INHIBITION OF MESSENGER RNA SYNTHESIS BY OXIDIZED SPERMINE

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Spermine, $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, a natural cationic compound, is oxidized by crude or purified serum amine oxidase (Hirsch, 1953; Tabor, Tabor and Rosenthal, 1954; Blaschko and Hawes, 1959) to a dialdehyde $H\overset{O}{\diagdown\!\!=}C(CH_2)_2NH(CH_2)_4NH(CH_2)_2C\overset{O}{\diagup\!\!=}H$ (Tabor, Tabor and Bachrach, 1964). This compound, oxidized spermine, inhibits the growth of various bacteria (Hirsch and Dubos, 1952; Tabor and Rosenthal, 1956; Bachrach and Persky, 1964) and inactivates bacteriophages (Bachrach, Tabor and Tabor, 1963; Bachrach and Leibovici, 1965) as well as certain plant viruses (Bachrach, Rabina, Loebenstein and Eilon, 1965). These findings have been interpreted in terms of the formation of a biologically inactive complex between oxidized spermine and microbial DNA (Bachrach and Leibovici, 1964). This hypothesis was supported by the observation that a complex between oxidized spermine and T5 DNA, is injected into Escherichia coli, but does not give rise to complementary RNA (Bachrach and Leibovici, in press). From these studies it could be inferred that synthesis of messenger RNA is sensitive to inhibition by oxidized spermine. Therefore it seemed of interest to investigate the effect of this agent on the induction of β-galactosidase in Escherichia coli. This system was chosen, since it has been thoroughly investigated in various laboratories and information

is available as to the kinetics of messenger RNA synthesis and breakdown (Mandelstam, 1961; Kepes, 1963; Nakada and Magasanik, 1964).

Materials and Methods

E. coli B was grown with aeration at 37° on a synthetic medium (Davis, 1949). The incubation temperature was changed to 30°, 1 - 2 hours prior to the beginning of the experiment. β-Galactosidase was induced and assayed at 30° by the conventional method (Pardee et al, 1959). Induction was terminated by filtration on Millipore filters (0.45 μ pore size) at room temperature, washing with 3 portions of 10 ml chilled 0.05 M potassium phosphate buffer pH 7.5, followed by 10 ml of the same buffer warmed to 30°. All subsequent incubations were at 30°. To determine incorporation of ^{14}C-uracil or ^{14}C-valine into trichloroacetic acid (TCA) - precipitable material, one ml aliquots of the culture were added at 4° to 2.0 ml of chilled 5% TCA solution, the precipitates collected on Millipore filters and washed with 30 ml of 5% TCA supplemented with uracil or valine (100 μg/ml). The filters were mounted on planchets and the radioactivity was determined in a Nuclear Chicago Gas flow counter. Oxidized spermine was prepared in a Warburg flask by incubating 10 μmoles of spermine with 100 μmoles potassium phosphate buffer pH 7.4; 600 units of purified amine oxidase, 82 units/mg (Tabor, Tabor and Rosenthal, 1954); 100 units of catalase in a final volume of 3.0 ml. The rate of oxidation was followed monometrically; the reaction was usually completed within 120 min.

Results and Discussion.

As shown in Figure 1, oxidized spermine arrested the incorporation of ^{14}C-uracil almost immediately, the synthesis of β-galactosidase after approximately 4 minutes and the incorporation of ^{14}C-valine after approximately 12 minutes. Thus, oxidized spermine acts on E. coli as actinomycin D does on Bacillus subtilis (Kirk, 1960; Levinthal, Keynan and Higa, 1962) or on EDTA - treated E. coli cells (Leive, 1965) in both cases RNA synthesis

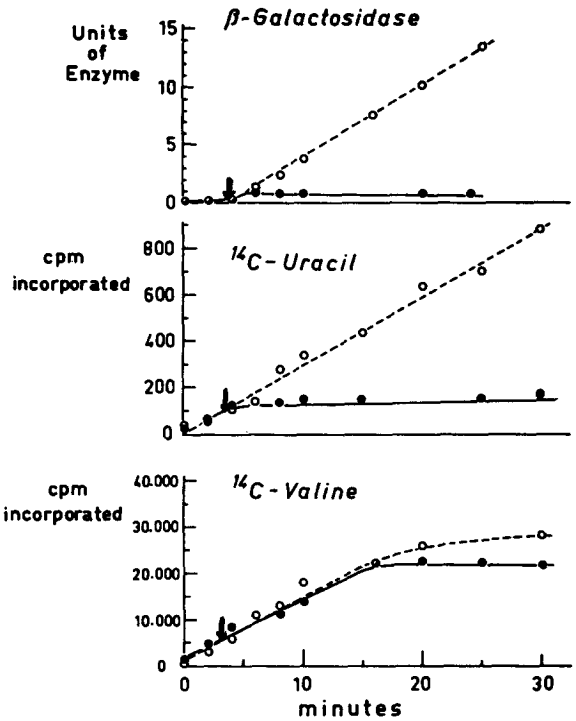

Fig. 1. The action of oxidized spermine on E. coli. E. coli B was grown as described in the text to a density of 5x10^8 cells/ml. Portions of 20 ml were incubated at 30° and induction was started (zero time on the graph) by the addition of methyl β-D-thiogalactoside (TMG), to a final concentration of 5x10^{-4} M. ^{14}C Uracil (5 μc, 1.2 mc/μmole) and ^{14}C valine (5 μc, 4.5 μc/μmole) were added respectively. At 3 min (indicated by arrow on each graph), 5 ml of oxidized spermine were added (final concentration 120 μg/ml) and incubation continued. Samples were taken at the time shown and assayed as described in the text. β-Galactosidase activity is expressed in units (1 unit of β-galactosidase splits 1 mμmole of ONPG per min at 30°) per 1.0 ml of original culture.
Oxidized spermine, ● ——●; control, ○- — ○.

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ceases at once and protein synthesis soon thereafter.

β-Galactosidase synthesis terminated sooner than did protein synthesis, as measured by incorporation of ^{14}C-valine into TCA-precipitable material. This may be explained by differences in half life times of the various messenger RNA's in the cell (Engelberg and Artman, 1964; Leive, 1965; Moses and Calvin, 1965). An alternative explanation would attribute the inhibition of

β-galactosidase synthesis to repression by carbon sources, which do not hinder enzyme formation during normal growth (Magasanik, 1961; Mandelstam, 1961; Nakada and Magasanik, 1964). Accordingly, cessation of ^{14}C-valine incorporation would reflect the life time of bulk bacterial messenger RNA. The inhibition of β-galactosidase formation and of ^{14}C-valine incorporation, could have been explained by assuming an inhibition of expression (translation) of messenger RNA by oxidized spermine. This possibility was ruled out by an experiment in which the inducer was removed by filtration. It has been inferred, from previous work (Kepes, 1963; Nakada and Magasanik, 1964), that the loss of β-galactosidase-synthesizing capacity, on removal of the inducer, reflects the destruction of specific messenger RNA. If this inference is correct, and if oxidized spermine inhibits the synthesis of messenger RNA, this agent should not affect the decay in β-galactosidase production after removing the inducer. On the other hand, inhibition of messenger RNA expression (translation), by oxidized spermine should lead to an immediate inhibition of β-galactosidase production. It is evident from Fig. 2 that this is not the case; after removing the inducer, the half-life time of the decay in enzyme production was not significantly affected by the addition of oxidized spermine. The half-life time of decay in the presence of oxidized spermine was about 2.3 minutes. Whereas a half-life time of 1.7 minutes was obtained in a control experiment in which no oxidized spermine was added (Fig. 2). On the other hand after resuspending the cells in a medium containing both inducer and oxidized spermine the half-life time was 3.5 minutes (Fig. 2).

Recently, Ben-Hamida and Schlesinger (1965), working with 5-fluorouracil, reported that the stability of β-galactosidase messenger RNA increases in the presence of the inducer. Our results also point to this possibility, although a slow (1-2 minutes) penetration of oxidized spermine into the

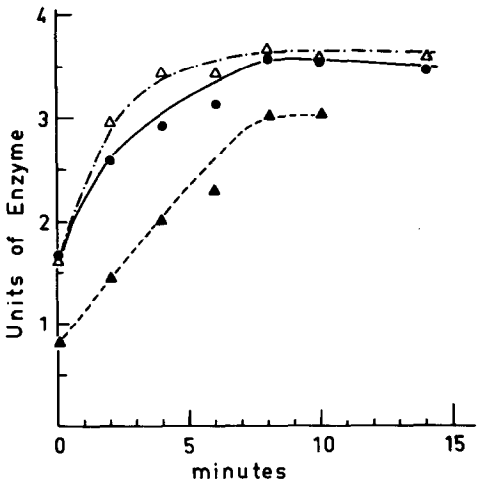

Fig. 2. Production of β-galactosidase. E. coli B was grown as described in the text to a density of 5×10^8 cells/ml. Portions of 40 ml were incubated at 30° and induction was started (zero time on the graph) by the addition of TMG (final concentration 5×10^{-4} M). At 4 min, the inducer was removed by filtration on a Membrane filter and the cells washed and resuspended in inducer free medium △-·-△, or in a medium containing oxidized spermine (final concentration, 120 µg/ml) ●——●; In a parallel experiment, cells were resuspended in a medium containing TMG (final concentration 5×10^{-4} M) and oxidized spermine (final concentration, 120 µg/ml) ▲ - - ▲.

cells might render an alternative explanation for the delayed effect.

If our explanation is correct and oxidized spermine interferes with messenger RNA synthesis, this agent might serve as a useful tool for the studying the rate, life time and time of appearance of messenger RNA. Oxidized spermine has an advantage over actinomycin D, which was used for a similar purpose, by being active against both gram positive and gram negative bacteria. Actinomycin affects E. coli, only after treating the cells with EDTA (Leive, 1965). This treatment might affect the normal behaviour of the bacterial cell. Furthermore, the use of actinomycin D for the determination of life time of messenger RNA has been criticised by various authors (Acs, Reich and Valanju, 1963; Coleman and Elliot, 1965), who claimed that this antibiotic induces or stimulates the formation of nucleases, thus enhancing the degradation of messenger RNA.

In our experiment (Fig. 1), no degradation of labeled RNA was noticed following oxidized spermine addition. In other experiments, considerable variation was observed and oxidized spermine caused loss up to 20% of the counts. The reason for this slow degradation is as yet unknown, however a similar effect was reported by Leive (1965), who studied the action of actinomycin D on E. coli. One possible explanation to this observation could be - inhibition of nucleases by oxidized spermine, or the formation complexes between RNA and the drugs, which are not hydrolyzed by the nucleases.

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