RIBOSOME SIZE DISTRIBUTION IN EXTRACTS OF POTASSIUM-DEPLETED ESCHERICHIA COLI

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Received May 23, 1972

SUMMARY

During K^+ depletion of E. coli strain B207, a mutant which is defective in the accumulation of K^+, the intracellular K^+ is replaced by Na^+, protein synthesis is inhibited, and polysomes break down completely. The ribosome distribution in extracts derived from K^+-depleted cells was studied. The data indicate that 70S ribosomes are present in cells depleted of their intracellular K^+, in which Na^+ has replaced the K^+.

INTRODUCTION

The question whether run-off ribosomes are present in the 70S form or as subunits has been a subject of dispute (1-5). The distribution of ribosomes and subunits observed in extracts of growing bacteria apparently depends on the cationic composition of the buffer used during gradient analysis (1-8). When extracts of growing cells are analyzed in the presence of Na^+, the ribosomes sediment largely in the form of 50S and 30S subunits. On the other hand, 70S ribosomes are observed when K^+ containing buffers are used. By varying the concentration of K^+ in the growth medium, one can readily and precisely regulate the intracellular concentration of K^+ in an Escherichia coli mutant, strain B207, defective in the accumulation of this cation (9,10). When cells of this mutant are depleted of K^+, the intracellular K^+ is replaced by approximately 100 mM Na^+ (10), protein synthesis stops (10) and polysomes break down completely (11). This gives us the opportunity to study the size distribution of ribosomes in vivo under conditions of run off during K^+ depletion. Furthermore, because growing cells and K^+-depleted cultures contain spermidine and putrescine (12,13) we studied the effect of these polyamines on the size distribution of ribosomes.

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MATERIALS AND METHODS

Bacterial strains, media and growth conditions. E. coli strain B207, a mutant of E. coli B that is defective in its ability to concentrate and accumulate K^+ from the growth medium was used (9). The cells were grown in Medium A (14) supplemented with glucose (0.25%) and histidine, leucine and methionine (100 μg/ml each). In medium lacking K^+ (Medium Na^+ A), the K^+ phosphates were replaced by an equimolar concentration of Na^+ phosphates. All cultures were grown at 37° with vigorous aeration. All cultures were exponentially growing at approximately 5 x 10^8 cells/ml when used.

K^+ depletion and preparation of extracts. Cultures growing in Medium A were washed free of K^+ and suspended at 5 x 10^8 cells/ml in Medium Na^+ A. The cultures were shaken at 37° for 20 min. Then the cells were poured over frozen medium Na^+ A, and centrifuged at 5000 x g for 5 min in a Sorvall Centrifuge at 1° C. The pellet was washed 2 times with cold Tris-Na-Mg buffer. The cells were then suspended in the buffer indicated in each experiment and sonicated. The debris and unbroken cells were sedimented by centrifugation at 10,000 x g for 5 min at 1° C. The supernatant which contained the ribosomes was decanted and analyzed as indicated in each experiment. Where indicated glutaraldehyde (2.5%) was added to the extracts as previously described (15). Polysomes were prepared and treated with RNAse as previously described (11).

Buffers. The buffers used were: (a) Tris-K-Mg, which contains 10 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate and 100 mM potassium chloride; (b) Tris-Na-Mg, which is the same as Tris-K-Mg except that 100 mM sodium chloride is substituted for the potassium chloride; and (c) Tris-Mg, which is the same as the above buffers but without potassium or sodium chloride.

RESULTS

Distribution of ribosomes in extracts of K^+-depleted cells in the presence of K^+ or Na^+. The following experiments were done to examine the intracellular distribution of ribosomes and subunits during K^+ depletion. Extracts of K^+-depleted cells were made in Tris-Na-Mg or Tris-K-Mg buffers. An aliquot of each extract was layered on sucrose density gradients prepared in Tris-Na-Mg or Tris-K-Mg buffers. Fig. 1A shows that K^+-depleted cells extracted and analyzed in Tris-K-Mg buffer contained 70S ribosomes and 50S and 30S subunits. The same extract sedimented in Tris-Na-Mg buffer, displayed only 50S and 30S

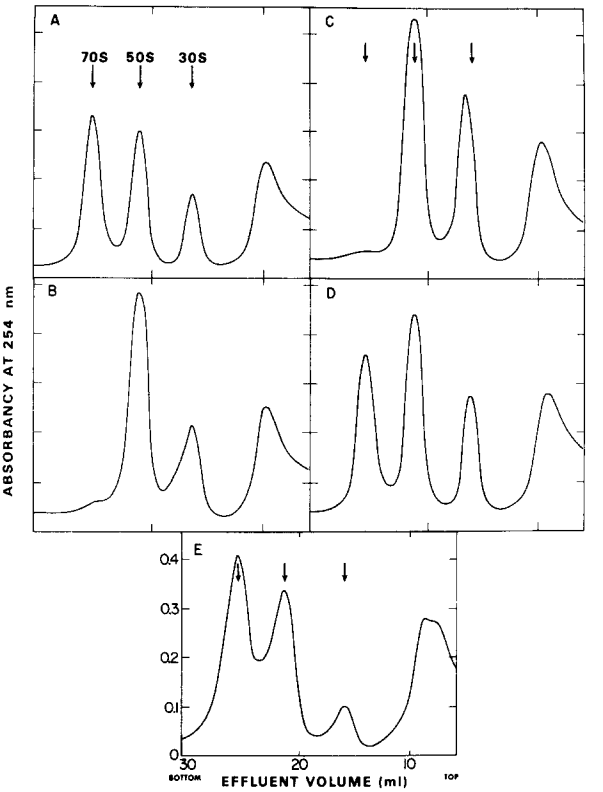

FIGURE 1: Effect of cationic composition of buffer during lysis and during analysis on distribution of ribosomes in lysates derived from K^+-depleted cells. Cells were depleted and lysed as outlined in Materials and Methods. The extract (1 ml of approximately 2-3 $A_{260\ nm}$ units) was layered on 29 ml of a 15-30% sucrose gradient made up in the indicated buffer. The tubes were centrifuged in a Spinco SW 25.1 rotor at 1° C for 17 hours at 20,000 rpm. The gradients were analyzed automatically at 254 nm by use of an ISCO fractionator connected to a Sargent recorder. All the optical density profiles shown are actual tracings. (A) Lysed in Tris-K-Mg and analyzed in sucrose density gradients containing Tris-K-Mg. (B) Lysed in Tris-K-Mg and analyzed in Tris-Na-Mg. (C) Lysed in Tris-Na-Mg and analyzed in Tris-Na-Mg. (D) Lysed in Tris-Na-Mg and analyzed in Tris-K-Mg. (E) Lysed and analyzed in Tris-Mg.

subunits (Fig. 1B). An extract of K^+-depleted cells prepared and analyzed in Tris-Na-Mg buffer contained only 50S and 30S subunits (Fig. 1C). However, this same extract analyzed on a gradient containing Tris-K-Mg, contained 70S ribosomes in addition to subunits (Fig. 1D).

These results indicate that the association and dissociation of ribosomes

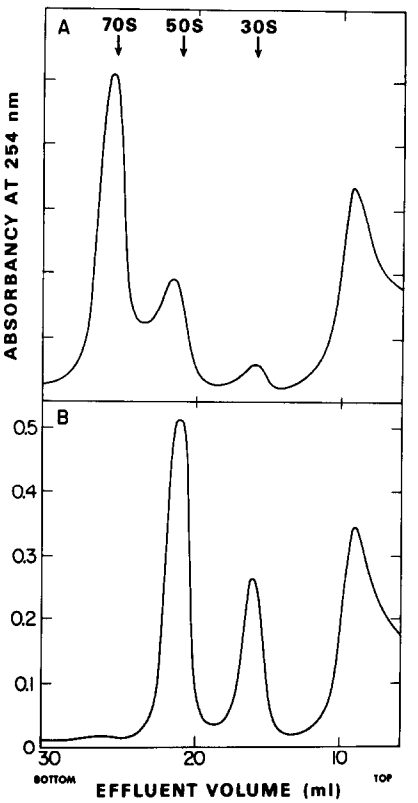

Fig. 2.

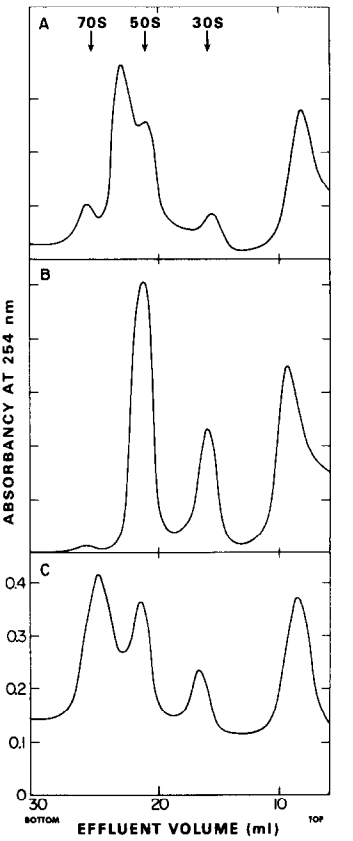

Fig. 3.

FIGURE 2: Distribution of ribosomes in extracts of K^+-depleted cells in the presence of spermidine and putrescine. The extracts were prepared and analyzed as described in the legend to Fig. 1. (A) Lysed and analyzed in Tris-Na-Mg containing spermidine (2 mM). (B) Lysed and analyzed in Tris-Na-Mg containing putrescine (10 mM). The control for this experiment, lysed and analyzed in Tris-Na-Mg is the same as shown in Fig. 1C.

FIGURE 3: Stability of 70S ribosomes in extracts isolated from K^+-depleted cells in the presence of high K^+. The extracts were prepared and analyzed as described in the legend to Fig. 1. (A) Lysed and analyzed in Tris-K-Mg containing 200 mM K^+. (B) Lysed and analyzed in Tris-K-Mg containing 400 mM K^+. (C) Same extract as B except spermidine (2 mM) was present in both the extract and in the sucrose density gradient. The gradient analyzed in 100 mM K^+ is not given but is the same as Fig. 1A.

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in K^+ and Na^+ containing buffers, respectively, may be a freely reversible process, and therefore, the results of the experiments by themselves cannot prove the actual state of ribosomes in the cell. In order to study the size

distribution in K^+-depleted cells, we made use of glutaraldehyde which prevents dissociation of 70S ribosomes (15,16) and does not promote association of 50S and 30S subunits into 70S ribosomes; and Tris-Mg buffer lacking both Na^+ and K^+ ions. When the extracts from K^+ depleted cells were made in Tris-Na-Mg buffer in the presence of glutaraldehyde and analyzed in sucrose gradients containing the same buffer, 70S ribosomes in addition to 50S and 30S subunits were observed (data not shown but similar to Fig. 1D). Cells extracted and analyzed in Tris-Mg buffer (lacking K^+ or Na^+) also gave a sedimentation profile showing 70S ribosomes (Fig. 1E).

Distribution of ribosomes in extracts of K^+-depleted cells in the presence of spermidine and putrescine. Besides K^+ and Mg^{2+}, other major cations in normally growing and K^+-depleted cells are spermidine and putrescine (12,13). Consequently, we made extracts of K^+-depleted cells in buffer containing spermidine (2 mM) or putrescine (10 mM) to determine the effect of these cations on ribosome association. The concentrations used are those normally found intracellularly in E. coli (12). As can be seen in Fig. 2, 70S ribosomes are found in extracts made and analyzed in the presence of Tris-Na-Mg containing spermidine (Fig. 2A) but not in the presence of Tris-Na-Mg containing putrescine (Fig. 2B). Only 50S or 30S subunits were found in the extracts made and analyzed in the presence of Tris-Na-Mg (see Fig. 1C). The formation of 70S ribosomes from 50S and 30S subunits in the presence of spermidine is reversible. Extracts treated as in Fig. 2A but then either dialyzed against Tris-Na-Mg lacking spermidine or merely sedimented through a sucrose density gradient made in Tris-Na-Mg buffer lacking spermidine, showed only 50S and 30S subunits (data not shown). The effect of spermidine cannot be explained merely by aggregation of ribosomes because no dimers, trimers or larger particles are formed under this condition. Purified 50S and 30S subunits also associate to form 70S ribosomes in Na^+ containing buffer in the presence of spermidine (data not shown).

Effect of high concentrations of K^+ on distribution of ribosomes. In these experiments extracts were made using Tris-K-Mg buffers in which the K^+ concentration was 100 mM, 200 mM, or 400 mM. Two types of ribosomes were compared: 70S ribosomes in extracts from K^+-depleted cells prepared in the presence of K^+ (see Fig. 1A); and 70S ribosomes in extracts prepared by RNAse treatment of polysomes derived from growing cells. Fig. 3 shows the results

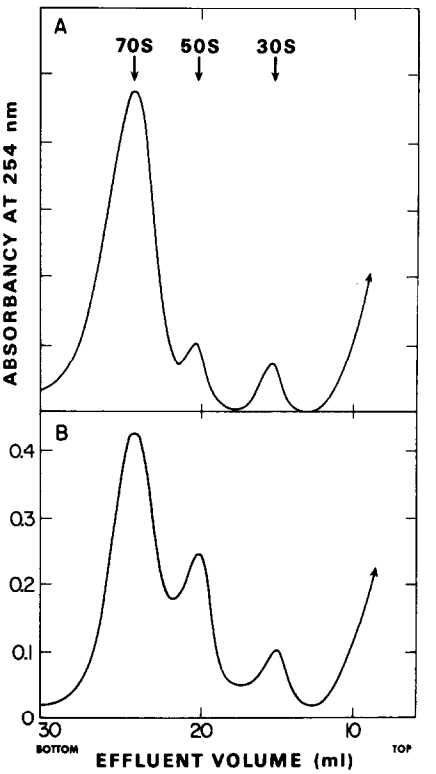

FIGURE 4: Stability of 70S ribosomes in extracts isolated by RNAse treatment of polysomes derived from growing cells. This experiment is the same as that described in the legend to Fig. 3. Polysomes were isolated from growing cells and were fragmented with RNAse as previously described (11). (A) Analyzed in Tris-K-Mg containing 200 mM K^+. (B) Analyzed in Tris-K-Mg containing 400 mM K^+. The gradient analyzed in 100 mM K^+ was the same as in Fig. 4A.

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obtained using ribosomes isolated from K^+-depleted cells. The extracts were made and analyzed on gradients in the indicated K^+-containing buffer. In the presence of 100 mM K^+, 70S ribosomes and 50S and 30S subunits are evident (see Fig. 1A). At 200 mM K^+ the profile is markedly changed. Few 70S ribosomes are present, 50S and 30S subunits are increased over that found in the presence of 100 mM and a new intermediate peak between 70S and 50S is seen (Fig. 3A). At 400 mM K^+ only 50S and 30S subunits are seen (Fig. 3B). The dissociation of the 70S ribosomes into subunits is due to the sedimentation through buffer containing 400 mM K^+. The same extract as analyzed in Fig. 3B treated with glutaraldehyde before sedimentation, still contained 70S ribosomes, in addition to subunits (data not shown). Similarly, an extract as in Fig. 3B

prepared and analyzed in the presence of spermidine plus 400 mM K^+ also contained 70S ribosomes (Fig. 3C).

Fig. 4 shows the results obtained using 70S ribosomes obtained by fragmentation of polysomes by RNAse. It is clear that these ribosomes are much less readily dissociated in the presence of high K^+ than ribosomes obtained from K^+-depleted cells. Even at 400 mM K^+ (Fig. 4B) the former ribosomes are still mainly 70S. Furthermore, high K^+ concentration did not effect the polysomes isolated from normally growing cells (data not shown).

DISCUSSION

During K^+ depletion of strain B207, which has lost the normal capacity to concentrate K^+, polysomes break down (11). Our previous work had already shown that the 70S ribosomes observed in extracts of K^+-depleted cells, prepared and analyzed in the presence of K^+, are devoid of peptidyl-tRNA (11). In this respect, they are similar to run-off ribosomes, ribosomes which have terminated protein synthesis (1). In the present investigation we have further characterized these ribosomes formed after the breakdown of polysomes due to K^+ depletion. As with other systems which have been studied (1-8), the ribosome profile, as displayed by sucrose density gradient analysis, depends on the cations used in the analysis of the cell extracts and the type of ribosomes used. Extracts of K^+-depleted cells (whether prepared in K^+ or Na^+) analyzed in K^+ containing buffer display 70S, 50S and 30S ribosomes. On the other hand, extracts (whether prepared in Na^+ or K^+) analyzed in Na^+ containing buffer show only 50S and 30S subunits. Spermidine, at a concentration (2 mM) which is normally found in *E. coli* can almost quantitatively convert the 50S and 30S subunits present in Na^+ prepared extracts to 70S ribosomes. This conversion is reversible and when the spermidine is removed from the extract only 50S and 30S subunits are observed. Putrescine has no effect on association of subunits in the presence of Na^+. Centrifugation of 70S ribosomes from extracts of K^+-depleted cells in gradients containing high concentrations of K^+ causes their dissociation to 50S and 30S subunits. 70S ribosomes prepared by the fragmentation of polysomes using RNAse, and which still contain mRNA and peptidyl-tRNA, are much less susceptible to dissociation by Na^+ (1) and by high concentrations of K^+ than ribosomes from K^+-depleted cells.

In summary, we have shown that 70S ribosomes found in K^+-depleted cells dissociate to subunits only after sedimentation through Na^+ containing sucrose density gradients. This is similar to previous work using run-off ribosomes (1). Furthermore, spermidine which can associate subunits to 70S ribosomes (6,8,17,18), is present in K^+-depleted cells (13). Consequently, these data and the results obtained with glutaraldehyde favor the existence of 70S ribosomes in the cells depleted of their intracellular K^+, in which Na^+ has replaced the K^+.

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