Communication to the Editors

EFFECT OF ANTIBIOTICS ON MAGNESIUM ION REMOVAL FROM *E. COLI* RIBOSOME SUSPENSIONS

Sir:

The naturally-occurring polyamines, spermine and spermidine, are known to show the same effect as Mg^{++} on the association of ribosome subunits to 70 S ribosomes[1,2]. Furthermore, it has been reported that basic antibiotics such as streptomycin and neomycin partially prevent 70 S ribosomes from dissociating into subunits at a reduced concentration of Mg^{++}[3]. We investigated the effect of reduction of Mg^{++} concentration on the sedimentation profiles of ribosomes in the presence of several antibiotics and other reagents.

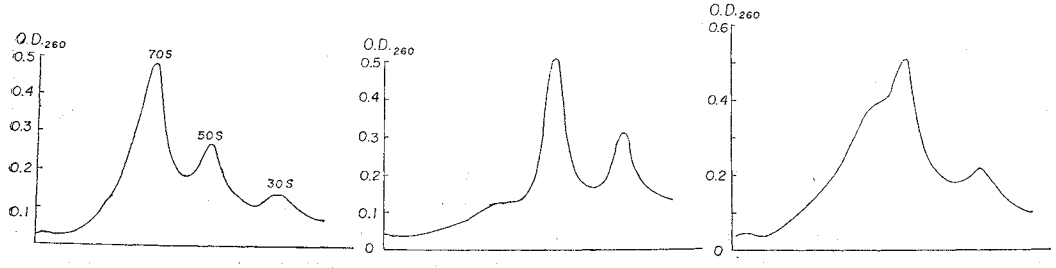
Ribosomes were prepared from *E. coli* B cells as described by MATTHAEI and NIRENBERG[4], except that quartz sand (Wako Pure Chemical Industries) was employed to grind frozen cells and the buffer used to prepare and store ribosomes was Tris-KCl-buffer* containing 10 mM $MgCl_2$ and 6 mM mercaptoethanol. Ribosomal preparations were dialyzed against Tris-KCl-buffer containing 10 mM $MgCl_2$ prior to use to remove mercaptoethanol.

The antibiotics and other reagents were mixed with ribosomes in Tris-KCl-buffer containing 10 mM $MgCl_2$, then Tris-KCl-buffer without $MgCl_2$ was added to lower the concentration of Mg^{++} to 4 mM. The final concentration of the antibiotics was 100 μg/ml. These steps were performed in an ice bath. An aliquot of the sample which contained about 0.6 O. D. units of ribosomes was placed on 5~20 % sucrose gradient (4.8 ml) which consisted of sucrose and the same ingredients as in the sample and centrifuged at 40,000 r.p.m. for 130 minutes at 2°C in a Hitachi RPS 40 rotor. The gradient was taken from the bottom through a needle inserted at the top of tube and was led to an Ohtake density gradient autoanalyzer. The ultraviolet absorption was recorded automatically.

The sedimentation pattern of the untreated *E. coli* ribosomes is shown in Fig. 1a. When Mg^{++} concentration was reduced to 2 mM, 70 S ribosomes were completely dissociated into 50 S and 30 S subunits (Fig. 1b). When the concentration of Mg^{++} was reduced to 4 mM (Fig. 1c), the 70 S peak again disappeared and a partially dissociated material[5] appeared as a shoulder on the heavier side of the 50 S peak. Its relative height and position in the gradient varied slightly from preparation to preparation, but it was always recognized at least as a shoulder of 50 S peak such as shown in Fig. 1c.

Kanamycin in the mixture seemed to protect 70 S from the dissociation (Fig. 2). When Mg^{++} was reduced to 4 mM in the presence of kanamycin, the partially dissociated particle persisted in the heavier side and became a separate peak from 50 S peak. At 100 μg kanamycin/ml the heaviest peak remained at the area of 70 S peak.

Fig. 1. Effect of Mg^{++} concentration on the sedimentation profiles of ribosomes.
(a) Mg^{++} 10 mM (b) Mg^{++} 2 mM (c) Mg^{++} 4 mM



* Tris-KCl-buffer: 50 mM Tris buffer (pH 7.9) containing 50 mM KCl.

Fig. 2. Effect of kanamycin.

The concentration of Mg^{++} was reduced to 4 mM in the presence of kanamycin.

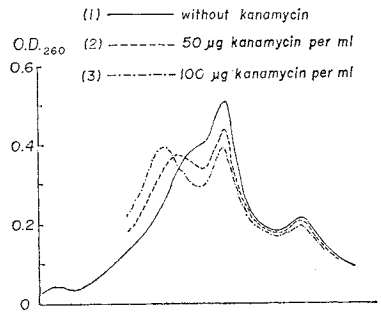


Fig. 3. Effect of neomycin.

The concentration of Mg^{++} was reduced to 4 mM in the presence of neomycin.

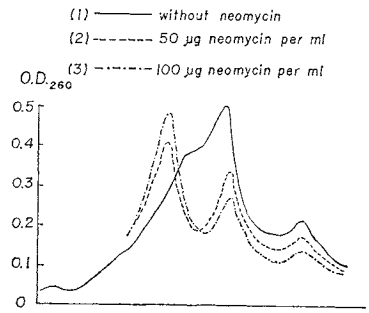


Table 1. Protective effect on ribosomes

Antibiotics	Protective effect
Neomycin	+++
Kanamycin	++
N-Acetyl kanamycin	−
Kanamycin-P-I	−
Streptomycin	+
Dihydrostreptomycin	+
Kasugamycin	−
Blasticidin S	++
Cycloheximide*	−
Chloramphenicol*	−
Erythromycin	−
Tetracycline	−
Spermine	+++
Glucosamine	−

Protective effects indicated are based upon the sedimentation profiles of ribosomes after Mg^{++} concentration is reduced to 4 mM in the presence of 100 μg/ml of antibiotics. When the profile looks like Fig. 1a, the effect is indicated as +++. In the similar manner, ++, + and − correspond to Fig. 2(3), Fig. 2(2) and Fig. 1c, respectively.

* The concentration used was 50 μg/ml instead of 100 μg/ml.

Iucubation of the sample mixture at 30°C for 30 minutes did not cause any further change in the sedimentation profiles of ribosomes. The effect of kanamycin could not be observed if the drug was added only to the sample mixture and omitted from the sucrose gradient.

Recently it was discovered that a multiple drug resistant *E. coli* K-12 (R-5) inactivates kanamycin by acetylation[6]. The inactivated products were isolated and their chemical structures were studied by KONDO *et al.* of our laboratory. One is N-acetyl kanamycin in which amino group of 6-amino-6-deoxy-D-glucose moiety was acetylated[7] and the other is kanamycin-P-I in which the hydroxyl group on C-3 of 6-amino-6-deoxy-D-glucose moiety was phosphorylated[8]. These inactivated kanamycins neither inhibit protein synthesis nor cause miscoding in a cell-free system[9]. These inactivated kanamycins did not cause any change in sedimentation profiles and at the concentration of 100 μg/ml ribosomes showed the same pattern as shown in their absence when Mg^{++} concentration was reduced to 4 mM.

For many antibiotics the primary site of action is considered to be the ribosomes. We checked some of them and the results are summarized in Table 1. The effect of neomycin was more marked than that of kanamycin (Fig. 3), and 50 μg/ml of this antibiotic was almost as effective as 100 μg kanamycin/ml. At 100 μg neomycin/ml the sedimentation profile could be superimposed completely on that of ribosomes in 10 mM Mg^{++}.

The protective effect of streptomycin and dihydrostreptomycin is much weaker than that of kanamycin and in the presence of 100 μg/ml of either the sedimentation profile was almost the same as that observed with 50 μg/ml of kanamycin. Blasticidin S was as effective as kanamycin. Spermine and glucosamine were chosen as examples of basic compounds other than antibiotics. Spermine was very effective and almost comparable to neomycin. Glucosamine showed no effect at 100 μg/ml.

Neomycin, kanamycin and streptomycin are known to cause miscoding but their patterns of miscoding are varied[10]. Blasticidin S which is a pyrimidine analogue containing two amino groups[11] and kasugamycin which is also a basic antibiotic[12] do not cause miscoding although they are inhibitors of protein synthesis[13,14]. Thus, it may be said that all basic antibiotics causing miscoding showed the protective effect to some extent.

Recently it has been suggested that 70 S ribosomes are artefacts which have been produced by degradation of polyribosomes[15] and the biological significance of the dissocia-

tion and re-association of 70 S particles is still obscure. Yet it is beyond doubt that the protective effect of several antibiotics as discussed above reflect their binding to ribosomes. Kanamycin seems to bind to ribosomes through its whole molecular structure because kanamycin-P-I and N-acetyl-kanamycin show no protective effect.

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