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RNA Tumor Virus Phosphoproteins: Primary Structural Analysis and Identification of Phosphopeptides

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Two-dimensional tryptic peptide mapping was used to compare the peptide sequences of the phosphoprotein (pp12) of cloned ecotropic and amphotropic wild mouse leukemia viruses, strains 1504 and 292. The maps of two ecotropic isolates were very similar to one another, as were the maps of two amphotropic isolates. There was also extensive similarity between the maps of this protein from ecotropic and amphotropic viruses, although characteristic peptide differences were readily recognized. These differences were consistent with the general type specificity of oncovirus phosphoproteins. The pp12 of the field isolate of 292 virus contained five phosphopeptides, and the non-phosphorylated and variously phosphorylated species of this pp12 showed identical peptide maps, indicating differential phosphorylation of a single polypeptide.

Structural proteins of type C RNA tumor viruses consist of a major high-molecular-weight glycoprotein and several low-molecular-weight non-glycosylated proteins. One of the non-glycosylated proteins has been shown to be the major structural phosphoprotein of the virion (13, 14). In viruses of lower mammalian species, including mice, rats, cats, minks, and pigs, the 12,000-dalton protein (p12) is always phosphorylated (pp12) (13, 14; our unpublished data). However, the analogous phosphoprotein in viruses of primate origin, such as RD-114 and baboon type C virus, is pp15 (13), and in avian viruses it is pp19 (12). These phosphoproteins are present in the virion in multiple but specific phosphorylated states (9, 10, 13), all of which are located in the core structure of the virion (14a). Viral phosphoproteins exhibit the most highly type-specific immunological characteristic among all the *gag* gene-coded structural proteins (6, 19, 20; J. R. Stephenson, S. G. Devare, and F. H. Reynolds, Jr., Adv. Cancer Res., in press), and they show specificity in binding to homologous viral RNA (17, 18).

Recently we examined the pp12 of different field, ecotropic, and amphotropic clonal isolates (1, 8, 16) of wild mouse leukemia viruses for the distribution patterns of the variously charged molecular species (10). Here we extend those studies with the wild mouse leukemia virus phosphoprotein and report our results of two-dimensional tryptic peptide analysis of the pp12 from ecotropic and amphotropic isolates. We have also identified the phosphopeptides and compared the peptide maps of the variously charged species of the pp12 of a field isolate.

(A preliminary account of these findings was presented at the Cold Spring Harbor Meeting on RNA Tumor Viruses, 25-29 May 1977.)

Tryptic peptide maps of the pp12 of cloned ecotropic and amphotropic components of wild mouse leukemia virus, strain 1504, and their mixture are shown in Fig. 1a through c. Fourteen major radioiodinated tryptic peptides were obtained with the amphotropic virus (Fig. 1b), and 13 were obtained with the ecotropic virus (Fig. 1a). These peptides may not necessarily be all tyrosine-containing peptides, as the formation of iodohistidine, iodotryptophan, and stable sulfenyl iodide has been reported under the experimental conditions used (5). Ten of the iodinated peptides were common between the pp12 polypeptides of these two host range classes, as shown in the schematic drawing of the composite maps (Fig. 1d). The unique peptides of the pp12 from ecotropic and amphotropic 1504 viruses were mapped in mirror-image positions (Fig. 1d). Although the significance of this interesting property is not clear, it may be related to primary structural differences in these unique peptides. The possibility that the mirror-image migration may be a result of the degree of phosphorylation is not very likely, because under the experimental conditions of electrophoresis at pH 1.8 and chromatography with relatively nonionic solvents, phosphorylation of peptides should not have a significant effect on their mobility. Moreover, some of the common rather than the unique peptides were detected as phosphorylated peptides, as described below.

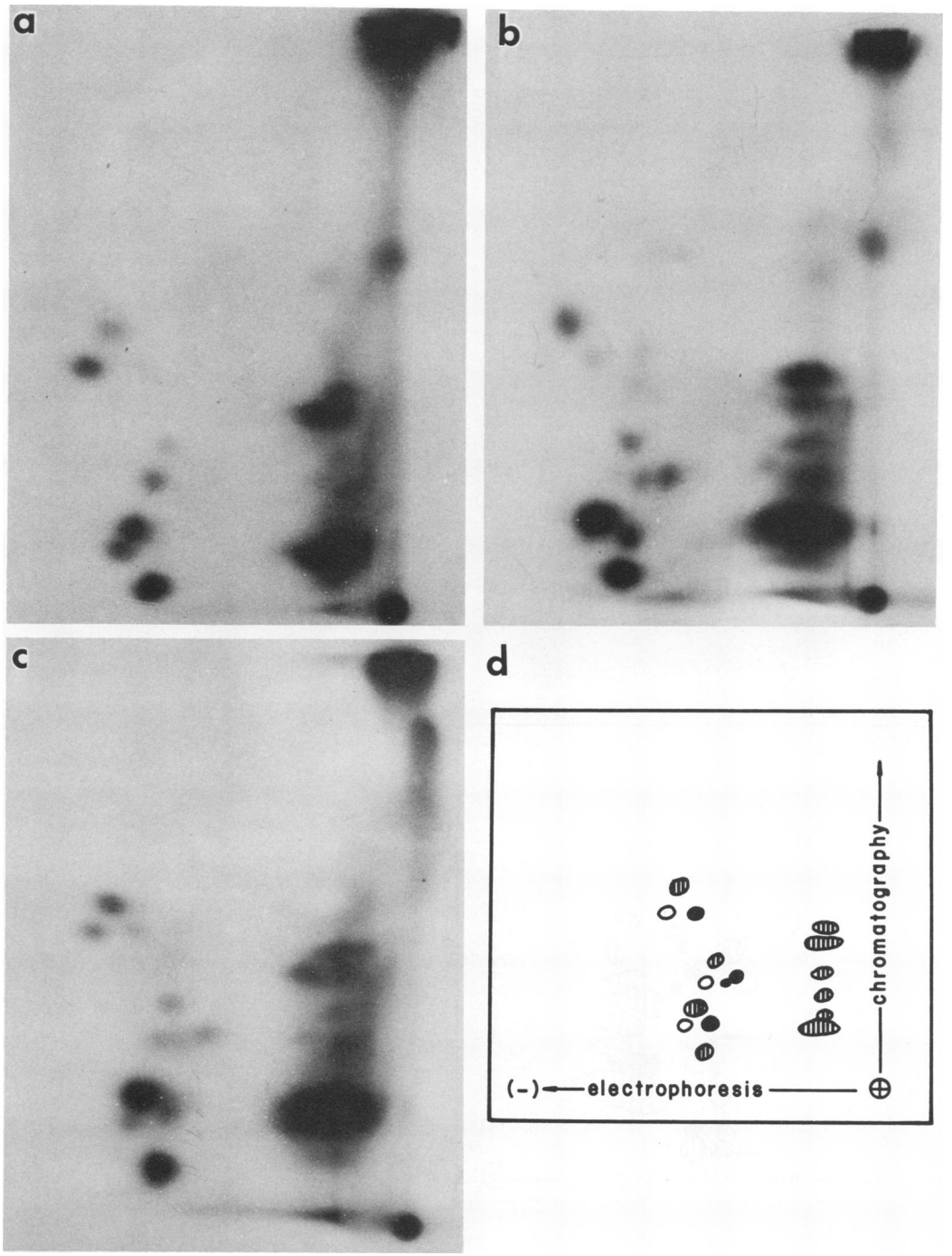

FIG. 1. *Two-dimensional tryptic peptide maps of ^{125}I-labeled pp12 from ecotropic and amphotropic 1504 virus. The pp12 fraction of cloned ecotropic and amphotropic 1504 virus labeled with ^{3}H-amino acid mixture was purified by guanidine-agarose chromatography (15). Isolated pp12 fraction was iodinated by the chloramine T method (7), and the purity of the radioiodinated pp12 was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (11); it showed a single major band of about 12,000 daltons. The radioiodinated pp12 fraction was then analyzed by two-dimensional tryptic peptide mapping procedure, as described previously (3). About 1 to 3 μl of tryptic digest of radioiodinated pp12 containing approximately 10^{6} cpm was spotted on a cellulose-coated thin-layer chromatography plate (E.M. Labs; 10 by 10 cm) and subjected to electrophoresis at 600 V for 30 min with a buffer system of formic acid-acetic acid-water (5:15:80; pH 1.8). The plates were dried at room temperature and chromatographed in the second dimension with a buffer system of n-butanol-pyridine-acetic acid-water (32:25:5:20). After the run, the plates were dried and analyzed by autoradiography with Kodak RP X-ray film. (a) pp12 of ecotropic 1504 virus; (b) pp12 of amphotropic 1504 virus; (c) mixed pp12 sample (1:1) from 1504 ecotropic and amphotropic viruses; (d) schematic drawing of the composite map as shown in (c). Symbols, ◍, common peptides; ○, peptides unique to ecotropic virus; ●, peptides unique to amphotropic virus.*

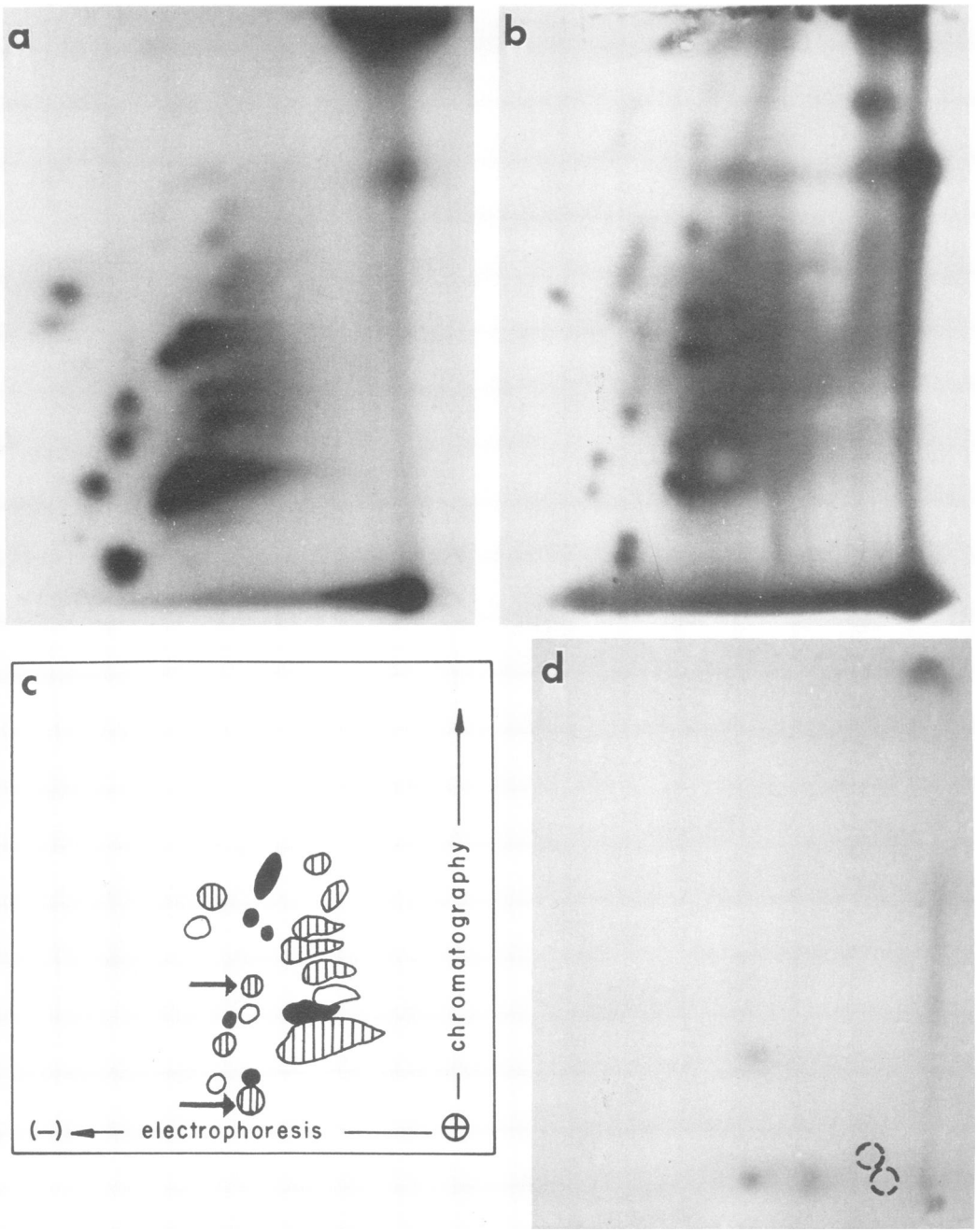

FIG. 2. *Two-dimensional tryptic peptide analysis of the pp12 of 292 virus and identification of phosphopeptides. Tryptic peptide mapping was carried out as described in the legend to Fig. 1. (a) ^{125}I-labeled pp12 from ecotropic 292 virus. (b) ^{125}I-labeled pp12 from amphotropic 292 virus. (c) Schematic composite drawing of pp12 of ecotropic and amphotropic 292 virus. Symbols: ◍, common peptides; ○, peptides unique to ecotropic virus; ●, peptides unique to amphotropic virus. Arrows identify the iodinated peptides corresponding to major phosphopeptides. For phosphopeptide mapping, pp12 of 292 field isolate, labeled with [^{32}P]phosphate, was subjected to a similar mapping procedure without going through the radioiodination step. (d) Phosphopeptides containing [^{32}P], detected by autoradiography. ○ indicates the positions of the two minor phosphopeptides.*

These structural characteristics of the phosphoprotein of wild mouse ecotropic and amphotropic viruses were also observed in the tryptic peptide maps of the pp12 of cloned subpopulations of another isolate, strain 292 (Fig. 2a-c). In addition, a comparison of the peptide maps shown in Fig. 1 and 2 revealed that, whereas the peptide patterns were almost identical for the pp12 of ecotropic 1504 and 292 viruses (Fig. 1a and 2a), the maps of the polypeptide of the amphotropic 292 virus contained three additional radioiodinated peptides that were not detected in the pp12 of amphotropic 1504 virus (Fig. 1b and 2b).

The autoradiogram of the tryptic peptide maps generated from pp12 of the 292 field isolate, labeled only with [^{32}P]phosphate, showed three major phosphopeptides (Fig. 2d). Of these three major phosphopeptides, two were readily recognized in the maps of the iodinated peptides (Fig. 2c, shown with arrows). In addition to these phosphopeptides, the positions of two other peptides containing low levels of ^{32}P radioactivity are indicated by dotted circles (Fig. 2d). Although these two minor spots were visible on the original X-ray plates, they were only faintly reproduced in the photograph. Identification of these three major and two minor phosphopeptides in the pp12 of 292 virus suggested that there are at least five and more likely a higher number of phosphorylation sites on this polypeptide. The intensity differences between the spots may indicate that the phosphopeptides contained variable amounts of [^{32}P]phosphate; and, therefore, more than one phosphorylation site may be present in the major phosphopeptides detected.

The pp12 of 292 field isolate was also used to determine peptide homology between the non-phosphorylated and variously phosphorylated pp12 species. The pp12 purified from 292 virus, labeled with ^{3}H-amino acids and [^{32}P]phosphate (13, 14), was resolved by DEAE-cellulose chromatography (18) into three major peaks. One was non-phosphorylated, and the other two varied in the degree of phosphorylation (data not shown). The molecular size of these non-phosphorylated and phosphorylated species was about 12,000 in sodium dodecyl sulfate polyacrylamide gels (11, 15), indicating that the subspecies were not due to protein degradation. These individual pp12 species were radioiodinated and analyzed for two-dimensional tryptic peptide patterns. The results, exhibiting identical maps (data not shown), suggested that the various charged species of pp12 represented the same polypeptide sequence. Similar results have also been reported with the lowly and highly phosphorylated species of avian pp19 (4, 9).

The data reported in this paper show that in spite of the presence of extensive homology, characteristic differences are present in the peptide maps of the phosphoprotein of ecotropic and amphotropic wild mouse leukemia viruses. The unique peptides, which are not phosphorylated, move in the two-dimensional maps in a mirror-image fashion. These observations are consistent with, but not proof of, the possible amino acid sequence differences in parts of the pp12 molecule of the ecotropic and amphotropic viruses. Previous analysis of the tryptic peptides of pp12 of Gross, Moloney, and Rauscher murine leukemia viruses also suggested that the unique peptides might be related to the strong type-specific serological reactivity of this protein (2). The peptide differences in pp12 of ecotropic and amphotropic wild mouse leukemia viruses may, therefore, predict the presence of immunological type specificity in this polypeptide of these two host range classes of virus. Furthermore, the close similarity between the tryptic peptide maps of two different wild mouse ecotropic isolates and between the maps of two different amphotropic isolates suggest that such maps of pp12, like those of gp70 (1a, 3), may be used as a distinguishing feature of ecotropic and amphotropic wild mouse virus classes.

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LITERATURE CITED

1. **Bryant, M. L., and V. Klement.** 1976. Clonal heterogeneity of wild mouse leukemia viruses: host range and antigenicity. Virology **73**:432-436.
1a. **Bryant, M. L., B. K. Pal, M. B. Gardner, J. H. Elder, F. C. Jensen, and R. A. Lerner.** 1978. Structural analysis of the major envelope glycoprotein (gp70) of the amphotropic and ecotropic type C viruses of wild mice. Virology **84**:348-358.
2. **Buchhagen, D. L., O. Stutman, and E. Fleissner.** 1975. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. IV. Biochemical typing of murine viral proteins. J. Virol. **15**:1148-1157.
3. **Elder, J. H., F. C. Jensen, M. L. Bryant, and R. A. Lerner.** 1977. Polymorphism of the major envelope glycoprotein (gp70) of murine type C viruses: virion associated and differentiation antigens. Nature (London) **267**:23-28.
4. **Erikson, E., J. S. Brugge, and R. L. Erikson.** 1977. Phosphorylated and nonphosphorylated forms of avian sarcoma virus polypeptide p19. Virology **80**:177-185.
5. **Glazer, A. N.** 1970. Specific chemical modification of proteins. Annu. Rev. Biochem. **39**:101-130.
6. **Green, R. W., D. P. Bolognesi, W. Schafer, L. Pister, G. Hunsmann, and F. deNoronha.** 1973. Polypeptides of mammalian oncornaviruses. I. Isolation and serological analysis of polypeptides from murine and feline C-type viruses. Virology **56**:565-579.
7. **Greenwood, F. C., W. M. Hunter, and J. S. Glover.**

1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. **89**:114–123.

8. **Hartley, J. W., and W. P. Rowe.** 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. J. Virol. **19**:19–25.
9. **Hayman, E. G., B. K. Pal, M. M. C. Lai, and P. Roy-Burman.** 1977. Major phosphoprotein of avian sarcoma virus: peptide analysis of the variously charged species. Biochem. Biophys. Res. Commun. **78**:1156–1161.
10. **Hayman, E. G., B. K. Pal, and P. Roy-Burman.** 1977. RNA tumor virus phosphoproteins: evidence for virus specificity of phosphorylation. J. Gen. Virol. **36**: 459–469.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Lai, M. M. C.** 1976. Phosphoproteins of Rous sarcoma virus. Virology **74**:287–301.
13. **Pal, B. K., R. M. McAllister, M. B. Gardner, and P. Roy-Burman.** 1975. Comparative studies on the structural phosphoproteins of mammalian type C viruses. J. Virol. **16**:123–131.
14. **Pal, B. K., and P. Roy-Burman.** 1975. Phosphoproteins: structural components of oncornaviruses. J. Virol. **15**:540–549.

14a. **Pal, B. K., and P. Roy-Burman.** 1977. RNA tumor virus phosphoproteins: subvirion location of the multiple phosphorylated species. Virology **83**:423–427.

15. **Pal, B. K., M. Wright, J. E. Officer, M. B. Gardner, and P. Roy-Burman.** 1973. Subviral components of a wild mouse embryo-derived type C oncornavirus. Virology **56**:189–197.
16. **Rasheed, S., M. B. Gardner, and E. Chan.** 1976. Amphotropic host range of naturally occurring wild mouse leukemia viruses. J. Virol. **19**:13–18.
17. **Sen, A., C. J. Sherr, and G. J. Todaro.** 1976. A specific binding of type C viral core protein p12 with purified viral RNA. Cell **7**:21–32.
18. **Sen, A., C. J. Sherr, and G. J. Todaro.** 1977. Phosphorylation of murine type C viral p12 proteins regulates their extent of binding to the homologous viral RNA. Cell **10**:489–496.
19. **Stephenson, J. R., R. K. Reynolds, and S. A. Aaronson.** 1976. Comparisons of the immunological properties of two structural polypeptides of type C RNA viruses endogenous to old world monkeys. J. Virol. **17**:374–384.
20. **Tronick, S. R., J. R. Stephenson, and S. A. Aaronson.** 1973. Immunological characterization of a low molecular weight polypeptide of murine leukemia virus. Virology **54**:199–206.