A CORRELATION BETWEEN THE BUOYANT DENSITY AND THE SEDIMENTATION COEFFICIENT OF EMC VIRAL POLYRIBOSOMES

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1. Introduction

We have shown earlier [1,2] that in extracts of encephalomyocarditis (EMC) virus-infected cells the buoyant density of polyribosomes is lower than that of 80 S ribosomes. Such a difference between mono- and polyribosomes had been reported by Spirin [3] and was attributed to the presence of mRNA-associated protein [3,4]. We have also shown [1] that EMC virus-specific polyribosomes have a slightly lower buoyant density than polyribosomes of uninfected cells. It has been suggested that this difference might

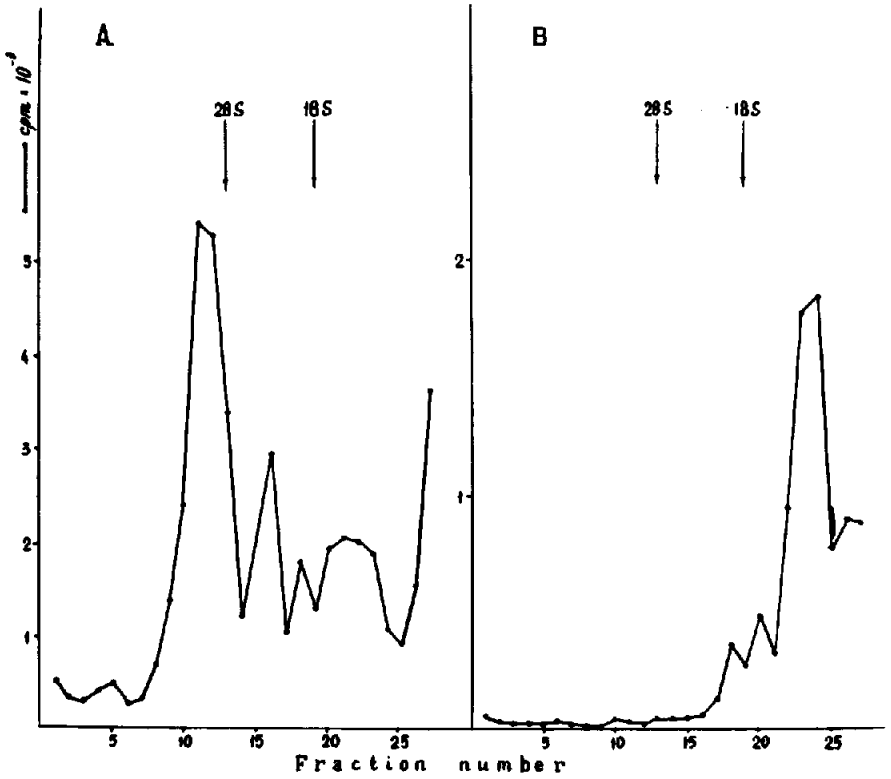

Fig. 1. Sucrose gradient fractionation of [^{3}H]uridine-labeled RNA extracted from cytoplasmic extract. (A) EMC-infected cells, (B) uninfected cells. Actinomycin D (5 μg/ml) added 2 hr prior to labeling. RNA was layered on top of 50 ml 15–30% sucrose gradient prepared in STE buffer (0.01 M Tris–HCl, 0.1 M NaCl, 0.001 M EDTA) containing 0.5% SDS and centrifuged in SW 25.2 rotor of Spinco L2 ultracentrifuge for 16 hr at 21 000 rpm and 25°C.

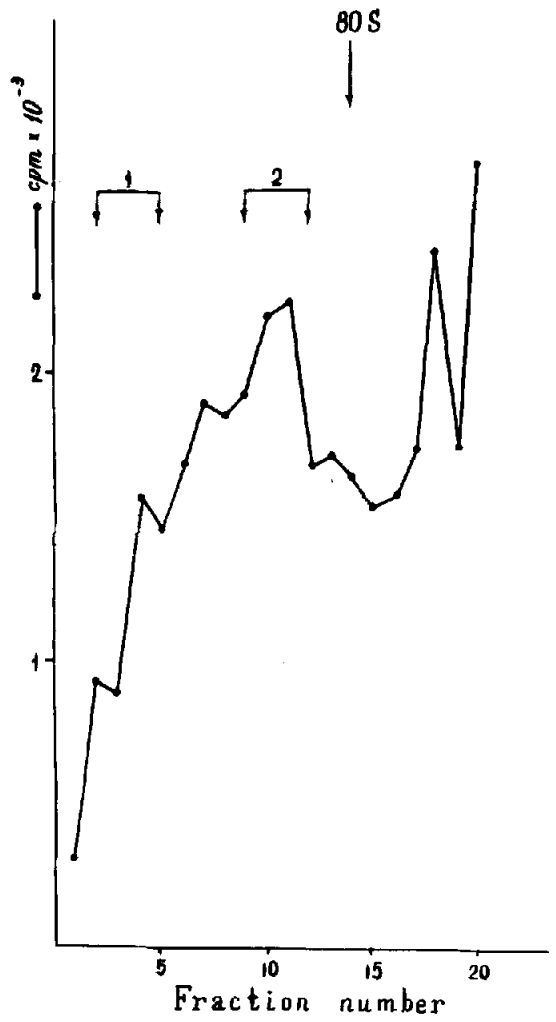

Fig. 2. The sedimentational distribution of virus-specific polyribosomes in formaldehyde-containing sucrose-density gradient. S15 preparation (2 ml) was layered over 1 ml of 5% sucrose on top of 16 ml of 15–30% sucrose gradient containing 4% formaldehyde. Centrifugation for 1.25 hr in 3 × 23 rotor of Superspeed 65 ultracentrifuge at 24 000 rpm and 6°C. Radioactivity was determined in aliquots and chosen fractions pooled for CsCl analysis.

also be due to the presence of mRNA-bound protein, since picornaviral RNA is much larger than the average normal mRNA [5,6], and the amount of mRNP per ribosome may be higher in virus-specific polyribosomes than in the normal ones.

In the present paper we describe experiments designed to compare the buoyant density of EMC polyribosomes of different sediment classes. The rationale for this type of experiment is given by the uniform size of viral mRNA in picornaviral systems [5]. For this reason the polyribosomes with a higher sedimentation coefficient (i.e., those bearing more ribosomes) should contain less mRNP per ribosome. If mRNA-bound protein plays a role in determining the buoyant density of polyribosomes, those with a higher sedimentation coefficient should also have a higher buoyant density. The data presented below indicate that this really is the case.

The experiments along this line seemed all the more justified as Fenwick and Wall [7] recently claimed that mRNA-bound protein plays no role in lowering the buoyant density of poliovirus polyribosomes.

2. Materials and methods

EMC virus-infected Krebs II cells were treated with Actinomycin D and labelled as described earlier [1,2]. One batch of the cells was labelled with [^{14}C]uridine, another with [^{3}H]uridine. Cytoplasmic extracts (S 15) were fractionated in 15–30% sucrose density gradient, the fractions were collected, treated with 1: 10 volume of 40% formaldehyde (pH 6.8), and radioactivity was determined in aliquots. Chosen fractions were pooled, dialyzed to remove sucrose and analysed in CsCl-density gradient. Before the analysis the 'heavy' 260–320 S ^{14}C-labeled material was mixed with the 'light' 120–180 S ^{3}H-labeled material and vice versa. For RNA extraction cytoplasmic extract was prepared in triethanolamine (TEA)–SM buffer (0.01 M TEA–HCl, 0.01 M NaCl, 0.003 M magnesium acetate, pH 7.5), treated with EDTA (final concentration 0.006 M) and Sodium dodecyl sulphate (SDS) (final concentration 1%).

3. Results and discussion

In the preliminary experiments we had to check whether the size distribution of EMC polyribosomes in sucrose-density gradients [1,2] is not a trivial result of viral mRNA breakage during the preparation of cell extract. The identification of the labeled virus-specific material in cytoplasmic extract (S15) as viral polyribosomes has been reported in an earlier publication

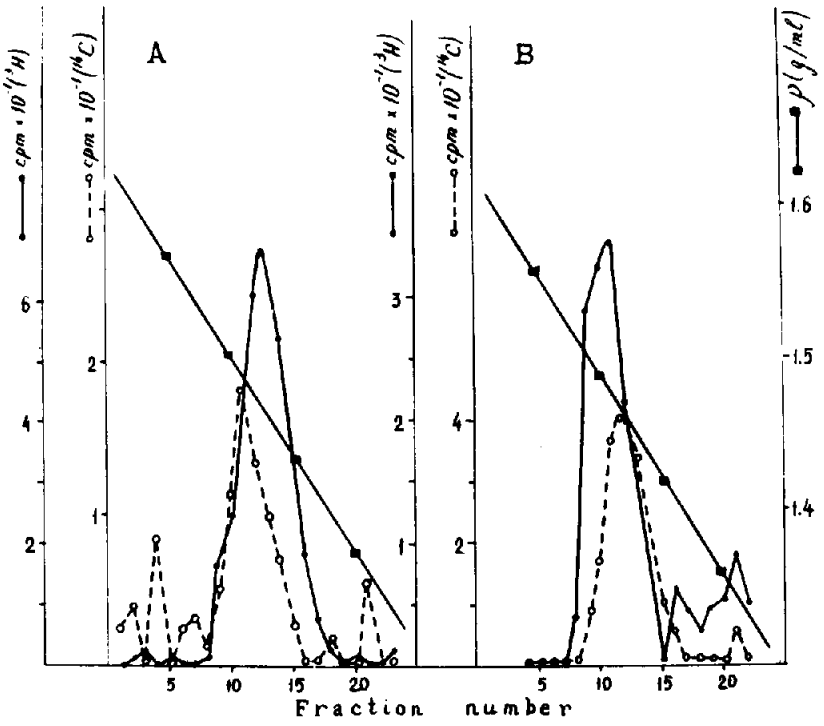

Fig. 3. A comparison of the buoyant density of EMC-virus-polyribosomes with different sedimentation coefficients. The cells were exposed either to [^{3}H] uridine (——) or to [^{14}C] uridine (——). (A) buoyant density distribution of 'heavy' (260–320 S) ^{14}C-labeled and 'light' (120–180 S) ^{3}H-labeled polyribosomes. (B) buoyant density distribution of 'heavy' (260–320 S) ^{3}H-labeled and 'light' ^{14}C-labeled polyribosomes. Centrifugation at 35 000 rev/min in 3 × 6.5 rotor of Superspeed 65 ultracentrifuge for 16 hr at 6° C.

[1]. As one can see from the results of fractionation of RNA preparation extracted from S15, the breakage does occur to a certain extent (fig. 1) but the major part of virus-specific RNA has a sedimentation coefficient characteristic for intact viral RNA.

The fractionation of S15 in formaldehyde-containing sucrose gradient, results in a broad distribution of the label in the polyribosomal region (fig. 2). When the polyribosomal material taken from the 'heavy' part of the gradient (260–320 S) was mixed with 'light' (120–180 S) material and analysed in CsCl gradient a small but definite difference was observed (fig. 3). This result is not due to the presence of formaldehyde in sucrose gradient: a similar difference was obtained when the fractions of sucrose gradient were subjected to formaldehyde treatment after the fractionation. In this case all the values of the buoyant density were slightly (0.01–0.02 g/ml) higher than after fractionation in formaldehyde-containing gradient

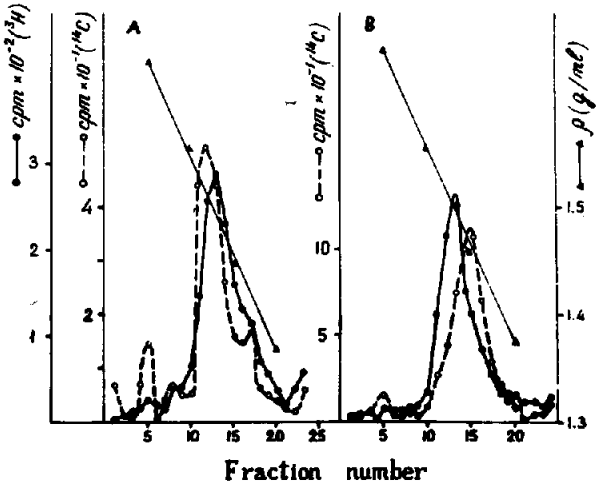

Fig. 4. Buoyant density of 'light' and 'heavy' polyribosomes taken from sucrose gradient without formaldehyde. The designations and the conditions of fractionation are the same as in fig. 3.

but the difference between the 'light' and 'heavy' polyribosomes was still evident (fig. 4).

The data presented in this communication suggest that mRNA-bound protein is a significant factor (though possibly not the only one) in determining the value of the buoyant density of polyribosomes. The data are in agreement with the view first expressed by Spirin [3] on the cause of lower density of polyribosomes as compared to monoribosomes.The results presented by Fenwick and Wall [7] may perhaps be explained by a very close distribution of ribosomes along mRNA in polio-polyribosomes or by a partial deproteinization of mRNA by 0.25% sodium deoxycholate used by these authors.

References

[1] Zaslavsky, V.G., Kaverin, N.V., Smirnov, Yu.A. and Syurina, N.V. (1971) FEBS Letters 17, 289.
[2] Zaslavsky, V.G., Smirnov, Yu.A., Syurina, N.V. and Kaverin, N.V. (1972) Molekul. Biol. 6, 128.
[3] Spirin, A.S. (1969) Eur. J. Biochem. 10, 20.
[4] Olsnes, S. (1970) Eur. J. Biochem. 15, 464.
[5] Summers, D.F. and Levintow, L. (1965) Virology 27, 44
[6] Dalgarno, L., Cox, R.A. and Martin, E.M. (1967) Biochim. Biophys. Acta 138, 316.
[7] Fenwick, M.L. and Wall, M.J. (1972) J. Gen. Virol. 17, 143.