SHORT COMMUNICATION

Fatty Acid-Conjugated Polyamines that Alter Cell Permeability and Active Transport Properties of *Escherichia coli*

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(Received February 1, 1969)

SUMMARY

A class of plant alkaloid tumor inhibitors were described by Kupchan *et al.* [*J. Amer. Chem. Soc.* **89**, 5718 (1967)] which are *N*-acyltriamines (fatty acid-conjugated aliphatic triamines). These substances are also growth-inhibitory for *Escherichia coli* at very low concentrations. The mode of action of the triamines appears to be disruption of surface properties (the cells do not lyse), since at inhibitory concentrations the cells become leaky and lose radioactive potassium and thiomethyl galactoside. The specific energy-dependent accumulation of potassium and thiomethyl galactoside is also inhibited.

Kupchan *et al.* (1) isolated and characterized a class of tumor-inhibitory plant alkaloids which are *N*-acyltriamines (fatty acids conjugated to aliphatic triamines). Mahler and Baylor[1] found that these materials are exceedingly toxic for cells of *Escherichia coli*, and, by analogy with the effects of another class of plant alkaloids, the steroidal diamines (2–4), suggested that the *N*-acyltriamines might alter cell permeability properties. Samples of four triamines, one natural (solapalmitine, compound I) (1) and three synthetic (compounds II, III, and IV[2]), were provided by Dr. Kupchan, and their structures are shown in Fig. 1.

To make sure of the generality of the effects of the triamines, we tested these compounds on two very dissimilar active transport systems: that transporting potassium into the cells, and that transporting thiomethyl galactoside. The experiments were similar to those previously described with the steroidal diamines (3, 4), and it was found that the triamines do alter cell permeability properties and cause accelerated leakage of potassium and thiomethyl galactoside from the cells. The cells do not visibly lyse or change in appearance in the phase contrast microscope on exposure to the triamines. Either because of direct inhibition of the active transport systems or because the material accumulated is rapidly lost by leakage, the bacterial cells cannot concentrate potassium or thiomethyl galactoside in the presence of the triamines.

Figure 2A shows this inhibition of the ability to accumulate ^{14}C-thiomethyl galactoside. All four triamines inhibit thiomethyl galactoside uptake by 80% or more when added at 100 μM; at 13 μM the inhibition is between 30 and 80% (data not shown). In a series of five independent experiments, we have been unable to find reproducible differences among the triamines with regard to "potency." The ex-

This investigation was supported by Research Grant GB 5922 from the National Science Foundation and by Research Grant AI 08062 from the United States Public Health Service.

[1] H. R. Mahler and M. B. Baylor, personal communication.

[2] S. M. Kupchan, G. Bondesson, and A. P. Davies, manuscript in preparation.

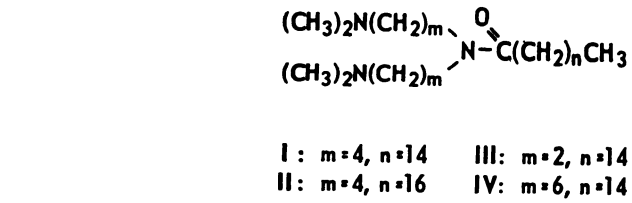

FIG. 1. *Structures of the N-acyltriamines*

periment in Fig. 2A was carried out at 4° because the rapidity of function of the thiomethyl galactoside transport system at room temperature makes it impractical to measure flux rates in addition to equilibrium concentrations (3). The concentration dependence of inhibition of the potassium accumulation system by compound III is shown in Fig. 2B. At 3.3 μM, the initial rate of ^{42}K uptake is reduced about 30%, while at 13 μM the rate is reduced at least 100-fold. Similar results with ^{42}K uptake were obtained with the other triamines. The difference in the concentrations of inhibitor required to prevent potassium uptake (Fig. 2B) and thiomethyl galactoside uptake (Fig. 2A) is due to the difference in the incubation temperatures in the two experiments. Cold temperatures alter cell membrane permeability properties (5–7), and higher concentrations of the steroidal diamines are required to alter potassium permeability at 4° than at 25°.[3]

The experiments in Fig. 2 show an inhibition of cellular uptake which could be due either to a direct effect on the metabolically active accumulation systems or to an increased rate of leakage induced by the triamines, or both. By loading the cells with ^{42}K or ^{14}C-thiomethyl galactoside, centrifuging, and resuspending the cells in a nonradioactive medium prior to adding the triamines, we have found an effect on

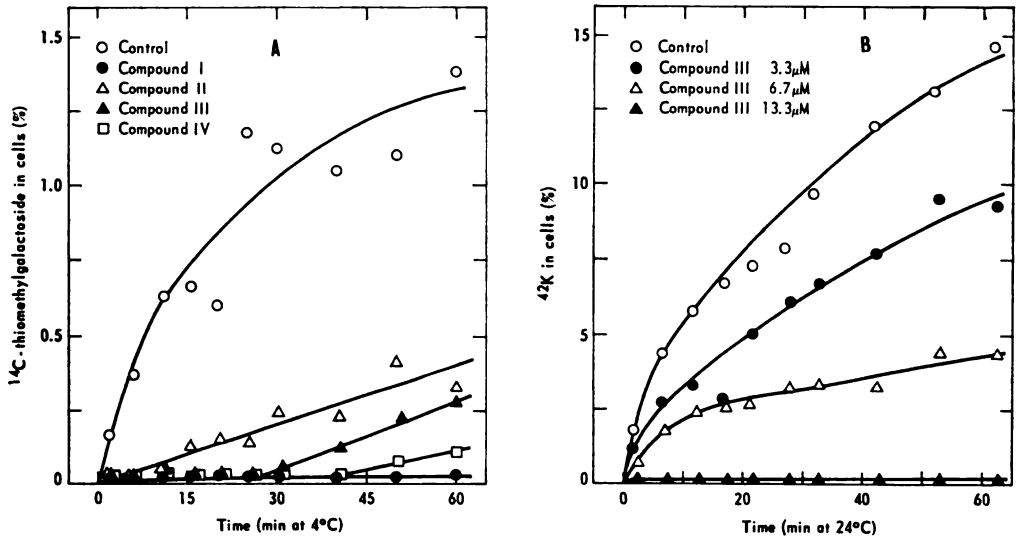

FIG. 2. *Triamine inhibition of the accumulation of ^{14}C-thiomethyl galactoside and ^{42}K*

E. coli strain B was grown at 37° with aeration in a dilute tryptone broth (2 g of Difco Bacto-tryptone and 1.25 g of NaCl per liter).

A. The synthesis of the β-galactoside active transport system was induced by the addition of 100 μM nonradioactive thiomethyl galactoside during growth. The cells were centrifuged and washed to remove the thiomethyl galactoside, and resuspended at 4° in fresh dilute broth at 3.6×10^8 cells/ml. Triamines at 100 μM were added 2 min before the addition of ^{14}C-thiomethyl galactoside (0.03 μC/ml; 1.0 μM). Samples were removed, filtered on Millipore HA filters, washed with 10 ml of broth, and counted in a Nuclear-Chicago gas flow counter.

B. Broth-grown cells were cooled to room temperature. Triamine III was added 2 min prior to the addition of ^{42}K (0.05 μC/ml; 1.0 μM).

[3] L. Wendt, unpublished observations.

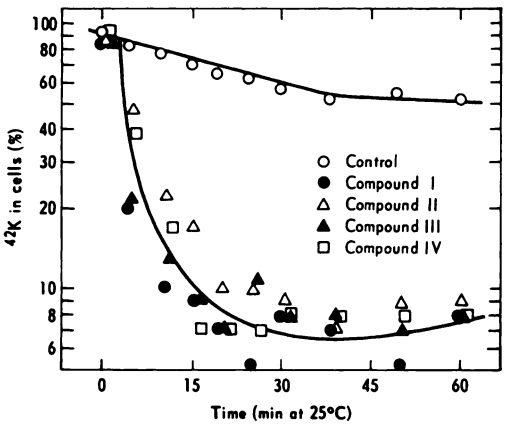

FIG. 3. *Triamine-induced leakage of ^{42}K*

E. coli were grown in broth with added ^{42}K (0.5 μC/ml) at 37°, centrifuged, and resuspended at 5×10^8 cells/ml in fresh broth at 25°. Samples were filtered, and both the filters and dried aliquots of the filtrates were counted. Triamines were added at 13 μM 2 min after the first samples were filtered.

the rate of loss of radioactivity from the cells. All four triamines cause a rapid loss of cellular potassium when added at 13 μM (Fig. 3). Analogous experiments with cells loaded with ^{14}C-thiomethyl galactoside showed accelerated leakage in the presence of the triamines.

Polyamines are well known for their effects in neutralizing, stabilizing, and/or labilizing lipid and membrane structures (3, 8, 9). Aliphatic polyamines such as spermidine, $H_2N(CH_2)_4NH(CH_2)_3NH_2$, and spermine, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, are without effect on *E. coli* active transport systems unless added in concentrations approaching 10^{-2} M. It seems reasonable to attribute the efficacy of the *N*-acyltriamines at 1000-fold lower concentrations to the lipophilic fatty acids covalently bound to the triamines. This is similar to the explanation we used for the potency of the steroidal diamines as inhibitors of active transport systems (3). In fact, the fatty acid-conjugated triamines are inhibitory at concentrations an order of magnitude lower than the steroidal diamines.

The experiments described here pertain to bacterial cells (*E. coli*). It is, of course, reasonable to expect that alterations of the cell membrane properties of mammalian cells is the basis for the growth inhibition reported by Kupchan *et al.* (1) for compound I.

ACKNOWLEDGMENTS

We are grateful to Drs. H. R. Mahler and M. B. Baylor for telling us about the *N*-acyltriamines, and to Dr. S. M. Kupchan for providing them. Mrs. Geraldine Knuckles assisted in the experiments.

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