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Model for Vesicular Stomatitis Virus

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Vesicular stomatitis virus contains single-stranded ribonucleic acid of molecular weight 3.6×10^6 and three major proteins with molecular weights of 75×10^3, 57×10^3, and 32.5×10^3. The proteins have been shown to be subunits of the surface projections, ribonucleoprotein, and matrix protein, respectively. From these values and from estimates of the proportions of the individual proteins, it has been calculated that the virus has approximately 500 surface projections, 1,100 protein units on the ribonucleoprotein strand, and 1,600 matrix protein units. Possible models of the virus are proposed in which the proteins are interrelated.

Vesicular stomatitis virus is a bullet-shaped, lipid-containing virus which measures approximately 175×65 nm and which has well defined surface projections. The virus ribonucleic acid (RNA) is generally regarded as existing in the form of a highly organized helix of ribonucleoprotein (RNP), but the exact length of the RNP strand and the precise form it takes within the virus particle are not known. For example, Simpson and Hauser (25) proposed a model for the virus in which the RNP was arranged as two concentric coils with a length of 7.3 μm, from which they calculated that the RNA had a molecular weight of 7×10^6. In contrast, Nakai and Howatson (21) showed that the RNP strands obtained by disruption of the virus with sodium deoxycholate had an average length of 3.6 μm. On the basis of this value, they suggested that there was only one coil of RNP in the virus particle. Such a strand of RNP would contain RNA with a molecular weight of 3.4×10^6 to 3.8×10^6. This value is in fair agreement with those calculated from sedimentation data (3, 13, 16, 20, 23, 26). The virus contains three major proteins (18, 29), each of which is associated with a distinct structural unit (6). This paper reports on the proportions of the three proteins, and, from the molecular weights of the RNA and of the individual proteins, we suggest further details regarding the architecture of the virus.

MATERIALS AND METHODS

Virus. The Indiana serotype was used in all experiments. Unlabeled virus and virus labeled with ^{3}H-uridine were grown in BHK-21 cell monolayers in Eagle's medium. When virus labeled with ^{14}C-amino acids was required, Earle's medium was used. The virus was purified by the method described previously (2).

Preparation of virus substructures. Surface projections were removed by incubating virus pellets in 0.04 M phosphate, *p*H 7.6, with trypsin (1 mg/ml) for 15 min at 37 C. The residual projection-free particles were isolated by layering onto 1 ml of 30% sucrose in 0.04 M phosphate, *p*H 7.6, and by centrifuging in the SW39 rotor of the Spinco ultracentrifuge at 30,000 rev/min for 1 hr. The pellet was drained well and suspended in water. Virus skeletons, i.e., particles from which the surface projections and lipid coat had been removed by treating at room temperature with Nonidet P-40 (2 mg/ml final concentration), were isolated by centrifuging through a layer of 30% sucrose in 0.04 M phosphate, *p*H 7.6. RNP was isolated in a similar manner after first treating the virus with 0.5% sodium deoxycholate.

Estimation of RNA and protein. Virus RNA was extracted from preparations of purified virus by mixing with 0.1% sodium dodecyl sulfate and shaking with phenol saturated with 0.1 M NaCl. The separated phenol layer was extracted with 0.5 volume of water, and this aqueous extract was combined with the aqueous layer obtained in the first phenol extraction. The combined layers were extracted twice with ether and then precipitated with 2 volumes of cold ethanol. After being stored for several hours at -20 C, the RNA was separated by centrifuging at $15{,}000 \times g$ for 1 hr and then was dissolved in a small volume of water. The amount of RNA in this solution was estimated by the orcinol method.

Protein was estimated by the modified Folin-Ciocalteau method (19). Estimations were made on intact virus, i.e., virus which had been incubated with trypsin to remove the surface projections and RNP. In each case, equal volumes of the preparations were layered over a cushion of 30% sucrose and were centrifuged in the SW39 rotor for 1 hr at 30,000 rev/min. Estimates were then made on samples of the resuspended pellets.

Electron microscopy. Samples of virus, RNP, and skeletons, isolated by sucrose gradient centrifugation, were mixed with bovine serum albumin to a final concentration of 0.05%, and fixed crystalline ox liver

catalase was added (30). Drops of the samples were applied to Formvar-carbon grids, negatively stained with 1% potassium phosphotungstate at *p*H 7.0, or with 1% ammonium molybdate, *p*H 7.0, and examined in a Siemens Elmiskop I. Measurements were made directly on the photographic plates either by a traveling microscope or by a recording microdensitometer.

RESULTS

Relative proportions of the major structural proteins of the virus. A purified virus preparation containing 1,020 μg of protein/ml was disrupted according to the scheme in Table 1, and protein estimations were made on the fractions. Estimations were made on virus, virus treated with trypsin (to remove surface projections), and isolated RNP. The material used in each estimation was derived from equal volumes of the untreated virus preparation. The amount of protein in virus (P_2, P_3, P_4) minus the amount in trypsin-treated virus (P_3, P_4) gives the protein content of the surface projections. Similarly, the amount in trypsin-treated virus (P_3, P_4) minus that in the RNP (P_3) gives P_4. From the calculations in Table 1, the percentage of the total virus protein in each subunit was used to calculate the molar ratios of each protein. These are very close to those given recently by Helleiner and Wunner (9).

RNA of the virus. A portion of the preparation of virus containing 1,020 μg of protein/ml was mixed with a trace amount of virus which had been grown in the presence of ^{14}C-amino acids and ^{3}H-uridine, and then was disrupted with 0.1% sodium dodecyl sulfate. After shaking with phenol as described above, the aqueous layer contained less than 2% of the ^{14}C counts in the virus, and precipitation with ethanol removed this small amount of ^{14}C from the RNA preparation. All the ^{3}H radioactivity was recovered in the aqueous layer after sodium dodecyl sulfate-phenol treatment, and 85% of the ^{3}H counts were present in the resuspended ethanol precipitate. After allowing for this loss, the RNA present in the virus preparation was estimated to be 24 μg/ml, an amount equal to 2.3% of the virus protein.

Electron microscopy. The measurements which were made on electron micrographs of the virus particles, using crystals of catalase as internal standards (30), are summarized in Table 2, where they are compared with those obtained by Simpson and Hauser (25) and Nakai and Howatson (21).

DISCUSSION

We have used the data presented above and that published by other workers (4, 6, 18, 20,

TABLE 1. *Calculation of the number of protein subunits in one vesicular stomatitis virus particle*

Sample for analysis	Component	Mol wt	Wt (mg)	Total protein (%)	No. of units per virus particle[a]	Ratio of units
Virus RNA	RNA	3.6×10^{6}[b]	24			
Virus (P_2 P_3 P_4)	P_2 P_3 P_4		1,020			
Trypsin-treated virus (P_3 P_4)	Surface projection: total − (P_3 + P_4) = P_2	75×10^{3}	245	24	$(1.53 \times 10^{8}/75 \times 10^{3}) \times 0.24 = 492$	1
Ribonucleoprotein from deoxycholate-disrupted virus (P_3)	RNP = P_3	57×10^{3}	430	42	$(1.53 \times 10^{8}/57 \times 10^{3}) \times 0.42 = 1,130$	2
	Matrix protein (P_3 + P_4) − P_3 = P_4	32.5×10^{3}	345	34	$(1.53 \times 10^{8}/32.5 \times 10^{3}) \times 0.34 = 1,600$	3

[a] Weight of protein in each virus particle = $3.6 \times 10^{6} \times (1,020/24) = 1.53 \times 10^{8}$.

[b] The use of a different molecular weight for the RNA would alter the total weight of the protein but would have no effect on the ratio of the polypeptides.

TABLE 2. *Dimensions of vesicular stomatitis virus and some of its constituents*

Dimension measured	Present work	Nakai and Howatson (1968)	Simpson and Hauser (1966)
Overall length of virus (excluding surface projections)	175 nm	173 nm	178 nm
Overall diameter of virus (excluding surface projections	65 nm	72 nm	80 nm
Number of striations of RNP helix	35	34	35
Distance between striations	4.5 nm	4.6 nm	5 nm
Diameter of striated structure	40–49 nm	49 nm	50 nm
Number of protein units per coil of RNP	34–35		
Size of protein units of RNP		3 by 3 by 9 nm	5 by 2 nm diameter

29) to develop a model for the virus. To calculate the number of protein subunits in the virus, it is necessary to know their molecular weights and the molecular weight of the RNA. Nakai and Howatson (21) calculated from measurements of the strand length of the RNP that the RNA had a molecular weight of 3.6×10^6, a value in good agreement with those obtained by sedimentation studies (3, 13, 16, 20, 23, 26). The molecular weights of the protein subunits were taken to be 75×10^3 for the surface projection, 57×10^3 for the RNP protein, and 32.5×10^3 for the matrix protein. These are average values calculated from our own determinations (6) and from the values published in the literature (4, 18, 20, 29).

Using these values, our estimate of approximately 1,100 for the number of protein units on the RNP strand is similar to that obtained by electron microscopy (21). The surface projections account for 24% of the virus protein, equivalent to a molecular weight of 37×10^6 and approximately 500 molecules of protein (Table 1). The surface projections are about 10.0 nm in length and approximately 3.5 nm in diameter, which suggests that each projection contains only one protein unit of molecular weight 75×10^3. This would mean that there are approximately 500 surface projections, a figure in good agreement with the figure of 550 given recently for influenza virus (27), the PR-8 strain of which has a surface area about 12% less than that of vesicular stomatitis virus.

The residue, after removing the surface projections and RNP, accounts for 34% of the total protein of the virus. If this protein is assumed to be entirely P_4, then we estimate that there are 1,600 units of the matrix protein in each virus particle. However, several authors have shown by polyacrylamide gel electrophoresis that small amounts of a large polypeptide (P_1) and a smaller polypeptide of molecular weight ca. 30×10^3 (20) are present in the virus. In our experiments, these polypeptides account for only a small proportion of the total radioactivity in the protein of the virus. The polypeptides would be found in the P_4 fraction when the method of fractionation described above is used. This would reduce the proportion of P_4 and could mean that there are rather fewer than 1,600 matrix protein units in the virus particle.

Our finding that the proportions of the three proteins of vesicular stomatitis virus are in the approximate ratio of 1:2:3, together with the fact that the matrix protein and RNP form a stable skeletonlike structure (5), led us to examine the feasibility of the proteins being structurally interrelated. The published electron micrographs show that the RNP is helically arranged in the virus with 35 turns spaced at 4.75-nm intervals (Table 2). We have no direct evidence concerning the arrangement of the matrix protein subunits and the surface projections, but electron micrographs of the virus particles suggest that they are not helically arranged. The most probable arrangement would seem to be a hexagonal lattice and, in fact, several groups of workers have already found that viruses with surface projections and a lipid envelope often show hexagonal structures when viewed in the electron microscope. Hexagonal structures have been observed in influenza virus (e.g., 1, 8, 12, 22, 24), Rous sarcoma virus (7), monkey kidney vacuolating agent (17), and, probably more significantly, in rabies virus (15) and in broccoli necrotic yellows virus (10), both members of the rhabdovirus group.

In designing a model for the virus, the main problem is to relate the helically arranged RNP to a hexagonal arrangement of the matrix protein and surface projections. We have considered two alternative models. In the first, each surface projection is situated between three matrix protein subunits (Fig. 1) in such a way that the

FIG. 1. *Hexagonal lattice in which each surface projection* (×) *is surrounded by three matrix protein subunits* (●). *These are arranged to fit a tubular structure with the distribution of matrix protein subunits based on a 92-subunit icosahedron cut across its inter-lattice axis. The distribution of the surface projections is based on a 32-subunit icosahedron cut across its twofold axis.*

matrix protein subunits of the tubular part of the particle and the surface projections are arranged as separate hexamers. This is in accord with the previously described hexagonal arrangement of protein subunits (e.g., 11, 28).

The rounded ends of tubular or bullet-shaped particles can be formed by the use of pentamer subunits instead of certain hexamers. Hull, Hills, and Markham (14) suggested that the tubular portion can be considered as being based on a half-icosahedron, with the arrangement of hexamers in the tubular portion depending on the axis across which the icosahedron is cut. From the dimensions of the virus particles and the numbers of surface projections and matrix protein subunits per particle (Table 1), the sizes of the hexamers shown in Fig. 1 can be estimated as about 13.5 nm center-to-center for the surface projection hexamers and about 7.5 nm center-to-center for the matrix protein hexamers. These sizes suggest that the surface projection structure of this model might be based on a 32-subunit icosahedron, and that of the matrix protein might be based on a 92-subunit icosahedron. If the interrelationship between the surface projections and matrix protein subunits is as in Fig. 1, there are only two possible structures for the tubular portion: (i) a matrix protein layer based on an icosahedron cut across the twofold axis with the surface projection layer on an inter-lattice axis, and (ii) a matrix protein layer based on an inter-lattice axis with the surface projection layer on the twofold axis. However, a helically arranged RNP of the dimensions given in Table 2 will not fit regularly with the matrix protein layer on either of these two structures.

In the second model, the units of matrix protein are linked directly to the RNP to form a hexagonal array (Fig. 2) in which the matrix protein hexamers are linked. The placing of each surface projection in the center of a matrix protein hexamer (Fig. 2) gives the ratio of one surface projection to three matrix protein subunits. A three-dimensional model incorporating these features (Fig. 3) shows that the matrix protein hexamers are in a helix following that of the RNP coil. End caps for this model can be formed by the substitution of hexamers by pentamers, but they would not have the icosahedral symmetry described for those in the first model.

At present, there is insufficient evidence on vesicular stomatitis virus to allow us to decide between these two models. Nevertheless, we think that some of the features of the models

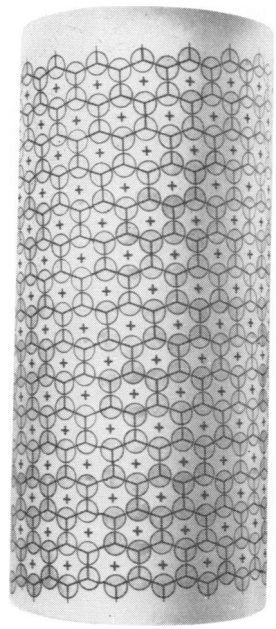

FIG. 2. *RNP* (○) *linked with a hexagonal array of matrix protein subunits to fit a tubular structure. The surface projections* (×) *are placed at the centers of the matrix protein hexamers.*

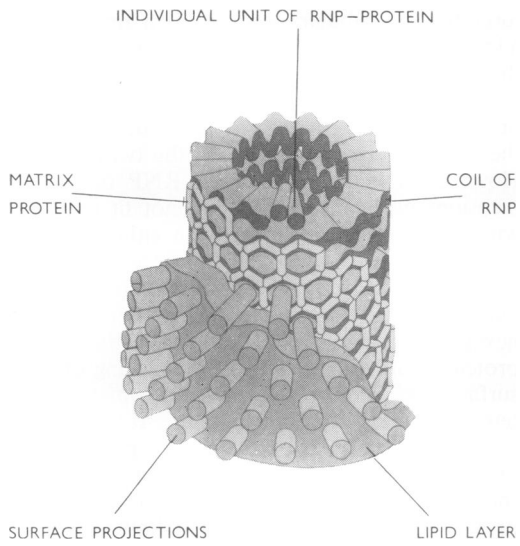

FIG. 3. *Virus particle in which a three-dimensional relationship between the three proteins and the lipid layer is suggested.*

proposed in this paper could be applied to other rhabdoviruses and even to other enveloped viruses.

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