Studies on the Action of Nalidixic Acid in the Yeast Saccharomyces cerevisiae

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The effect of the antibiotic nalidixic acid on macromolecular metabolism in the yeast Saccharomyces cerevisiae has been studied. It was found that, upon the addition of nalidixic acid to a logarithmically growing culture, there is a transient inhibition of total cell ribonucleic acid, deoxyribonucleic acid, and protein synthesis, after which the cells show an almost complete recovery. In addition, there is no preferential inhibition of yeast mitochondrial deoxyribonucleic acid synthesis.

Nalidixic acid has been shown to be a selective inhibitor of in vivo deoxyribonucleic acid (DNA) synthesis in bacteria (2, 5); however, its mechanism of action remains unknown. Cell-free systems synthesizing DNA have thus far been found to be resistant to the antibiotic (16). In vitro studies with DNA polymerase I, membrane preparations from polA1 mutants, endonuclease I, exonuclease I, 11, and 111, deoxyribonuclease I, deoxyribosyl transferase from Escherichia coli, and DNA polymerase from Bacillus subtilis have shown that these enzymes are resistant to nalidixic acid (16). Some studies of the effects of nalidixic acid on eukaryotic organisms have been made. In Euglena gracilis, nalidixic acid blocks the replication of chloroplasts (but not plastid DNA) without affecting cell division (4, 13). Nalidixic acid has also been shown to inhibit the growth of the yeast Kluyveromyces lactis on both fermentable and nonfermentable carbon sources as well as to considerably and selectively lower the level of mitochondrial DNA (12).

This investigation describes the effect of nalidixic acid on macromolecular synthesis in the yeast Saccharomyces cerevisiae. We found that, upon the addition of nalidixic acid to a logarithmically growing culture, there is a transient inhibition of total cell RNA, DNA, and protein synthesis, after which the cells show an almost complete recovery. In addition, there is no preferential inhibition of yeast mitochondrial DNA synthesis.

MATERIALS AND METHODS

Strains. The strain of S. cerevisiae used for this study was Sa-c, a, ρ+, his−. It was isolated in this laboratory for its sensitivity to chloramphenicol and neomycin by recombination between two mutants of A664a/18A, α, ρ+, ura+, sensitive to these antibiotics and A2111 Ps/41 (obtained from B. Dorfman), α, ρ+, his−.

Cell growth and labeling. The liquid growth medium used, called NYPP, contained per liter of 0.05 M sodium phosphate (pH 7.0), 2% dextrose, Difco yeast nitrogen base (6.7 g), histidine (100 mg), Difco peptone (2.0 g), Difco yeast extract (1.0 g). Cell growth was followed by using a Klett-Summerson colorimeter fitted with a 660-nm filter. The cultures were grown to saturation overnight, diluted to a turbidity of 20, and allowed to regrow to a turbidity of 30 (except where noted) before the nalidixic acid was added. The cells were labeled as noted in each experiment with [3H]-adenine (New England Nuclear Corp.) at 1 to 5 μCi/ml. YPD plates contain 1% Difco yeast extract, 2% Difco bacto-peptone, 2% dextrose and 2% Difco agar; in YPG plates 2% glycerol is added instead of glucose. The petite or grande yeast phenotype was determined by replica plating colonies from those grown on YPD to YPG and by checking growth after 2 days of incubation at 30 C.

Assay of RNA, DNA, and protein synthesis. Ribonucleic acid (RNA) synthesis was followed by the incorporation of label from [3H]-adenine into cold, acid-precipitable material. A 0.5-ml sample of the culture was added to 0.5 ml of cold, 10 or 20% trichloroacetic acid, chilled, and collected onto glass fiber filters. The filters were then washed with cold, 5% trichloroacetic acid (5 ml), cold, 95% ethanol (2 ml), and dried. Radioactivity was measured by counting each filter in a toluene Liquidfluor scintillant (New England Nuclear Corp.) with an Intertechinique SL-4001 scintillation counter.

DNA synthesis was followed by measuring the incorporation of [3H]-adenine into hot-alkali-stable,
cold-acid-precipitable material. A 0.5-ml sample of the culture was treated in either of two ways. (i) The cells were added to 0.5 ml of 1 M KOH, hydrolyzed at 60°C for 2 h, precipitated with 0.9 ml of cold, 20% trichloroacetic acid, chilled, and collected on glass fiber filters as described above. (ii) The cells were harvested by centrifugation, suspended in 0.5 ml of a 0.4 M KOH and 20% dimethylsulfoxide solution (1), hydrolyzed for 2 h at 60°C, precipitated with 0.4 ml of 20% cold trichloroacetic acid, chilled, and collected on glass fiber filters as described above.

Samples were assayed for protein by the method of Lowry, et al. (11). The samples were prepared in the following way. A 2-ml sample of culture was centrifuged, and the cell pellet was resuspended in 20% trichloroacetic acid and boiled for 20 min, and hot-trichloroacetic acid-insoluble material was pelleted by centrifugation and resuspended in 2 ml of 0.5 M NaOH. A 0.5-ml sample of this solution was then used for the assay.

**Spheroplast formation and preparative CsCl density gradient centrifugation.** Cells were harvested by centrifugation and washed once with distilled water. They were then suspended in 0.5 M sodium thioglycolate-0.1 M tri(hydroxymethyl)aminomethane buffer (pH 8.9) and incubated for 1 h at 30°C. The cells were centrifuged and washed twice with distilled water, suspended in 1 M sorbitol-0.001 M ethylenediaminetetraacetic acid (EDTA) and 2% glusulase (Endo Laboratories, Garden City, N.Y.) and incubated for 30 min at 30°C. The spheroplasts were collected by centrifugation, and the pellet was suspended in 1.65 ml of 0.1 M NaCl-0.15 M EDTA (pH 9.0) and lysed by Sarkosyl (Geigy Chemical Co.) which was added to a concentration of 2%. This lysate was then transferred to a nitrocellulose centrifuge tube, and a saturated CsCl solution (4.1 ml) was used to adjust the density to between 1.680 g and 1.695 g/ml. The samples were overlaid with mineral oil and centrifuged in the 40 rotor of a preparative ultracentrifuge (Spinco) at 18°C at 31,000 rpm for 65 h. Fractions (0.15 ml) were collected from the bottom of the tube by puncturing it with a no. 22 gauge needle. Each fraction was adjusted to 0.5 M KOH and incubated for 2 h at 60°C to digest RNA. The labeled DNA was precipitated with bovine serum albumin (100 µg/ml) and 0.5 ml of 20% trichloroacetic acid at 0°C. The fractions were chilled, collected by filtration on glass fiber filters (Whatman GF-A), washed with 5% trichloroacetic acid followed by 95% ethanol, dried, and counted for radioactivity as before.

**Chemicals.** Nalidixic acid was generously supplied by the Sterling-Winthrop Research Institute, Rensselaer, N.Y. It was dissolved in and neutralized with 0.1 N NaOH before use.

**RESULTS**

**Effect of nalidixic acid on growth and protein synthesis.** Figure 1 shows the effect of nalidixic acid (500 µg/ml) on a logarithmically growing culture of *S. cerevisiae* in NYPP medium. The response may be divided into two phases: an inhibitory phase (initial 2 h) and a recovery phase. After the addition of nalidixic acid, both total yeast protein and turbidity continued to increase, but at a somewhat reduced rate. After approximately 30 min to 1 h, net protein synthesis stopped in the drug-treated culture, and there was no further increase in total yeast protein for about 90 min. This was also reflected in a slight increase in turbidity of the culture during the 2nd h. The viable count on YPD medium showed that, during the first two hours after the addition of the drug, the colony-forming ability of the
culture was reduced by about 40%. Two hours after the nalidixic acid was added, the cells recovered from the effects of the antibiotic. The number of colony-forming units began to increase, protein synthesis resumed, and the culture began to grow logarithmically with the number of colony-forming units increasing at approximately the same rate as in the control. No increase in the number of petites was found during the first 6 h of growth or after overnight growth of the culture to saturation in the presence of nalidixic acid. A similar inhibition of yeast cell growth and recovery of the cells from the effects of the drug has also been reported by Wehr et al. (20) with another strain of *S. cerevisiae* which is sensitive to low concentrations of nalidixic acid.

**Effect of nalidixic acid on nucleic acid synthesis.** The effect of nalidixic acid on the synthesis of DNA and RNA (Fig. 2) produced results similar to those obtained for protein. There was some synthesis of RNA and DNA in the 1st h after the addition of nalidixic acid, but the synthesis of both nucleic acids then stopped for about 1 h and, at approximately 2 h, the culture recovered its RNA and DNA synthetic capacity, but at reduced rates. The lowered rate of synthesis most likely reflects the loss in the viable cell count during the inhibitory phase.

In their studies of the effects of nalidixic acid on macromolecular synthesis in bacteria, Goss et al. (6) reported no inhibition of RNA or protein synthesis during short incubation times in the presence of the antibiotic, but at slightly longer time periods, some inhibition was evident. The effect of nalidixic acid on *E. coli* nucleic acid synthesis was immediate. Figure 3 shows the results of an RNA and DNA synthesis experiment during the first 60 min after the addition of nalidixic acid to a growing yeast culture. In this experiment, the cells were prelabeled for 30 min in order to saturate the internal pool with 3H-adenine and its nucleosides and nucleotides. Incorporation of label into DNA and RNA continued for about 30 min in the drug-treated culture and then stopped. The initial rate of incorporation was slightly less than that of the control for both RNA and DNA synthesis. In contrast to the results obtained with bacteria, the inhibition of DNA synthesis by nalidixic acid cannot be separated from its effect on RNA synthesis.

**Recovery phase.** In order to determine whether the recovery phase of the culture was due to the breakdown or inactivation of nalidixic acid in the medium by the cells, a 10-fold variation in cell concentration was compared for the time of the onset of the recovery phase. In these experiments, the time of recovery was determined by following the uptake of 3H-adenine from the growth medium (Fig. 4). By this method, in which the recovery began at 2 h, the uptake of label was not linear until 3 h after addition of the antibiotic. If the uptake of label was followed in a series of cultures in which the concentration of cells was varied (up to a sevenfold increase in cell concentration), the time of recovery remained constant. Thus it appears that the nalidixic acid was not inactivated by the cells, but that they somehow adapted to the drug or became impermeable to it. It should be noted that this adaptation occurred during a period when there was no net

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**Fig. 2.** Synthesis of RNA and DNA after the addition of nalidixic acid. The culture was treated as described in Fig. 1 except that at zero time 1 μCi of 3H-adenine and 8 μg of cold adenine per ml were added to both the control and drug-treated cultures. Triplicate samples were taken at the indicated times and assay for incorporation into DNA and RNA as described in Materials and Methods. Symbols: ⋄, control; ○, nalidixic acid (500 μg/ml)-treated culture.

**Fig. 3.** Synthesis of RNA and DNA during the first hour after the addition of nalidixic acid. The cultures were treated as described in Fig. 1, except that the culture was prelabeled with 6 μCi of 3H-adenine and 2 μg of cold adenine per ml and divided 30 min before the addition of nalidixic acid to the experimental culture. Samples were taken at the indicated times and assayed for incorporation into DNA and RNA as described in Materials and Methods Symbols: ⋄, control; ○, nalidixic acid (500 μg/ml)-treated culture.
protein synthesis. This, of course, does not preclude the synthesis of small amounts of specific proteins which enable the cell to adapt to growth in the presence of nalidixic acid.

Cesium chloride density gradient analysis of DNA from nalidixic acid-treated yeast. The finding that exposure of the yeast cells to nalidixic acid did not produce respiratory-deficient colonies (petites), even after growth to saturation in the presence of the antibiotic, suggests that, at least under these growth conditions, mitochondrial DNA synthesis is not preferentially inhibited. To demonstrate this, we compared the synthesis of mitochondrial and nuclear DNA during different time periods after the addition of nalidixic acid to a growing culture of yeast. In yeast, mitochondrial and nuclear DNA have different base compositions and therefore band at different positions in CsCl density gradients. Figure 5 shows CsCl density gradients of yeast DNA synthesized in the presence of nalidixic acid during overlapping time periods of the inhibitory phase. The incorporation of 3H-adenine into both nuclear and mitochondrial DNA was greatly inhibited, but to approximately the same final extent. It seemed, though, that the synthesis of mitochondrial DNA was affected somewhat more rapidly, and could indicate that either the mitochondrial DNA synthesizing system is more sensitive to nalidixic acid than is the nuclear system or that the mitochondrial membrane is more permeable to nalidixic acid than is the nuclear envelope. CsCl gradients of the DNA synthesized after the recovery of the culture (Fig. 6) showed that the rate of mitochondrial DNA synthesis, as compared with nuclear DNA synthesis, was lower than in the control but that both classes of DNA were synthesized at significant rates.

![Fig. 4. Uptake of 3H-adenine from the growth medium after the addition of nalidixic acid. A growing culture of Saccharomyces cerevisiae was divided in half, and both cultures were prelabeled with 5 μCi of 3H-adenine and 10 μg of cold adenine per ml for 1 h. Nalidixic acid was then added to one culture, (indicated by the arrow) and the uptake of labeled adenine by the cells was measured. A 0.5-ml sample of culture was centrifuged to remove the cells, and the counts remaining in the supernatant fluid were determined. Symbols: ○, control; O, nalidixic acid (500 μg/ml)-treated culture.](image)

![Fig. 5. CsCl density gradients of the DNA synthesized by Saccharomyces cerevisiae during the first 3 h after the addition of nalidixic acid. A growing culture was divided into six, 10-ml cultures, and nalidixic acid (500 μg/ml) was added to five of these at zero time. At different times after the addition of the nalidixic acid, 3H-adenine (1 μCi/ml) was added to each culture. One hour after the addition of label, the cells were made up to 0.1 M azide, chilled, and prepared for CsCl density-gradient analysis as described in Materials and Methods. Marker 14C-labeled total yeast DNA was run in each gradient. The arrow indicates the position of the marker mitochondrial DNA in the density gradient.](image)

DISCUSSION

It can be concluded from the experiments described that nalidixic acid is not a specific inhibitor of DNA synthesis in yeast as it is in bacteria. Instead, what is found is a coordinate, short lived, inhibition of the synthesis of DNA, RNA, and protein. It is not possible to come to any conclusions concerning the primary site of
action of this inhibitor in yeast. It is unlikely that nalidixic acid is specifically inhibiting DNA replication and that the inhibition of RNA synthesis is necessarily and closely coupled to this inhibition. This can be inferred from the fact that temperature-sensitive mutants for DNA replication and initiation in yeast, isolated by Hartwell (7), show no inhibition of RNA synthesis at the nonpermissive temperature. If nalidixic acid were acting as an inhibitor of RNA or protein synthesis, pleiotrophic effects on macromolecular synthesis similar to those found in this study would be expected. Cycloheximide, an inhibitor of protein synthesis in yeast, secondarily inhibits DNA synthesis and affects RNA synthesis, but only after longer times of incubation (10, 17, 18). No specific inhibitors of RNA synthesis in yeast are now available, but temperature-sensitive mutants defective in RNA production have been described (9). After shifting to the nonpermissive temperature, RNA synthesis ceased very rapidly. This was followed, after a brief lag, by the gradual decay of DNA and protein synthesis. Although it has not been possible in these experiments to separate the times of onset of inhibition of DNA, RNA, and protein synthesis, we cannot exclude the possibility that nalidixic acid is functioning specifically as an inhibitor of either RNA or protein synthesis in our system.

In addition to its effects on macromolecular metabolism, it was found that nalidixic acid caused a decrease in the number of colony-forming units during the inhibitory phase. Cook et al. (3) found that the bactericidal action of nalidixic acid resulted from the degradation of E. coli DNA. Unfortunately, the analogous experiment cannot be carried out in yeast because of the existence in this organism of large, internal nucleotide pools; the removal of external radioactive precursor does not prevent the continued labeling of the DNA.

An additional finding from these studies should be stressed. Nalidixic acid does not preferentially inhibit mitochondrial DNA synthesis, even in the inhibitory phase, nor does growth in the presence of the antibiotic result in an increase in the number of respiratory-deficient cells. In yeast, nalidixic acid has been shown to inhibit the induction of petites by ethidium bromide (8, 9, 19). The results of Vidova and Kovac (19) enabled them to infer that the inhibition by nalidixic acid of the induction of petites by ethidium bromide did not result from the specific inhibition of mitochondrial DNA synthesis by the antibiotic. This analysis is corroborated by the results of our experiments. It has been reported that nalidixic acid also inhibits the respiratory adaptation of yeast to aerobic growth and the biogenesis of respiratory enzymes (14, 15). These effects could not result from the specific inhibition of mitochondrial DNA synthesis unless it is postulated that during respiratory adaptation the macromolecular synthesizing systems of the yeast are different from those found in the repressed yeast cell. Clearly, much more work is required to define the action of this antibiotic in yeast as well as in bacteria.

LITERATURE CITED


