

Characteristics of Growth Inhibition of *Lactobacillus casei* by 4-Nitroquinoline-*n*-Oxide

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Received for publication 4 June 1970

The bacteriostatic action of 4-nitroquinoline-*n*-oxide (4-NQO) for *Lactobacillus casei* is substantially reversed by D- and L-cysteine, glutathione, and 2,2-dihydroxy-1,4-dithiolbutane (dithioerythritol). The action appears to involve a chemical reaction between carbon atom 4 of 4-NQO and nucleophilic centers, such as -SH groups, located on essential cell constituents. The evidence presented indicates that the protective effect of D- and L-cysteine, glutathione, and dithioerythritol against the action involves reactions between 4-NQO and -SH compounds.

The diversity of biochemical effects (1-3) demonstrated by 4-nitroquinoline-*n*-oxide (4-NQO) in a variety of test systems has generated interest in studying the characteristics of the interaction of this compound with essential cellular components. It has been reported that 4-NQO is not only a powerful carcinogen [comparable to the more potent polycyclic hydrocarbons (6)], but also that it demonstrated *in vitro* anticancer activity on the NF sarcoma (12). It has also been reported that 4-NQO is bacteriostatic and fungistatic (3), demonstrates irregular inhibition of protein and nucleic acid synthesis in *Escherichia coli* (4), causes irregular inhibition of growth and ribonucleic acid (RNA) synthesis in shake cultures of *E. coli* (8), and induces bacteriophage formation in *E. coli* (10).

In this paper, certain characteristics of the inhibiting action of 4-NQO on the growth of *Lactobacillus casei* will be reported.

MATERIALS AND METHODS

Microbiological experiments. Diminished growth responses of *L. casei* in a nutritionally adequate medium containing graded amounts of 4-NQO were used as a measure of its bacteriostatic action.

L. casei (ATCC 7469) was carried routinely in stock, on Difco yeast extract and glucose agar.

All chemicals were commercial grade used without further purification.

Prior to use in an experiment, *L. casei* from stock cultures was grown in a liquid medium (14). After 24 hr of incubation at 37 C, the cells were centrifuged, washed once with a saline solution, and resuspended in 10 ml of the culture medium.

Aqueous solutions of 4-NQO and potential reversing agents were filter-sterilized and added aseptically in graded amounts of sufficient sterile water to make 5 ml. A 5-ml amount of sterilized, double-

strength culture medium was added to bring the volume to 10 ml. A medium control was prepared by adding 5 ml of the sterilized medium to 5 ml of sterile water. Immediately before use, a 1:200 dilution of the 24-hr culture was prepared in fresh culture medium. Each tube was inoculated with one drop from a 1-ml pipette of this dilution which contained approximately 10^6 cells per ml, estimated turbidimetrically. After 23 hr of incubation at 37 C, the growth in each tube was determined turbidimetrically.

Reaction of 4-NQO with -SH compounds. The relative ease of displacement of the 4-NO₂ group by certain -SH compounds and by essential cellular nucleophiles (13) suggests that these nucleophiles might be the sensitive targets involved in the inhibition of *L. casei* by 4-NQO. The chemical mechanism is a nucleophilic substitution of the 4-NO₂ group by -SH compounds and proceeds readily under physiological conditions to yield a 4-SR substituted quinoline-*N*-oxide derivative and nitrous acid. Since nitrous acid is produced by the reaction, the colorimetric identification of nitrite ion by the method described by Snell and Snell (15) was used to follow the reaction.

The possible displacement of the 4-NO₂ group in an *in vitro* system employing ruptured *L. casei* cells as a nucleophile source was studied in an attempt to obtain qualitative evidence for the interaction of 4-NQO with intracellular compounds.

The cells from a 24-hr culture of *L. casei* were centrifuged and resuspended in 20 ml of 0.15M NaCl. One μ mole of 4-NQO in 1 ml was added to each 9 ml of the saline suspension of cells and to 9 ml of saline. The latter solution served as a 4-NQO control. After incubation at 37 C for 30 min, the cells were centrifuged, washed twice with physiological saline, and resuspended in saline. The nitrite test was negative in both supernatant solutions, indicating that nitrite ion was not released. A saline suspension of the twice-washed cells was sonically treated for 15 min at 9 kc with a Raytheon sonic generator. The nitrite test was positive.

RESULTS AND DISCUSSION

Variations in the growth of *L. casei* with the inhibitor from experiment to experiment were observed. However, the trend was always in the direction of inhibition by 4-NQO and reversal by the -SH compounds, hence qualitative interpretations seem justified.

Without any reversing agent, the bacteriostatic action was complete between 0.1 and 0.3 $\mu\text{mole}/10\text{ ml}$ (Table 1). Under the same experimental conditions, quinoline-*n*-oxide (QO) demonstrated essentially no inhibitory effect at 3.0 $\mu\text{mole}/10\text{ ml}$.

A comparison of Tables 1 and 2 shows that the four -SH compounds demonstrated some variation in protective capability against 4-NQO. D-Cysteine and glutathione appear to be the best. Glutathione and Cleland's Reagent (2,3-dihydroxy-1,4-dithiolbutane) are slightly inhibitory at high levels. However, the relatively small inhibition exhibited by these two compounds does not significantly influence their effectiveness as protective agents.

The growth of *L. casei* in 4-NQO and -SH compounds is concentration-dependent and an essentially constant molar ratio of inhibitor to reversing agent is required to maintain a defined inhibition. Evidence against -SH group rejuvenation as a protective mechanism was obtained when other experiments showed that a reversal effect did not occur when -SH compounds were added after the first 2 hr of incubation. The supernatant solutions of sonically disrupted *L. casei* cells, exposed to 4-NQO for 30 min before sonic treatment, gave evidence of a reaction

TABLE 1. Effect of *L*- and *D*-cysteine on 4-NQO toxicity for *Lactobacillus casei* after 23 hr at 37 C in a semisynthetic medium^a

| 4-Nitroquinoline- <i>n</i> -oxide ($\mu\text{moles}/10\text{ ml}$) | L-Cysteine ^b ($\mu\text{moles}/10\text{ ml}$) | | | | | D-Cysteine ^b ($\mu\text{moles}/10\text{ ml}$) | | | |
|--|--|-----|-----|------|------|--|-----|------|------|
| | 0 | 1.0 | 3.0 | 10.0 | 30.0 | 1.0 | 3.0 | 10.0 | 30.0 |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 0.1 | 63 | 80 | 72 | 100 | 80 | 83 | 89 | 100 | 100 |
| 0.3 | 0 | 0 | 0 | 76 | 89 | 0 | 87 | 94 | 100 |
| 1.0 | 0 | 0 | 0 | 0 | 51 | 0 | 0 | 0 | 92 |

^a Composition given in text.

^b Values indicate per cent of normal growth. Turbidity reading at zero concentration of inhibitor was taken as the normal growth in each series. Figures are rounded off to whole numbers and represent per cent of normal growth. Each figure represents an average of the results from three or four experiments with $\pm 10\%$ variation.

TABLE 2. Effect of glutathione and Cleland's reagent on 4-NQO toxicity for *Lactobacillus casei* after 23 hr at 37 C in a semisynthetic medium^a

| 4-Nitroquinoline- <i>n</i> -oxide ($\mu\text{moles}/10\text{ ml}$) | Glutathione ^b ($\mu\text{moles}/10\text{ ml}$) | | | | Cleland's reagent ^b ($\mu\text{moles}/10\text{ ml}$) | | | |
|--|---|-----|------|------|---|-----|------|------|
| | 1.0 | 3.0 | 10.0 | 30.0 | 1.0 | 3.0 | 10.0 | 30.0 |
| 0 | 100 | 100 | 92 | 90 | 100 | 82 | 68 | 81 |
| 0.1 | 37 | 90 | 76 | 96 | 75 | 78 | 72 | 75 |
| 0.3 | 0 | 5 | 78 | 95 | 0 | 49 | 88 | 89 |
| 1.0 | 0 | 0 | 0 | 90 | 0 | 0 | 82 | 68 |

^a Composition given in text.

^b See Table 1, footnote b.

between intracellular -SH compounds and 4-NQO by the production of nitrite.

These findings of a reaction between 4-NQO and -SH compounds through the release of nitrite are in qualitative agreement with the findings of Searle and Woodhouse (13), who used animal tissues and a different test system. The evidence indicates that a relationship exists between the relative ease of displacement of the 4-NO₂ group and the bacteriostatic property of 4-NQO, since the amount of NO₂ resulting from the displacement of the 4-NO₂ group by known -SH compounds and by the cellular nucleophiles from disrupted *L. casei* cells was similar. Furthermore, quinoline-*n*-oxide did not demonstrate antibacterial activity in this test system which supports previously reported evidence that a replaceable NO₂ group is essential to certain of the antibacterial properties of the quinoline derivatives (5).

At this point, it appears that the bacteriostatic action of 4-NQO for *L. casei* results from its reaction with intracellular, essential nucleophiles and that the protective effect of -SH compounds results from a reaction between 4-NQO and the -SH compounds added to the culture medium. However, the interaction between 4-NQO and essential cell constituents or of the products formed must be determined before a precise explanation of the antibacterial action of 4-NQO can be proposed.

ACKNOWLEDGMENT

This work was supported by a grant from The Robert A. Welch Foundation, Houston, Texas.

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