THE INFLUENCE OF CO-PRECIPITANTS ON THE SOLVENT DENATURATION OF DOUBLE-STRANDED RNA

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The development of the technique of solvent denaturation of double-stranded RNA (Katz & Penman, 1966; Applebaum, Ebstein & Wyatt, 1966) has provided us with a test for molecular integrity of a variety of biologically significant species of RNA (Katz & Penman, in prep.). This test presumably demonstrates whether an RNA species is a single covalently linked polynucleotide chain, or whether it is a non-covalently linked aggregate of two or more chains. By changing the nature of the solvent, the non-covalent forces which are responsible for the formation of regular double-stranded structures and of other non-regular aggregates are altered and disruption of the secondary structure of the polynucleotide strands occurs. The solvation conditions are altered by the addition of dimethylsulfoxide to the solution of the RNA under study. After a short incubation period, cold ethanol is added to precipitate the polynucleotides. The precipitate is collected by centrifugation, resuspended in aqueous buffer and analyzed for its sedimentation properties in zone sedimentation. In order to insure the precipitation of the RNA under study, additional polynucleotides are added to the sample as co-precipitants and carried through the experiment. This "carrier" acting as a co-precipitant gives rise to a macroscopically visible precipitate. We wish to describe explicitly here how the demonstration of strand separation for double-stranded RNA depends on the nature of this "carrier". Some types of RNA used as carrier

apparently permit the reformation of the original complex and an absence of "melting" or permit the formation of new aggregates of previously unaggregated material.

Materials and Methods

Purified poliovirus RNA and double-stranded RNA from poliovirus infected HeLa cells were prepared as described previously (Baltimore, 1966; Katz & Penman, 1966). Ribosomal RNA was prepared from HeLa cell ribosomes by the hot phenol-SDS method (Scherrer & Darnell, 1962). Polyadenylic acid (poly-A) was obtained from Miles Chemical Co. and used without further treatment. t-RNA (Grand Island Biological Co.) was deproteinized with hot phenol, alcohol precipitated and resuspended in SDS buffer (0.5% sodium dodecylsulfate, 0.1M NaCl, 0.01M Tris, pH 7.4, 0.001M EDTA). The strand separation technique by the addition of 6 volumes of dimethylsulfoxide (DMSO) to the RNA solution is essentially that described previously (Katz & Penman, 1966), except that SDS buffer was used throughout. Other variations will be explicitly noted.

Results

The initial demonstration of separation of double-stranded RNA from poliovirus infected HeLa cells into two complementary strands of identical sedimentation behavior was accomplished in the presence of HeLa ribosomal RNA (r-RNA) as the carrier species (Katz & Penman, 1966). In the presence of t-RNA, however, strand separation cannot be demonstrated either by sedimentation or by RNAase sensitivity. Figure 1 shows the results of identical treatment of two aliquots of double-stranded RNA with t-RNA present as carrier in one case and ribosomal RNA present in the other. The double-stranded material treated in the presence of ribosomal RNA (squares) sediments more rapidly than if treated in the presence of t-RNA (circles), while its RNAase resistance (dotted lines) has been eliminated. The hypothesis was made that the apparent failure to achieve strand separation when t-RNA is used as carrier is due to the inability of the small compact t-RNA molecules to prevent reformation of the double-helix form during precipitation. In

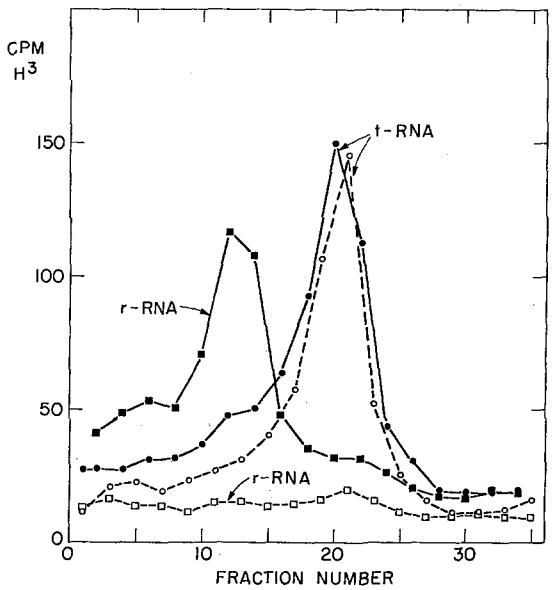

Figure 1. Zonal sedimentation profiles of double-stranded RNA treated with DMSO in the presence of t-RNA (-●-) and in the presence of r-RNA (-■-). The samples were sedimented through 15-30% sucrose-SDS gradients for 14 1/2 hours at 25,000 rpm in a Spinco SW 25.3 rotor at 25°C. Alternate fractions were assayed for radioactivity (——) and for precipitable activity after RNAase treatment (- - -) as described previously (Katz & Penman, 1966).

this case, any large polynucleotide should prevent helix reformation and permit the demonstration of strand separation. To test this hypothesis, a solvent denaturation was carried out using high molecular weight poly-A as the carrier polynucleotide species. The results depicted in Figure 2 show that strand separation and concomitant loss of RNAase resistance occurs when poly-A is used.

As a consequence of this experimental result, polyadenylic acid is now used routinely as carrier for precipitating RNA after solvent denaturation.

It was felt that the demonstration of strand separation when polyadenylic acid is used as carrier strongly supported the hypothesis that the reformation of the double-helix probably occurs during precipitation. The propensity to reanneal could be a general property of small complementary molecules when precipitated out of organic solvents or could be a result of incomplete strand

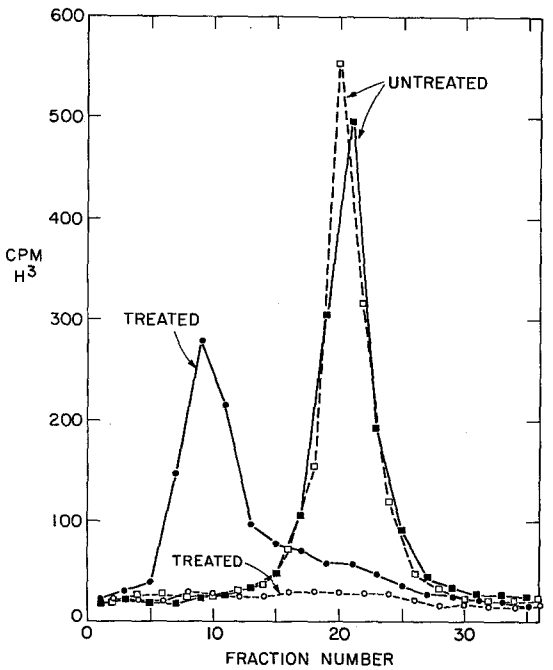

Figure 2. Zonal sedimentation profiles of double-stranded RNA before and after denaturation with DMSO in the presence of poly-A. Untreated samples (-■-) and treated samples (●) were assayed for total (——) and RNAase resistant (– – –) radioactivity as described previously.

separation after double-helix denaturation in the organic solvent. In order to test which is the more likely explanation, double-stranded RNA was melted in dimethylsulfoxide in the presence of a small amount of single-stranded viral RNA. These experiments did not demonstrant any tendency of the single-stranded RNA to enter a slowly sedimenting ribonuclease resistant structure, despite the fact that there was a large molar excess of double-stranded RNA present. In view of some earlier results of Geiduschek & Herskovits (1961), it seemed possible that the rapid renaturation of the double-stranded form was due to an incomplete strand separation. If this were true, it should be possible to show a flow of double-stranded RNA into the single-strand form after long periods of incubation in dimethylsulfoxide even when t-RNA is used

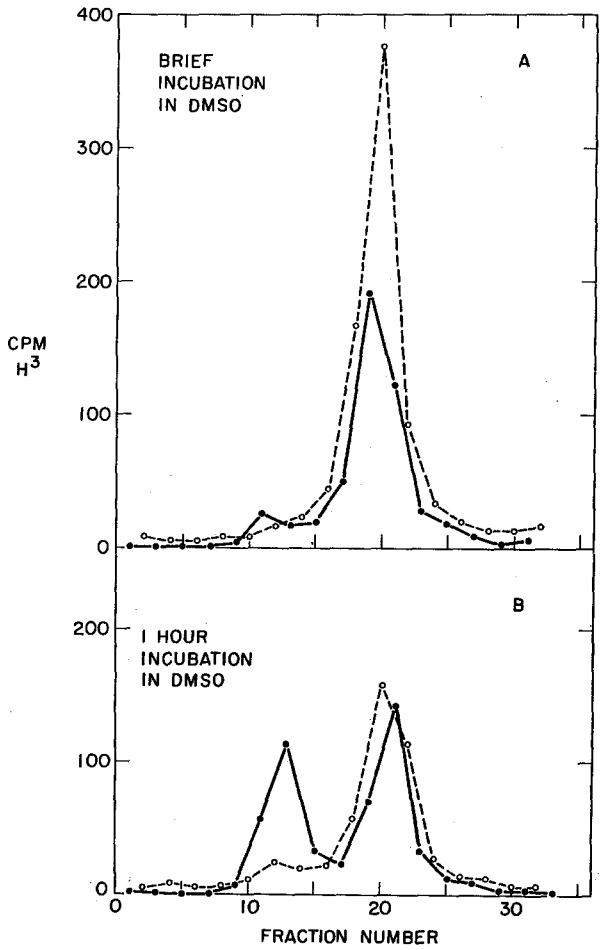

Figure 3. Effect of brief and extended treatment of double-stranded RNA by DMSO in the presence of t-RNA carrier. Samples were treated as described above, except that for the "brief" treatment, alcohol was added immediately after adding the DMSO, and the sample placed at -20°C. The RNAase resistant radioactivity (- - -) was determined as described previously.

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as carrier. The results indicate that no single-strand RNA is produced after brief exposure to dimethylsulfoxide when t-RNA is used as carrier (Figure 3A), but after an hour of incubation in the organic solvent, approximately half of the double-strand is converted to the single-strand form (Figure 3B). This result is consistant with the hypothesis that the rapid renaturation of the double-stranded RNA is due to incomplete strand separation in the organic solvent.

Discussion and Conclusions

The experiments described above indicate that the demonstration of the separation of the two molecules of double-stranded RNA by DMSO treatment may not be observed. It seems likely that physical strand separation does not occur immediately in DMSO but rather that the disruption of secondary structure leads to the production of entangled chains (Geiduschek & Herskovits, 1961). Whether reformation of the double-stranded structure can take place depends on the nature of the co-precipitant species present: with t-RNA the original structure is reformed, but with ribosomal RNA or poly-A, the secondary structure remains disrupted and single strands are observed. It seems probable that the crucial step in this process is the formation of the macroscopic aggregate during the alcohol precipitation. The addition of poly-A will also inhibit the formation of new ribosomal RNA aggregates after DMSO treatment, whereas t-RNA will not (Katz & Penman, in prep.). The inability of t-RNA to prevent reannealing or aggregation is presumably due to its relatively compact secondary structure or to a comparatively slow rate of precipitation.

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