The Proteins of Biologically Active Sub-units of Vesicular Stomatitis Virus

(Accepted 12 February 1970)

Our experiments on the stepwise degradation of vesicular stomatitis virus into well-characterized structural components have enabled us to relate some of the biological properties of the virus to certain of its structural units (Cartwright, Smale & Brown, 1969, 1970). For example, incubating the virus with trypsin produces a bullet-shaped structure similar to the virus except that it no longer possesses the surface projections. Unlike the virus, the projection-free component has low infectivity and does not produce neutralizing antibodies when inoculated into guinea-pigs, thus providing decisive evidence that the immunizing activity of the virus is associated with the surface projections.

By treating the virus with Tween + ether or Nonidet P40 or sodium deoxycholate (Brown, Cartwright & Smale, 1967; Cartwright et al. 1970), the immunizing antigen can be released in a biologically active form, sedimenting at about 6s. An infective skeleton-like structure with the same size and shape as the virus is also produced by treating the virus with Tween + ether or with Nonidet. This skeleton lacks the lipid envelope and surface projections but contains the virus RNA in the form of a helical RNP. The RNP is released by sodium deoxycholate as filaments which still retain infectivity. These observations suggested to us that the filamentous RNP is held in a helical configuration within the bullet-shaped skeleton by a component which is disrupted by sodium deoxycholate. The present communication provides evidence in support of this concept and demonstrates unequivocally that the surface projections, the RNP and the component maintaining the RNP in its helical conformation contain distinct proteins.

Virus grown in monolayers of BHK 21 cells in the presence of [3H]uridine (4 μC/ml.), [14C] amino acids (algal protein hydrolysate, 1.6 μC/ml.) and Actinomycin D (0.1 μg/ml.) was purified by precipitating with 60% saturated ammonium sulphate, pelleting at 15,000 g for 100 min. and centrifuging in a sucrose gradient (Brown, Cartwright & Almeida, 1966). Samples of the virus were mixed with 0.2% Nonidet P40 or 0.1% sodium deoxycholate or 0.05% trypsin and centrifuged in 15 to 45% sucrose gradients for 2 hr at 20,000 rev./min., using the SW 25.1 rotor. The positions of the components in the gradient were determined by measuring the radioactivity in 0.1 ml. samples of the one ml. fractions in a Packard scintillation counter, no. 3310. The skeletons produced by Nonidet P40 formed aggregates which were deposited as a pellet at the bottom of the tube, well separated from the immunizing antigen which remained near the top of the gradient (Cartwright et al. 1970). The RNP released by sodium deoxycholate sedimented at 140 s, the remainder of the protein remaining near the top of the gradient. The surface projection-free component obtained by incubation with trypsin sedimented to approximately the same position as the untreated virus.

The different fractions were first examined by immunodiffusion in 1% agar gels. The intact virus is too large to diffuse through this concentration of agar, but after disruption with 0.1% sodium deoxycholate three bands were obtained (Fig. 1a). In most experiments the two bands nearest to the antiserum well were superimposed, so that the fainter of the two bands was clearly observed only when the more intense band was absent (Fig. 1b). The band nearest to the antigen well (band 3) is identical with that obtained with the RNP separated...
Short communications

by sucrose gradient centrifugation of deoxycholate-disrupted virus (Fig. 1 a). The slowly sedimenting fractions gave the two superimposed bands 2 and 4. The projection-free structure obtained by trypsin treatment gave bands 3 and 4. The fainter band 4 was seen clearly in the absence of the band (2) produced by the spike projections (Fig. 1 b). The fractions obtained by treatment of the virus with Nonidet did not give such clear-cut precipitin patterns (Fig. 1 c) because, as is shown below, the skeleton disintegrates to some extent during its isolation so that the component which holds the RNP in its helical configuration within the skeleton is found in the slowly sedimenting fraction in addition to the spike projections.

![Immunodiffusion analysis of biologically active subunits of vesicular stomatitis virus.](image)

Fig. 1. Immunodiffusion analysis of biologically active subunits of vesicular stomatitis virus. (A) Virus; (B) RNP isolated from virus disrupted with 0.1% sodium deoxycholate; (C) slowly sedimenting fraction from deoxycholate-disrupted virus; (D) projection-free structure separated from sucrose gradients of trypsin-treated virus; (E) skeleton separated from Nonidet-disrupted virus; (F) slowly sedimenting fraction from Nonidet-disrupted virus; (G) hyperimmune guinea-pig serum. Each fraction was mixed with 0.1% sodium deoxycholate before adding to the cups to ensure total disruption of the structures. Three bands were regularly obtained with the virus, bands 2 and 4 usually being superimposed, so that the fainter band 4 was seen clearly only in the absence of band 2, as in D and E.

The protein constituents of the different fractions were then examined by electrophoresis in polyacrylamide gels. Samples of untreated virus, aggregated skeleton, RNP, projection-free component and the fractions containing the slowly sedimenting proteins were mixed with 1/10 vol. glacial acetic acid and then made to 0.5M-urea and 1% sodium dodecyl sulphate. After incubating for 1 hr at 37°, the solutions were dialysed overnight against 500 vol. of a solution containing 0.5M-urea, 0.1% sodium dodecyl sulphate and 0.1% mercaptoethanol in 0.01M-phosphate, pH 7.2, before analysing by electrophoresis in polyacrylamide gels. The gels, containing 7.5% acrylamide and 0.2% methylene bisacrylamide, were prepared in 0.5M-urea, 0.1% sodium dodecyl sulphate and 0.1M-phosphate, pH 7.2.
Samples (0.2 ml.) were made to 30% with sucrose before applying to the gel and electrophoresis was performed for 7 hr at a constant current of 5 mA. The gels were then removed from the tubes, stained for 16 hr with 0.25% Coomassie Blue solution containing 40% methyl alcohol and 9% acetic acid, and destained in a 10% methyl alcohol–9% acetic acid solution.

![Polyacrylamide gel electrophoresis of vesicular stomatitis virus and biologically active subunits derived from the virus by treatment with detergents or with trypsin. The gels were stained with Coomassie Blue: (1) virus; (2) slowly sedimenting fraction from virus treated with trypsin; (3) projection-free structure separated from sucrose gradients of trypsin-treated virus; (4) slowly sedimenting fraction from deoxycholate-treated virus; (5) RNP separated from deoxycholate-treated virus; (6) slowly sedimenting fraction from Nonidet-treated virus; (7) skeleton separated from Nonidet-treated virus; (8) virus.](image)

The stained protein patterns obtained with the virus and with the different fractions are shown in Fig. 2. The virus gave three major protein bands and one minor band. After densitometer tracings had been made, the gels were sectioned into 1 mm. lengths, each section placed in a scintillation vial and incubated with 0.5 ml. Nuclear Chicago solubilizer at 65°C for 2 hr. After cooling, 7.5 ml. of scintillation fluid (5 g. PPO and 0.3 g. dimethyl-POPOP in 1 l. of toluene) were added to each vial and the samples allowed to equilibrate for 12 hr at 4°C before they were counted in a Packard scintillation counter. The distribution of radioactivity in the gels provided striking confirmatory evidence for the visual observations (Fig. 3, 4). The pattern obtained for the virus is identical with that found by Kang & Prevec (1969), but differs slightly from the one described by Wagon et al. (1969a) in that only one minor band (P1) was found. For convenience in comparing the results, therefore, we have numbered the bands according to the scheme of Kang & Prevec. Using bovine serum albumin, trypsin and haemoglobin as marker proteins, the molecular weights of the three major bands were estimated to be approximately $73 \times 10^8$ (P2), $60 \times 10^8$ (P3) and
32 × 10^8 (P4). These values are in fair agreement with those given by Wagner et al. but rather lower than the estimates of Kang & Prevec.

We found, in agreement with the two groups mentioned above, that the RNP component of the virus contained only one protein, P3 (Fig. 4). Production of RNP by disrupting the virus with deoxycholate also released a slowly sedimenting fraction which contained proteins P2 and P4. All the P2 and P4 content of the virus was present in this fraction but P3 was never detected.

![Polyacrylamide gel electrophoresis of vesicular stomatitis virus showing the effect of trypsin on the protein constituents.](image)

Fig. 3. Polyacrylamide gel electrophoresis of vesicular stomatitis virus showing the effect of trypsin on the protein constituents. The top half of the figure shows the densitometer tracing of the stained bands and the bottom half shows the distribution of radioactivity: (a) virus; (b) virus plus trypsin; (c) slowly sedimenting fraction; and (d) projection-free structure isolated by sucrose gradient centrifugation of trypsin-treated virus. Note that P2 is absent from all preparations which had been treated with trypsin. The position of the trypsin in the gels is indicated by T.

Virus which has been treated with trypsin sediments in sucrose gradients at about the same rate as the untreated virus. This fraction contained P3 and P4 but was completely devoid of P2. Comparison of the radioactivity profiles in polyacrylamide gels of trypsin-treated and untreated virus showed that P3 and P4 were retained completely after enzyme treatment, but we could find no evidence for P2 (Fig. 3). This observation shows that P2 corresponds to the projections on the virus surface which are removed by the enzyme. We have also failed to detect P2 in unfractionated preparations of trypsin-treated virus,
indicating that the projections are degraded into small molecules by the enzyme. This is in accordance with the loss of immunizing activity which occurs when the virus is treated with the enzyme (Cartwright et al. 1969). Our conclusion is at variance with that reached by Wagner et al. (1969 b). On the basis of their examination of digitonin-disrupted virus, they considered that P4 was the surface projection.

![Fig. 4. Polyacrylamide gel electrophoresis of subunits of vesicular stomatitis virus showing (top) the densitometer tracing of the stained bands and (bottom) the distribution of radioactivity. (a) slowly sedimenting fraction, and (b) RNP isolated by sucrose gradient centrifugation of deoxycholate-disrupted virus: (c) slowly sedimenting fraction and (d) skeleton isolated by sucrose gradient centrifugation of Nonidet-disrupted virus.](image)

The skeleton separated from Nonidet-disrupted virus contained only P3 and P4. Since P4 is present in the skeleton fraction but not in the RNP, we have concluded that this protein is a constituent of the component which holds the RNP in its helical configuration within the bullet-shaped skeleton. The slowly sedimenting fraction separated from the Nonidet-disrupted virus contained P2 together with a small amount of P4. We have shown that the presence of this small amount of P4 is due to partial disintegration of the skeleton by detergent during the isolation procedure. Attempts to obtain a more clear-cut disruption of the virus by varying the concentration of Nonidet resulted in either incomplete removal of the protein P2 or increased removal of P4. In all cases, the protein component of the RNP (P3), was retained completely in the skeleton.
Short communications

The significance of the minor band P1 has not been ascertained. It occurred in the intact virus, skeleton and spike-free structure but was not found in either of the fractions isolated from deoxycholate-disrupted virus. The observations provide evidence, however, for the identity of the three major proteins and show unequivocally that the RNP, spike projection and component holding the RNP in its helical configuration contain distinct proteins with different molecular weights.

The Animal Virus Research Institute
Pirbright, Woking
Surrey, England

REFERENCES


(Received 2 February 1970)