BACTERIOPHAGE MS2 RNA AND *ESCHERICHIA COLI* 23 S RIBOSOMAL RNA HAVE A SIMILAR CONFORMATION AFTER REACTION WITH FORMALDEHYDE AT LOW pH

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1. Introduction

Formaldehyde has several important applications in ribonucleic acid research. On the one hand, it is often used to obtain conformation-independent structures, allowing the estimation of molecular weights from physical techniques such as sedimentation [1] and electrophoresis [2]. On the other hand the ability of formaldehyde to form methylene-bridges between purines can be used for the fixation of specific nucleic acid structures [3, 4]. The 2 different types of application are directly related to the 2-stage reaction of RNA with formaldehyde and to the particular reaction conditions used such as pH and ionic strength [3, 5]. The acid structure of bacteriophage MS2 RNA [6] can be fixed by formaldehyde when the reaction is carried out at pH 3.8 in the presence of low concentrations of divalent ions [5]. The fixed structure sediments at 37.5 S. Also the 23 S and 16 S ribosomal RNA's form higher sedimenting structures of 38.2 S and 24.9 S, respectively [7].

In contradiction to our earlier conclusions, however, the 37.5 S structure of MS2 RNA is not a dimer [5]. Indeed, we have recently been able to measure more directly the partial specific volume for the formaldehyde-fixed RNA by means of the apparatus described by Kratky [8]. The value obtained amounts to only 0.44 cc/g and when this is introduced into the Mandelkern-Scheraga equation instead of the previously assumed value of 0.55 cc/g, a molecular weight corresponding to the monomer form is obtained [5].

In this paper we report that the 38.2 S structure derived from the 23 S rRNA during the reaction with formaldehyde in acid medium, forms an almost identical structure as the cross-linked MS2 RNA and consequently it corresponds also to a fast-sedimenting, contracted monomer.

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2. Materials and methods

Ribosomal RNA was isolated from *E. coli* MRE 600 ribosomes, and the extracted 23 S and 16 S rRNA were separated from each other as described previously [7].

The reaction was carried out in 7.7% formaldehyde at pH 3.8 in buffer containing 10^{-3} M formiate and 10^{-4} M $MgCl_2$ at an RNA concentration of 1.6 OD/ml. After reaction at 60° for 20 min and quick cooling in melting ice, the RNA was concentrated by Diaflo-ultrafiltration in order to obtain RNA concentrations suitable for viscosity measurements and gradient centrifugation. An EM 20E Diaflo membrane was used, pretreated with 5% formaldehyde to prevent nuclease degradation. After ultrafiltration at 5° the RNA was brought to pH 6.8 by addition of sodium phosphate buffer to a final concentration of 8×10^{-2} M and 4.6% formaldehyde. The concentrated RNA reaction mixtures were analyzed by centrifugation on a 5–20% glycerol gradient in 4.6% formaldehyde and 8×10^{-2} M sodium phosphate pH 6.8. Centrifugation was at 40,000 rpm for 4 hr at 5° in the Beckman SW-41 rotor.

The specific viscosity was measured as a function of RNA concentration at 20° in a Cannon-Ubbelohde semi-micro dilution viscometer. Sedimentation coefficients were measured in the Spinco Model E analytical

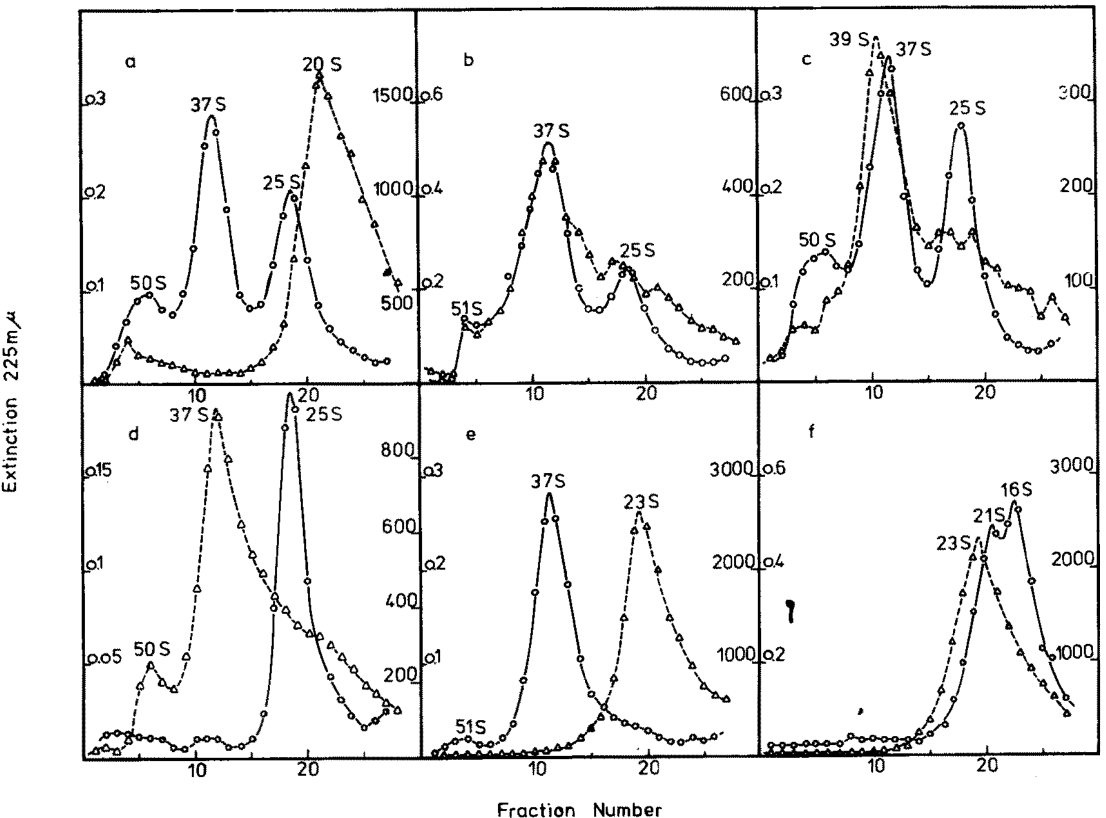

Fig. 1. Gradient centrifugation of formaldehyde-fixed ribosomal RNA and MS2 RNA. The RNA-conformation, obtained at pH 3.8 and low Γ/2, was fixed with formaldehyde as described in the text. Subsequently it was neutralized, and if necessary concentrated. Analysis was on a glycerol gradient in pH 6.8 phosphate buffer containing 4.6% formaldehyde. Left ordinates refer to optical density (o—o—o) and right to radioactivity (^{32}P-MS2 RNA) (△—△—△). a) The reaction mixture contained 16 S and 23 S ribosomal RNA (58 μg/ml). After centrifugation, 190 μg rRNA were loaded together with 8 μg untreated ^{32}P-MS2 RNA. b) The reaction mixture contained 36 μg/ml ribosomal RNA and 36 μg/ml ^{32}P-MS2 RNA. After concentration, 300 μg were loaded. c) The same reaction mixture as for b but the ^{32}P-MS2 RNA concentration was 20-fold lower (but of higher specific activity). After concentration, 300 μg were loaded. d) The reaction mixture contained 58 μg/ml ^{32}P-MS2 RNA, of which 11 μg were loaded together with 180 μg unlabeled, unreacted MS2 RNA. e) The reverse of d: The load contained 133 μg formaldehyde-reacted, unlabelled MS RNA and 2 μg unreacted ^{32}P-MS2 RNA. f) Control: 288 μg unreacted ribosomal RNA and 2 μg unreacted ^{32}P-MS2 RNA were loaded.

ultracentrifuge at 20° and at 52,640 rpm. They were corrected to standard conditions in the usual way. ^{32}P-labelled MS2 RNA was prepared as previously described [9].

3. Results

In order to compare the sedimentation of the fast-sedimenting RNA-forms, we have analyzed the reacted ribosomal RNA on glycerol gradients, using ^{32}P-MS2 RNA forms as internal markers (fig. 1). The reaction mixtures, which were analyzed on the gradients a, b and c, had been concentrated as described above. The reference is ^{32}P-MS2 RNA, which after reaction with formaldehyde at low pH and low ionic strength, sediments at 37.5 S. The untreated viral RNA partially unfolds due to the 4.6% formaldehyde present in the gradient solution and sediments at 20–23 S (fig. 1, a, e and f). This partial unfolding also occurs with untreated ribosomal RNA (fig. 1, f).

As previously reported [7], the 23 S ribosomal RNA changes after reaction with formaldehyde at appropriate pH and ionic strength to a form sedimenting at 37.5 S, while the 16 S is converted to a 25 S form (fig. 1, a, b and c). At the higher RNA concentrations used here, some intermolecular cross-linking occurs as well, which is undoubtedly responsible for the 50 S component. It is evident from the results that the compact form of the 23 S ribosomal RNA sediments at almost the same rate as the reacted viral RNA,

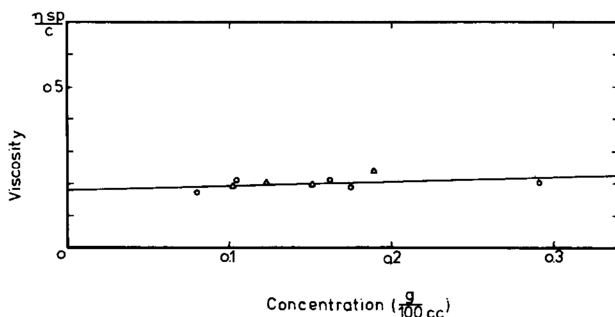

Fig. 2. The reduced, specific viscosity of 37.5 S MS2 RNA (○) and 36.8 S rRNA (△), obtained after reaction in the presence of 7.7% formaldehyde and 1×10^{-4} M $MgCl_2$ at pH 3.8, as a function of RNA concentration. The measurements were carried out in 8×10^{-2} M sodium phosphate buffer pH 6.8 and 1.5 M formaldehyde.

although the exact sedimentation rate seems to be slightly dependent on the RNA concentration at which the formaldehyde reaction is carried out.

This similarity in hydrodynamic properties between compact 23 S-ribosomal RNA and viral RNA is also born out by a comparison of their reduced specific viscosity in function of the RNA concentration (fig. 2). The maximum error in the measurements is approximately 2%. No significant difference exists between the specific viscosity of both structures. The intrinsic viscosity of the 2 fast sedimenting conformations amounts to 0.182 dl/g. From the Mandelkern-Scheraga relation, and using the recently measured partial specific volume of 0.44 cc/g obtained for 37.5 S MS2 RNA [6], a molecular weight of 1.14×10^6 is calculated for 36.8 S rRNA using a shape factor $\beta = 2.5 \times 10^6$, and 1.49×10^6 using a shape factor $\beta = 2.12 \times 10^6$ (the latter assumes a spherical particle).

4. Discussion

Under identical conditions of pH and ionic strength, MS2 RNA and the ribosomal RNA's undergo structural transitions during the reaction with formaldehyde and form fast sedimenting species [5, 7]. The structural difference which exists between 27 S MS2 RNA and 23 S rRNA is eliminated after reaction with 7.7% formaldehyde at pH 3.8, as evident by the change of reduced specific viscosity with RNA concentration (fig. 2) and by the change of $s_{20,w}$ with pH [7], which are identical for both RNA's. Also in gradient centrifugation the fast-sedimenting structures of 27 S MS2 RNA and 23 S rRNA are present in the same fractions when the reaction is carried out at comparable RNA concentrations. Indeed, the sedimentation rate is somewhat dependent on the RNA concentration in the preceding formaldehyde reaction; under the conditions used here the most compact RNA shape is not yet obtained [6].

It is evident from the molecular weight estimates that these fast-sedimenting species are in fact monomers. They have a very compact conformation, linked by methylene-bridges, and the hydration is considerably less than in the more native conformation. These fast sedimenting structures, whose shape is almost independent of the solvent, can also be useful as internal references for gradient centrifugation.

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References

[1] H. Boedtker, J. Mol. Biol. 35 (1968) 61.
[2] H. Boedtker, Biochim. Biophys. Acta 240 (1971) 448.
[3] M.Ya. Feldman, Biochim. Biophys. Acta 149 (1967) 20.
[4] A.S. Spirin, N.V. Belitsina and M.I. Lerman, J. Mol. Biol. 14 (1965) 611.
[5] H. Slegers and W. Fiers, Biopolymers 9 (1970) 1373.
[6] H. Slegers and W. Fiers, Biopolymers, in preparation.
[7] H. Slegers and W. Fiers, FEBS Letters 7 (1970) 55.
[8] O. Kratky, H. Leopold and H. Stabinger, Z. Angewandte Phys. 27 (1969) 273.
[9] W. Fiers, L. Lepoutre and L. van den Driessche, J. Mol. Biol. 13 (1965) 451.