VALANIMYCIN ACTS ON DNA IN BACTERIAL CELLS

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We recently isolated a new azoxy antibiotic, valanimycin, and described its biological and physico-chemical properties[1], and its biosynthesis[2]. Its stronger antibacterial activity against a DNA-repair deficient strain (BE1121) of *Escherichia coli* than against DNA-repair proficient counterparts suggested that the mode of action of the antibiotic might involve an interaction with DNA[1]. We conducted some experiments to test this possibility and the results are presented in this paper.

In shaken cultures of BE1121, valanimycin showed a unique growth-inhibition profile when the growth was monitored by reading the turbidity of the culture. After addition of valanimycin at a concentration of 1.6 μg/ml, which was later shown to reduce the colony-forming ability of the cell population to 10^{-4} within 4 hours, the turbidity of the culture increased throughout the observation period even faster than that of the control culture (Fig. 1). Microscopic inspection of a sample taken 4 hours after the addition of valanimycin showed filamentous cells. Elongation of bacterial cells is a characteristic of drugs inhibiting the synthesis of either DNA or some component of the cell surface. The latter group, exemplified by penicillin, usually causes cytolysis. In this respect, it should be noted that the plateau of turbidity of the valanimycin-supplemented culture lasted until the final observation (4 hours after the addition of valanimycin) suggesting the lack of a cytolytic effect (Fig. 1). These results support the possibility that valanimycin primarily acts on DNA. This conclusion was strengthened by the results obtained when its effect on the synthesis of cellular macromolecules (Fig. 2) and its mutagenic activity (Table 1) were determined.

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Fig. 1. *Escherichia coli* BE1121 was shake-cultured in 10 ml portions of nutrient broth (Eiken Co.) in L-shaped tubes at 37°C.

The cell growth was followed by reading the turbidity (A_{660}). When A_{660} reached 0.1 (3.5 hours of incubation), valanimycin was added to a culture at 1.6 μg/ml (arrow A) and incubation was resumed (closed circles). The same volume of water was added to the control culture (open circles). At 7.5 hours of incubation, 0.1 ml samples were taken (arrow B), appropriately diluted, and the number of viable cells determined by the colony counting method. The density of viable cells was 1×10^8/ml for the control culture but less than 1×10^4/ml for the valanimycin culture.

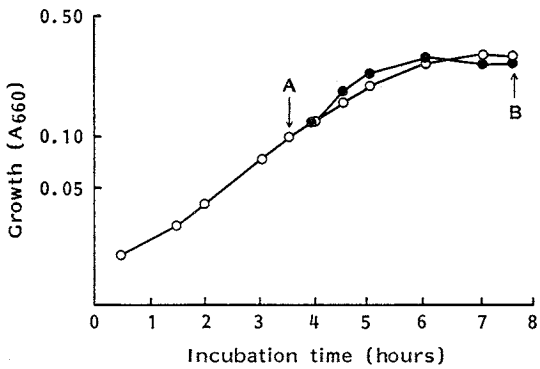


Table 1. Numbers of revertant colonies of *Salmonella* strains.

Valanimycin (μg/ml)	Strains	
	TA98*	TA100*
0	23	200
10	NT	1,000
20	37	1,560
40	NT	2,400
80	NT	1,330
100	73	370

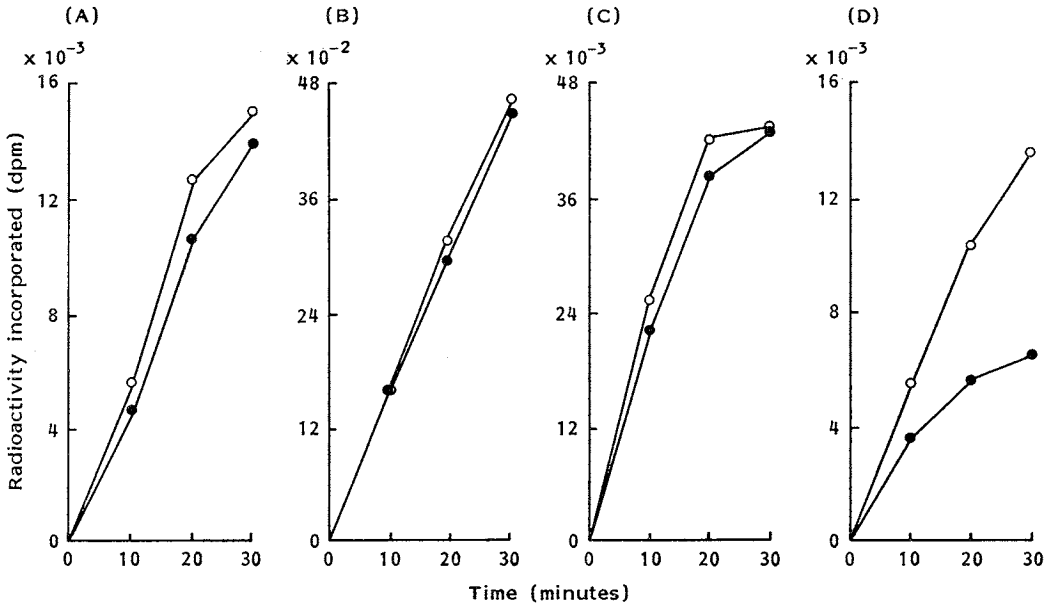
NT: Not tested.

* TA98 (*rfa*, *uvrB*, *hisD*3052, amp^r), TA100 (*rfa*, *uvrB*, *hisG*46, amp^r).

The test was conducted by the method of B. N. AMES *et al.*[3] without the mammalian microsome system. Each value for revertant colonies represents the mean of 3 plates.

Fig. 2. *Escherichia coli* BE1121 was cultured as described in the legend to Fig. 1.

When A_{660} reached 0.1, 2′-deoxyadenosine was added at 0.1 mM to inhibit thymidine phosphorylase and the culture was divided into 0.9 ml portions in short test tubes; 2 tubes in each of 4 sets. To a tube of each set, 50 μl of valanimycin solution was added to give a final concentration of 2 μg/ml (closed circles) while 50 μl of water was added to the other tube (control, open circles). After shaking for 3 minutes at 37°C, a 50-μl solution containing either 0.5 μCi of [1-^{14}C]-*N*-acetyl-D-glucosamine (A), 0.2 μCi of L-[4,5-^{3}H]leucine (B), 1 μCi of [6-^{3}H]uridine (C), or 0.5 μCi of [6-^{3}H]thymidine (D) was added to both tubes of each set. Shake culture was continued at 37°C. At 10-minute-intervals, 100 μl samples were withdrawn and radioactivity incorporated into the acid-insoluble fraction of cells was determined as reported[5].



The incorporation of [^{3}H]thymidine into the acid-insoluble fraction of BE1121 cells was inhibited by valanimycin while those of [1-^{14}C]-*N*-acetyl-D-glucosamine, L-[4,5-^{3}H]leucine and [6-^{3}H]uridine were not inhibited (Fig. 2). A separate experiment indicated that valanimycin did not inhibit the membrane-transport of [6-^{3}H]-thymidine (data not shown). Thus, the blockade should be located at a later stage. That this is probably the polymerization step of DNA synthesis is discussed below. A number of compounds reacting with DNA are mutagenic. We examined valanimycin for possible mutagenicity by the AMES' test[3]. As Table 1 shows, mutagenic activity of valanimycin was especially strong with strain TA100.

The conclusion drawn from these results is that valanimycin reacts with the DNA template resulting in inhibition of DNA synthesis, induction of DNA repair, and mutation. It has been reported that methylazoxymethanol, a structural moiety of cycasin, reacts with isolated DNA yielding 7-methylguanine[4]. Attempts were made to demonstrate the following possible interactions of valanimycin with isolated DNA: (1) scission of DNA strands, (2) alteration of the electrophoretic mobility of DNA, (3) inhibition of the DNA-directed RNA polymerization reaction, and (4) inhibition of restriction endonuclease reactions. None was found to be positive, however. Valanimycin may be converted by bacterial metabolism to an active metabolite capable of reacting with DNA.

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