Ribonuclease Sensitivity of Semliki Forest Virus Nucleocapsids

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Treatment of Semliki Forest virus nucleocapsids with pancreatic ribonuclease (1 μg/ml, 37 C) digests the ribonucleic acid to acid-soluble fragments; the nucleocapsid protein forms a rapidly sedimenting aggregate.

The nucleocapsid of Semliki Forest virus, a group A arbovirus, is a spherical particle, 30 to 40 nm in diameter, which contains the viral ribonucleic acid (RNA) and probably a single protein having a molecular weight of approximately 32,000 (2, 3, 8). Nucleocapsids, free of the viral envelope, can be isolated (8) and purified (2) from homogenates of infected chick embryo cells by repeated sedimentation on sucrose gradients. We have found that the RNA within isolated Semliki Forest virus nucleocapsids is degraded in situ by mild treatment with bovine pancreatic ribonuclease (EC 2.7.7.16). The extent of this reaction and the fate of the RNA and protein of ribonuclease-treated nucleocapsids are reported in this communication.

Ribonuclease sensitivity of nucleocapsids in cell homogenates. Nucleocapsids sediment as a single peak at about 145S in sucrose gradients (2, 8). To determine whether the RNA within Semliki Forest virus nucleocapsids is accessible to digestion by ribonuclease, homogenates of infected cells in which viral RNA was specifically labeled were incubated with or without ribonuclease and analyzed by sucrose gradient sedimentation. Primary cultures of chick embryo cells (approximately 6 × 10⁷ cells) in 100-mm plastic petri dishes were infected with 20 to 50 plaque-forming units (PFU) of Semliki Forest virus (Kumba strain) per cell as described previously (1, 2) and incubated with 2 μg of actinomycin D per ml from 0 to 4 hr after infection. At 4 hr, 20 μCi of uridine-5³H per ml (1,000 mc/mnmole, Amer- sham-Searle Corp., Des Plaines, Ill.) was added, and 1 hr later the cells were scraped from the dishes, pelleted, and Dounce homogenized in 3 ml of cold reticuloocyte standard buffer [RSB: 0.01 M tris(hydroxymethyl)aminomethane, pH 7.4; 0.01 M NaCl; 0.0015 M MgCl₂]. Nuclei and debris were removed by pelleting. One-half of the supernatant was incubated with 1 μg of ribonuclease per ml (crystallized from ethanol or code RAF, electrophoretically pure, Worthington Biochemical Corp., Freehold, N.J.) at 37 C for 10 min, and the other half was incubated under the same conditions without enzyme. Both samples were chilled in ice, immediately layered over identical 5 to 20% (w/w) sucrose gradients prepared in RSB, and centrifuged for 2 hr at 24,000 rev/min (60,000 × g) in a Spinco SW-25.1 rotor at 5 C. The gradients were collected by displacement, absorbance at 254 nm was continuously recorded, and samples of 1-ml fractions were assayed for acid-precipitable radioactivity.

All of the labeled RNA in the 145S nucleocapsid peak was digested to acid-soluble fragments by ribonuclease treatment (Fig. 1). Most of the labeled RNA at the top of the gradient (free viral RNA) and in the pellet (probably replicating RNA species; 9) was also digested. In similar experiments in which more concentrated cell extracts (6 × 10⁸ cells) were used, a portion of the radioactivity in the nucleocapsid peak remained after ribonuclease digestion. This was probably due to the lower relative proportion of ribonuclease to nucleocapsid RNA in these experiments.

Ribonuclease treatment of partially purified nucleocapsids. In a further experiment, ³H-uridine-labeled nucleocapsids pooled from the 145S region of a 5 to 20% sucrose gradient were incubated with ribonuclease (1 μg/ml, 37 C, in RSB), and the amount of acid-precipitable label remaining after different times was measured. The RNA is these partially purified nucleocapsids was also rapidly digested to acid-soluble fragments (Fig. 2).

The RNA within nucleocapsids of two other group A arboviruses, Western equine encephalitis virus (16) and Sindbis virus (4, 17), has also been reported to be digested by ribonuclease treatment.
could be seen with the electron microscope. This result suggests that ribonuclease treatment of nucleocapsids leads to disintegration of the capsid structure, rather than leaving intact capsids deficient in RNA. To elucidate further the fate of the protein and RNA of nucleocapsids after ribonuclease treatment, we carried out sedimentation analysis of untreated and ribonuclease-treated nucleocapsids.

Nucleocapsids were labeled with 3H-uridine or a 3H-amino acid mixture (Schwarz BioResearch Inc., Orangeburg, N.Y.) and purified by a method described in detail elsewhere (2). Briefly, fractions from the 145S peak of a 5 to 20% sucrose gradient such as that in Fig. 1 were dialyzed against a buffer lacking magnesium (PN buffer: 0.01 M sodium phosphate, pH 7.3; 0.1 M NaCl) to dissociate the ribosome species which represent the major contaminants of such a preparation. The diazylated nucleocapsids were then sedimented through one or two further sucrose gradients.

However, Friedman and Berezesky (8) reported that Semliki Forest virus nucleocapsids are not ribonuclease-sensitive. The latter authors found a peak of 3H-uridine-labeled material at 145S in a sucrose gradient of a ribonuclease-treated infected cell homogenate, but they did not report the results of controls in which the homogenate was incubated without ribonuclease. Thus, it is possible that in their experiments, too, some of the RNA in Semliki Forest virus nucleocapsids was digested by ribonuclease.

**Fate of ribonuclease-treated nucleocapsids.** Electron microscopic examination of purified nucleocapsids (2) reveals numerous particles of uniform size, 40 nm in diameter, which are penetrated by negative stains (sodium phosphotungstate or uranyl acetate). However, after ribonuclease treatment, no intact nucleocapsids or other structures
prepared in the same buffer and were finally dialyzed against PN buffer to remove the sucrose. Prelabeling studies showed that approximately 5% of the RNA and 25% of the protein in these purified nucleocapsid preparations were of cellular origin (2). However, almost none of this contaminating cellular RNA or protein was labeled under the conditions used, as shown by sucrose gradient sedimentation of the nucleocapsid RNA and gel electrophoresis of the nucleocapsid protein (2, 3).

Samples of labeled nucleocapsids in PN buffer were treated with 1 μg of ribonuclease per ml for 10 min at 37°C, and identical samples were incubated without enzyme. In PN buffer, nucleocapsid RNA is digested more slowly than in RSB, and approximately one-third of the RNA remains acid-precipitable after 10 min of ribonuclease treatment. The samples were layered over 5 to 30% (w/w) sucrose gradients prepared in PN buffer, with a 50% potassium tartrate cushion at the bottom of each gradient, and centrifuged for 1.5 hr at 25,000 rev/min (90,000 × g) in an SW-27 rotor at 5°C. Radioactivity in samples of 1-ml fractions was measured, and the results are shown in Fig. 3 and 4.

Untreated nucleocapsids (Fig. 3A and 4A) sedimented as a single peak in fractions 10 to 16, which accounts for 95% of the label in the gradients. There was a small amount of label at the interface between 30% sucrose and the 50% potassium tartrate cushion, probably due to a very slight degree of aggregation of the purified nucleocapsids. A lower counting efficiency made the total label (acid-soluble plus acid-precipitable) in nucleocapsid RNA appear less than the acid-precipitable label alone.

After ribonuclease treatment, all of the protein in 3H-amino acid-labeled nucleocapsids sedimented to the bottom of the gradient and formed a narrow band in the tartrate cushion (Fig. 3B). None of the labeled nucleocapsid protein remained in the position of the nucleocapsid peak in Fig. 3A, nor was any protein found at the top of the gradient. Thus, ribonuclease treatment of nucleocapsids in PN buffer appears to lead to

![Graph](http://jvi.asm.org/)
aggregation of the nucleocapsid protein into a large structure which sediments very rapidly.

After ribonuclease treatment of 3H-uridine-labeled nucleocapsids (Fig. 4B), most of the remaining acid-precipitable RNA was found in the tartrate cushion at the bottom of the gradient. None of the acid-precipitable RNA sediments at the position of untreated nucleocapsids. Measurement of the total label (acid-soluble plus acid-precipitable) from the same gradient (Fig. 4B) shows that most of the digested RNA is released and remains at the top of the gradient in acid-soluble form.

Thus, ribonuclease treatment of nucleocapsids in PN buffer leads to the formation of a rapidly sedimenting aggregate containing all of the nucleocapsid protein and all of the remaining acid-precipitable RNA. No nucleocapsid protein or RNA sediments at the position of intact, untreated nucleocapsids. Most of the RNA is digested to slowly sedimenting acid-soluble fragments.

Most animal virus nucleocapsids are resistant to ribonuclease, although the helical nucleo-
capsid of influenza virus has recently been shown to be ribonuclease-sensitive (6, 14). Several small, RNA-containing plant viruses are, however, sensitive to ribonuclease (4, 7, 13). Ribonuclease
sensitive spherical nucleocapsids may have a loose capsid structure which allows the penetration of ribonuclease molecules to the viral RNA. Also, portions of the viral RNA strand may be located at the surface of the capsid.

Group A arbovirus virions consist of a nucleo-
capsid tightly wrapped in a lipid-containing envelope (1, 11). The RNA within complete virions of several group A arboviruses is resistant to ribonuclease digestion (12, 15, 18), although, as in Semliki Forest virus, their nucleocapsids are ribonuclease-sensitive (5, 16, 17). Thus, the envelope protects the viral RNA within the nucleocapsid from enzymatic attack.

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