RELATIONS BETWEEN SPERMIDINE CONTENT AND RNA STABILITY IN RIFAMPICIN TREATED ESCHERICHIA COLI*

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SUMMARY: During incubation in the presence of chloramphenicol (CM), E. coli synthesizes RNA (CM-RNA). When CM is removed and further RNA synthesis is blocked with rifampicin, cells lose spermidine and CM-RNA degrades with a 15 minute half-life. However, if RNA synthesis is blocked by starving for a required amino acid, spermidine is retained by the cells and CM-RNA degrades with a 24 minute half-life. Raising the intracellular spermidine concentration in rifampicin treated cultures by the addition of spermidine to the growth medium stabilizes CM-RNA to the level observed in histidine starved cultures.

Introduction

During logarithmic growth, bacterial messenger RNA is unstable but ribosomal RNA (rRNA) is not. The available evidence suggests that ribosomal proteins stabilize rRNA during ribosome formation. This evidence is derived from experiments in which rRNA is allowed to accumulate in bacteria during inhibition of protein synthesis. When the inhibitor of protein synthesis is removed ribosomal proteins are synthesized preferentially, and the rRNA becomes incorporated intact, into mature ribosomes (5,7,9,10,11,14). However, if RNA synthesis is blocked after removal of the inhibitor of protein synthesis, almost no ribosomal protein synthesis occurs and most of the rRNA degrades (1,5,10,14). In the experiments reported here, we allowed rRNA to accumulate in E. coli in the presence of chloramphenicol (CM), removed CM, and blocked further RNA synthesis.

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The kinetics of degradation of this accumulated RNA (CM-RNA) was used as a model system for studying those factors involved in rRNA stability independently of continuing ribosomal protein synthesis. We have found that spermidine and possibly putrescine are important in maintaining rRNA stability in vivo.

Materials and Methods

Bacteria, Growth Conditions, and Measurements of Radioactivity. E. coli B207, a derivative of E. coli B, which requires histidine, leucine, and methionine for growth, was used in this investigation. Growth conditions, accumulation of CM-RNA in the presence of 100 μg/ml chloramphenicol, and measurement of incorporation of ^{14}C-uracil into RNA and ^{14}C-leucine into protein in vivo by precipitation with trichloroacetic acid has been described previously (1,2). All experiments were performed at 37° with vigorous aeration of cultures.

Quantitative determination of spermidine and putrescine in E. coli B207. Putrescine and spermidine were extracted from 60 ml cultures of E. coli B207 at 1.0 x 10^9 cells/ml by the method of Raina and Cohen (12) with the exception that the cells were centrifuged for 5 minutes at 8000 x g at room temperature to avoid acetylation of the polyamines in vivo caused by chilling (17). The polyamine extracts were spotted on Whatman No. 1 paper strips and chromatography was performed using the "cellosolve" NaCl saturated solvent system of Herbst et al. (6). Putrescine and spermidine spots were developed by the method of Dubin and Rosenthal (3), eluted, and quantitated by the method of Raina and Cohen (12). ^{14}C-labelled spermidine (0.01 μCi, 9.3 x 10^{-4} μ moles) and ^{14}C-labelled putrescine (0.01 μCi, 1.4 x 10^{-3} μ moles) were added to crude cell extracts to determine loss of putrescine and spermidine during the purification procedure. In all experiments, the recovery of spermidine and putrescine from E. coli B207 cells was greater than 85%.

Chemicals. Uracil-2-^{14}C (30 mCi/m mole), L-leucine-1-^{14}C (25 mCi/m mole), spermidine-^{14}C trihydrochloride (10.7 mCi/m mole), and putrescine-1,4-^{14}C dihydrochloride (7.41 mCi/m mole) were purchased from New England Nuclear Corp., Boston, Mass. All other chemicals used were the best grade commercially avail-

able. Chloramphenicol was a gift from Parke, Davis, and Co., Detroit, Mich.

Results

CM-RNA stability in rifampicin treated and histidine starved cultures.

E. coli B207 is a stringent strain which ceases to accumulate RNA when starved for the required amino acid, histidine (1) or when treated with rifampicin, an inhibitor of RNA polymerase (15). When the rate of degradation of CM-RNA was measured in a B207 culture recovering from CM-inhibition, in a similar culture treated with rifampicin, and in another culture starved for histidine, CM-RNA was found to degrade faster in rifampicin treated cultures than in histidine starved cultures and was most stable in normally recovering cultures, presumably because it was incorporated into mature ribosomes (Fig. 1). In separate

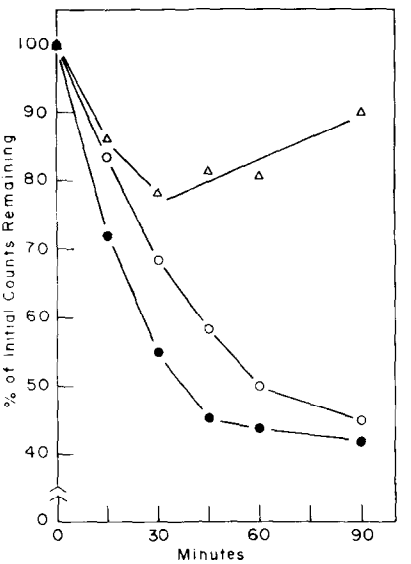

Fig. 1. Stability of CM-RNA. A culture of E. coli B207 at 3.6 x 10^8 cells/ml was allowed to accumulate CM-RNA in the presence of ^{14}C-uracil (0.01 µCi/ml, 4 µg/ml) for 1 hour at 37°. The cells were washed twice in fresh medium at room temperature to remove CM and ^{14}C-uracil. Equal portions of cells were resuspended at 1.0 x 10^9 cells/ml in the indicated media supplemented with non-radioactive uracil (50 µg/ml) at 37°. The cultures: (Δ), were allowed to recover normally; (●), were treated with rifampicin (100 µg/ml); (○), were starved for histidine. At the indicated times, samples were taken and the radioactivity remaining in CM-RNA was determined. Approximately 5000 counts/minute per ml of culture were present in CM-RNA at 0 minutes.

experiments it was shown that both rifampicin treatment and histidine starvation blocked RNA and protein synthesis almost completely in recovering cultures (data not shown).

Polyamine content of rifampicin treated and histidine starved cultures. Although the factors involved in the instability of CM-RNA are unknown, it has been found that the intracellular concentration of spermidine increases while the intracellular concentration of putrescine remains roughly constant in CM-treated E. coli 15 TAU (13). We find that during exponential growth, strain B207 contains 0.05 μ moles of spermidine and 0.10 μ moles of putrescine per 10^{10} cells. Cells treated with CM for 1 hour contain the same amount of spermidine as during logarithmic growth (0.05 μ moles/10^{10} cells) but retain only 25% of their putrescine (0.025 μ moles/10^{10} cells). After removal of CM, normally recovering cells or histidine starved cells lose only about 20% of their spermidine over a 90 minute incubation period, but rifampicin treated cells lose 70% (Fig. 2A). That the loss of spermidine from rifampicin treated cells is due to spermidine exiting the cells rather than being converted to other compounds and remaining intracellular is shown by the fact that CM-treated cells containing a small amount of intracellular ^{14}C-spermidine lose 70% of it to the growth medium when treated with rifampicin over the 90 minute incubation period (Fig. 2B). In contrast, histidine starved or normally recovering cells retain ^{14}C-spermidine (Fig. 2B).

The loss of spermidine from rifampicin treated cells is accompanied by an initial 50% increase in the intracellular putrescine concentration over the first 30 minutes of recovery followed by a drop in putrescine content over the next hour (Fig. 2C). In histidine starved cells the putrescine content remains constant throughout the recovery period (Fig. 2C). The intracellular putrescine concentration increases steadily in normally recovering cultures and reaches the normal log phase level of 0.10 μ mole per 10^{10} cells about 90 minutes into recovery.

CM-RNA stability in the presence of exogenous spermidine and putrescine.

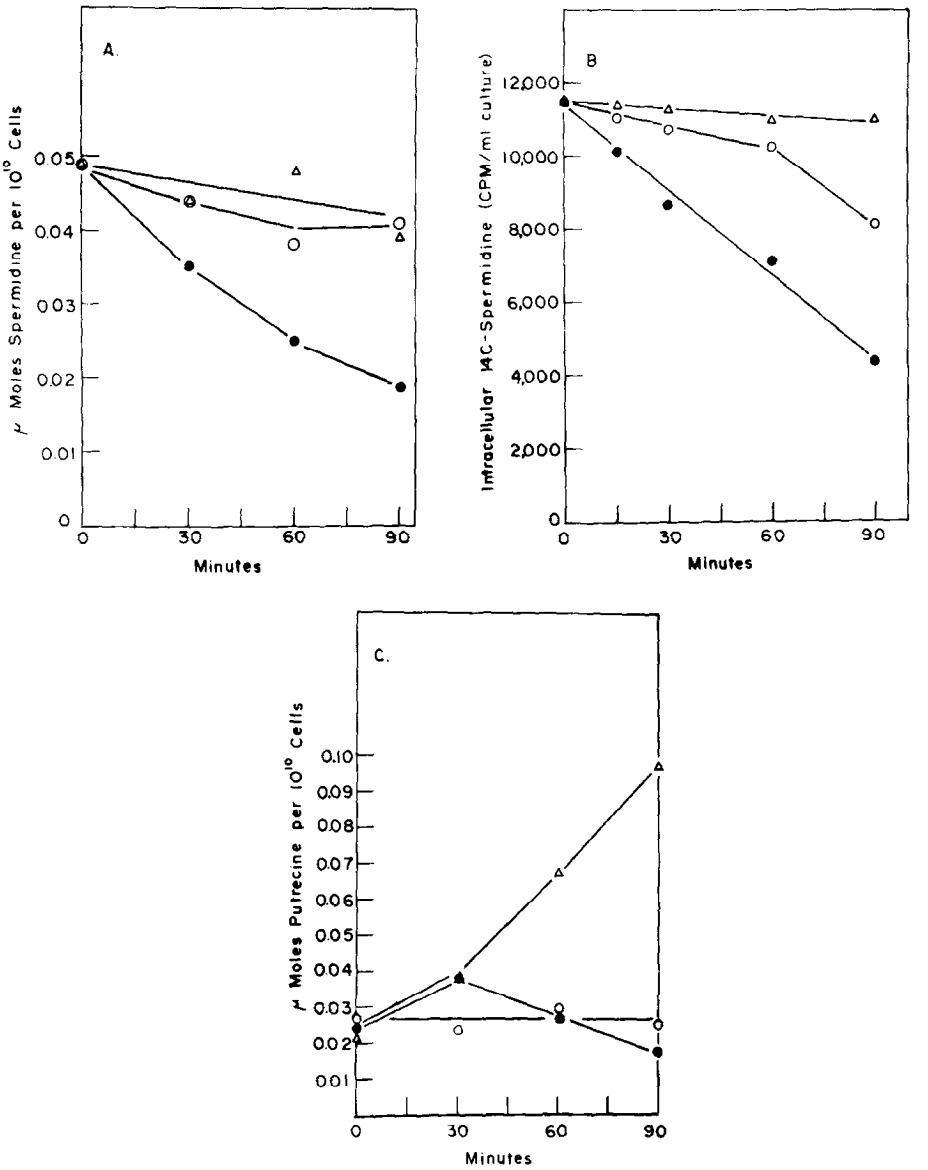

Fig. 2. Spermidine and putrescine content of recovering cultures.

(A) Spermidine content. A culture of E. coli B207 was incubated in CM for 1 hr at 37°, washed free of CM, and equal portions of cells were resuspended in fresh medium at a concentration of 1 x 10^9 cells/ml. The cultures: (Δ), were allowed to recover normally; (●), were treated with rifampicin (100 μg/ml); (○), or were starved for histidine. At the indicated times, 60 ml samples were taken and the spermidine content was determined (see Materials and Methods). Over the 90 minute incubation period the cell numbers in rifampicin treated and histidine starved cultures did not change, while in normally recovering cultures the cell number increased continuously. The calculation of μ moles of spermidine/10^{10} cells is based on the number of cells in a culture at the time of sampling.

(B) Retention of ^{14}C-spermidine by recovering cultures. E. coli B207 was grown in the presence of ^{14}C-spermidine (0.01 μCi/ml, 9 x 10^{-4} μ moles/ml) to a concentration of 3.6 x 10^{8} cells/ml, washed free of radioactive spermidine, treated with CM (100 ug/ml) for 1 hour at 37°, washed free of CM, and equal portions of cells were resuspended in fresh medium at 37° at a concentration of 1 x 10^{9} cells/ml. At the indicated times, 1 ml samples were taken, poured over millipore filters and ^{14}C-spermidine remaining in the cells was determined. Symbols are the same as in (A).

(C) Putrescine content. Conditions were the same as in (A) except at the indicated times the putrescine content of the cells was determined. Symbols are the same as in (A).

If the spermidine loss from rifampicin treated cells causes decreased CM-RNA stability, it should be possible to reduce the rate of CM-RNA degradation in rifampicin treated cultures to the rate observed in histidine starved cultures by increasing the intracellular spermidine concentration in rifampicin treated cells.

About 40% of the CM-RNA in rifampicin treated cells and histidine starved cells is stable (Fig. 1) and is mostly 4S RNA (data not shown). Degradation of the unstable CM-RNA with time follows first order kinetics (Fig. 3) and the half-lives of unstable CM-RNA in rifampicin treated and histidine starved cultures are 15 minutes and 24 minutes respectively. Although spermidine concentrations as low as 5 μ moles per ml of culture increase CM-RNA stability (data not shown), a spermidine concentration of 80 μ moles per ml of culture is required to increase the half-life of unstable CM-RNA from 15 minutes to 22 minutes (Fig. 3). At this extracellular concentration of spermidine, the intracellular concentration is 0.16 μ moles per 10^{10} cells, and RNA and protein synthesis are still completely blocked.

Extracellular putrescine concentrations as high as 125 u moles per ml of culture do not influence the half-life of CM-RNA in rifampicin treated cultures (Fig. 3). However, when both putrescine (125 μ moles/ml) and spermidine (80 μ moles/ml) are added simultaneously, the half-life of unstable CM-RNA increases from 15 minutes to 29 minutes (Fig. 3). At this extracellular putrescine concentration the intracellular putrescine content is 0.30 μ moles per 10^{10} cells.

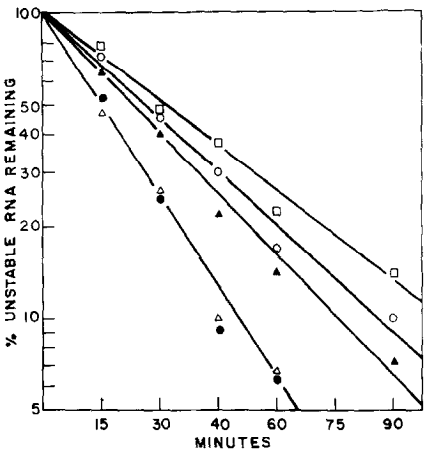

Fig. 3. Half-life of unstable CM-RNA. E. coli B207 was allowed to accumulate radioactive CM-RNA, was washed free of CM and radioactivity, and equal portions of cells were resuspended in fresh media as described in Fig. 1. In addition, the cultures were treated with: (●), rifampicin (100 μg/ml); (△), rifampicin (100 μg/ml) and putrescine (125 μ moles/ml); (▲), rifampicin (100 μg/ml) and spermidine (80 μ moles/ml); (□); rifampicin (100 μg/ml), spermidine (80 μ moles/ml), and putrescine (125 μ moles/ml); (○), or were starved for histidine. At the indicated times, samples were taken and the radioactivity remaining in unstable RNA was determined. Approximately 5000 counts/minute per ml of culture were present in CM-RNA at 0 minutes.

Discussion

In the present investigation, we allowed RNA to accumulate in CM-treated E. coli and studied its stability in the absence of further RNA and protein synthesis. In this way we were able to determine the following:

(a) The method used to block RNA and protein synthesis determines the rate of RNA degradation (Fig. 1). In rifampicin treated cells the half-life of unstable CM-RNA was 15 minutes, while in histidine starved cells it was 24 minutes (Fig. 3).

(b) The more rapid rate of RNA degradation in rifampicin treated cells is accompanied by the loss of intracellular spermidine due to excretion of this polyamine into the medium (Fig. 2). Histidine starved cells maintain their intracellular spermidine content and do not leak spermidine to the medium (Fig. 2)

(c) The rate of degradation of unstable CM-RNA in rifampicin treated cells can be decreased to that observed in histidine starved cells by the addition of

spermidine to the medium (Fig. 3). However, this increased stability is observed at an intracellular spermidine concentration of 0.16 μ moles per 10^{10} cells, three fold greater than the spermidine concentration of 0.05 μ moles per 10^{10} cells found in histidine starved cultures. Only intracellular spermidine was measured, but it is possible that rifampicin treatment causes a release of other RNA stabilizing cations to the medium. It is known that inorganic cations such as Mg^{2+} and Ca^{2+} can inhibit ribonuclease activity in vitro (4,8). If a general loss of cations does occur upon rifampicin treatment of E. coli, it would be expected that a higher than normal intracellular concentration of spermidine would be needed to stabilize RNA. In this regard, it is interesting that putrescine addition to the medium enhances the RNA stabilizing ability of spermidine (Fig. 3).

It is well established in E. coli and in animal cells undergoing rapid growth that an increase in the rate of RNA synthesis is associated with the intracellular accumulation of spermidine and that spermidine stimulates RNA polymerase activity (12,13,16). The experiments reported here suggest that the increased rate of spermidine biosynthesis accompanying an increased rate of rRNA synthesis would also be advantageous to the cell in maintaining stability of the newly synthesized rRNA until ribosomal proteins become available to provide further stability.

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