CHARACTERISTICS OF VIRION RNA OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

A. S. Agabalyan, et al

Army Medical Research Institute of Infectious Diseases
Frederick, Maryland

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CHARACTERISTICS OF VIRION RNA OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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Certain physico-chemical parameters of infectious RNA of the Venezuelan equine encephalomyelitis virus were determined. It was established that virion RNA sediments in sucrose gradient with sedimentation coefficient of 38-40 S. In fractionation of RNA in cesium sulfate density gradient, the RNA was found in one zone of 1.66 g/cm^3 density. Electrophoresis of virion 40 S RNA in 3.5% agarose-polyacrylamid gel supplied additional information on RNA preparations, making it possible to determine the molecular weight of RNA, which, in several different experiments, varied within the limits of 4.0×10^6 and 4.3×10^6 daltons.

Despite certain difficulties associated with obtaining infectious RNA of the arboviruses (the presence of lipids, low content of RNA in the virion, lability of the RNA), such RNA has been obtained from 13 different arboviruses of groups A and B.

The object of the present study was to investigate certain of the physico-chemical parameters of the RNA of the Venezuelan equine encephalomyelitis (VEE) virus, which is an arbovirus of group A.

Material and methods. The VEE virus used was obtained from the Museum of Viral Strains, D. I. Ivanovskiy Institute of Virology, USSR Academy of Medical Sciences, and subjected to periodic passages in mice. Infectious activity of the viruses was determined from their ability to form plaques under an agar cover [15]. The hemagglutination reaction was achieved with use of the Clark and Casals method [8].

Fibroblasts of chicken embryo were prepared according to the generally accepted method [2]. The culture of embryo fibroblast cells was infected with VEE virus (5-10 biol. toxic units/cell), treated with actinomycin (2 μg/ml), and combined with H^3-uridine (5 μc/ml). Eighteen hours following infection, the cells were removed by cen-

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trifuging at 3,000 g for 15 min, and the superfluous liquid was subjected to further cleaning by a method described earlier [5]. The cleansed virus contained 10^{10} - 10^{11} biol. toxic units/ml, and 6,400-12,800 units of hemagglutinating activity.

RNA from the cleansed and H^3-uridine-tagged virus was separated with use of triple boiling (65°C) phenol deproteinization in the presence of 0.5% SDS, using the method described earlier [1, 4]. The tagged RNA was separated from the uninfected embryo fibroblast cells, which had been previously incubated with H^3- or C^{14}-uridine (5 μc/ml) for a period of 18-20 hrs. The position of the tagged 28S and 18S RNA in the density gradients of the saccharose was determined spectrometrically.

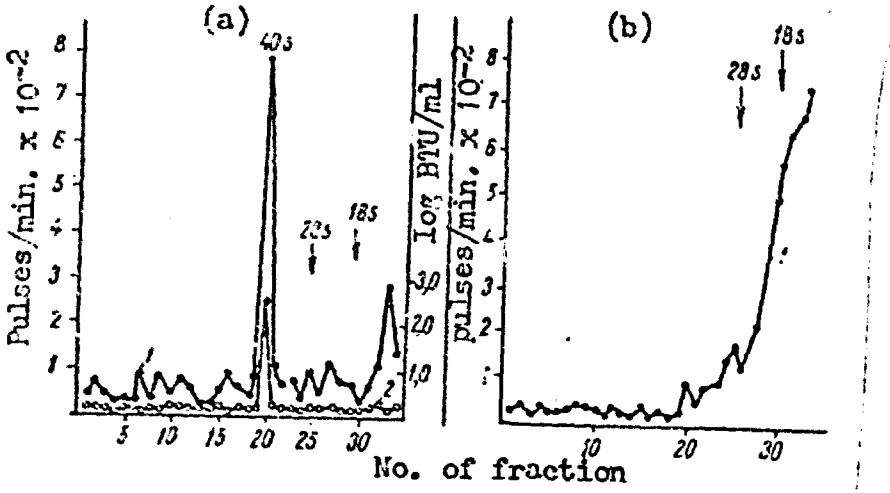

Figure 1. Sedimentation profile of RNA separated from purified virus. 1 - radioactivity; 2 - infectivity: a - without treatment with RNA-ase. Positions of tagged 28S and 18S RNA are shown with arrows.

The infectious properties of viral RNA were determined with the method of plaques under an agar cover. The titration method for viral RNA has been described in detail in [1]. Centrifuging of RNA in the saccharose gradient was carried out by the method described in [6]. For this purpose, the RNA preparations were dissolved in 1 ml of tris-buffer (0.01 M tris, 0.001 M EDTA, 0.1 M NaCl), pH 7.4. The pressure gradients of the saccharose were prepared by the commonly used method [14]. Centrifuging was conducted with use of hKE-Producter equipment (collector with drop counter, ultraviolet absorptiometer with flow cuvette), and self-

recording potentiometer PSR-1-0.8 (USSR). The values of the coefficients of sedimentation were determined by the Martin and Ames method [14].

Study of the density characteristics of the RNA was made by centrifuging the latter in the density gradient of cesium sulfate. For this purpose, 4 ml of saturated solution were mixed with 0.8 ml of RNA. The mixture was prepared on a tris-buffer of pH 7.4. Centrifuging was conducted in the rotor SW-50 of the Spinko L-2 centrifuge at 42,000 rpm for 48 hrs.

The fractions were collected following calcination of the bottom of the test tubes. Densities of the solutions of cesium sulfate in the fractions were determined from the refractometric magnitudes [11].

The polyacrylamid gels were prepared from 0.5 agarose, 3.5% acrylamid and N,N'-methylenebisacrylamid in tubes of 0.4 cm diameter and 6 cm length.

Electrophoresis of the RNA was accomplished with tris-buffer in the "Polyanalist" apparatus.

The molecular weight of the samples studied was determined with use of preparations of C^{14}-uridine of cellular 28S and 18S RNA, the molecular weights of which were 1.58×10^6 and 0.7×10^6, respectively [13]. Radioactivity was measured on the Tri-Carb Spectrometer Packard.

Results. The RNA preparations exhibited an ultraviolet absorption spectrum with absorption maximum at 260 Nm, characteristic of the nucleic acids. The E_{260}/E_{250} ratio was approximately 3. The amount of RNA in the samples was determined from equating 1 toxic unit at 260 Nm with 40 μg of RNA.

The RNA samples studied (0.5-1.0 mg/ml) were analyzed in saccharose density gradients (5-20 and 10-30%), the saccharose being prepared on tris-buffer. Following centrifuging, all fractions of the saccharose gradient were divided into two parts, for one of which the radioactivity was determined, and for the other the infectious activity of the material of the fractions.

Data of the sedimentation analysis, shown in Figure 1-a, indicate that RNA extracted from purified viral suspension has a sedimentation coefficient of 38-40S. Fractionation of RNA preparations which had been processed with RNA-ase (10 μg/ml, 30 min, 37°C) presented a different picture of the radioactivity distribution (Fig. 1-b). To determine the infectious activity of fractions of saccharose gradient, material of the fractions was titrated by the plaque method. The results, shown in Fig. 1-A, indicated that the infectious activity of the material was mainly associated with the fraction in which maximal radioactivity appeared, while the peaks of infectivity and radioactivity coincided

completely. Infectivity was absent from RNA preparations treated with RNA-ase.

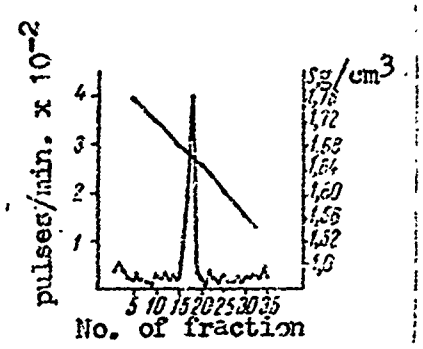

Figure 2. Distribution of radioactivity with equilibrium centrifuging of virion RNA in the cesium sulfate density gradient

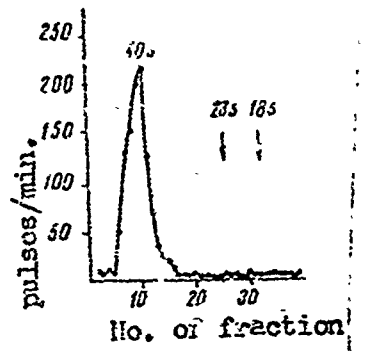

Figure 3. Electrophoresis of virion RNA in 3.5% agarose-polyacrylamid gel. The position of the tagged 28S and 18 S RNA is indicated by arrows. Entry into gel state on left.

In the density gradient of cesium sulfate, virion RNA is distributed in a single zone with buoyant density of 1.56 g/cm^3 (Fig. 2). Treatment of these RNA preparations with ribonuclease led to disappearance of radioactivity peaks.

To obtain additional information on the homogeneity of RNA preparations, and to study their electrophoretic mobility and determine the molecular weight of RNA, the appropriate fractions of the saccharose gradient (fractons Nos. 19-21, Fig. 1) were combined, the RNA newly treated with phenol, extracted with ethanol, and studied with the help of electrophosphoresis in 3.5% agarose-polyacrylamid gel.

On the accompanying electropherograms (Fig. 3) is shown the electrophoretic mobility of the viral RNA. It is quite evident here that viral 40S RNA have identical electrophoretic mobility and that the peaks of radioactivity distinctly coincide.

To determine the molecular weight of the VEE virus RNA, coelectrophesis of ribosome 28S and 18S RNA was run (See "Material and Methods"). To obtain C^{14} markers, chicken cell RNA was fractionated in the saccharose density gradient, and fractions corresponding to 28S and 18S RNA were removed, the position of these being determined by ultraviolet absorption.

Comparison of the electropherograms made it possible to determine the molecular weight of VEE virus RNA; the value of this varied within the limits of 4×10^6 and 4.3×10^6 daltons. A certain variation in the results obtained can evidently be explained by the position of the tagged RNA, which in the

various tests with electrophoresis was observed in the different fractions.

In computing molecular weight with the formula proposed by A. A. Spirin (mol. wt. = 1,500 x $S^{2.1}$, where S is the coefficient of sedimentation of the RNA), we obtained values close to the figure obtained with the help of electrophoresis of the RNA in a polyacrylamid gel.

Discussion. Only in the past few years has arbovirus RNA become an object of biochemical research. Most studied (as regards sedimentation rate, buoyant density, nucleotide content, molecular weight) have been the arboviruses of group A. It should be noted that the parameters just mentioned, in the majority of representatives of group A, have very similar values, such variation as is present being evidently explainable in terms of the differences in research methods used by the investigators.

Virion RNA of the VEE virus remained practically unstudied until very recently. The data obtained her in analysis of this RNA in the density gradient of saccharose made it possible to establish that it is exhibited by a a single homogeneous peak with sedimentation coefficient of 38-40S.

This homogeneous and RNA-ase-sensitive peak showed an infectivity peak whose maximal values coincided with those of the radioactivity maximum.

Similar results have been obtained by a number of investigators in the analysis of tagged RNA from other arboviruses from group A. It should be noted, however, that, despite very close similarities among arbovirus RNA, a certain variation in the values of their sedimentation coefficients is found. Those coefficients vary within the limits of 38S and 45S [9, 12, 16, 18]. This variation, probably, is associated with differences in the conditions of gradient centrifuging. However, infectivity in all of the works referred to coincided with the radioactivity peak.

Analysis of virion RNA with use of electrophoresis in agarose-polyacrylamid gel made it possible to obtain additional information on the homogeneity of the investigated RNA samples, and to determine their molecular weight as well. In general, deducing the molecular weight of arbovirus RNA is a difficult matter, on account of the very l r RNA content (1-2%) in the virions—a circumstance which naturally complicates the production of a large amount of RNA. Until recently, only in a single case, with use of sedimentation coefficient and specific density of the VEE virus, was it possible to establish the molecular weight of its RNA within the limits of 1×10^6 and 3×10^6 daltons [17].

Nevertheless, research recently conducted on the molecular weight of RNA from two other arboviruses (Sindbis and Semliki Forests*) has shown that RNA arboviruses are heavier than previously supposed [7, 10].

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*Both names adapted from the Russian.

The results referred to were applied to the data obtained by the present writers in their derivation of the molecular weight of VEE virus RNA.

The impossibility of any more precise determination of the molecular weight of RNA, in our view, is inevitable on account of a number of factors. Thus, no data have appeared indicating the effect of the buffers used in the processes of electrophoresis on the conformation and mobility of the RNA or the positions of tagged 28S and 18S RNA; and the final result will be influenced by the concentrations of the polyacrylamid gels. For example, in using 2.5% gels, we obtained, for the most part, lower values of molecular wieght (3.89×10^6) than with the use of 3.5% gels (4.3×10^6 daltons).

From results obtained, we may assume that the VEE virus RNA differs little in physico-chemical properties from the RNA derived from other representatives of group A of the arboviruses.

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