THE EFFECT OF 5-AZACYTIDINE ON THE SYNTHESIS OF RIBOSOMES IN ESCHERICHIA COLI

V.PAČES, J.DOSKOČIL and F.ŠORM
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Praha

Received 29 May 1968

1. Introduction

The accumulation of subribosomal particles has been observed in cells exposed to the action of various inhibitors of protein synthesis [1] or some analogs of purine and pyrimidine bases such as 8-azaguanine [2] or 5-fluorouracil [3-6]. This communication is concerned with the effect of 5-azacytidine [7-9] on the synthesis of ribosomes. In the presence of this compound both 23 S and 16 S ribosomal RNAs are formed, but are not incorporated into the ribosomes. If the inhibitory action of 5-azacytidine is interrupted by adding an excess of cytidine, the 50 S peak gradually appears while two undistinct peaks of 25 S and 34 S are formed in place of 30 S peak. A large proportion of the particles formed in the cells previously exposed to 5-azacytidine is incapable of forming 70 S ribosomes in media with high concentration of Mg^{2+}, thus resembling the "nascent ribosomes" [10] or abnormal ribosomes formed in the presence of ethionine [11]. Some experiments suggest that the lesion responsible for this effect might be located in tRNA rather than in the particles themselves.

2. Methods

The bacteria, *E. coli* B, were grown at 37° with shaking in a medium according to Spizizen [12] supplemented with 0.5% casamino acids. At a density of $3-5 \times 10^8$ cell/ml, the cultures (usually 25 ml) were used for the experiments. Cell-free extracts were prepared by sonicating the bacteria in buffers, containing 0.01M Tris, pH 7.3, 0.06M KCl and either 0.1 mM or 10 mM Mg-acetate. RNA was prepared according to Salser, Gesteland and Bolle [13], except that the phenol treatment was done at room temperature. The cell-free extracts or preparations of RNA were centrifuged at 37.000 rev./min in linear sucrose gradients (5-25% sucrose in the buffers indicated above). A SW-39 rotor of centrifuge Spinco L was used. The centrifugation times were 90 min for cell-free extracts in high-Mg^{2+} medium, 150 min for extracts in low-Mg^{2+} medium, and 330 min for RNA. The temperature was approx. 5°.

3. Results

If the cells of *E. coli* are exposed to 5-azacytidine for 10-20 min, the RNA formed during this period has a normal sedimentation profile, consisting mainly of 23 S, 16 S and 4 S material. All these species contain 5-azacytidine (fig. 1b). However, the analysis of crude extracts (fig. 1c) shows that most of the RNA synthesized in the presence of 5-azacytidine is not present in the ribosomes, being found in a heterogeneous fraction with lower sedimentation coefficient.

If cytidine is added to the culture, the incorporation of [^{14}C] 5-azacytidine ceases [9]; the sedimentation characteristics of RNA and its total 5-azacytidine content do not change during the next 30 min. A gradual and rather slow transfer of the labelled material into ribosomes is observed. While in the 50 S region the maximum radioactivity coincides with maximum optical density, two undistinct peaks of 5-azacytidine radioactivity appear and do not coin-

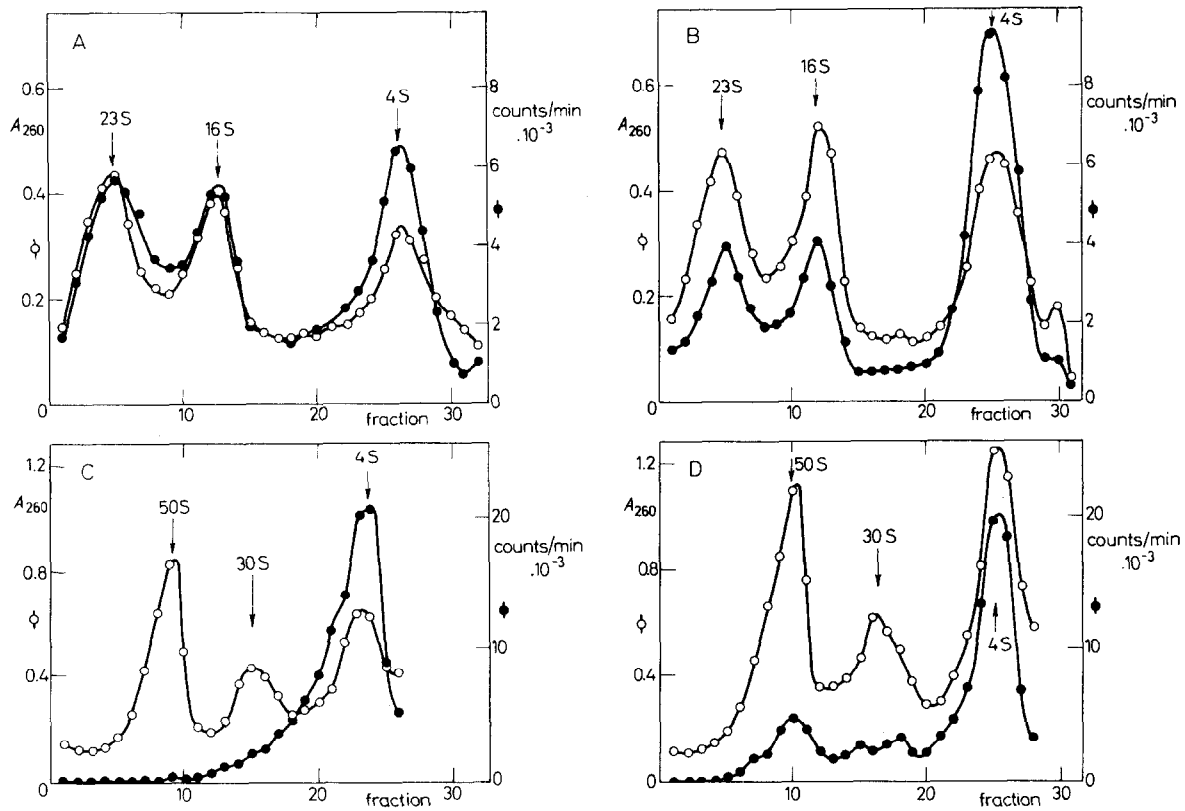

Fig. 1. Sedimentation profiles of RNA (A,B) and cell-free extracts (C,D) from cells labelled with [^{14}C] 5-azacytidine. A,C: The cultures were labelled for 20 min with [^{14}C] 5-azacytidine (4 mC/mmole, 5 μg/ml). B,D: The cultures were labelled similarly as in A,C, and subsequently treated with cytidine (100 μg/ml) for 30 min (B) or 45 min (D). Low-Mg^{2+} medium was used.

cide with the 30 S peak (fig. 1d). Similar results are obtained using [^{14}C] guanosine in the presence of unlabelled 5-azacytidine.

In high-Mg^{2+} medium the formation of 70 S ribosomes is incomplete. A large proportion of the labelled particles does not associate and remains in the 50 S or approx. 30 S stage, behaving like "nascent ribosomes". In a control experiment, with cells similarly labelled but not exposed to 5-azacytidine, the association is nearly complete (fig. 2).

In an experiment represented in fig. 3 the cells were doubly-labelled, using [^{14}C] guanosine during the treatment with 5-azacytidine, and [^{3}H] uridine in the subsequent period of cytidine chase. In this case both [^{14}C] and [^{3}H] sedimentation profiles show distinct peaks of "nascent ribosomes", indicating that even the ribosomes formed from the material incorporated during the chase period do not associate.

4. Discussion

According to Schlessinger et al. [10] mRNA, tRNA and high concentration of Mg^{2+} are necessary for the formation of 70 S ribosomes from nascent ribosomes. In our experiments both tRNA and rRNA contain 5-azacytidine, so that the lesion responsible for incomplete association could be located in either species of RNA. If the rRNA formed during the treatment with 5-azacytidine is degraded and its components reutilized for resynthesis, a uniform population of ribosomes would be formed during the period of cytidine chase, accounting for the result of the doubly-labelling experiment (fig. 3). However, we cannot exclude the possibility that tRNA, containing 5-azacytidine in the terminal sequence or in the interior of the molecule, is incapable of promoting the association, while the "healthy" part of the

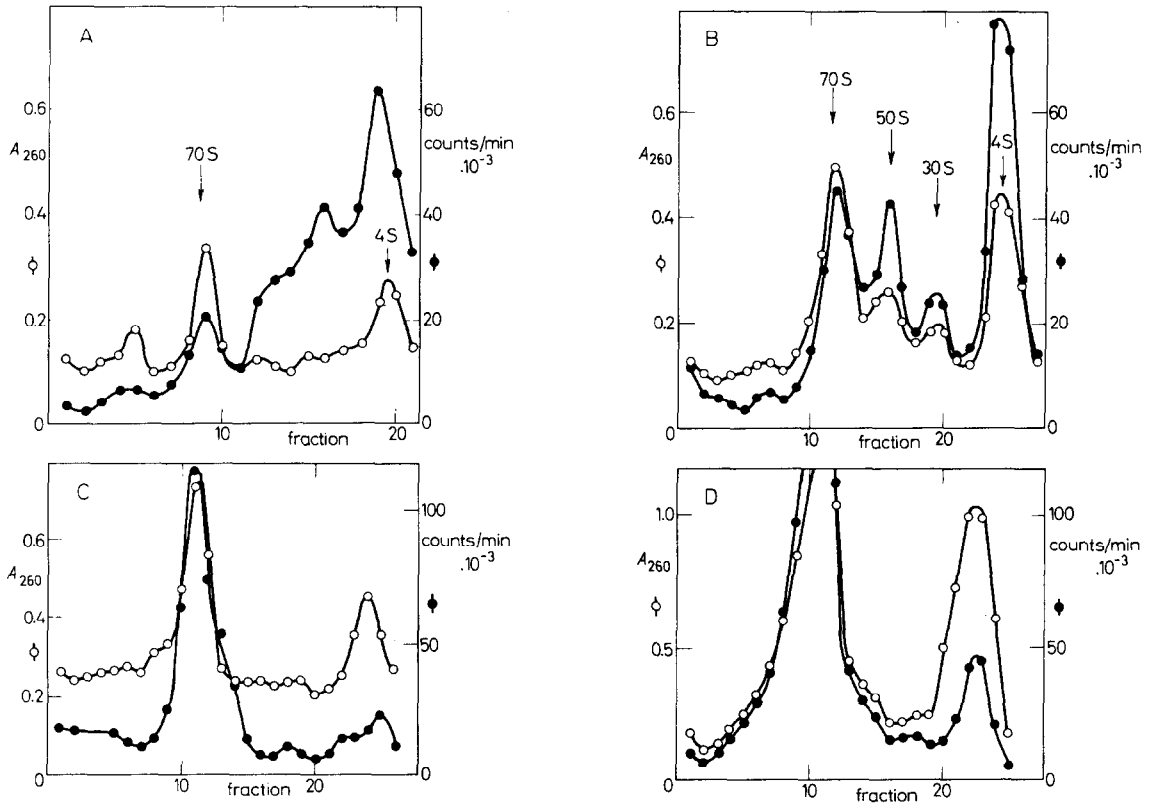

Fig. 2. Sedimentation profiles of cell-free extracts from cultures exposed to 5-azacytidine. The cultures were labelled with [^{14}C] guanosine (31.6 mC/mmole, 0.1 μC/ml) for 20 min. 5-Azacytidine (5 μg/ml) was present during the labelling period in A, B, while C and D served as controls without 5-azacytidine. A,C: The cell-free extracts were prepared immediately after the labelling; B, D: After the labelling, unlabelled guanosine (50 μg/ml) and cytidine (100 μg/ml) were added and the incubation continued for 45 min. High-Mg^{2+} medium was used for sucrose gradients.

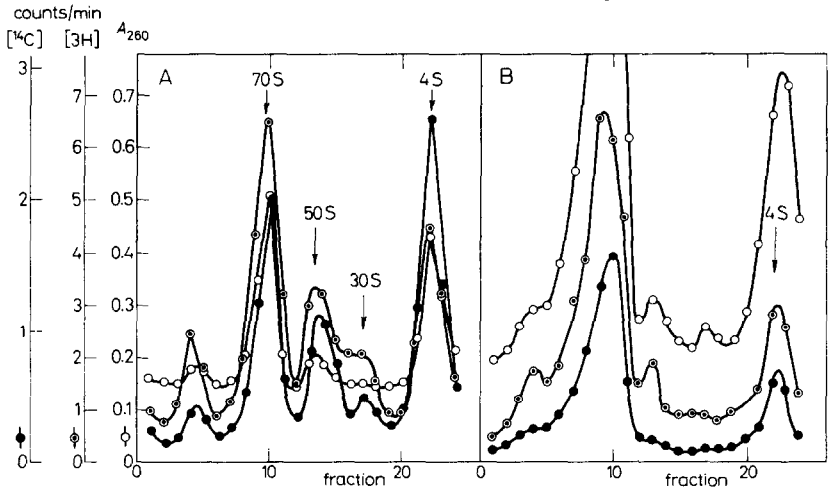

Fig. 3. Sedimentation profiles of cell-free extracts from cultures exposed to 5-azacytidine. The cultures were labelled with [^{14}C] guanosine (0.02 μC/ml) for 20 min. After this time unlabelled guanosine (100 μg/ml), cytidine (200 μg/ml) and [^{3}H] uridine (45 mC/mmole, 0.2 μC/ml) were added and the incubation continued for 45 min. After this time the cultures were harvested. A: 5-Azacytidine (15 μg/ml) was added simultaneously with [^{14}C] guanosine; B: Control without 5-azacytidine. The centrifugation was performed in high-Mg^{2+} media.

population (i.e. the molecules of tRNA persisting from the period before treatment, with no 5-azacytidine in the interior) could account for the partial association observed.

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