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Mode of Action of Albocycline, an Inhibitor of Nicotinate Biosynthesis

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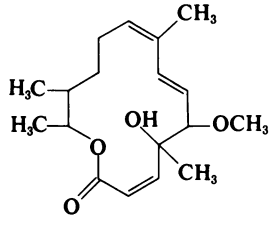
The antibiotic albocycline blocks the synthesis of nicotinate or nicotinamide in *Bacillus subtilis* cells. The inhibitory activity of the agent is fully reversed by nicotinic acid, nicotinamide, and, to a moderate extent, also by quinolinate. This suggests that in *B. subtilis* the antibiotic interferes with a reaction step occurring prior to the formation of quinolinate within the biosynthetic pathway leading to nicotinate.

Albocycline was isolated from the culture broth of *Streptomyces maizeus*. The agent inhibits a variety of gram-positive and gram-negative bacteria and fungi in vitro, but was ineffective in the treatment of experimental infections caused by bacteria in mice. The antibiotic is not toxic in mice and has a tolerated dose of >320 mg/kg. Albocycline has a very low solubility in water (10 to 20 μg/ml), but is soluble in most organic solvents. The antibiotic crystallizes in the form of colorless plates and has the empirical formula $C_{18}H_{28}O_4$. The proposed chemical structure is shown below (6):



The results presented in this paper show that the inhibitory activity of albocycline is fully reversed in *Bacillus subtilis* cells by the addition of nicotinate, nicotinamide, and, to a moderate extent, also by quinolinate.

MATERIALS AND METHODS

Cells of *B. subtilis* strain 23 were grown in a glucose-salt medium described previously (5). Shaken flasks containing 100 ml of medium were inoculated with 5 ml of seed derived from an overnight culture. The flasks were incubated on a rotary shaker at 37 C. Albocycline was dissolved in dimethylformamide and was added in 0.1-ml portions per 100 ml of culture medium. Cell growth was assessed by measuring the optical density of the bacterial suspensions at 570 nm.

Total nicotinic acid present in the cultures was assayed microbiologically (5). Preparation and assay of quinolinate phosphoribosyltransferase were described previously (5).

RESULTS

The effect of different albocycline concentrations in exponentially growing *B. subtilis* cells is shown in Fig. 1. At albocycline concentrations ranging from 0.5 to 5 μg/ml, cell growth remained unaffected for approximately 30 min following addition of the antibiotic. After this time, growth ceased abruptly in the presence of 5 μg/ml of drug. Concentrations of 0.5 to 2 μg/ml caused correspondingly less inhibition.

Albocycline did not interfere with macromolecular biosynthetic processes (nucleic acid, protein synthesis) in bacterial cell-free systems prepared according to the method of Reusser (4).

On the other hand, it was found that nicotinate effectively reversed albocycline inhibition in *B. subtilis*. In the presence of a constant amount of albocycline (5 μg/ml), an amount of 1 μg/ml of nicotinate caused full reversal of albocycline inhibition as shown in Fig. 2. Nicotinate concentrations of 0.1 or 0.01 μg/ml caused less reversal and 0.001 μg/ml remained without effect. This indicates that the extent of reversal of antibiotic activity (in the presence of a constant amount of albocycline) depends on the concentration of nicotinate in the culture medium.

When the concentration of albocycline was varied in the presence of a constant amount of nicotinate (1 μg/ml), cell growth remained completely unaffected by albocycline present in concentrations from 5–20 μg/ml (Fig. 3). Fifty μg/ml of albocycline caused only moderate inhibition.

Addition of nicotinate from 0 to 60 min after exposure of the cells to albocycline resulted in an immediate resumption of growth (Fig. 4). After

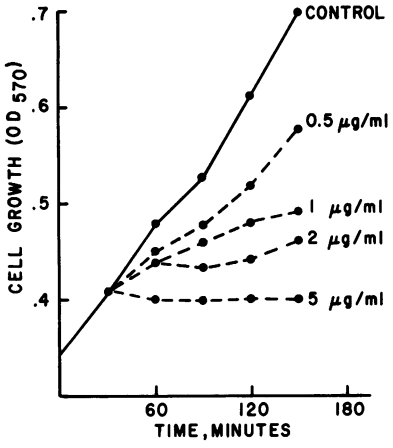


FIG. 1. *Effect of albocycline on B. subtilis cell growth. Antibiotic was added at the onset of exponential growth.*

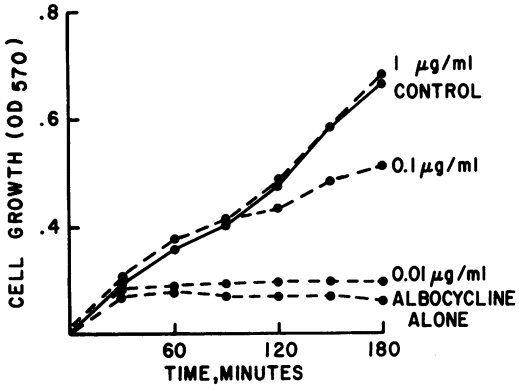


FIG. 2. *Effect of different concentrations of nicotinate on reversal of albocycline activity. Concentration of albocycline was 5 μg/ml.*

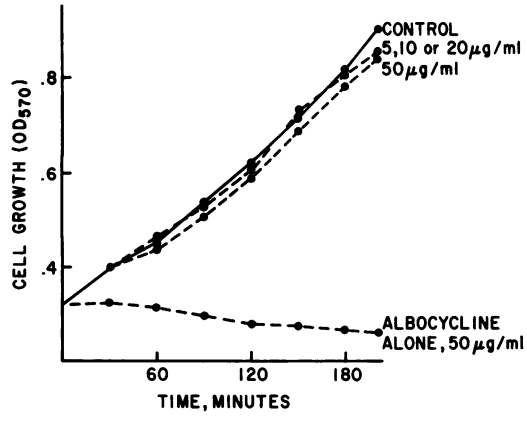


FIG. 3. *Effect of different concentrations of albocycline on B. subtilis cell growth in the presence of a constant amount of nicotinate (1 μg/ml).*

time periods of 90 or 120 min, lag periods from 30 to 60 min occurred before growth resumed.

Nicotinamide reversed growth inhibition induced by albocycline as effectively as nicotinate. Nicotinamide adenine dinucleotide (NAD) or reduced NAD (NADH) caused substantial reversal only after prolonged incubation of the cultures (12 to 16 hr). L-Kynurenine, L-3-hydroxykynurenine, and 3-hydroxyanthranilic acid did not cause any reversal of albocycline activity. Quinolinic acid caused some reversal but only after prolonged incubation of the cultures (Table 1). The extent of reversal obtained was dependent on the concentration of quinolinate present.

Cell growth and net nicotinic acid biosynthesis were measured simultaneously in the presence or absence of albocycline. In the presence of 10 μg/ml of albocycline moderate cell growth was apparent for 2 hr following addition of the drug, but nicotinic acid biosynthesis ceased immediately after addition of antibiotic (Fig. 5).

Quinolinic acid phosphoribosyl transferase

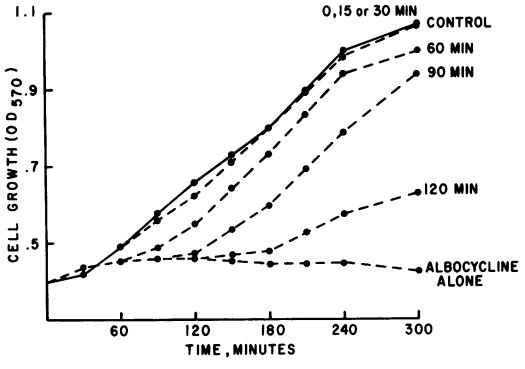


FIG. 4. *Effect of delayed addition of nicotinate in presence of albocycline. Concentration of nicotinate was 1 μg/ml; albocycline, 5 μg/ml. Antibiotic was added at the onset of exponential growth. The times of nicotinate addition are indicated in the figure.*

TABLE 1. *Reversal of albocycline growth inhibition of B. subtilis by quinolinate*

Sample[a]	OD_{570} of cell growth at 18 hr[b]
Control	1.80
Albocycline, 5 μg/ml	0.34
Albocycline + nicotinate, 1 μg/ml	1.80
Albocycline + quinolinate, 1 μg/ml	0.80
Albocycline + quinolinate, 2 μg/ml	0.81
Albocycline + quinolinate, 3 μg/ml	0.89
Albocycline + quinolinate, 4 μg/ml	0.90

[a] Albocycline and reversing agents were added at the onset of exponential growth.

[b] The optical density of the cultures at time of drug addition was 0.35.

catalyzes the formation of nicotinate ribonucleotide from quinolinate (1, 2, 3). Albocycline did not interfere with the activity of a purified preparation of this enzyme.

DISCUSSION

Cell growth of a *B. subtilis* culture usually ceases after 30 min exposure to 5 μg/ml of albocycline. This suggests that de novo synthesis of an essential metabolite is blocked by the antibiotic.

Addition of nicotinate or nicotinamide to *B. subtilis* cultures containing albocycline effectively reverses the inhibitory activity of the antibiotic. Quantitation of total nicotinate formed in the cultures established that synthesis of this vitamin ceases immediately upon addition of albocycline. No destruction of nicotinate is apparent.

In animals and fungi, nicotinate arises from tryptophan as a precursor. Several intermediates occurring within the tryptophan pathway (L-kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid) did not cause reversal of albocycline inhibition in *B. subtilis*. In most bacteria, nicotinate is formed via the condensation of a four-carbon dicarboxylic acid (succinate, aspartate) with a three-carbon compound (glycerol). One or several unknown steps lead to the formation of quinolinate which is then converted to nicotinate ribonucleotide and eventually to NAD, as shown in the scheme below (2, 3):

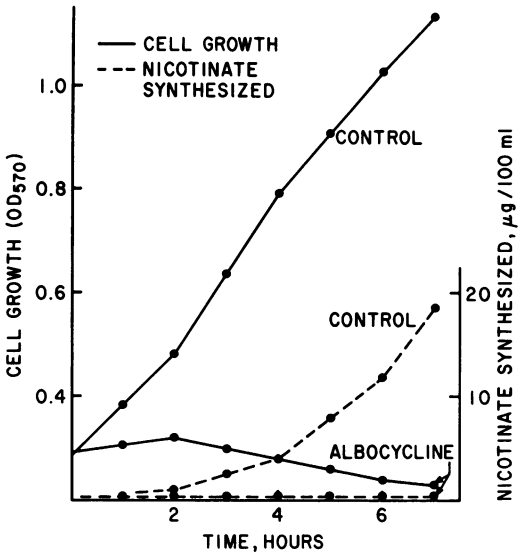


FIG. 5. *Effect of albocycline on cell growth and nicotinate biosynthesis in B. subtilis. Albocycline concentration was 10 μg/ml. Total nicotinate in the cultures at time 0 was 5 μg/100 ml.*

In a recent paper, we reported that the sulfur-containing antibiotic melinacidin acts as a very potent inhibitor of nicotinate biosynthesis in bacteria by blocking a reaction step presumably occurring prior to the formation of quinolinate

glycerol + succinate or aspartate → → quinolinate
↓
nicotinic acid → nicotinate ribonucleotide (nicotinate pathway) → deamido-NAD
↑ ↓
nicotinamide → nicotinamide ribonucleotide (nicotinamide pathway) → NAD

The fact that quinolinate reverses albocycline inhibition somewhat, although only after prolonged incubation of the cultures, indicates that albocycline blocks the synthesis of nicotinate in susceptible bacteria at a reaction step occurring prior to the formation of quinolinic acid. The slow reversal caused by quinolinate might be due to the poor penetration of the cell membranes by this acid. A similar lack of effective penetration of quinolinate has been observed in liver (2).

The very unrelated chemical structure of albocycline compared to quinolinate or nicotinate is of interest. It appears unlikely that the much larger and complicated albocycline molecule is structurally related to an unknown precursor occurring prior to the formation of quinolinate. This suggests that the antibiotic does not merely act as an antimetabolite of the postulated precursor but effectively blocks the synthesis or function of an enzyme occurring prior to the formation of quinolinate within the nicotinate biosynthetic pathway.

(5). The evidence presented in this paper suggests that albocycline interacts in a similar manner within the pathway of nicotinate biosynthesis.

ACKNOWLEDGMENT

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