THE EFFECT OF IONIC STRENGTH ON THE BUOYANT DENSITY OF POLYRIBOSOMES AND 80 S FREE RIBOSOMES IN EMC VIRUS-INFECTED AND UNINFECTED KREBS II CELLS

N.V. KAVERIN, Yu.A. SMIRNOV, N.V. SYURINA and V.G. ZASLAVSKY
D.I. Ivanovsky Institute of Virology, Academy of Medical Sciences, Moscow, USSR

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1. Introduction

We have reported [1] that polyribosomes in cytoplasmic extracts of mouse ascites carcinoma Krebs II cells have lower buoyant density in CsCl than free monosomes and that the viral polyribosomes have a slightly lower buoyant density value than normal polyribosomes. It had been shown that mRNA in polyribosomes is associated with protein [2, 3] and that some RNA-protein complexes are dissociated at elevated ionic strength [4–6]. In the present work an attempt was made to remove the mRNA-bound protein from polyribosomes by treatment with elevated ionic strengths. The results suggest that such treatment also strips some protein from ribosomes and that single ribosomes are more sensitive to this treatment than both normal and viral polyribosomes.

2. Materials and methods

Experimental procedures have been described earlier [1, 7] in detail. Briefly, they were as follows. EMC virus-infected Krebs II cells were incubated in the presence of actinomycin D (0–2 hr post infection) and labeled with ^{3}H-uridine (2–5.5 hr post infection). Uninfected cells were incubated with actinomycin D and labeled for 2 min with ^{14}C-chlorella hydrolysate. Cytoplasmic extracts were prepared in standard buffer [7] and layered on 15–30% (w/w) sucrose gradients prepared in the same buffer containing 0.06 M, 0.26 M or 0.4 M KCl. The gradients were centrifuged in a 3 × 23 rotor of a Superspeed-50 ultracentrifuge (MSE, England) at 25,000 rpm at 2° for 90 min. Fractions were collected and formaldehyde was added to every fraction to a final concentration 4%. The material of the 220–300 S and 80 S zones (poly- and monoribosomes) was dialysed against standard buffer with 4% formaldehyde and analysed in preformed CsCl gradients [8]

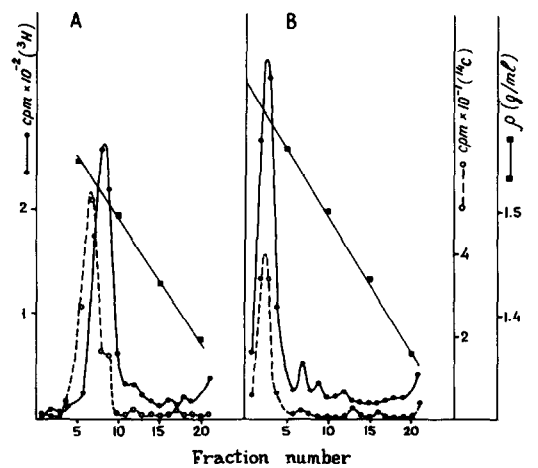

Fig. 1. The effect of ionic strength on the buoyant density of viral and cellular polyribosomes. Polyribosomal material containing virus-induced ^{3}H-labeled RNA was mixed with polyribosomal material containing ^{14}C-labeled nascent polypeptides and analysed in a CsCl density gradient. A, the mixture of polyribosomal materials after fractionation of the cytoplasmic extract in a sucrose gradient with 0.06 M KCl; B, the mixture of polyribosomal materials after fractionation in a sucrose gradient with 0.26 M KCl.

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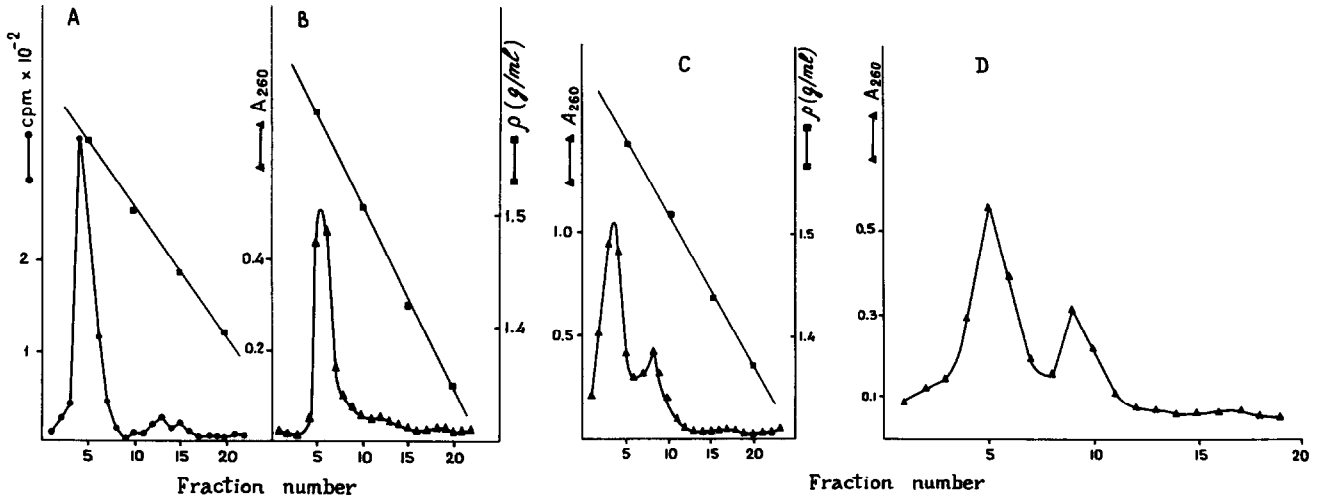

Fig. 2. The effect of ionic strength on the buoyant density of free ribosomes. Ribosomes from sucrose gradients with different ionic strengths were analysed in a CsCl gradient as described in fig. 1. A, the material from a sucrose gradient with 0.06 M KCl; B, with 0.26 M KCl, C, with 0.4 M KCl; D, the material from the sucrose gradient with 0.4 M KCl was layered on a 15–30% sucrose gradient containing 4% formaldehyde and centrifuged in a 3 X 23 rotor for 11 hr at 25,000 rpm.

(ρ = 1.36–1.7 g/ml) in a 3 X 5 rotor at 35,000 rpm for 16–18 hr.

3. Results and discussion

It had been pointed out [1] that polyribosomes after fractionation in formaldehyde-containing sucrose gradients [9] have buoyant densities of 1.51 g/ml (normal polyribosomes) or 1.49–1.50 g/ml (viral polyribosomes). Free ribosomes fractionated in such conditions have a buoyant density of 1.55 g/ml. To remove excess protein from polyribosomes we applied centrifugation through sucrose gradients containing KCl concentrations of 0.06 M and 0.26 M. By this treatment we sought to remove extra protein from mRNP in polyribosomes and to increase their buoyant density up to that of monoribosomes. The result was not quite the expected one. Moderately elevated ionic strength (0.06 M KCl) did not affect buoyant densities either of viral or of normal polyribosomes (fig. 1A) while the buoyant density of monoribosomes was increased up to 1.58 g/ml (fig. 2A). Further elevation of the ionic strength (0.26 M KCl) resulted in a sharp increase of buoyant densities of both viral and normal polyribosomes up to 1.58 g/ml (fig. 1B). The difference between normal and viral polyribosomes was abolished. Monoribosomes had the same buoyant density as after centrifugation through 0.06 M KCl, viz. 1.58 g/ml (fig. 2B); i.e. the treatment with 0.26 M KCl led to equalization of buoyant densities of viral polyribosomes, normal polyribosomes and monoribosomes at a high level. Further increase of KCl concentration (0.4 M) has no effect on the buoyant density of polyribosomes but leads to the dissociation of free ribosomes (fig. 2C, D) which is in agreement with the data obtained with HeLa cells [10].

The results of ionic strength treatment indicate that monoribosomes in the extracts of Krebs II cells contain loosely bound protein removable by a very gentle ionic strength treatment. For this reason any change in the buoyant density of polyribosomes should be considered in terms of loss not only of mRNA-bound protein but also of proteins loosely bound to ribosomes. It hampers the analysis of differences in buoyant density of monoribosomes, viral polyribosomes and normal polyribosomes. Nonetheless one may suppose that the more harsh treatment (0.26 M KCl) abolished not only "ribosomal" loosely bound proteins from poly- and monoribosomes but also the greater part of the mRNA-bound protein from polyribosomes. This is the most probable explanation of equalization of buoyant density at 1.58

g/ml. Thus, if the difference between viral and normal polyribosomes is caused by the different amount of mRNP per ribosome (as a result of different spacing of ribosomes along mRNP strand) one would expect that 0.26 M KCl treatment should remove the difference. This is in fact the case (fig. 1B). So the data of ionic strength treatment do not contradict the initial hypothesis on the cause of the difference. The effect of ionic strength on ribosomal proteins makes other explanations possible though not likely.

Another conclusion that can be drawn from the data presented is more definite. The treatment at slightly elevated ionic strength (0.06 M KCl) removes a large part of the protein from monosomes but does not affect polyribosomes. This indicates that loosely bound proteins are more easily removable from single ribosomes than from both viral and normal polyribosomes. This may be connected with the functional significance of such proteins [11] or it may be a result of binding proteins to the polyribosomes similar to the one described by Olsnes [12].

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