POLYRIBOSOMES OF *E.COLI*: THE DISTRIBUTION OF FREE 70 S PARTICLES AND SUBUNITS IN THE PRESENCE OF K^+ AND Na^+ IONS

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Received 8 September 1970
Revised version received 27 January 1971
Original figures received 8 February 1971

1. Introduction

When polyribosomes isolated from *E. coli* are sedimented in sucrose gradients, the absorbancy profile of the gradient is either characterized by a large 70 S peak or by large subunits peaks, depending on the type of monovalent ion used during isolation and centrifugation [1–3]. K^+ and NH_4^+ ions seem to generate high 70 S type profiles, whereas the presence of Na^+ ions leads to preparations which are rich in subunits. Which of these profiles is most in accordance with the in vivo situation is still not clear, although it has been suggested that the 70 S peak observed in the presence of K^+ is due to initiation monosomes formed during isolation [3, 4].

Here we report some observations on the differential effect of K^+ (or NH_4^+) and Na^+ on *E. coli* polyribosomes. It is shown that the 70 S present in the K^+ containing gradients consists primarily of free ribosomes (i.e. bearing no messenger and no peptidyl-tRNA). Little if any free 70 S ribosomes are found in gradients containing Na^+. Instead, a new ribosomal peak sedimenting at approximately 64 S appears in the gradients. The amount of subunits is also increased in the presence of Na^+.

2. Results

Typical gradient profiles of polyribosomes sedimented in the presence of K^+ or Na^+ are shown in fig. 1. The messenger RNA has been labeled by a 30 sec pulse label with ^{3}H-uridine and the gradients were centrifuged overnight in order to resolve more clearly the ribosomes and subunits. The Na^+ containing gradients show three obvious differences from K^+ gradients:

1) The amount of subunits is increased.
2) A broad peak of ribosomes sedimenting at approximately 64 S appears, whereas the amount of 70 S material is strongly reduced.
3) Polyribosomal peaks are broader although their total amount is not affected. The radioactivity present on polyribosomes sediments also in a broader range.

Planimetrical measurements show that the combined amounts of 70 S and subunits in K^+ gradients is the same as the total amount of 64 S and subunits in Na^+ gradients. This, together with the observation that there is no difference in the amount of polyribosomes, strongly suggests that the type of ion mainly affects the distribution of ribosomes and subunits. The suggestion of Phillips et al. [3], that the 70 S material seen in the presence of K^+ is mostly 'initiation monosomes', is ruled out by these data since this would imply an increase in ^{3}H-uridine counts parallel to the increase of 70 S absorbancy.

Another possibility which could account for the large amount of 70 S in the presence of K^+ – namely an artefactual association of subunits, peptidyl-tRNA and fragmented mRNA – needed a more elaborate experiment. We made use of the observation of Van Duin et al. [5] that free 70 S ribosomes dissociate completely into subunits at 2 mM Mg^{2+}, whereas particles complexed with mRNA and peptidyl-tRNA partly persist at this Mg^{2+} concentration. In a mixture of labeled monosomes and free ribosomes the per-

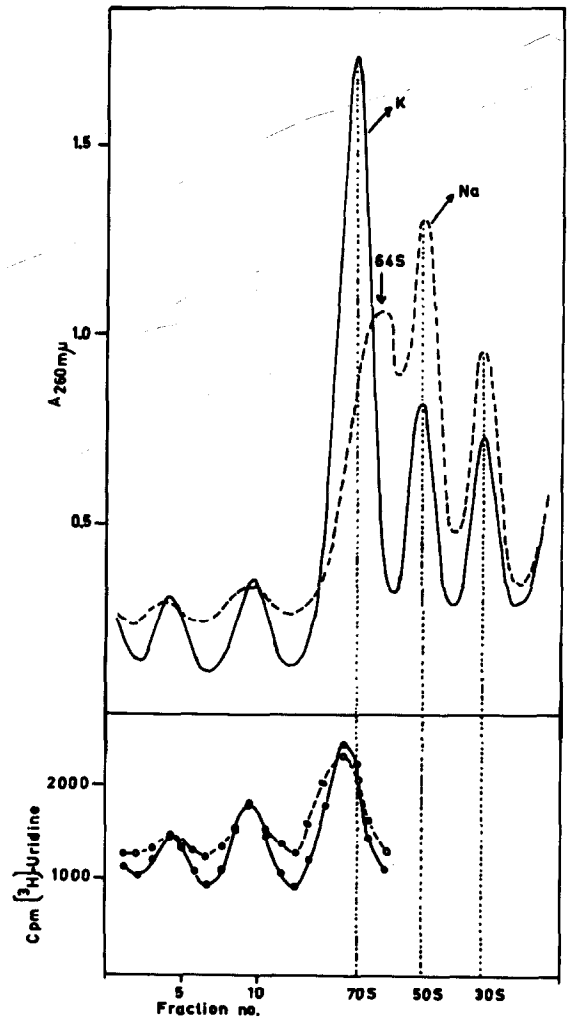

Fig. 1. Sucrose gradient centrifugation of polyribosome preparation in the presence of K^+ and Na^+ ions. *E. coli* K12, grown on a synthetic medium was labelled for 30 sec with ^{3}H-uridine (Uridine-5T, 25 Ci/mmole, 1 μCi/ml) and harvested by pouring in ice and chloramphenicol (100 μg/ml). Spheroplasts were made according to Dresden and Hoagland [9] and lysed in 0.01 M tris-HCl pH 7.6: 0.010 M Mg acetate (TM-buffer) containing 0.25% deoxycholate. The lysate was centrifuged for 10 min at 10,000 *g*. 0.8 ml aliquots of the supernatant were layered on two 15–30% sucrose gradients containing TM-buffer and 0.06 M KCl and 0.06 M NaCl, respectively. The gradients were centrifuged for 16 hr at 16,000 rpm. Absorbancy at 260 mμ was monitored with a Gilford spectrophotometer (*upper part*). Collected fractions were supplemented with 5% trichloroacetic acid, filtered on Millipore HA filters and counted (*lower part*). •——• cpm in K^+ gradient; ○ - - - ○ cpm in Na^+ gradient.

centage of monosomes can now be determined in the following manner (compare also the legend to table 1): the 70 S material isolated from a K^+ gradient of polyribosomes (dual labelled with ^{3}H-uridine in mRNA and ^{14}C-amino acid in the peptidyl-tRNA) is analyzed on two sucrose gradients containing 10 and 2 mM Mg^{2+}, respectively. The specific activity of the 70 S peak in the latter gradient is the true specific activity of the monosomes. By dividing the radioactivity of the 70 S peak at 10 mM Mg^{2+} by the specific activity obtained at 2 mM, the absorbancy due to monosomes at 10 mM Mg^{2+} is obtained. This can be expressed as percentage of total 70 S absorbancy (table 1). The independent calculations based on ^{3}H-uridine and ^{14}C-amino acid labeling, respectively, gives the same result: of all 70 S material present in K^+ gradients, only 28 to 29 percent is complexed with mRNA and peptidyl-tRNA. This means that at least 70% of the 70 S material does not bear mRNA or peptidyl-tRNA and must be considered to represent true free ribosomes. It seems that these ribosomes only are partly converted into 64 S and partly dissociated in the presence of Na^+ ions.

Table 1
Mg^{2+} dependent dissociation of dual labelled 70 S particles.

Measurement	10 mM Mg^{2+}	2 mM Mg^{2+}
70 S absorbancy	11.2	1.3
70 S cpm ^{3}H	10,500	4,250
cpm ^{3}H/absorb.	–	3,250
% 70 S containing ^{3}H	29	100
70 S cpm ^{14}C	640	265
cpm ^{14}C/absorb.	–	204
% 70 S containing ^{14}C	28	100

E. coli cells were pulse-labelled for 30 sec with ^{3}H-uridine and for 5 sec with ^{14}C-amino acids (^{14}C-amino acid mixture 200 mCi/mmole, 0.3 μCi/ml). Polyribosomes were isolated and centrifuged in a K^+ gradient as described in fig. 1. The 70 S area was pooled, pelleted by centrifugation and resuspended in TM-buffer containing 0.06 M KCl. Aliquots were diluted to make the final Mg^{2+} concentrations 10 and 2 mM, respectively. All other concentrations were the same. The samples were centrifuged for 16 hr at 20,000 rpm in gradients containing 10 and 2 mM Mg^{2+}, respectively. Radioactivities in the fractions were assayed as described in fig. 1. The counts present in the 70 S peak were determined. The absorbancy of the 70 S peaks was measured planimetrically. See text and [5] for details of calculations.

The polysomal preparations described above were all isolated from cells rapidly cooled in the presence of chloramphenicol. When these precautions are not taken, the amount of 70 S in K^+ gradients increases drastically (table 2). In Na^+ containing gradients, the large 70 S peak is again absent whereas 64 S and increased amounts of 50 S and 30 S are found (fig. 2). This indicates that lack of chloramphenicol results in a substantial runoff of ribosomes, which respond to Na^+ in the same way as normally occurring 70 S ribosomes.

Since it is known that 70 S particles present in standard *E. coli* extracts obtained by alumina grinding, are almost exclusively free 70 S ribosomes [5], the effect of Na^+ ions on the ribosomal distribution in such extract was also studied (fig. 3, this experiment was kindly

Table 2
The effect of chloramphenicol on the amount of 70 S present in polyribosomes.

	% 70 S
+ chloramphenicol	27
– chloramphenicol	61

A culture of *E. coli* was divided into two equal parts. One portion was harvested in ice and chloramphenicol (100 μg/ml), the other in ice alone. Polysomes were prepared as described in fig. 1 and analyzed by centrifugation for 2 hr at 27,000 rpm in 15–30% sucrose gradients containing 0.06 M KCl. The amount of 70 S is expressed as percentage of total polysomes and ribosomes determined planimetrically from the Gilford recording chart (subunits were excluded from the measurement as their amounts were the same in both gradients).

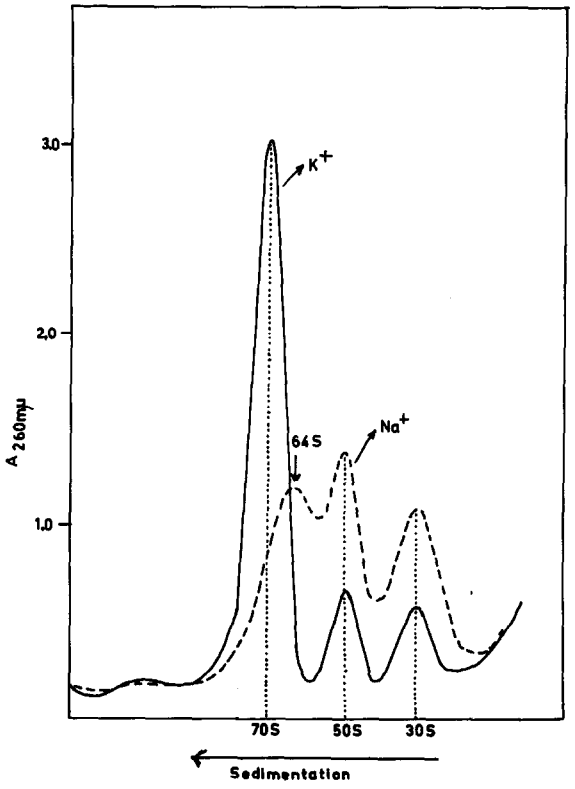

Fig. 2. Ribosomal distribution in polyribosome preparation from cells harvested in the absence of chloramphenicol. Unlabelled bacteria were harvested by pouring in ice without chloramphenicol. Spheroplast formation and lysis were as described in fig. 1. Preparations were centrifuged for 16 hr at 20,000 rpm in 15–30% gradients containing 0.06 M KCl and 0.06 M NaCl, respectively.

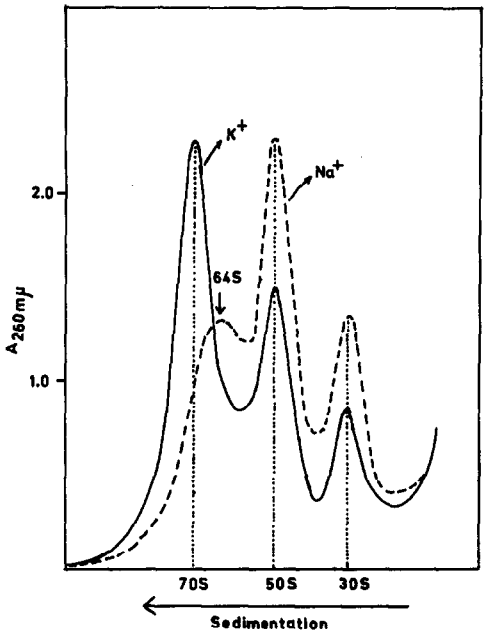

Fig. 3. The effect of K^+ and Na^+ on the sedimentation of ribosomes and subunits present in extracts obtained by alumina grinding. Extracts were prepared as described previously [5]. Equal amounts were dialyzed against TM-buffer containing either 0.06 M KCl or 0.06 M NaCl. These were centrifuged for 16 hr at 22,000 rpm in 15–30% sucrose gradients containing the corresponding salt ion concentration.

performed by G. van Dieijen). It can be seen that a similar effect of Na^+ is observed as in the previous experiments with polyribosomes.

4. Discussion

Our results indicate very strongly that the effect of different monovalent ions on polyribosome profiles is mainly due to the state of dissociation or association of ribosomes and subunits*. If the natural distribution is such that 70 S ribosomes are indeed present in substantial amounts as has been suggested [6], Na^+ seems to cause dissociation of the ribosomes into subunits through an intermediate 64 S particle. Intermediates in dissociation have been described before [5, 7], also when the dissociation is caused by the so-called dissociation factor [8]. It is a matter of speculation to what extent these intermediates are comparable, but a comformational change in the ribosomes as a prelude to dissociation is not unlikely.

* A similar conclusion was reached recently by Algranati [10].

A comformational change in the ribosomes may also be responsible for the effect of Na^+ on the sedimentation characteristics of the polysomes (broader peaks). The stabilizing forces of mRNA and peptidyl-tRNA, responsible for the higher resistancy to low Mg^{2+} concentration [5], might also counteract Na^+ induced dissociation.

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