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Reevaluation of the Proteins in Rabies Virus Particles

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Protein content and localization of individual proteins of rabies virus have been studied. Four major proteins (estimated molecular weights, about 65,000, 54,000, 37,000 and 21,000), one minor component (molecular weight, about 200,000), and one intermediate (as regards its molar concentration) component (molecular weight, about 43,000) were revealed in rabies virus particles. In subviral particles accumulating in virus-infected cells, the 200,000-, 54,000-, and 37,000-dalton components were revealed. Some properties of the subviral particles allow them to be considered as viral nucleocapsids and the proteins composing them as analogs of L, N, and NS proteins of other rhabdoviruses. Thus, the protein composition of the rabies virus strain studied does not differ from that of other rhabdoviruses.

The goal of this work was to reevaluate the existing notions concerning the differences in the set of structural proteins and in their location in virions between rabies virus and other rhabdoviruses. The typical representative of the latter, vesicular stomatitis virus, contains three proteins in the nucleocapsid (L, N, and NS), one protein in the inner membrane (M), and one glycoprotein (G) in the envelope (7). It is considered that the external envelope of rabies virus also contains G glycoprotein, whereas the inner membrane contains two proteins, M1 and M2, and the nucleocapsid has only one protein, N (3-6). Recently, one more protein was revealed in rabies virions that electrophoretically corresponds to L protein of vesicular stomatitis virus; however, the location of this component was not established (3).

This paper presents data indicating that the set and the location of structural proteins in rabies virus do not differ from those of other rhabdoviruses.

In the first series of experiments, proteins of purified rabies virus were analyzed. Rabies virus, Vnukovo-32 strain, was grown in BHK-21 cells. The virus concentrate from several decaliters of virus-containing fluid was prepared by centrifugation in a K-6 continuous-flow rotor of a Mark II Electronucleonics centrifuge. Purification of the virus was performed by the pelleting of viral particles from the concentrate and isopycnic centrifugation in 20 to 60% linear sucrose gradients in an SW27 rotor of a Spinco L5 centrifuge. The light-scattering material with a density of ~1.19 g/ml was collected, and the virus was sedimented, treated with Nonidet P-40 at various ionite strengths, and analyzed by electrophoresis in sodium dodecyl sulfate (SDS)-polyacrylamide gels.

Figure 1 shows the results of such experiments. The detergent treatment at an NaCl concentration of 0.15 M essentially removed G protein, which corresponds to an early observation (5) and contradicts a recent one (1). A further increase of the ionic strength did not significantly change the relative content of viral proteins, although there seemed to be a slight decrease in the amount of M protein as compared with NS protein. (The reason for the usage of the symbols M, NS, etc., will be clear from the consideration discussed below.)

The molecular weights of viral proteins were determined with 11 marker proteins having molecular weights ranging from 130,000 to 25,000. The following values were obtained: L, about 200,000; G, 65,000 ± 500; N, 54,000 ± 400; A, 43,100 ± 300; NS (M1), 37,100 ± 200; M (M2), 21,100 ± 200 (the former designation of the proteins are given within parentheses).

Because the protein(s) of the inner matrix was not dissociated from the nucleocapsids, experiments were performed with virus-infected cells labeled with ^{14}C-amino acids. As shown in Fig. 2, in the labeling for 24 h, only N and NS proteins were revealed in virus-infected cells, whereas all structural proteins were revealed in the harvest. These data are similar to observations of Madore and England (3), and they indicate differences in the accumulation of various structural proteins in rabies virus-infected cells.

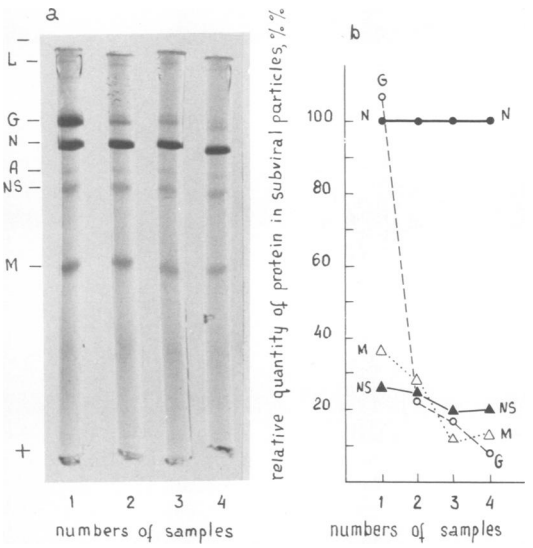

FIG. 1. *Structural proteins of rabies virus studied by electrophoresis in SDS-polyacrylamide gels. The suspension of purified virus in 0.1 NaCl-0.01 M Tris-hydrochloride (pH 7.4)-10^{-3} M EDTA was divided into four equal parts. Part 1 was not treated; parts 2 to 4 were treated with 0.5% Nonidet P-40 at 20°C for 20 min and with 0.15 M (part 2), 0.30 M (part 3) and 0.50 M (part 4) NaCl. The suspensions were then layered on the tops of 15% sucrose solutions in tubes for an SW50 rotor of a Spinco L5 centrifuge with the corresponding concentrations of NaCl and centrifuged at 35,000 rpm at 4°C for 1 h. The sediments were used for electrophoresis in SDS-polyacrylamide gels. (a) Proteins stained in the gels with amido black; (b) relative content of proteins after scanning and determination of areas of the peaks, the NP protein area being taken as 100%.*

Figure 3 shows results of the study of proteins of virus-specific structures that accumulate in the cytoplasm of virus-infected cells. For the buoyant density analysis, particulate fractions of the cytoplasmic extracts were used, from which cell membranes and free proteins were removed beforehand. Figure 3 shows that in the zone of the maximal concentration of particles containing viral proteins (ρ = 1.27 g/cm^3), the ratio of N, NS, and apparently L proteins was constant; this allowed us to exclude cosedimentation of different materials.

Several explanations of the possible origin of the virus-specific structures are worth considering. Their buoyant density and protein content clearly indicate that they are not virions. Their origin from virus particles as a result of detergent treatment is also extremely improbable, since such products should also contain M protein in addition to L, N, and NS proteins. One could suggest that ^{14}C-labeled M protein is substituted in the particles by unlabeled molecules formed before labeling of the cells. However, such a situation would require the synthesis of a large pool of matrix protein, so that its amount would be considerably higher than that of the nucleocapsid N protein. In all known systems for the replication of negative-stranded RNA viruses, envelope proteins are synthesized to a lesser extent than are nucleocapsid proteins. The same situation apparently occurred in the system studied here (Fig. 2).

One might argue that the 1.27-g/cm^3 particles are complexes of viral nucleocapsids, with the envelope protein designated as NS in this paper and M1 in previous papers. The only basis for

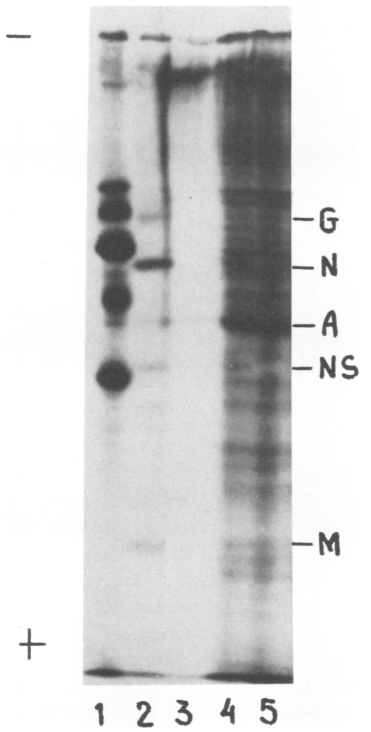

FIG. 2. *Structural proteins in rabies virus-infected cells and in the virus progeny. BHK-21 cells were infected at a multiplicity of infection of about 1 mouse 50% lethal dose per cell. At 3 days after infection with the virus, the cells were incubated in 0.05% lactalbumin hydrolysate with ^{14}C-amino acids (10 μCi/ml) for 24 h. The medium was used as the source of the virus, the cells were lysed with 1% SDS, and both were studied by electrophoresis in SDS-Laemmli polyacrylamide gels. The gels were dried, and the positions of ^{14}C-labeled proteins were determined by autoradiography. (1) Sendai virus ^{14}C-labeled proteins (marker) (2); (2) rabies virus proteins; (3) proteins from the culture fluid of mock-infected cells; (4) proteins of lysates of virus-infected cells; (5) proteins of lysates of mock-infected cells.*

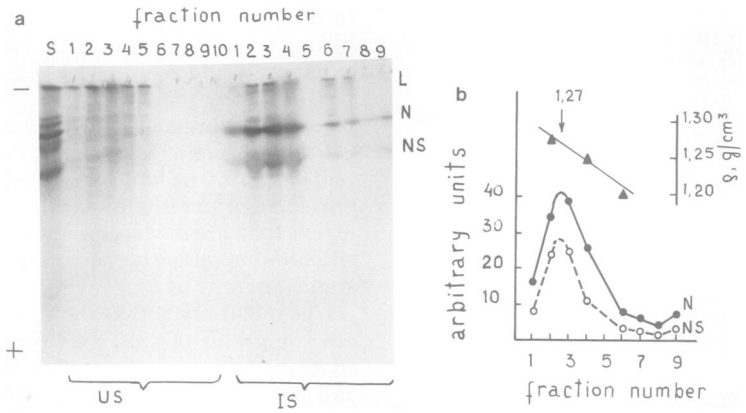

FIG. 3. *Proteins in subviral particles accumulating in the cytoplasm of infected cells. At 3 days after infection, mock-infected and infected cells were labeled with 50 μCi of ^{14}C-amino acids in Hanks balanced salt solution per ml for 3 h. The cells were then suspended in a buffer solution containing 0.01 M NaCl, 0.01 M Tris-hydrochloride (pH 7.5) and 0.003 M $MgCl_2$ and disrupted by treatment with 1% Nonidet P-40. After disruption, the nuclei were deposited by low-speed centrifugation, cytoplasmic extracts were treated with 0.01 M EDTA, and particulate fractions were obtained by pelleting of the particles from extracts through a 20% sucrose solution (centrifugation at 140,000 × g for 2 h). The pellets were fractionated by isopycnic centrifugation in linear sucrose gradients (20 to 70% [wt/wt]; centrifugation in an SW50 rotor at 35,000 rpm for 18 h). The gradient fractions were collected from the bottoms of the tubes, and proteins from each fraction were precipitated with 5% trichloroacetic acid in the presence of carrier protein. Precipitates were washed with acetone, and proteins were used for electrophoresis in polyacrylamide slab gel. The slab was then dried and exposed to X-ray film, and the densitometric scanning was performed with a Joyce-Loebl device. (a) Autoradiograph of the gel: S, Sendai virus ^{14}C-labeled proteins; US, ^{14}C-labeled proteins in the uninfected sample; IS, ^{14}C-labeled proteins in the infected sample. (b) Buoyant density profile of rabies virus proteins in particles. After scanning of ^{14}C-labeled proteins in tracks 1 to 9 of the infected sample (a), the relative quantities of N and NS proteins were determined by weighing.*

this suggestion is the hypothetical membrane function of this protein in rabies virions. This was concluded from the fact that nucleocapsids, if isolated from rabies virions by sodium deoxycholate treatment, contain N protein only (1, 4, 5). However, it is well known that such treatment may remove loosely bound nucleocapsid proteins of negative-stranded RNA viruses (for example, see reference 8). It is also known that the assembly of nucleocapsids with membrane proteins is correlated with the release of virions from the cell by a budding mechanism.

Thus, the most plausible nature of the subviral particles is that they are nucleocapsids. Their buoyant density (1.27 g/cm^3) correlates with prior data (5), a small difference in the density being attributable to the use of sucrose gradients in our experiments instead of cesium chloride used by Sokol and co-workers. Also, these authors studied nucleocapsid preparations containing only N protein. Finally, essential support for the nucleocapsid nature of the 1.27-g/cm^3 particles is that they contain three classes of proteins, L, N, and NS, with molecular weights and molar ratios similar to those of nucleocapsid proteins of other rhabdoviruses.

LITERATURE CITED

1. **Kawai, A.** 1977. Transcriptase activity associated with rabies virus. J. Virol. **24**:826–835.
2. **Lamb, R. A., B. W. Mahy, and P. W. Choppin.** 1976. The synthesis of Sendai virus polypeptides in infected cells. Virology **69**:116–131.
3. **Madore, H. P., and J. M. England.** 1977. Rabies virus protein synthesis in infected BHK-21 cells. J. Virol. **22**: 102–112.
4. **Schneider, L. G., and H. Diringer.** 1976. Structure and molecular biology of rabies virus. Curr. Top. Microbiol. Immunol. **75**:153–180.
5. **Sokol, F., H. D. Schlumberger, T. J. Wiktor, and H. Koprowski.** 1969. Biochemical and biophysical studies on the nucleocapsid and on the RNA of rabies virus. Virology **38**:651–665.
6. **Sokol, F., D. Stancek, and H. Koprowski.** 1971. Structural proteins of rabies virus. J. Virol. **7**:241–249.
7. **Wagner, R. R., L. Prevec, F. Brown, D. F. Summers, F. Sokol, and R. McLeod.** 1972. Classification of rhabdovirus proteins: a proposal. J. Virol. **10**:1228–1230.
8. **Zaides, V. M., O. G. Nikolaeva, L. M. Selimova, O. P. Zhirnov, and A. G. Bukrinskaya.** 1974. Deoxycholate-mediated cleavage of the protein of Sendai virus nucleocapsids derived from infected cells. Biochem. Biophys. Res. Commun. **59**:1018–1022.