THE EFFECT OF NALIDIXIC ACID ON THE EXPRESSION OF SOME GENES IN ESCHERICHIA COLI K-12

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SUMMARY

The synthesis of maltodextrin phosphorylase and the phage λ receptor of Escherichia coli K-12 is substantially inhibited by the presence of 50 μg nalidixic acid/ml in the culture medium. β-galactosidase synthesis is inhibited to a lesser extent and no inhibition of L-tryptophanase synthesis is observed. The inhibition of enzyme synthesis is apparently not due to the effect of nalidixic acid on deoxyribonucleic acid synthesis.

INTRODUCTION

Nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1, 8-naphthydrine-3-carboxylic acid) is an inhibitor of bacterial deoxyribonucleic acid synthesis whose mode of action is poorly understood (2,8). Many reports have appeared in the literature which describe the use of nalidixic acid to stop DNA synthesis assuming that there is no effect on protein synthesis (13,14, for example). This assumption is based on the observation that in the presence of the drug, bacteria do not incorporate thymine into DNA while they do incorporate amino acids into protein at a rate not significantly different from that observed in the absence of the drug. (2).

We wish to describe experiments which show that in Escherichia coli K-12 cells, a concentration of nalidixic acid (50μg/ml) commonly used to interrupt DNA synthesis also

inhibits the appearance of maltodextrin phosphorylase (EC2,4,1,1), the product of the malP gene, and the receptor for the bacteriophage lambda, which is determined, at least in part, by the lamB gene (10). The malP gene is located in the malA region of the Escherichia coli chromosome and the lamB gene is located in the malB region (3,4). The expression of the malA and malB regions is positively controlled by the malT product and is subject to catabolite repression (1,3,4,15,Hofnung and Schwartz, unpublished results). The synthesis of the products of both the malA and malB regions can be induced in Escherichia coli K-12 cells which are using glucose as carbon source by adding maltose and 3':5' cyclic-adenosine monophosphate (cAMP) to the medium (Fig.Ia and Ib).

RESULTS AND DISCUSSION

The induction of the λ receptor by cAMP and maltose, both in the absence and presence of nalidixic acid is shown in Figure 1a. An 80% reduction in the rate of synthesis of the receptor was observed in the presence of the drug. In the same experiment, a 70% reduction in the rate of synthesis of maltodextrin phosphorylase was observed in the presence of the drug. (Fig.1b). The above effects were observed with 50 µg of nalidixic acid per ml. At a concentration of 10µg of nalidixic acid per ml no significant effect was observed on the appearance of either maltodextrin phosphorylase or the lambda receptor, although incorporation of ^{14}C-thymine into trichloroacetic acid insoluble material was completely inhibited,(data not shown). This suggests that the effect of nalidixic acid on the maltose system is not a consequence of inhibition of DNA synthesis. In order to test this hypothesis

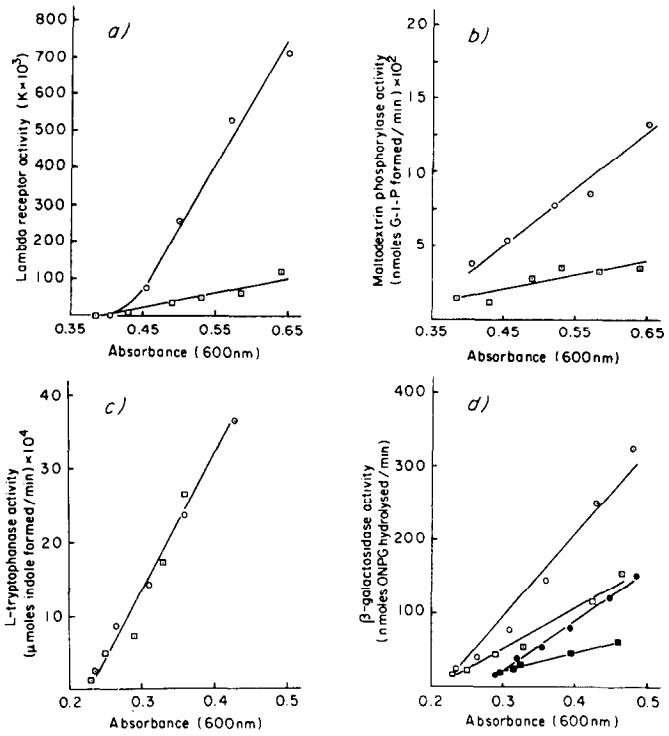

FIGURE 1 : The effect of nalidixic acid on the synthesis of some inducible proteins.

The activity of each protein per ml of culture is plotted as a function of the absorbance of the culture at 600 nm.

a) Lambda receptor. A culture of exponentially growing Escherichia coli K-12 cells, using glucose (2×10^{-2}M) as carbon source was divided in half. Nalidixic acid was added to one half of the culture at a final concentration of 50 μg/ml. After shaking at 37 C for 15 minutes, cAMP and maltose were added to both halves of the culture, at final concentrations of 4×10^{-3}M and 10^{-2}M, respectively. One ml samples were taken from each culture into chloramphenicol (100 μg/ml) at 0 C, at 0, 10, 20, 30, 45, and 60 min after addition of cAMP and maltose. After the A_{600} was determined for each sample, the cells were centrifuged, resuspended in 0.1ml of tris-cholate-EDTA buffer, (10) and extracted for 30 min at 37 C. Lambda receptor activity was determined in the extracts as described by Randall-Hazelbauer and Schwartz (10). (O-O without nalidixic acid, □-□ with nalidixic acid).

b) Maltodextrin phosphorylase. The procedure is the same as that used in a) except 2ml samples were taken and maltodextrin phosphorylase activity was determined in sonicated extracts of the bacteria. The sensitive method of Schwartz and Worcel (12) which measures incorporation of ^{32}P inorganic phosphate into glucose-1-phosphate was used to determine the amount of maltodextrin phosphorylase activity in the extracts. (O-O without nalidixic acid, □-□ with nalidixic acid).

c) L-tryptophanase. The procedure used was the same as in a) except that L-tryptophan (1mg/ml final concentration) was added to the culture instead of maltose, to induce synthesis of L-tryptophanase. L-tryptophanase activity was determined in toluenized cells using

the procedure of Newton et al.(7). (O-O without nalidixic acid). □—□ with nalidixic acid).

d) β-galactosidase. The procedure was as in a) except that the culture was divided into 4 parts before treatment with nalidixic acid. Nalidixic acid was added to two of the aliquots and after shaking for 15 min at 37 C, cAMP and isopropyl-β-D-thiogalactoside (IPTG) ($5x10^{-3}$M final concentration) were added to one treated and one untreated aliquot. IPTG ($5x10^{-3}$M, final concentration) alone was added to the two remaining aliquots. β-galactosidase activity was determined in toluenized cells by the method of Horiuchi et al (5).

IPTG + cAMP O-O
IPTG + cAMP + nalidixic acid □—□
IPTG ●-●
IPTG + nalidixic acid ■—■

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we examined the synthesis of maltodextrin phosphorylase and lambda receptor in a strain which carries a dnaB^{ts} mutation. In such a strain, DNA synthesis stops immediately after transfer of the cells to 42 C. In the absence of nalidixic acid,incubation of the bacteria at 42 C had little effect on the rate of synthesis of maltodextrin phosphorylase or lambda receptor. In the presence of nalidixic acid, the synthesis of both maltodextrin phosphorylase and lambda receptor was inhibited severely during incubation either at 30 C or 42 C. In view of these results it seems likely that the effect of nalidixic acid on the expression of the maltose system is not a consequence of the effect of the drug on DNA synthesis.

In preliminary experiments we have tested the possibility that nalidixic acid interferes with the ability of the bacteria to accumulate enough internal maltose to induce the system. This was done by examining the effect of nalidixic acid both on the induction of the maltose transport system and on the ability of the bacteria to transport maltose. The results of of these experiments suggest that nalidixic acid does not directly interfere with maltose transport but does prevent the induction of the maltose transport system in addition

to lambda receptor and maltodextrin phosphorylase. The results shown below for the effect of nalidixic acid on the induction of β-galactosidase and L-tryptophanase indicate that nalidixic acid does not interfere with cAMP transport.

Figure 1c and 1d show the effect of nalidixic acid on the synthesis of L-tryptophanase and β-galactosidase. There is a 40% reduction in the rate of β-galactosidase synthesis in the presence of the drug when induced either with or without cAMP. Nalidixic acid has no apparent effect on the rate of synthesis of L-tryptophanase. In addition, nalidixic acid has no effect on the synthesis of homoserine dehydrogenase or glucose-6-phosphate dehydrogenase (data not shown). These results do not suggest any involvement of catabolite repression in the effect of nalidixic acid since the drug does not affect synthesis of L-tryptophanase (an enzyme whose synthesis is very sensitive to catabolite repression) nor does cAMP reverse the effect of the drug on β-galactosidase synthesis.

Puga and Tessman have observed that nalidixic acid causes an 80% reduction in the amount of RNA produced by *in vivo* transcription of the phage S-13 genome. They postulate that nalidixic acid, or a metabolic product of it, may act as an intercalating agent in supercoiled DNA (9).

Senkaran and Pogell have shown that drugs which intercalate supercoiled DNA such as acridine orange and ethidium bromide inhibit the expression of some catabolite sensitive operons (11).

Following these lines of reasoning it could be proposed that nalidixic acid, or a metabolic product of it, acting as an intercalating agent may preferentially inhibit the transcription of some genes such as those of the maltose

system. Whatever their interpretation , our observations suggest that nalidixic acid, besides blocking DNA synthesis can have an effect on the expression of some genes.

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