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# Structural Proteins of Equine Infectious Anemia Virus

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Equine infectious anemia virus was found to be comprised of fourteen polypeptides of molecular weight ranging from 10,000 to 79,000. Eighty percent of the virion protein was accounted for by five polypeptides, including two non-glycosylated components (p29 and p13) comprising one-half of the virion protein and three glycoproteins (gp77/79, gp64, and gp40).

Equine infectious anemia virus (EIAV) is a persistent RNA virus of horses which causes a recurrent anemia of immunopathological origin (10, 11). EIAV has properties characteristic of the RNA tumor virus group and related slow viruses (family *Retroviridae* [8]), including (i) initiation of an in vitro noncytocidal persistent infection of fibroblasts of the natural host (14), (ii) ultrastructural appearance (15, 21), (iii) sensitivity to iododeoxyuridine (13), (iv) virion-associated RNA-dependent DNA polymerase (1, 3), and (v) subunit structure of the viral RNA (4).

We report here a study by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the structural proteins of EIAV produced by persistently infected equine fibroblasts. The Wyoming strain of EIAV, adapted to continuous replication in equine fibroblasts (14), was used. Virus was produced in roller bottle cultures of equine dermal cells as previously described (1). Medium was collected at intervals of 48 to 72 h, stored at 4°C, and used as the source of unlabeled virus. For some experiments virus was grown in primary cultures of equine macrophages (13) or obtained from plasma of acutely infected horses.

Figure 1a shows a polyacrylamide gel stained with Coomassie brilliant blue after electrophoresis of SDS-disrupted EIAV. The absorbancy tracing at 570 nm of this gel is shown in Fig. 1c. Fourteen bands were resolved. The most prominently stained bands are 1, 2, 3, 8, 9, and 12. Polypeptides 4, 5, 10, 11, 13, and 14 are present in very low amounts and are difficult to demonstrate photographically; they are, however, readily apparent from the densitometer tracing and by visual examination of the gels.

The molecular weights of EIAV polypeptides were estimated from their electrophoretic mobility in SDS-polyacrylamide gels relative to the migration of marker proteins of known molecular weight (20). The mobility of the marker proteins is shown in Fig. 1b. The results of gels on four virus preparations, presented in Table 1, indicate that the 14 polypeptides released by SDS-disruption of EIAV range in molecular weight from approximately 10,000 to 79,000.

Because of variations in the amount of SDS bound (19), some proteins, notably glycopeptides, do not conform to a linear relationship between mobility and the logarithm of molecular weight in SDS-acrylamide gel electrophoresis (20). Such anomalous migration may be detected by comparing electrophoretic mobilities at a va-

TABLE 1. *Quantitation of EIAV proteins*

| Band no. | Apparent mol wt ($\times 10^{-3}$)[a] | % of radioactivity[b] |
|---|---|---|
| 1 | 78.8 (75–84) | 9.5 (7.2–10.7) |
| 2 | 77.5 (74–82) | |
| 3 | 64.1 (63–65.5) | 12.2 (10.9–14.4) |
| 4 | 57.9 (57–58.5) | 7.3 (7–7.7) |
| 5 | 51.3 (50–53) | |
| 6 | 47.8 (46.5–49) | |
| 7 | 42.9 (42–43.5) | |
| 8 | 39.5 (38–40.5) | 9.3 (8.5–10.6) |
| 9 | 28.9 (27–30) | 26.9 (25.3–27.9) |
| 10 | 23.3 (23–23.5) | 4.2 (3.7–4.6) |
| 11 | 17.8 (17.5–18) | 5.4 (3.4–7.2) |
| 12 | 12.6 (10–14.5) | 21.1 (16.9–24.5) |
| 13 | 12.5 (12–13) | 2.4 (1.8–3.2) |
| 14 | 9.3 (9–9.5) | 1.7 (1.4–2.2) |

[a] Molecular weights of EIAV proteins were estimated from electrophoretic mobility in 10% SDS-polyacrylamide gels in comparison to marker proteins subjected to electrophoresis in parallel gels or together with EIAV proteins in split gels (6). Results are the average of four gels. The range of molecular weights obtained is given in parentheses.

[b] Calculated from polyacrylamide gel electrophoresis of EIAV proteins labeled with $^{3}H$- or $^{14}C$-amino acid mixtures. Average of three experiments. Ranges are given in parentheses.

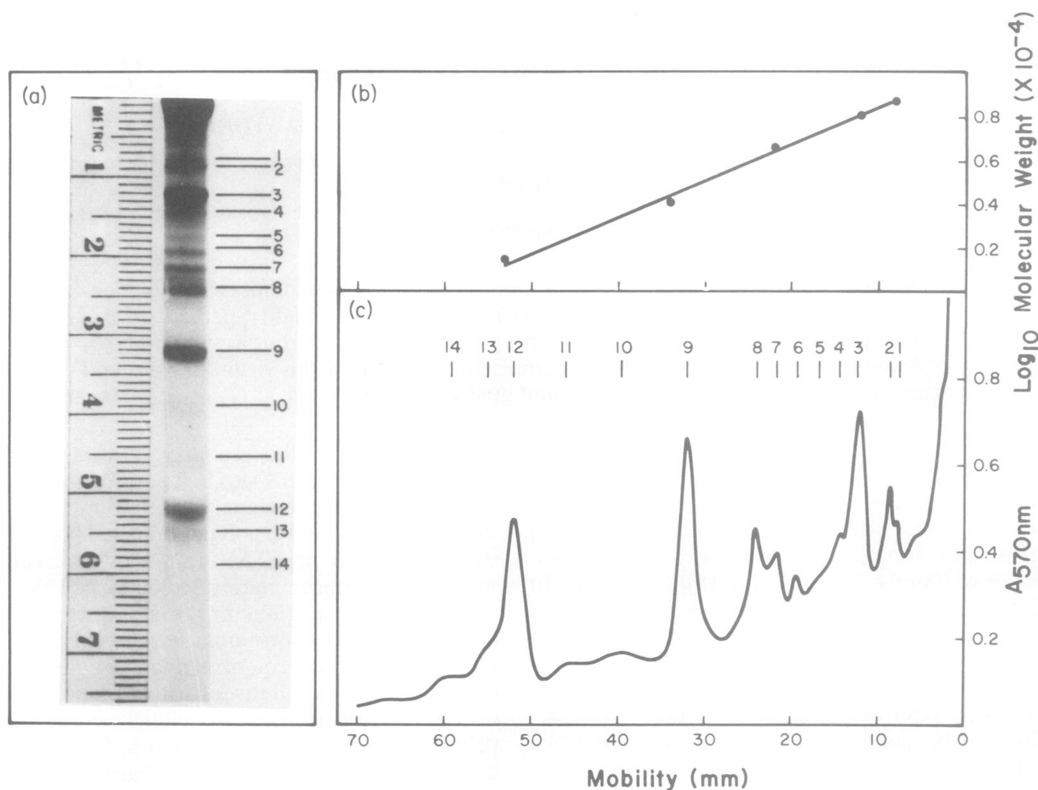


FIG. 1. *Polyacrylamide gel electrophoresis of EIAV proteins. Virus was sedimented from culture medium and purified by differential centrifugation and isopycnic banding in sucrose density gradients as previously described (5). Purified virus was disrupted by boiling for 3 min in 100 μl of 10% SDS-1% 2-mercaptoethanol-10% glycerol. Electrophoresis of viral proteins was performed in phosphate-buffered continuous SDS-polyacrylamide gels by the method of Dunker and Rueckert (6). (a) Gel was stained with 0.05% Coomassie brilliant blue in 25% isopropanol-10% acetic acid and destained as described by Fairbanks et al. (7). (b) Marker proteins (transferrin, molecular weight 74,000; bovine serum albumin, molecular weight 64,500; ovalbumin, molecular weight 45,500; α-chymotrypsinogen A, molecular weight 25,500; lysozyme [monomer], molecular weight 14,000) were subjected to electrophoresis with EIAV proteins in parallel gels. (c) The absorbance of stained bands from gel in (a) was measured at 570 nm with a Beckman model 25 recording spectrophotometer.*

riety of acrylamide concentrations (16), in which the relative mobilities at the different concentrations obey the relationship $\log R_F = \log Y_o - K_R T$, where $R_F$ is the relative mobility of a protein in a gel of concentration $T$, $Y_o$ is the limiting relative mobility, and $K_R$ is the retardation coefficient (5). The mobilities of the prominent EIAV proteins (bands 1, 2, 3, 8, 9, and 12) were compared with monomers, dimers, and trimers of lysozyme in split gels ranging from 5 to 15% acrylamide. These procedures indicated that EIAV polypeptides 1, 2, and 3 deviate markedly, and polypeptide 8 deviates slightly, from their expected rate of migration over a range of acrylamide concentrations. All of these bands are glycopeptides (see below), and the results indicate that their molecular weights as listed in Table 1 are inaccurate. Nevertheless, following the convention suggested by August et al. (2), we shall refer to these proteins according to their apparent molecular weights.

The relative proportion of EIAV proteins was estimated by computation of the amount of radioactivity in each polypeptide relative to the total radioactivity present in all virion proteins labeled for at least 12 h with $^{3}H$- or $^{14}C$-amino acid mixtures. The electrophoretic mobilities of these labeled polypeptides (Fig. 2) corresponded well with the patterns obtained with stained gels (Fig. 1c). Quantitation of the radioactivity associated with each peak from three such experiments is presented in Table 1. Eighty percent of the virion protein is accounted for in bands 1, 2, 3, 8, 9, and 12. Half is confined to bands 9 and 12. This protein composition is not remarkably different from that of other mammalian type C

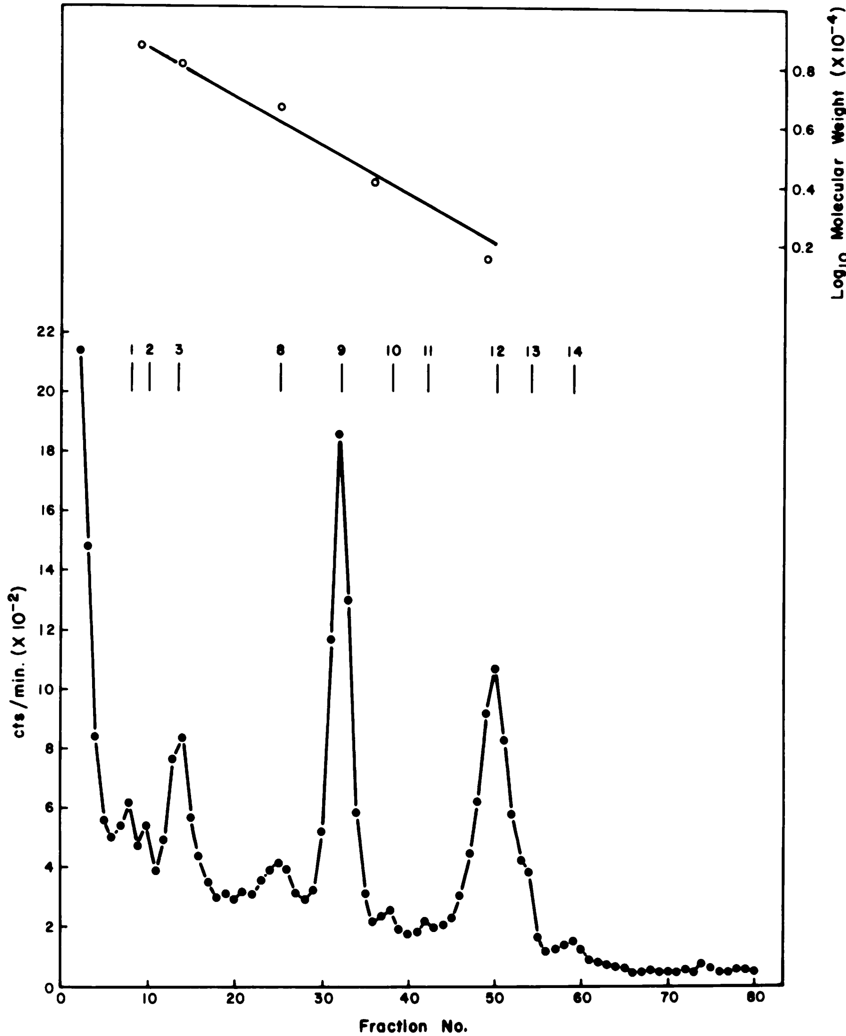


FIG. 2. *Polyacrylamide gel electrophoresis of EIAV labeled with $^{3}$H-amino acids. EIAV was labeled for 12 h with amino acid-free medium containing L-$^{3}$H-amino acid mixture (10 μCi/ml; specific activity, 6.25 mCi/mg). Labeled virus was purified, disrupted with SDS, and subjected to electrophoresis in a 10% SDS-polyacrylamide gel as described in Fig. 1. The gel was fractionated into 1-mm sections with a Gilson gel fractionator and analyzed for radioactivity in 3a70 scintillation cocktail (Research Products International Corp.). Marker proteins were subjected to electrophoresis in a parallel gel. Symbols: ●, $^{3}$H-labeled EIAV polypeptides; ○, migration of marker proteins.*

retroviruses (2, 9, 17; J. R. Stephenson, S. G. Devare, and F. H. Reynolds, Adv. Cancer Res., in press), except for the prominent band 3.

Glycopeptides of EIAV were identified by labeling with [$^{3}$H]glucosamine. The radioactivity profile of glucosamine-labeled components subjected to co-electrophoresis with $^{14}$C-amino acid-labeled components is shown in Fig. 3. In this gel, correspondence of $^{3}$H and $^{14}$C radioactivity peaks indicate that polypeptides 1, 2, 3, 8, and 14 are glycosylated. The relative prominence of glycopeptides 1, 2, and 8 is consistent with results obtained from other mammalian type C viruses (Stephenson et al., in press), and they are therefore considered to be analogous to the gp69/71 and gp45 glycoproteins. Glycopeptide 3 (gp64), comprising 10 to 12% of the total protein and 15% of the glucosamine label, has not been previously described in retroviruses. The minor glycopeptide gp10 (band 14) has not been described in type C retroviruses; however, an apparently analogous protein was noted in Mason-Pfizer monkey virus, a type D mammalian retrovirus (18).

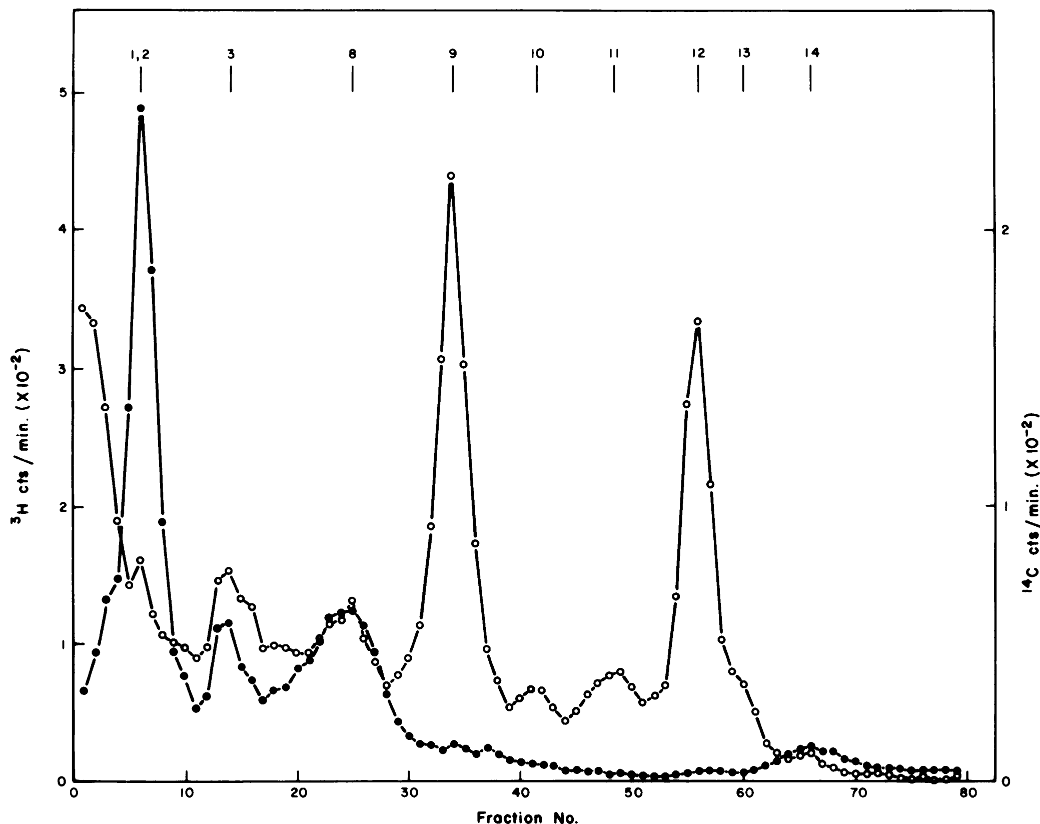


FIG. 3. *Polyacrylamide gel electrophoresis of EIAV glycopeptides. EIAV produced during a 24-h period in the presence of [$^{3}$H]glucosamine (20 µCi/ml; specific activity, 6.3 Ci/mmol) or L-$^{14}$C-amino acid mixture (2 µCi/ml; specific activity, 54 mCi/mAtom of carbon) was purified by isopycnic centrifugation in sucrose gradients, disrupted with SDS, and subjected to co-electrophoresis. Symbols: ●, $^{3}$H radioactivity; ○, $^{14}$C radioactivity.*

In summary, the results of this work indicate that EIA virions produced by persistently infected equine dermal fibroblasts are composed of 14 polypeptides ranging in molecular weight from ~10,000 to 79,000. Eighty percent of the virion protein is comprised of five polypeptides, designated gp77/79, gp64, gp40, p29, and p13. In a recent, similar study, Ishizaki et al. (12) reported three major non-glycosylated proteins with molecular weights of 25,000, 14,000, and 11,000 and two glycoproteins with molecular weights of 80,000 and 40,000. The prominent gp64 observed in our gels was not noted, and evidence was not cited for the minor glycopeptide gp10.

Current work in this laboratory is directed toward defining the relationships between the major structural proteins of EIAV by peptide mapping, immunological cross-reactivity, and in vitro iodination reactions. With regard to the minor bands obtained after electrophoresis of SDS-disrupted EIAV, our present working hypothesis is that they are not coded by the viral genome, with the possible exceptions of p12 (band 13) and gp10 (band 14) (Stephenson et al., in press). The aggregate molecular weight of the major EIAV proteins plus p12, gp10, and RNA-dependent DNA polymerase, with a presumptive monomeric molecular weight of 70,000 (Stephenson et al., in press), account for the coding capacity of EIAV RNA, based on a subunit molecular weight of $2.8 \times 10^{6}$ (4). Purified virus was not contaminated by cellular membrane structures as judged by electron microscopy. Extensive purification by several cycles of sucrose gradient centrifugation did not change the electrophoretic profile of SDS-disrupted EIAV. Purification of unlabeled EIAV in the presence of extracts of uninfected cells incubated with $^{3}$H-amino acids (9) did not result in localization of label in any of the presumptive contaminants. The polypeptide profiles of virus produced by

lytically infected equine macrophage cultures and of virus isolated from the plasma of acutely infected horses were not significantly different from that of virus produced by persistently infected fibroblasts. Thus, despite the fact that the coding capability of EIAV RNA suggests that some of the constitutive structural proteins of EIAV are derived from host cells, we have not been able to prove that this is the case. The answer to this question must await a comparison of the proteins of virus grown in cell cultures derived from several species. This option is not as yet open, because EIAV is not known to replicate in other than equine cells.

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