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Inhibition of bacterial macromolecular syntheses by the polyamine POX-3

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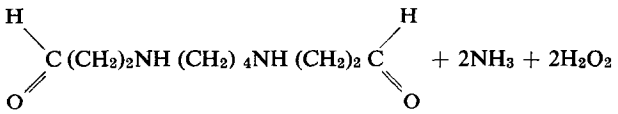
THE NATURALLY occurring polyamine, spermine, is oxidized by purified serum amine oxidase (amine: O_2 oxidoreductase; deaminating) as follows[1]:

$$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2 + 2H_2O + 2O_2 \rightarrow$$



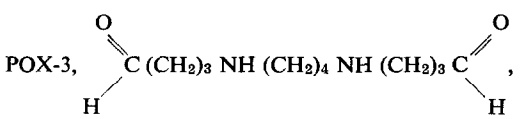
$$+ 2NH_3 + 2H_2O_2$$

(oxidized spermine).

The product, oxidized spermine, was shown to inhibit bacterial growth,[2] to inactivate bacterial, plant and mammalian viruses[3–6] and to be toxic for Ehrlich ascites cells.[7] Very recently, the chemical synthesis of oxidized spermine and related iminodialdehydes has been described by Fukami *et al.*[8] These investigators also confirmed our finding that oxidized spermine inactivates coliphages of the T-odd series.

The antimicrobial action of enzymatically prepared oxidized spermine has been explained on the basis of the interaction of the drug with microbial DNA.[9–12] This interaction was shown to lead to an immediate inhibition of RNA synthesis;[9,13] on the other hand, protein synthesis was arrested only after a definite lag period.[14]

The antiviral and antitumor activities of oxidized spermine make it a potential therapeutic agent. However, further pharmacological studies were hampered by the instability of the biologically prepared iminodialdehyde and by the tediousness of the enzymatic oxidation. The chemical synthesis of stable diacetals eliminates these difficulties and also permits the examination of related oxidized polyamines. The present work deals with the effect of the commercially available oxidized polyamine,



on the synthesis of macromolecules in *Escherichia coli*. It will be shown that POX-3 behaves like oxidized spermine and inhibits the synthesis of bacterial nucleic acids within 3 min. On the other hand, protein synthesis is inhibited by POX-3 only after a lag period.

POX-3, in the form of the diacetal of the oxalic acid salt, was synthesized by Fine Organics Inc., Lodi, New Jersey. The compound, in the form of the oxalate salt, was recrystallized from aqueous ethanol; it decomposed at 230–240°. The phosphate salt, however, had a melting point of 148–150°. The diacetal was converted into the free aldehyde by incubating for 3 hr at 37° with 0·05 N H_2SO_4

followed by neutralization with 1 N NaOH. (^{3}H)-thymidine-5-monophosphate (^{3}H-TMP; 40 c/m-mole, 0·5 mc/ml), (5-^{3}H)-uridine (20 c/m-mole, 1 mc/ml) and (^{3}H)-L-lysine (0·48 c/m-mole, 1 mc/ml) were obtained from Schwarz BioResearch Inc. *E. coli* B was grown in glycerol-lactate medium[15] to a density of 2×10^8 cells/ml. Radioactive precursors and POX-3 (or 0·15 M NaCl. solution) were added simultaneously to portions of the cultures. One-ml aliquots were withdrawn at the indicated time intervals and added to 0·1-ml quantities of 50% trichloroacetic acid solution (TCA). After standing in the cold for at least 30 min, samples were filtered through membrane filters (type B-6, Carl Schleicher & Schuell and Co., Keene, N.H.) and washed with 5% TCA solution. The filters were then dried, transferred to vials containing 10 ml of scintillation solution (4 g of omnifluor, New England Nuclear Corp., per 1000 ml toluene) and counted in a Packard Tri-Carb scintillator.

Table 1 shows that the incorporation of (^{3}H)-uridine into TCA-insoluble materials was most sensitive to the action of 0·9 mM POX-3, being reduced by 99 per cent. The incorporation of ^{3}H

TABLE 1. INHIBITION OF MACROMOLECULAR SYNTHESES BY POX-3*

	Control (cpm)	POX-3 (cpm)	Inhibition (%)
(^{3}H)-uridine incorporation	73,309	770	99
(^{3}H)-TMP incorporation	2,592	399	84·5
(^{3}H)-lysine incorporation	3,153	786	75

* *E. coli* B was grown in 2·0-ml quantities of glycerol–lactate medium to a density of 2×10^8 cells/ml. (^{3}H)-uridine (1·0 μc, 0·4 μmole), (^{3}H)-thymidine-5-monophosphate (^{3}H)-TMP; 0·5 μc, 0·8 mμmole) or (^{3}H)-lysine (1·0 μc, 0·5 μmole) was added to the respective culture along with POX-3 (final concentration 0·9 mM) or with 0·05 ml of 0.15 M NaCl. After shaking at 37° for 25 min, incorporation was stopped by addition of 0·1 ml of 50% trichloroacetic acid and the radioactivity incorporated into each sample was determined.

TMP and of ^{3}H-lysine was inhibited to a lesser extent. As expected, the unhydrolyzed diacetal did not inhibit the growth of *E. coli*, did not inactivate T-odd bacteriophages and did not have a significant effect on macromolecular biosyntheses.

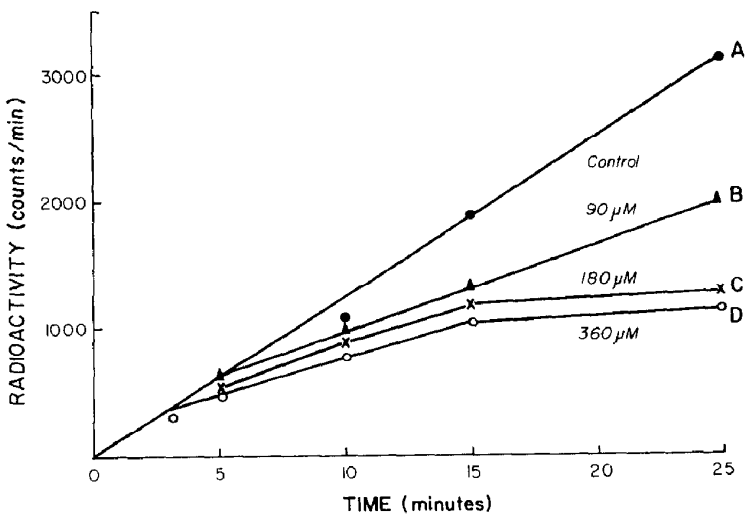


FIG. 1(a)
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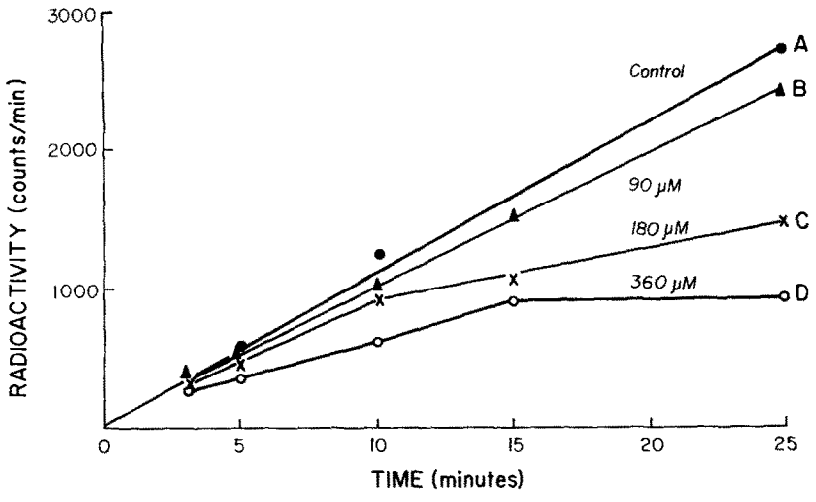
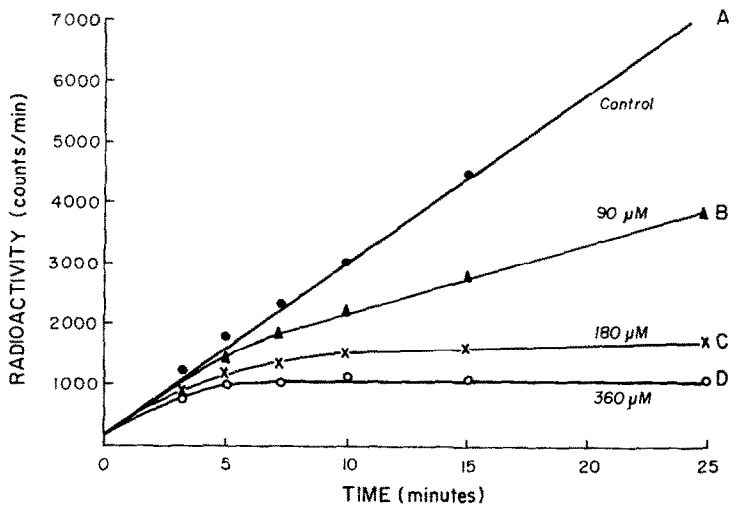


(b)



(c)

FIG. 1. Effect of POX-3 on bacterial protein and nucleic acid syntheses. Cultures of *E. coli* (20 ml) in glycerol–lactate medium were brought to the exponential growth phase, at which time 20 μl of radioactive precursors and graded amounts (0·5 ml) of POX-3 were added; the control culture received 0·15 M NaCl. Incorporation was stopped by the addition of 0·1 ml of 50% trichloroacetic acid to 1·0 ml-portions of cultures. Curve A, control; curve B, POX-3, 0·09 mM; curve C, POX-3, 0·180 mM; curve, D, POX-3, 0·36 mM.

(a) Incorporation of (^{3}H)-uridine (10 μc, 4 μmole).
(b) Incorporation of (^{3}H)-thymidine-5-monophosphate (5 μC, 8 mμmole).
(c) Incorporation of (^{3}H)-lysine (10 μc, 5 μmoles).

In the above experiment, the concentration of POX-3 was relatively high. It was therefore decided to test the effect of small amounts of POX-3 on macromolecular syntheses in *E. coli*. Fig. 1*a* shows that 0·9 mM POX-3 inhibited uridine incorporation by approximately 50 per cent after 25 min of incubation. When a concentration of 0·36 mM was used, substantial inhibition was observed even during the first 3 min after the addition of the drug; incorporation was completely arrested after 5 min. The incorporation of TMP into bacterial DNA was inhibited by 50 per cent only when levels of POX-3 as high as 0·18 mM were used (Fig. 1b); at 0·09 mM, only a slight effect on the incorporation was noticed. DNA synthesis was not completely inhibited even by 0·36 mM POX-3. Fig. 1*c* shows that the incorporation of L-lysine into bacterial proteins was not significantly inhibited by POX-3 during the first 5 min of the experiment, even though high concentrations of the drug were used (0·36 mM). On further incubation, protein synthesis was inhibited and the rate of lysine incorporation was very slow.

These experiments thus suggest that POX-3 interferes primarily with RNA synthesis and this in turn leads to a delayed arrest of protein synthesis. DNA synthesis is also inhibited by POX-3, although higher concentrations are required. This inhibition of DNA synthesis may be a reflection of the regulatory role of RNA in DNA production.[16, 17]

Our experiments also confirm the notion that the iminoaldehyde moieties are the active principles of the oxidized polyamines, and not acrolein, as has been suggested by Alacron.[18]

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