NOTE

Molecular Weight of Sindbis Virus Ribonucleic Acid as Measured by Polyacrylamide Gel Electrophoresis

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The molecular weight (MW) of Sindbis virion ribonucleic acid (RNA) determined by gel electrophoresis was in a range of 3.89×10^6 to 4.45×10^6 daltons. Upon denaturation with urea, it separated into fragments having an MW of 1.76×10^6 daltons. Viral-specific Sindbis 26S RNA contained a major species having an MW of 1.76×10^6 daltons.

Measurement of the molecular weight (MW) of the viral genome in group A arboviruses is hampered by the relatively low nucleic acid content of these viruses [5.9% ribonucleic acid (RNA) in Sindbis virus; 8] which makes it difficult to obtain sufficient amounts of undegraded viral RNA for biophysical measurements. Only in the case of Eastern equine encephalomyelitis have biophysical studies based on determination of sedimentation coefficient and specific viscosity led to an estimated MW of 10^6 to 3×10^6 (13). Recent experiments indicate that the MW of the arbovirus genome is substantially greater than the widely accepted figure of 2×10^6 daltons [3, 12; J. H. Strauss, 1969, *unpublished data*, quoted in H. B. Levy (ed.), *Biochemistry of Viruses*, Marcel Dekker, London].

It is known that the RNA of a group A arbovirus has a higher degree of secondary structure than polio RNA (10). Denaturation of Western equine encephalomyelitis 42S RNA by heat, dimethylsulfoxide (DMSO), or 8 M urea resulted in a decrease in sedimentation coefficient to 26S (11). Similar observations were made when the effects of heat were examined on the sedimentation coefficients of Sindbis RNA (5) and Semliki Forest virus (SFV) RNA (3). Since a 26S single-stranded RNA species of unknown function is also present in arbovirus-infected cells (4, 6, 11) and this "natural" 26S RNA may be a precursor to the RNA found in the virion (3), knowledge of the MW of the viral genome is important in attempting to evaluate these findings. Recently, it has been found that, based on its relative electrophoretic mobility, 45S SFV RNA has an MW of 3.4×10^6 ; it has been proposed

that the viral genome is made up of two 26S RNA pieces of MW 1.74×10^6 held together by hydrogen bonds (3).

We compared the behavior of Sindbis 42S RNA from virions and "natural" 26S from infected cells on polyacrylamide gels and estimated the MW relationships by using chick ribosomal RNA (rRNA) as reference markers.

Polyacrylamide gel electrophoresis (PAGE) of Sindbis 42S RNA in 3% gels (Fig. 1A) revealed a single major component. The 26S viral RNA isolated from the 65S particles of virus-infected cells (4) moved with a higher mobility in gels (Fig. 1B). The profile in gels observed for viral 26S RNA indicates a heterogeneity in the fraction. It is not known whether this heterogeneity represents the natural condition of 26S RNA in the cytoplasmic particle or whether breaks may have been induced during the extraction procedure. By assuming that electrophoretic mobility of RNA is proportional to log MW (2) and by using chick 28 and 18S rRNA (MW, 1.58×10^6 and 0.7×10^6 ; 7) as reference markers, a value of 4.45×10^6 was obtained for the MW of 42S Sindbis RNA and a value of 1.76×10^6 was obtained for the slowest moving peak in 26S RNA. Values as low as 3.89×10^6 for 42S RNA have been obtained with 2.5% acrylamide-0.5% agarose gels where higher mobilities of RNA species are obtained (Arif and Faulkner, *unpublished data*).

We denatured Sindbis 42S RNA by treatment with 8 M urea and examined the product on 3% gels containing 8 M urea and containing 8 M urea in the electrophoresis buffer. Although mobilities of RNA species are lower in urea, the electrophoretic profile is not altered (9). The profile in Fig. 1C shows that the mobility of 42S

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FIG. 1. Electrophoresis of Sindbis 42S and 26S RNA in 3% acrylamide gels with 0.2% sodium dodecyl sulfate (SDS; A and B) and 3% acrylamide gels with 0.2% SDS and 8 M urea (C and D). ^3H - or ^{32}P -42S was prepared from purified virions, and ^3H -26S RNA was prepared from 65S cytoplasmic particles (4). In A and B, RNA dissolved in 10 to 30 μl of STB [100 mM NaCl, 50 mM tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.2] was layered on 6-cm gels in EPB buffer (4 mM Tris, 2 mM sodium acetate, 1 mM EDTA, pH 7.2, containing 0.2% SDS). Electrophoresis proceeded at room temperature for 2.5 hr at 8 v/cm of gel. Gels were fixed in 7% acetic acid, sliced into 2-mm pieces, digested in H_2O_2 , and counted as described (5). Chick ^{32}P rRNA (5) was present as indicated. In C and D, RNA samples obtained from sucrose gradients (4) were made 12 M by addition of the solid urea. Samples were incubated at 37 C in a water bath for 30 min and applied to gels which contained 8 M urea. Electrophoresis was in EPB containing 8 M urea and was continued for 12 hr at 8 v/cm of gel. Processing of the gels was as for A and B.

RNA in gels containing 8 M urea was very close to that of 28S rRNA. Thus, upon denaturation, 42S RNA was converted to a species of RNA having an MW of about 1.76×10^6 daltons. Coelectrophoresis of ^{32}P -42S Sindbis RNA and ^3H -26S RNA from 65S cytoplasmic particles (Fig. 1D) showed that (i) the major peak observed with 26S RNA (Fig. 1B) is not denatured by urea and (ii) its MW is the same as that of

denatured 42S RNA since both species of RNA were coincident on the gel after electrophoresis.

The results indicate that Sindbis virion RNA has an MW in a range of 3.89×10^6 to 4.45×10^6 daltons and that it contains fragments, having an MW of 1.76×10^6 daltons, which can be separated by a denaturing agent.

Estimations of the numerical value of the MW of the genome and its fragments are subject to

uncertainties which arise from incomplete knowledge of the effects of electrophoresis buffers on the conformation and mobilities of RNA and upon the lack of general acceptance of absolute values for the MW of ribosomal RNA species used as markers. An unpublished report [J. H. Strauss, 1969, quoted in H. B. Levy (ed.), *Biochemistry of Viruses*, Marcel Dekker, London] puts the MW of the Sindbis genome at 4×10^6 to 5×10^6 daltons, based on sedimentation in DMSO. This high value, obtained in the presence of a denaturing agent, may have resulted from incomplete removal of divalent ions before the measurement was made. It is known that the melting temperature of RNA may be increased by as much as 25 C in the presence of 10 mM $MgCl_2$ (1). It is possible that separation of viral RNA fragments would not occur in DMSO in some ionic environments.

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