhibitors (Aaronson & Stephenson, 1973; Aaronson & Dunn, 1974). However, host range and seroneutralization tests failed to distinguish between the various xenotropic viruses subsequently characterized. This was achieved by the detection of type-specific antigenic
determinants on the p12 polypeptide by radioimmunological techniques (Stephenson et al. 1974). Each mouse endogenous virus isolate could thus be placed in one of three classes (Stephenson et al. 1974, 1975). The present results show that these classes can also be distinguished by the pI of their major isop3o, namely 6.1 for class I (BALB: virus 1), 5.7 for class II (BALB: virus 2), and 5.5 for class III (NZB, NIH, and ATS124). The NZB virus stock studied, which was grown in rat cells, was found to contain a fair amount of the 6.1-isop3o. In contrast, the human cell-grown NIH virus sample had none (Fig. 1). The presence of BALB: virus 1 in this NZB virus stock is in agreement with the reported infectivity of BALB: virus 1 for rat (NRK) cells (Aaronson & Stephenson, 1973; Stephenson & Aaronson, 1973), which can support the growth of both ecotropic and xenotropic viruses. None of the prototype endogenous viruses was found to form the major component of the established strains investigated in parallel studies. The better resolution afforded by TLIEF, as compared to the column method, for isop3os with neighbouring pI values, has increased the number of laboratory strain subgroups delineated in our previous studies (Chuat et al. 1975), in particular in showing that the major isop3o shared by the Friend and Rauscher strains had a pI slightly lower than that of Gross virus. The subgroups recognized at the present time on the basis of the pI of the major isop3o as determined by the combined column and gel methods of isoelectric focusing include the following: wild-type (pI 7.0), Gross-AKR (pI 6.6), Friend–Rauscher (pI 6.5), Moloney (pI 5.9), and Graffi (pI 5.2).

The results of column (Table 1) and of TLIEF alignment studies in 6 M-urea (Fig. 1 b) show that many of the established strain harvests examined in the present work also contain the 6.1-isop3o in addition to their major isop3o. This is in keeping with the reported frequency of spontaneous expression of BALB: virus 1 in several mouse cell lines (Aaronson et al. 1969; Todaro, 1972; Aaronson & Dunn, 1974). The relative amount of BALB: virus 1 seemed to vary greatly in the course of time in the different harvests of a given cell line. This was observed in particular with the wild type virus-producing cell line 1504E (Gardner et al. 1973), where the incorporation of the 6.1-isop3o into virus particles sometimes overcame that of the 7.0-isop3o characteristic of wild-type virus (Table 1). The presence of class I endogenous virus in harvests of laboratory strains serving as a source of p3o for immunization or for the preparation of labelled or competing antigen in radioimmunological assays might introduce a bias in the specificity of the reaction observed. In particular, this might account in some measure for the differences in concentration of group-specific determinants or in antibody affinity noted for p3os isolated from different laboratory strains of mouse C-type viruses (Strand & August, 1974).

In conclusion, the three subgroups of murine endogenous viruses are quite distinct from the laboratory virus subgroups defined on the basis of the major isop3o characteristic of each strain. In view of its simplicity and elegance, TLIEF might constitute a valuable method for the routine typing of virus isolates. In comparison to radioimmunological techniques and isoelectric focusing in gel rods (Pfeffer, Pincus & Fleissner, 1976), it has the advantage of permitting a strictly comparative study of many different virus samples, without prior radioactive labelling. Isop3o composition should thus prove useful for evaluating the contribution of endogenous virus gene expression in virological studies involving mouse cells.

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