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Denaturation of Rat Liver Ribosomal Ribonucleic Acid with Dimethyl Sulfoxide†

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ABSTRACT: Dimethyl sulfoxide is an effective denaturing agent for rRNA. However, this solvent can also promote *aggregation* of RNA, presumably at the time its concentration is lowered. The phenomenon has been investigated and this has led to the development of empirical methods which circumvent the problem.

High molecular weight RNA molecules, such as rRNA, possess some degree of secondary and tertiary structure which is stabilized by hydrogen bonds and by base stacking and other less well-defined interactions (Spirin, 1963; Cox, 1970; Attardi and Amaldi, 1970). As well as influencing the sedimentation and electrophoretic properties of an RNA molecule, these forces may hold together separate polynucleotide chains (*e.g.*, the replicative form of RNA viruses) or fragments of a larger molecule which arise due to "hidden" breaks. It has been possible to disrupt noncovalent bonds and dissociate chains and fragments using high concentrations of

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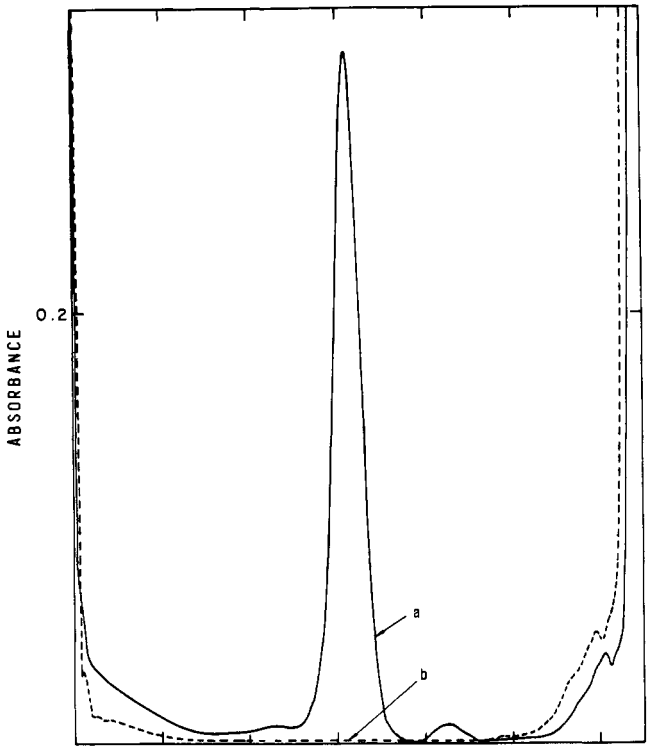

FIGURE 1: (a) Sedimentation analysis of untreated 28S rRNA. In this and in the following figures, 14 μg of RNA was analyzed by sucrose gradient centrifugation as described in Methods; direction of sedimentation is from left to right and the abscissa is in arbitrary units. (b) Absorbance profile of a blank sucrose gradient.

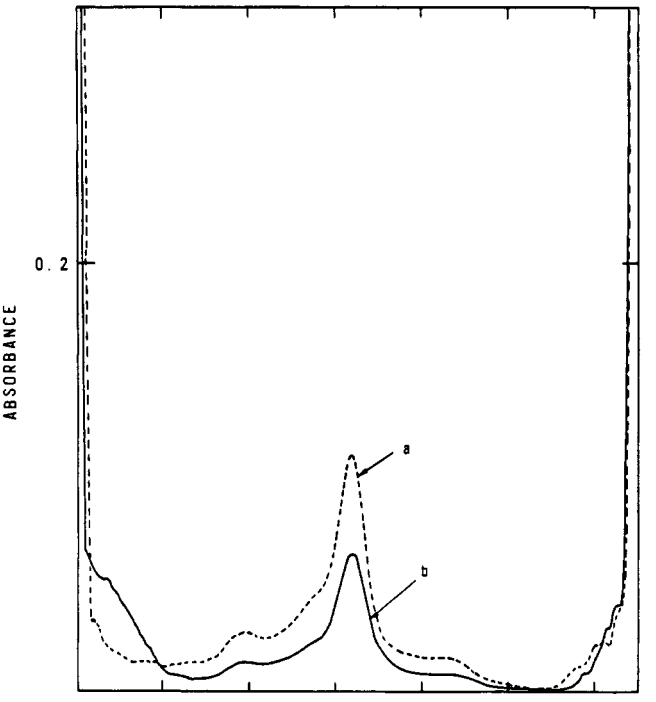

FIGURE 2: Sedimentation analysis of 28S RNA, treated with Me_2SO as follows. RNA (14 μg) dissolved in 10 μl of either NETS buffer (a) or 0.1 NETS buffer (b) was mixed with 100 μl of Me_2SO and incubated at 37° for 20 min. Two volumes of cold ethanol was added and the precipitate was collected by centrifugation at 30,000g for 20 min at 0°. It was dissolved in 0.5 ml of NETS buffer, incubated at 37° for 15 min, then applied to a sucrose gradient. Other conditions as in Figure 1.

Me_2SO, a solvent which is an effective denaturing agent for nucleic acids (Helmkamp and Ts'o, 1961; Applebaum *et al.*, 1966; Katz and Penman, 1966; Strauss *et al.*, 1968). Me_2SO treatment has also been used as a test for the covalent integrity of an RNA species, *i.e.*, the absence of a detectable effect on its sedimentation properties has been taken to indicate that the RNA molecule in question is a single polynucleotide chain. However, this test is complicated by the observation that separated viral RNA strands may in some cases reassociate (Katz and Penman, 1967). This probably occurs when the solvent concentration is lowered, such as when samples are applied to aqueous sucrose gradients for centrifugation. We now report that rRNA can also reassociate (aggregate) after exposure to Me_2SO. Because of the importance of this solvent as a denaturant for RNA, we have explored conditions which minimize this potential difficulty.

Materials and Methods

Preparation of Rat Liver rRNA. rRNA was prepared by phenol extraction of a cytoplasmic extract from rat liver. Individual rRNA species were separated by centrifugation in a Beckman Ti-15 zonal rotor and the 18S and 28S RNA peaks were precipitated with ethanol and redissolved in NETS[1] buffer.

Sedimentation Analysis of RNA. Sucrose density gradient centrifugation was used for analysis of RNA samples. Linear gradients of sucrose (15–30%, w/w) in NETS buffer (12.9 ml) were overlayed with 0.5 ml of sample and centrifuged for 16 hr at 23,000 rpm in a Beckman SW40 rotor at 20°. After centrifugation, the RNA profiles were monitored at 260 nm by displacing the gradient solution from below through an ISCO flow cell (Instrument Specialties Co., Inc., Lincoln, Neb.) adapted to a Gilford Model 240 recording spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The top of the gradient was detected as a pen deflection when the meniscus passed through the flow cell (left side in the figures); the bottom was detected as a deflection caused by a dye marker in the dense displacing solution (right side in the figures).

Other Materials. Me_2SO was Fisher reagent grade. tRNA was "soluble" RNA, type IV, from Sigma Chemical Co., St. Louis, Mo. Poly(adenylic acid) was purchased from P-L Biochemicals, Inc., Milwaukee, Wis. Both tRNA and poly(adenylic acid) were phenol extracted before use.

Results and Discussion

In the following experiments, rat liver 28S rRNA was used to demonstrate that Me_2SO is capable of both denaturing RNA and, under unfavorable conditions, causing aggregation. The RNA sample which was used sedimented as a single sharp peak (Figure 1a); however, it contained "hidden" breaks which were exposed when the RNA was denatured (see below). The breaks are presumed to have been introduced during preparation of the RNA, since the purified sample showed no detectable change in its sedimentation profile over a period of several months. Aggregation was detected by

[1] Abbreviations used are: NETS buffer, 0.1 M NaCl–0.01 M EDTA–0.01 M Tris-HCl–0.2% sodium dodecyl sulfate (pH 7.5); 0.1 NETS buffer, 0.01 M NaCl–0.001 M EDTA–0.001 M Tris-HCl–0.2% sodium dodecyl sulfate (pH 7.5).

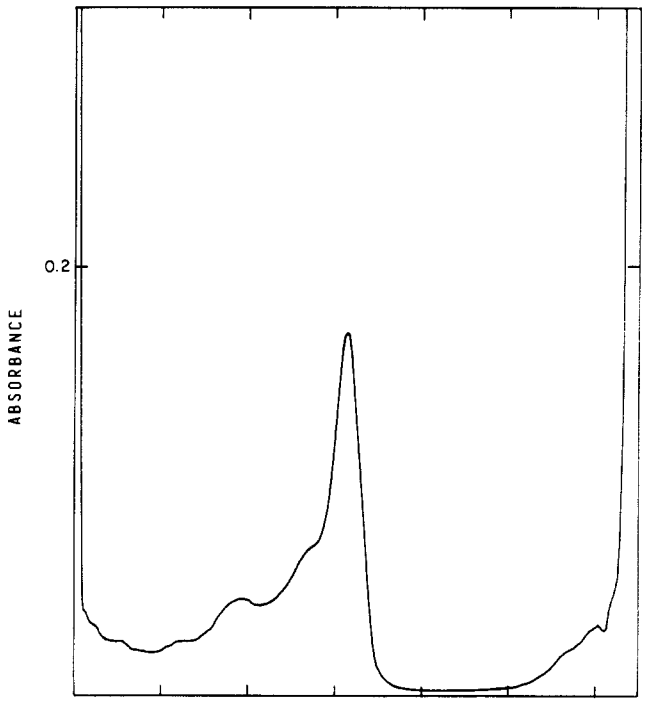

FIGURE 3: Sedimentation analysis of 28S RNA, treated with Me_2SO as in Figure 2a with the following modifications. After incubation with Me_2SO, 200 μl of NETS buffer and 600 μl of cold ethanol were added. The precipitate was dissolved in 0.5 ml of 0.1 NETS buffer and incubated. NaCl was added (10 μl of 5 M) and the sample applied to a sucrose gradient. Other conditions as in Figure 1.

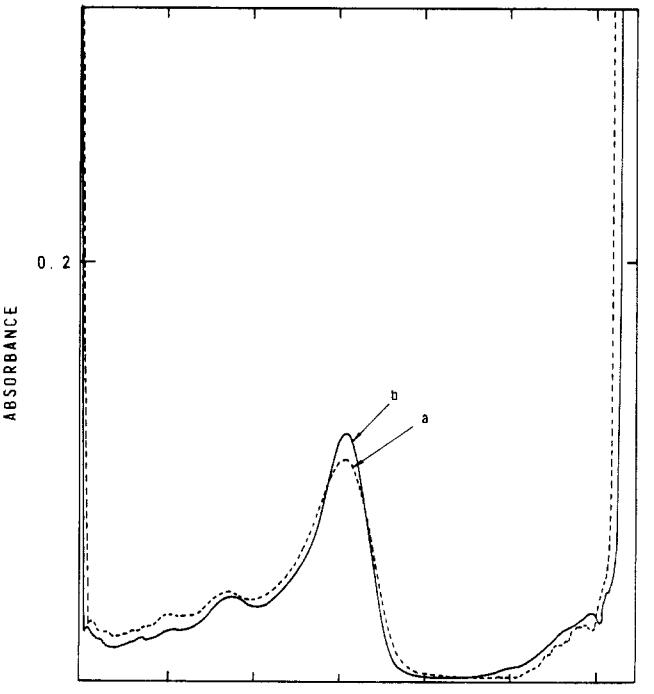

FIGURE 4: Sedimentation analysis of 28S RNA applied directly to sucrose gradients after Me_2SO treatment. After incubation with Me_2SO as in Figure 2a, samples were diluted with either two volumes (a) or four volumes (b) of NETS buffer and layered directly onto sucrose gradients. Other conditions as in Figure 1.

the presence of material in a sucrose gradient which sedimented more quickly than the main band. In Figure 1a, a small amount of RNA aggregate can be seen in addition to

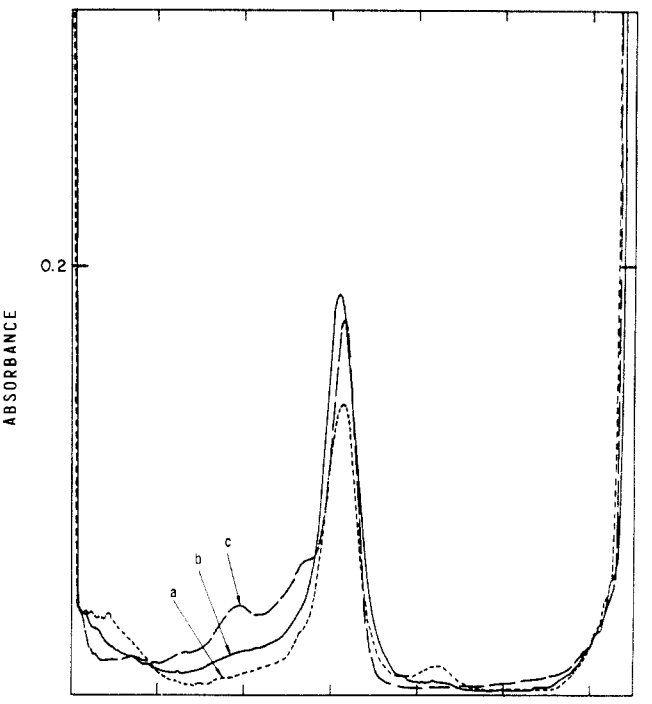

FIGURE 5: Sedimentation analysis of 28S RNA, treated with Me_2SO as follows. Alcohol-precipitated RNA was dissolved directly in 100 μl of one of the following solvents: (a) Me_2SO–NETS buffer (90:10, v/v), (b) Me_2SO–1 mM EDTA (99:1, v/v), and (c) Me_2SO–0.1 NETS buffer (90:10, v/v). Thereafter samples were treated as described in Figure 3. Other conditions as in Figure 1.

the main band of material. The absorbance profile of a density gradient with no RNA sample is shown in Figure 1b for reference.

Conditions of Me_2SO Treatment Which Cause Aggregation (Figure 2). Denaturation of the 28S RNA was readily accomplished using Me_2SO, but aggregation of the RNA was seen when the RNA was precipitated directly with ethanol and dissolved in NETS buffer (Katz and Penman, 1966) (Figure 2a). The sedimentation profile shows a distinct shoulder of material sedimenting more rapidly than the main band, in addition to several peaks of slower sedimenting material. The slower sedimenting material represents fragments of 28S RNA which were released by the denaturing agent. The more rapidly sedimenting material is aggregated RNA, probably analogous to that obtained by other denaturing agents (Wagner *et al.*, 1967). It should be emphasized that rRNA aggregates can be overlooked under the usual conditions of sedimentation where 28S RNA is centrifuged to a position nearer the bottom of the tube.

Similar results were obtained when the salt concentration was decreased during Me_2SO treatment (Figure 2b). The sedimentation profile is very similar to that of Figure 2a, but the efficiency of the alcohol precipitation was lower. Denatured material, main peak, and aggregates are again seen.

Conditions of Me_2SO Treatment Which Minimize Aggregation. Aggregation could be avoided by (i) dilution of the Me_2SO with NETS buffer before ethanol precipitation and (ii) dissolving the ethanol-precipitated RNA in 0.1 NETS instead of NETS buffer. Satisfactory results were obtained if, after Me_2SO treatment, the sample was diluted with one to three volumes of NETS buffer, precipitated with ethanol and dissolved in 0.5 ml of 0.1 NETS buffer (Figure 3). The sedimentation profile shows little or no aggregated RNA. 28S RNA

(70 μg) treated in this way also gave satisfactory results.

Other Conditions Tested. The inclusion of either tRNA or poly(adenylic acid) alone (Wagner *et al.*, 1967) without the steps described above gave results which were less satisfactory than those of Figure 3. In another experiment, samples of rRNA in Me_2SO were diluted with either two volumes (Figure 4a) or four volumes (Figure 4b) of NETS buffer and layered directly onto sucrose gradients (Lindberg and Darnell, 1970). Although there was no difficulty in layering the samples, the sedimentation profiles were very broad, compared to methods in which ethanol precipitation of the RNA was used.

A different problem was encountered in attempting to dissolve ethanol-precipitated RNA directly in aqueous Me_2SO solvents. The solvents used are indicated in the legend to Figure 5. Relatively small changes in the composition of the solvent had a pronounced effect on the results. It is clear that in Figure 5a,b the RNA had not dissolved adequately, since the Me_2SO-induced denaturation was not complete. Only in Figure 5c are the results comparable to those of Figure 3.

The present experiments were designed to provide practical information concerning the use of Me_2SO as a denaturing agent for rRNA. One of the major problems encountered was aggregation which seems to be due to a tendency of denatured RNA molecules to form base-paired regions (intermolecular as well as intramolecular) when the denaturing condition is removed. This phenomenon resembles aggregation of rRNA which has been induced by heat treatment (Stanley and Bock, 1965; Marcot-Queiroz and Monier, 1965; Wagner *et al.*, 1967; Lovett and Leaver, 1969). Our results suggest that intermolecular bond formation can be minimized by dilution of the RNA and the use of low ionic strength solutions as described. Another potential complication was that precipitated RNA did not dissolve readily in some solvents containing high concentrations of Me_2SO. This could be misleading since denaturation of the RNA would likely be incomplete.

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Exciton Interaction in the Photosystem I Reaction Center from Spinach Chloroplasts. Absorption and Circular Dichroism Difference Spectra†

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ABSTRACT: Preparations enriched 10-fold in the photosystem I reaction center chlorophyll, P700, have been studied by observing the changes which occur in both the absorption and circular dichroism upon illumination. The results suggest that there are at least two chlorophyll a molecules within the reaction center and that a significant exciton interaction exists among them. Upon photoactivation, one molecule within the array becomes oxidized, leading to the loss of exciton interaction. It is proposed that these intimate associations among the chlorophyll molecules are an integral part of the operation of the photosystem I reaction center.

The chlorophyll molecules found within the chloroplasts of higher photosynthetic organisms are of two distinct functional types. The large majority of pigment molecules are antenna, or bulk, chlorophylls, and a smaller fraction is contained in what is known as the reaction center. The function of the antenna chlorophylls is to absorb incident light

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