JOURNAL OF VIROLOGY, Nov. 1970, p. 695-698
Vol. 6, No. 5

Proteins of Avian Tumor Viruses with Different Coat Antigens

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Received for publication 29 June 1970

Isoelectric focusing of avian tumor viruses with distinct type-specific envelope antigens demonstrated no differences in isoelectric points. Viruses with different type-specific antigens were found to contain different glycoprotein components when virion proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The type-specific antigen of the avian tumor virus (ATV) is considered to be on the virion surface because it reacts with virus-neutralizing antiserum (8). The ATV have been placed in subgroups on the basis of their host range in genetically different types of chicken cells and in other avian cells (4, 18). The type-specific antigens of viruses in subgroups A, B, and C generally do not cross-react (4, 8, 10), and specific interference is generally not observed between viruses with different type-specific antigens (4, 5, 19). Viruses with different type-specific antigens not only differ in host range but may also differ in the efficiency with which they infect cells. For example, Rous associated virus-1 (RAV-1) appears to infect cells with great efficiency. RAV-2, Schmidt-Ruppin Rous sarcoma virus (SR-RSV), and RSVβ(0) (7, 16) appear to infect susceptible cells with an intermediate efficiency which can be enhanced by the presence of the polycation diethylaminoethyl (DEAE)-dextran (17) and by extracts of RAV-1 infected cells (6, 7). RSVα(0) (7) has not been shown to be infectious on any cell type tested.

Little work has been done to characterize the ATV type-specific antigens. There is a recent report of the detection of a trypsin- and chymotrypsin-sensitive component in Tween 20-disrupted ATV which reacted with homologous virus-neutralizing antiserum in agar-gel diffusion, adsorbed homologous virus-neutralizing antiserum, induced neutralizing antibody for homologous virus in rabbits, and induced early interference for homologous viruses (15). No further chemical characterization of this antigen was done.

To compare the overall charge of viruses with distinct type-specific antigens, viruses labeled with amino acids containing ^{14}C or ^{3}H were compared by electrofocusing in LKB ampholine *p*H gradients (14). Figure 1 shows that the Bryan high-titer strain (BH) RSV(RAV-1) labeled with a mixture of ^{14}C-amino acids did not differ significantly in isoelectric point from ^{3}H-amino acid-labeled (a) SR-RSV, (b) BH RSV(RAV-2), or (c) RSVα(0). The A_{280} tracing shows the position of hemoglobin with an isoelectric point of *p*H 7.5. Multiple experiments failed to show any measurable differences in the isoelectric points of these viruses. ^{3}H-BH-RSV (RAV-2) was indistinguishable from ^{14}C-RSVβ (0) (Fig. 1d). Thus any differences in over all charge between viruses with different type-specific antigens were too small to be detected by this method and over all surface charge differences probably do not account for the differences in the efficiency with which avian tumor viruses with different type-specific antigens infect cells. The isoelectric point of each virus was about *p*H 3.9 to 4.0, indicating the presence of acidic groups on the virus surface. Phospholipid in the viral envelope could contribute such acidic groups.

Electrofocusing represents a method for further virus purification after sedimentation in sucrose density gradients (12). Unfortunately, these viruses lose infectivity at *p*H 4.0 so that infectious virus could not be recovered. Prolonged incubation in the electrofocusing column beyond 36 to 48 hr resulted in gradual breakdown of virus. The first protein components to be released were two proteins with isoelectric points around *p*H 9.0 and 7.0 (Fig. 1b and c). The 70*S* viral ribonucleic acid was also degraded to 4*S* pieces.

To compare the dissociated protein components of two viruses with distinct type-specific antigens, RAV-1 and RSVβ(0) labeled with ^{14}C- or ^{3}H-amino acid mixtures and with ^{3}H-

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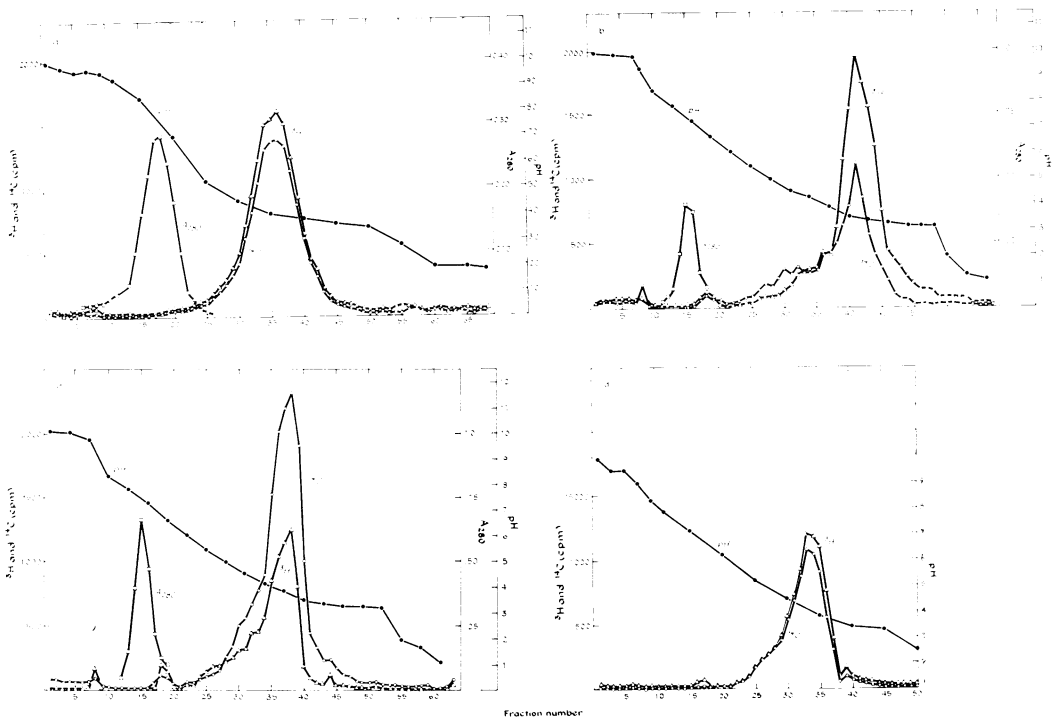

FIG. 1. *Isoelectric focusing of purified viruses with different type-specific antigens. Tissue culture methods for growing cells and viruses (9) and isolating RSV(0) have been described (11). RSVβ(0) and RSVα(0) were distinguished by their ability to make foci on Japanese quail cells (7). A description of the biological and antigenic properties of the viruses used will be published (unpublished data). Viruses were labeled in tissue culture with similar mixtures of 15 ^{3}H (1 to 10 Ci/mM) or ^{14}C (10 to 100 mCi/mM) L-amino acids (New England Nuclear Corp.) added to 199 based growth medium 1 (9) containing one-tenth the regular amino acid concentration (^{14}C, 1 to 2.5 μCi/ml and ^{3}H, 10 to 20 μCi/ml). A 6-ml amount of radioactive medium per 100-mm culture dish was changed at 12-hr intervals four times. Viruses were purified from culture medium (12) for isoelectric focusing (14) with 2 mg of human hemoglobin carrier in a 110-ml LKB column by using 1% ampholine (LKB) in a 5 to 20% sucrose density gradient at 600 v, 2 C, for 48 hr. Fractions were collected and pH (●), A_{280} (□), and trichloroacetic acid-precipitable ^{14}C (○) and ^{3}H (△) were determined. (a) ^{14}C-RSV(RAV-1) and ^{3}H-SR-RSV, (b) ^{14}C-RSV(RAV-1) and ^{3}H-RSV(RAV-2), (c) ^{14}C-RSV(RAV-1) and ^{3}H-BH-RSVα(0), and (d) ^{14}C BH-RSVβ(0) and ^{3}H RSV(RAV-2).*

glucosamine were prepared. It is known that ATV contain carbohydrate (2), and radioactive glucosamine has been shown to be readily incorporated into avian myeloblastosis virus (AMV) grown in tissue culture (1).

Figure 2a shows the results of coelectrophoresis of ^{14}C-amino acid-RSVβ(0) and ^{3}H-amino acid-RAV-1 in a polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) after dissociation of the viruses in 1% SDS, 4 M urea, 1% 2-mercaptoethanol at 37 C for 30 min. Under these conditions, all of the radioactivity penetrated the gel. At least eight protein components can be identified in these viruses and they are numbered P-1 to P-8. P-5 and 6 and P-7 and 8 were not completely separated in this experiment, but the two proteins of each pair have clearly distinct isoelectric points in electrofocusing (*unpublished data*). Of the slowly moving, amino acid-labeled proteins, P-1 was the major protein in RSVβ(0) (peak in fraction 23) and P-2 was the major protein in RAV-1 (peak in fraction 30). In addition, the protein component from RAV-1 in the region of P-3 (peak in fraction 55) migrated slightly faster than the closely migrating protein from RSVβ(0) (peak in fractions 58 to 59). The electrophoretic profiles of ^{14}C-amino acid-RAV-1 and ^{3}H-amino acid-labeled RAV-1 were shown to be indistinguishable as were the profiles of RSVβ(0) labeled with each isotope. Components P-4, P-5 + 6, and P-7 + 8 correspond, respectively, to the previously described components RSV-3, RSV-2, and RSV-1 and show no difference in electrophoretic mobility for proteins isolated from viruses with different type-specific antigens (3).

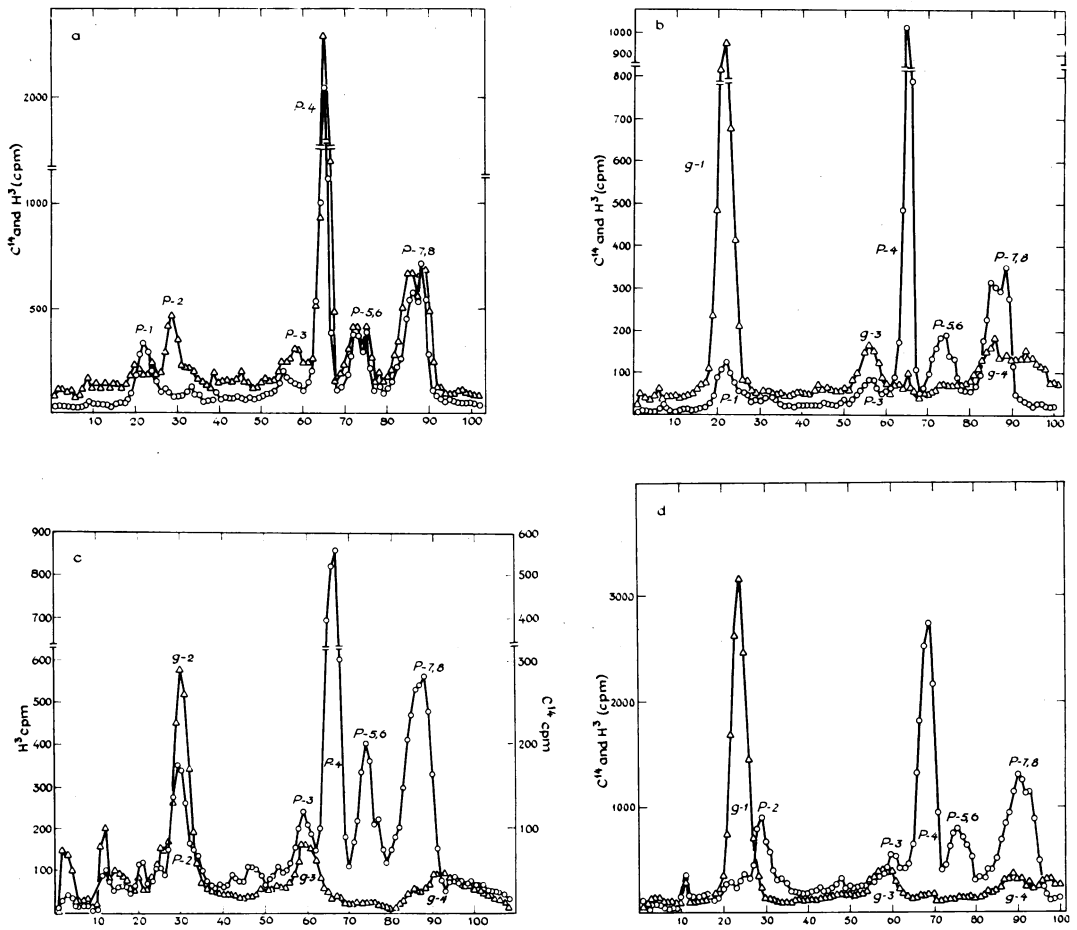

FIG. 2. *Polyacrylamide gel-SDS electrophoresis of dissociated RAV-1 and RSVβ(0). RAV-1 and RSV-β(0) were labeled with a mixture of* ^{14}C *or* ^{3}H *L-amino acids and purified as described in Fig. 1. In a similar fashion, virus was labeled with* ^{3}H*-glucosamine (10 Ci/mM), 10 μCi/ml in growth medium 1 (9). Viruses were dissociated with 1% SDS, 4 M urea, and 1% 2-mercaptoethanol at 37 C for 30 min. Electrophoresis was carried out in 7% bisacrylamide gels, 11 cm long and containing 0.1% SDS and 0.01 M sodium phosphate (pH 7.2) at 10 ma per gel column (13). Gel slices were shaken in 0.7 ml of water for 2 hr and then 10 ml of Aquasol (New England Nuclear) was added for scintillation counting. (a)* ^{14}C*-amino acid-RSVβ(0), ○, and* ^{3}H*-amino acid RAV-1, △; (b)* ^{3}H*-glucosamine-RSVβ(0), △, and* ^{14}C*-amino acid RSVβ(0), ○; (c)* ^{3}H*-glucosamine-RAV-1, △, and* ^{14}C*-amino acid RAV-1, ○, and (d)* ^{3}H*-glucosamine-RSVβ(0), △, and* ^{14}C*-amino acid RAV-1, ○.*

Figure 2b shows the results of coelectrophoresis of ^{14}C-amino acid-RSVβ(0) and ^{3}H-glucosamine-RSVβ(0). Three major components labeled with ^{3}H-glucosamine in this virus are designated g-1, 3, and 4. G-1 had the same mobility as P-1 and g-3 the same as P-3. G-4 was more heterogeneous and migrated in a broad band with P-7 and 8.

Figure 2c shows the results of coelectrophoresis of ^{3}H-glucosamine-RAV-1 and ^{14}C-amino acid-RAV-1. The major ^{3}H-glucosamine-labeled components, g-2 and g-3, had the same mobilities as ^{14}C-amino acid-labeled components P-2 (peak in fraction 30) and P-3 (peak in fraction 59) respectively. As with RSVβ(0), there was some fast moving heterogeneous ^{3}H-glucosamine-labeled material (g-4, fractions 85 to 105). The radioactive peaks in fractions 2 and 12 did not appear in other experiments with these viruses and may represent aggregated material. ^{3}H-glucosamine-labeled components g-1, g-2, and g-3 migrated with amino acid-labeled P-1, P-2, and P-3, respectively, in electrofocusing as well as in SDS-acrylamide gel electrophoresis indicating that P-1, P-2, and P-3 are probably glycoproteins (*unpublished data*). They contain about

5% of the radioactive amino acid in the total virion protein. ^{3}H-labeled g-4 did not follow P-7 or P-8 in electrofocusing and does not appear to be a glycoprotein (*unpublished data*).

Figure 2d shows the results of coelectrophoresis of ^{3}H-glucosamine-RSVβ(0) and ^{14}C-amino acid-RAV-1. In agreement with the results in Fig. 2a and b, the major ^{3}H-glucosamine-labeled component of RSVβ(0) with a peak in fraction 24 (g-1) moved more slowly than the ^{14}C-amino acid-labeled RAV-1 component P-2 (peak in fraction 30). Similarly, glucosamine-labeled component g-3 (peak in fraction 58) moved more slowly than amino acid-labeled P-3 (peak in fraction 60 and 61).

We have also found significant differences in the electrophoretic mobilities of glycoproteins in RSV(RAV-1), RSV(RAV-2), and SR-RSV (10). Duesberg, Martin, and Vogt (*personal communication*) have also recently shown differences in electrophoretic mobilities of glycoprotein components in RSV(RAV-1) and RSV(0).

These experiments indicate that viruses with different type-specific surface antigens have glycoprotein components with different mobilities in SDS-polyacrylamide gel electrophoresis, suggesting that the glycoproteins are related to the type-specific antigen.

We thank Virginia Rimer and Nona Stone for excellent technical assistance.

This work was supported by Public Health Service grant CA 10467 from the National Cancer Institute. D. D. R. was supported by Public Health Service training grant AI 0185 from the Institute of Allergy and Infectious Diseases.

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