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Venezuelan equine Encephalomyelitis virus: structural components.

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Summary. Venezuelan equine encephalomyelitis (VEE) virus, purified in sucrose density gradients was examined with the electron microscope before and after sodium desoxycholate (DOC) treatment. The structure of the nucleocapsid revealed 10 to 12 nm ring shaped units organized into an icosahedral symmetry. Density gradient fractions exhibiting hemagglutinating activity after DOC treatment show 12 to 18 nm particles lined by short projections. These isolated hemagglutinating subunits are though to correspond to the superficial spikes of VEE virus.

INTRODUCTION

The development of Venezuelan Equine Encephalomyelitis (VEE) virus in tissue cultured cells (3, 9) and in the brain of newborn mice (4), has been examined with the electron microscope. These studies revealed that VEE virus, like many other enveloped viruses, maturate at cellular membranes. While the virus is assembled, the capsomeric structure is masked by the lipoprotein membranous envelope.

Attempts to determine the architecture and symmetry of the capsid structural units and to examine the ultrastructural details of the envelope and the spikes studding the surface of several group A arbovirus have been reported (5, 6, 10, 14). However the structural arrangement and exact chemical composition of the nucleocapsid and the envelope of group A arbovirus is still uncertain.

The present investigation represents an attempt to examine the ultrastructural organization of VEE virus capsid and to correlate the hemagglutinating properties of sucrose density gradient fractions to the presence of structural units isolated from VEE virus envelope.

MATERIAL AND METHOD

Virus: VEE virus, Guajira strain, originally obtained from a human case of encephalitis (IVIC P846), was passed six times intracerebrally in 3-day-old mice. The titer used was over 10^{10} DL50/ml intraperitoneally in weanling mice.

Preparation of purified virus stock: Suckling mice were intracerebrally inoculated with 0.02 ml of VEE virus suspension and they were sacrificed when paralysis was evident. Brains were collected by suction into tubes inmersed in a dry-ice bath. The frozen brains were homogenized in a Potter-Elvejehm homogenizer with 5 volumes of ice-cold 0.02 M Tris (tris-hidroximetil-amine-metane), 5 x 10^{-4} M EDTA, and 0.1 M NaCl buffer, adjusted to pH 8.5 by the addition of HCl (Tris-saline). The homogenate was clarified by centrifugation at 1.400 x g for 20 minutes in a Sorvall RC2B centrifugue. The supernatant was spun at 7.000 x g for 20 minutes. Concentration of the virus was effected by precipitation of the supernatant fluid with ammonium sulphate at 50% saturation. The pellet was collected by centrifugation at 26,400 x g, suspended in Tris-saline, and subsequently dialyzed against borate-buffer pH 9.0 overnight at 0ºC.

Density gradient centrifugation: The sample was layered on the top of a 15 to 30% (w/v) sucrose gradient in Tris-saline in lusteroid cellulose tubes and was spun for 4 hours at 41.400 x g in a SW25.1 rotor of the Spinco Model L ultracentrifugue. Fractions were collected through a hole on the bottom of the tube and optical densities at 260 and 280 nm were measured using a Zeiss PMQII spectrophotometer. Hemagglutinating (HA) activity was determined using goose erithrocytes, applying the micromethod described by Sever (13).

Fractions with hemagglutinating activity were pooled and concentrated by centrifugation at 100.000 x g for 1 hour. The sediment was resuspended in Tris saline.

Detergent treatment: Purified virus suspensions were treated with 0.2% sodium desoxycolate (DOC) in Tris-saline for 1 hour at room temperature and layered on the top of a 5 to 30% sucrose gradient containing 0.2% DOC. After centrifugation for 2 hours at 51.000 x g in a SW50L rotor of a Spinco Model L ultracentrifugue, fractions were taken from the bottom of the tube. Optical densities at 260 and 280 nm were measured and hemagglutinating activity was determined as previously described.

Fractions from both the bottom and the top were pooled separately and concentrated by centrifugation at 100.000 x g for 1 hour. The sediments were resuspended in Tris-saline.

Electron Microscopy (EM): Purified virus preparations, before and after DOC treatment, were applied on carbon coated copper or nickel grids for 1 minute. A drop of a saturated aqueous solution of uranyl acetate was applied on the grid and the excess of fluid was blotted with

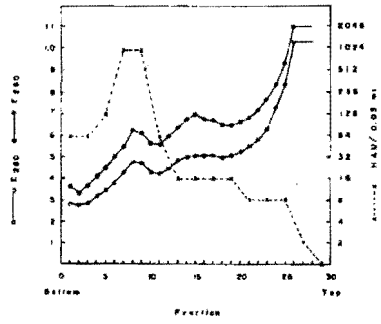

Fig 1. Purification of VEE virus from suckling mice brain tissue through a sucrose density gradient (15% to 30%). The gradient was centrifuged for 4 hr at 41.400 x g.

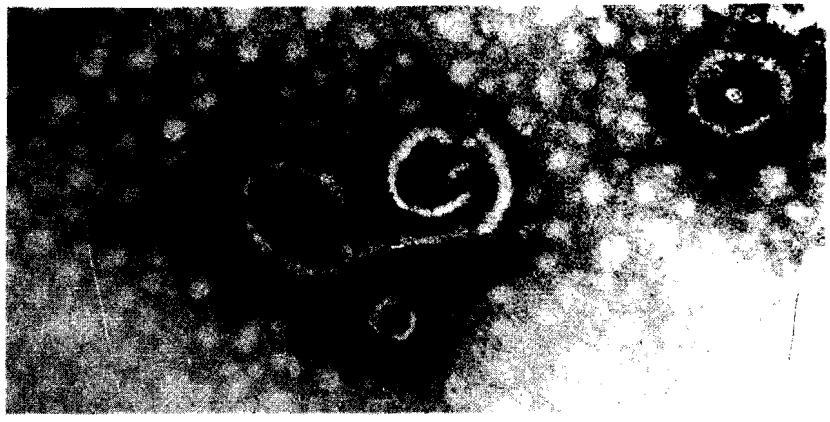

Fig. 2. Two virus particles. Swollen and unfolded membranous forms are also seen. X 162.500.

filter paper. Preparations were examined in a JEM 7A electron microscope with an accelerating voltage of 80 Kv and direct manification ranging from 8.000 to 65.000 X.

RESULTS

Purification of VEE Virus:

Spectrophotometric analysis of purified VEE virus preparation revealed three protein bands distributed at the bottom, the middle part, and the top of the sucrose gradient (Fig. 1). Hemagglutinating activity was detected only in the fraction at the bottom of the gradient. Viral particles observed with the EM are shown in Fig. 2.

In order to clarify the virus preparation, fractions exhibiting HA activity were pooled and concentrated by centrifugation at 100.000 x g for 1 hour. The sediment was placed on a sucrose gradient similar to the one previously

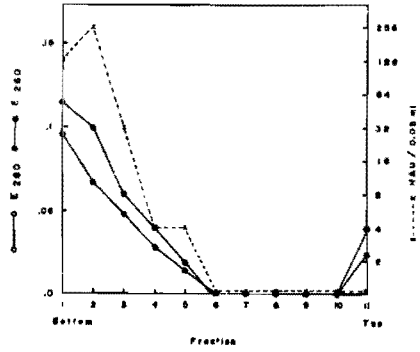

Fig. 3. Sucrose density gradient of partially purified VEE virus. The preparation was layered on the top of a 5% to 30% gradient and centrifuged for 2 hr at 51.000 x g.

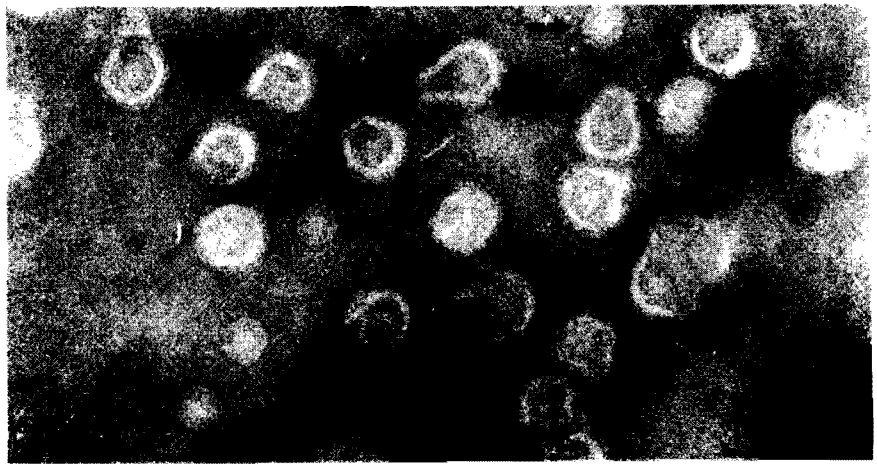

Fig. 4. Various forms of the virus. Opening of viral envelope and fusion of 2 o 3 virus particles. The arrows point to uncoated nucleocapsids. X 162.500.

described. Results show that protein and HA activity were concentrated at the bottom of the gradient (Fig. 3).

This procedure provided a clearer preparation with abundant viral particles as can be observed in Fig. 4.

Dissociation of Viral Particles:

After DOC treatment two bands of protein were clearly separated (Fig. 5). Fractions from the top of the gradient carried the HA activity. EM observations showed the nucleocapsids in the bottom of the gradient (Fig. 6-8) .

Electron Microscopy:

Purified virus preparation show round particles measuring 55 to

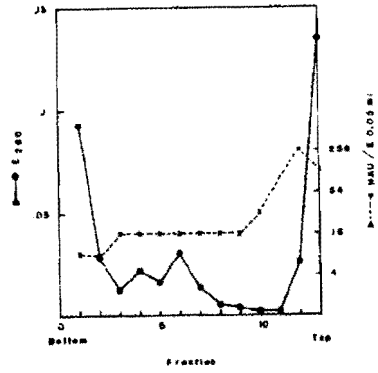

Fig. 5. Density gradient centrifugation of 0.2% DOC-treated virus. The treated virus was centrifuged for 2 hr at 51.000 x g through a 5% to 30% sucrose gradient containing 0.2% DOC.

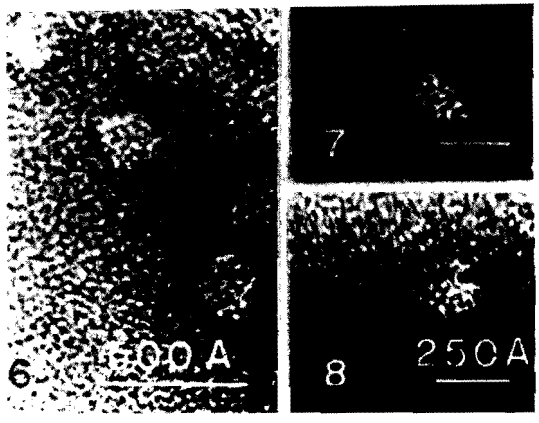

Fig. 6, 7, 8. Nucleocapsids of VEE virus after DOC treatment show ring shaped hollow subunits with icosahedral symmetry. X 455.000.

66 nm. in diameter with a 30 to 40 nm spherical inner core and surrounded by a irregular envelope bearing short proyections (Fig. 2). In some virus particles initial steps of disruption was observed which were characterized by swelling and rupture of the envelope. Fragmented or unfolded membranous forms were also seen. When the fraction was clarified in a second sucrose gradient, viral particles were concentrated and no fragments of disrupted envelopes were observed. Viral particles were slightly larger and the structure of the envelope with superficial spikes was clearly shown (Fig. 4). Opening of viral envelope was frecuently seen. Agglomerates of several virions formed by fusion of 2 or 3 viral particles and naked nucleocapsids measuring 40 nm in diameter were also seen.

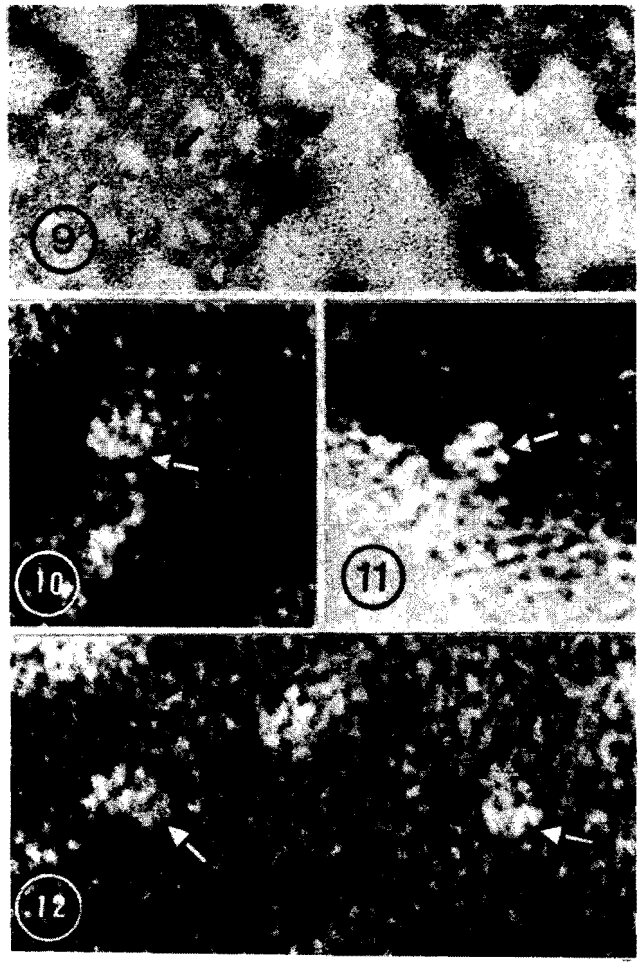

Fig. 9. After DOC treatment density gradient fractions exhibiting HA activity show 12 to 18 nm structures (arrows). X 162.500.

Figs. 10, 11, 12. Round and curved structures with short projections in fractions with HA activity. X 455.000.

Preparations for VEE virus obtained from the bottom of the gradient after DOC treatment show roughly spherical particles measuring from 25 to 32 nm in diameter. These particles appeared to be formed by 10 to 12 nm ring-shaped structural units with a icosahedral arrangement (Fig. 6-8).

Fractions from the top of the sucrose gradient after DOC treatment show masses of amorphous material. Among these electron lucent areas there were abundant 12 to 18 nm structures with round or curve shape, bearing projections which measured 8 to 10 nm, in lenght. (Figs.9-12).

DISCUSSION

A characteristic of enveloped viruses is that they are susceptible to be disrupted into subviral constituents. We have studied the ultrastructure and some physicochemical properties of the nucleocapsid from VEE virus. The presence of 10 to 12 nm ring- shaped structures in the nucleocapsid is in agreement with the observations of Horzinek and Mussgay reported for Sindbis virus (6), and also confirms the cubic symmetry previously suggested for several group A arbovirus (14). The regularly organized ring-shaped hollow subunits may represent groupings of polypeptide chains organized into a icosahedral capsid.

Complete and disrupted VEE virus particles, purified in potassium tartrate density gradients, were examined by Horzinek and Munz (5), an special attention was given to the arrangement of the superficial spikes on the virus envelope. The spherular appearance of the spikes and the shape of some structures isolated after treating VEE virus with Tween-ether suggested a looped configuration for the projections studding the surface of the virion. In our material VEE virus was purified in sucrose gradient and a very clear separation of the viral particles was achieved. Negative stained particles of VEE virus frequently exhibit globular protrusions on the surface envelope, however, we could not identify looped structures on the surface of the virions before and after DOC treatment.

The hemagglutinating properties of the superficial spikes in the envelope of several group A arbovirus has been examined (1, 5, 8, 10). After DOC treatment of VEE virus we observed in the gradient fractions with hemagglutinating activity, 12 to 18 nm round particles surrounded by short projections. This star-like or rossette configuration of the isolated particles is similar to biologically active hemagglutinating subunits separated from the surface of mixovirus (7), mammary tumor virus (11), and Sindbis virus (8), after they were treated with Tween ether or DOC.

Hemagglutinins of mixovirus are known to be glycoproteins and when aggregated, their star-like shape is explained as a consequence of natural repulsion caused by electric charges in the molecules (12). On the basis of the physicochemical and ultrastructural similarities of the hemagglutinin subunits of VEE virus to mixovirus hemagglutinins, the former may also be constitued by glycoprotein molecules. This hypothesis is reinforced by the recent finding of glycopeptides in the envelope of Sindbis virus (2).

Studies to determine the exact chemical composition of the hemagglutinating subunits obtained by disruption of VEE virus envelope are now in progress in our laboratory. The exact relationship between the electric charges of the glycoprotein molecules on the surface of the cell membrane and the hemagglutinat-

ing activity adquired by VEE virus during their assembly remains to be established. Knowledge of the structure of the proteins in the envelope of VEE virus will contribute to clarify the nature of the hemagglutinating phenomenon.

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