THE SOLVENT DENATURATION OF DOUBLE-STRANDED RNA FROM POLIOVIRUS INFECTED HELA CELLS

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A double-stranded RNA intermediate has been implicated in the replication of viruses containing a single-stranded RNA genome for animal (Montagnier and Sanders,1963; Baltimore, Becker and Darnell, 1964), bacterial (Weissman, Borst, Burdon, Billeter and Ochoa, 1964; Kelly and Sinsheimer, 1964; Nonoyama and Ideda, 1964) and plant (Shipp and Haselkorn, 1964) viruses. In most systems, the duplex nature of this species of RNA has been demonstrated indirectly by its resistance to enzymatic cleavage by RNAase, although hyperchromism (Amman, Delius and Hofschneider, 1964) and X-ray diffraction (Langridge, Billeter, Borst, Burdon and Weissman, 1964) have been utilized when very large quantities were available. Because of the very high melting temperature (90-105°C) exhibited by these structures, some degradation invariably accompanies melting, making it difficult to assess the physical integrity of the virus-like strand and of its compliment. We present here a method for achieving strand separation under mild conditions which does not degrade the single strands thus released, and permits one to examine the duplex structure for single-strand breaks. Using this method, we show that the replicative form of poliovirus RNA, which sediments as a single species at 20S can be completely transformed into a more rapidly sedimenting species, whose behavior is the same as single-stranded RNA extracted from mature virus, sedimenting at 35S.

MATERIALS AND METHODS

Single-stranded viral RNA was prepared from purified poliovirus grown in HeLa cells in the presence of C^{14}-uridine (Levintow and Darnell, 1960). The double-stranded form was prepared as described by Baltimore (1966) from infected cells and purified by two zonal centrifugations in sucrose solutions of different ionic strengths. The double-stranded form was labeled with H^3-uridine. Strand separation was achieved by incubating the RNA in the presence of dimethyl sulfoxide (DMSO) with the addition of carrier RNA prepared by phenol extraction of the cytoplasm of uninfected HeLa cells. Aliquots of purified C^{14}-single-stranded viral RNA and H^3-double-stranded RNA dissolved in SDS-buffer (0.5% sodium dodecylsulfate, 0.1M NaCl, 0.01M Tris, pH 7.4, 0.001M EDTA) were added

to a solution containing cold carrier RNA to give a final concentration of about 50 μg/ml. Dextran sulfate was added to a concentration of 1 mg/ml. Three milliliters of dimethyl sulfoxide were then added to 0.5 ml of the above mixture, which was then incubated at 37°C for 18 minutes. The RNA was precipitated with 7 ml of ethanol at -20°C for one hour, the precipitate collected by centrifugation, and resuspended in 0.5 ml of SDS-buffer by stirring at 37°C for 30 minutes. Sedimentation analysis of this RNA was carried out by layering the solution on a 15-30% sucrose SDS-buffer gradient and centrifuging for 15 hours at 22,000 rpm in a Spinco SW25.1 rotor.

Fractions of 0.5 ml were collected and alternate fractions placed into two sets. The odd numbered fractions were precipitated with 10% TCA after the addition of 200 μg of protein carrier, collected on millipore filters and placed in glass scintillation vials. The samples were then treated with NH_4OH as previously described (Penman, Becker and Darnell, 1964) and Bray's scintillating mixture added. The ionic composition of the even numbered fractions was changed by the addition of 0.1 ml of a 5M KCl, 0.05M $MgCl_2$ solution and then the samples were refrigerated for 15-20 minutes. Most of the SDS was precipitated in this manner permitting rapid digestion of RNA by added RNAase. Ten micrograms of pancreatic RNAase was added to each of these samples and the mixtures incubated for 20 minutes at room temperature. The samples were then precipitated with 10% TCA and assayed as described above. Radioactivity was measured in an Ansitron scintillation counter.

RESULTS

Previous experiments in this laboratory have shown that the double-stranded RNA retains its secondary structure in concentrations of DMSO up to 70%. Figure 1a shows the sedimentation profile of an untreated sample of the double-stranded species. Fig. 1b shows the profile of acid-precipitable radioactivity in a mixture of H^3-labeled double-stranded RNA from poliovirus infected HeLa cells and C^{14}-labeled mature poliovirus RNA after treatment in 85% DMSO.

It is clear that the double stranded form has been transformed by this treatment from a slowly sedimenting (20S) species to a rapidly sedimenting form (35S), a transformation which precludes degradation. The rapidly sedimenting species produced by this treatment has a profile coincident with RNA from mature virus particles. Since the radioactivity in the H^3-35S peak represents all the material introduced into the sample, there has been no net loss of material, indicating that both the virus-like strand and its complement are intact and have the same sedimentation profile. Furthermore, the original double-stranded form is completely RNAase resistant, while the converted form, as well as the original viral RNA is completely degraded by RNAase.

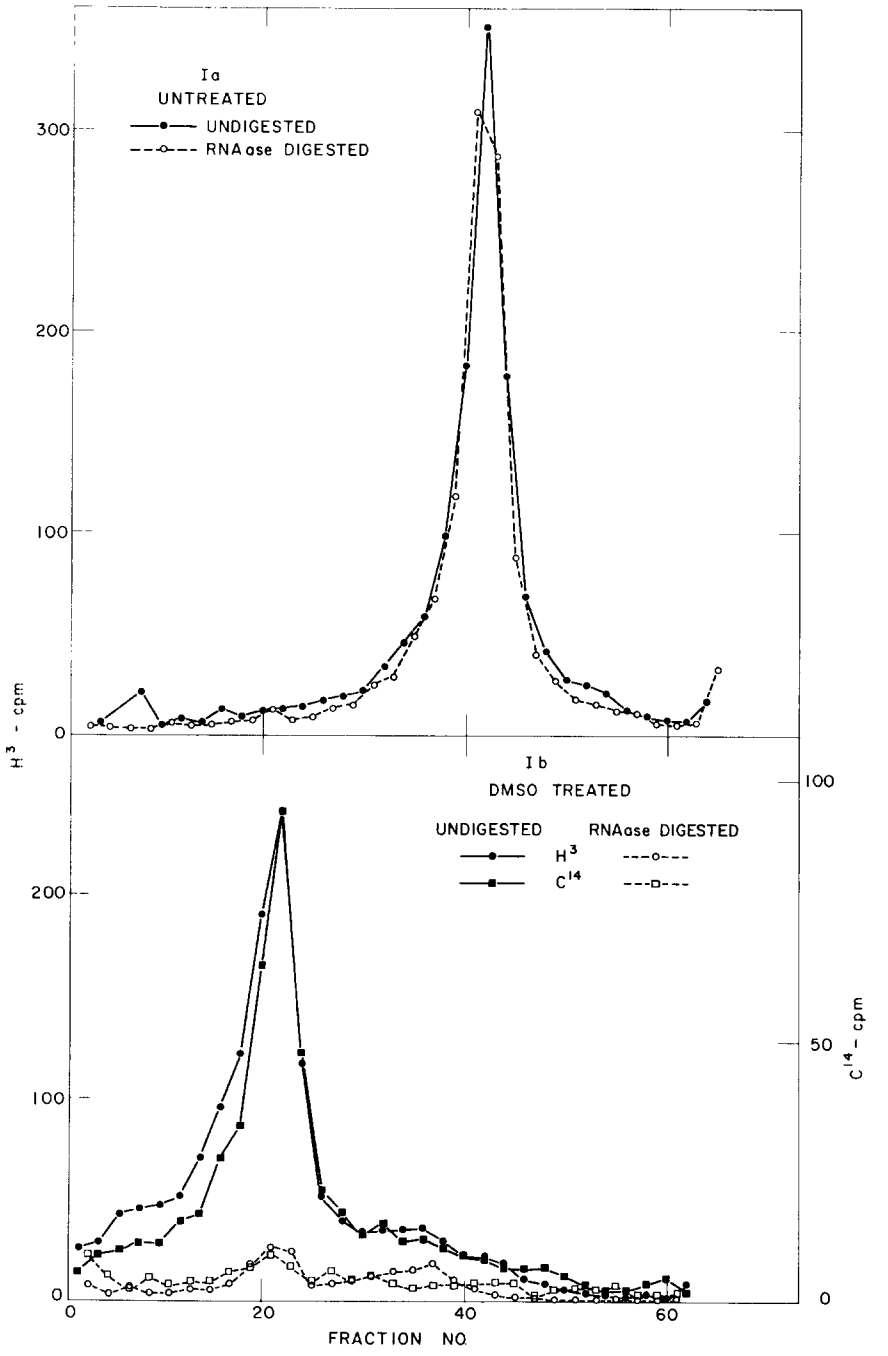

Figure 1. Sedimentation profile of acid-precipitable radioactivity in single and double-stranded RNA. Solid lines and filled symbols represent total acid precipitable radioactivity. Dashed lines and open circles represent RNAase resistant, acid precipitable radioactivity.

a) Untreated H^3-double-stranded RNA

b) DMSO treated H^3-double-stranded RNA (circles) and C^{14}-single-stranded RNA (squares).

Centrifugation of the 15-30% sucrose-SDS-buffer density gradients was for 15 hours at 22,000 rpm, at 25°C in a Spinio SW25.1 rotor.

DISCUSSION AND CONCLUSIONS

Various organic solvents, such as dimethyl sulfoxide and formamide (Marmur and Ts'o, 1961; Helmkamp and Ts'o, 1961) have been shown to disrupt the secondary structure of polynucleotides, and to interfere with the stacking of the bases necessary for the integrity of these structures (Fasman, Lindblow and Grossman, 1964; Katz and Penman, 1966). By adding dimethyl sulfoxide to a solution containing a double-stranded polynucleotide, the stabilization by non-covalent forces responsible for the secondary structure is destroyed, and the strands separate into individual polynucleotide chains. By treating the RNAase resistant species formed in poliovirus infected HeLa cells with dimethyl sulfoxide, we have been able to demonstrate its duplex nature. The very large increase in sedimentation coefficient precludes a degradative process occurring during the course of this treatment. Aggregation of unseparated double strands is ruled out by the transformation from resistance to sensitivity to enzymatic attack by RNAase. The transformation of all of the radioactivity to this 35S form demonstrates the integrity and homogeneity of both the viral RNA and of its complement. The data also strongly suggest that the double-stranded RNA is not in the form of a circle or hairpin but is simply a hydrogen-bonded association of two polynucleotide strands.

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