MESSENGER-SELECTIVE INHIBITOR FOR THE INITIATION OF TRANSLATION IN *ESCHERICHIA COLI:* NITROFURANTOIN

Erwin WAGNER, Manfred SCHWEIGER, Helmut PONTA and Peter HERRLICH
Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem and Institut für Biochemie (nat. Fak.) der Universität Innsbruck, Innsbruck, FRG

Received 22 September 1977

1. Introduction

Nitrofurantoin (5-nitro-2-furfurylidene-1-amino-hydantoin) interferes with the synthesis of inducible proteins such as β-galactosidase or galactokinase much more than with the synthesis of most *Escherichia coli* proteins or of phage proteins [1]. We have previously shown, mainly from experiments in vitro, that this inhibition is exerted at the initiation of translation. In vitro, the synthesis of galactokinase was inhibited by nitrofurantoin to the same extent whether DNA or RNA was used as template. The synthesis became resistant, however, if polysomes were permitted to read out from the same mRNA [1]. In order to broaden the basis of these conclusions, a series of in vivo experiments has been performed. The new data not only confirm the selective action of nitrofurantoin on mRNA, but are suggestive of a direct interaction of nitrofurantoin with mRNA sequences.

2. Experimental

2.1. *Materials*

7-Aza-tryptophan and 5-methyl-tryptophan were obtained from Sigma. Nitrofurantoin was a gift of Boehringer, Mannheim. Nitrofurantoin was dissolved at 5 mg/ml in 0.4 M triethanolamine, pH 8.5.

2.2. *Strains of bacteria and viruses*

As described [2].

2.3. *In vivo translation of preformed lac mRNA*

Measured according to [3].

2.4. *T7 DNA ligase synthesis*

T7 DNA ligase synthesis was either determined by following the accumulation of ATP-dependent DNA ligase activity (not shown) or by labeling the DNA ligase peptide with amino [^{14}C]acids. For this, cells were irradiated with ultraviolet light to inhibit host protein synthesis [2], and infected with a T7 mutant (T7 H280) inducing only four proteins (the amber peptide of T7 RNA polymerase, T7 DNA ligase, M protein and 1.1 protein). The infected culture was immediately mixed with 25 μCi/ml [^{14}C]protein hydrolysate plus 0.01% casamino acids and aliquots of the culture were treated with nitrofurantoin at various times after the infection as described in fig.2. After further incubation until 15 min past infection, each cell sample was dissolved in gel sample buffer and total protein resolved by SDS polyacrylamide gel electrophoresis [2,4]. Autoradiograms of these gels were evaluated by microdensitometry. The arbitrary units plotted were derived from densitometer readings (peak area).

3. Results and discussion

The principle of this series of experiments is to study the action of nitrofurantoin on the translation of preformed mRNA. *Lac*-specific mRNA is accumulated in the presence of a tryptophan analogue. Transcription is then stopped by the addition of rifampicin and the action of nitrofurantoin on the translation of preformed lac-mRNA is followed. The aminoacid analogue is incorporated into β-galactosidase, rendering the enzyme inactive but

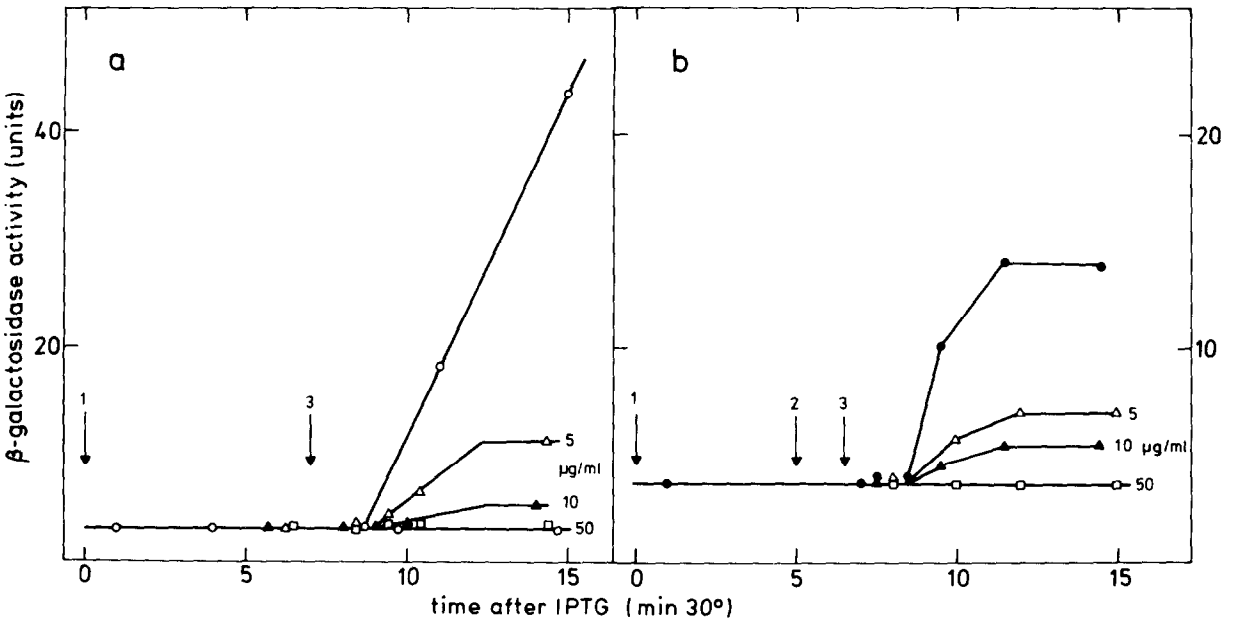

Fig.1. Nitrofurantoin inhibits translation of preformed β-galactosidase mRNA in vivo. To an exponentially growing culture of *E. coli* B_{s-1} in M9 minimal medium supplemented with 10^{-4} M $MgSO_4$ and 0.4% glycerol, the following components were added at $A_{600\ nm}$ 0.5: 50 μg/ml 7-aza-tryptophan plus 5 μg/ml 5-methyltryptophan at 4 min before start of the experiment; 1 mM isopropyl-thiogalactoside (IPTG) at time zero (arrow one); at 5 min, 200 μg/ml rifampicin (arrow two), and at 6.5 min, 1.25 μg/ml tryptophan was added. The cultures were divided and increasing concentrations of nitrofurantoin were given at 6.5 min. Aliquots were withdrawn, extracted with toluene three times in order to remove rifampicin and assayed for β-galactosidase activity (according to [7]). (a) Coupled DNA-directed synthesis, without rifampicin. (b) Translation of preformed mRNA. 5-Methyltryptophan is not incorporated into protein but inhibits tryptophan biosynthesis thus enforcing exclusive incorporation of 7-aza-tryptophan [3]. (o——o) Control – nitrofurantoin and control – IPTG (a). (•——•) Control – nitrofurantoin (b).

permitting normal polysome formation with lac-mRNA [3,5]. The influence of nitrofurantoin on the rate of translation is measured by permitting the formation of active enzyme in the presence of excess tryptophan.

In an experiment omitting rifampicin we first convinced ourselves that nitrofurantoin inhibited β-galactosidase synthesis under the new experimental conditions as specifically and in the same dose range as observed [1] (see fig.1a). In the critical experiment, we added nitrofurantoin only after mRNA synthesis had stopped and found that nitrofurantoin did indeed inhibit translation of preformed β-galactosidase mRNA (fig.1b). The dose response curves of mRNA-directed β-galactosidase synthesis were similar to those obtained in the coupled transcription–translation reaction. This indicates that nitrofurantoin acted mainly or even exclusively on translation.

A discrimination between a mechanism affecting initiation or elongation of translation was achieved by kinetic measurements. In principle, we are asking at what time after the start of translation does enzyme synthesis become resistant to nitrofurantoin. If the initiation of enzyme synthesis were the inhibited step, then maximal inhibition could only occur if the inhibitor were added at time zero. At any later time, peptides which had already been initiated, would be completed in the presence of the inhibitor. If nitrofurantoin interfered with the elongation of translation, it would inhibit any appearance of enzyme chains not yet completed. β-Galactosidase synthesis is particularly well suited for this type of examination since the subunit peptide is long, requiring about 60 s for completion. The action of chloramphenicol, a typical inhibitor of elongation, revealed the expected kinetics (fig.2a,b). The experiments with nitrofurantoin, at the lowest dose leading to total inhibition of

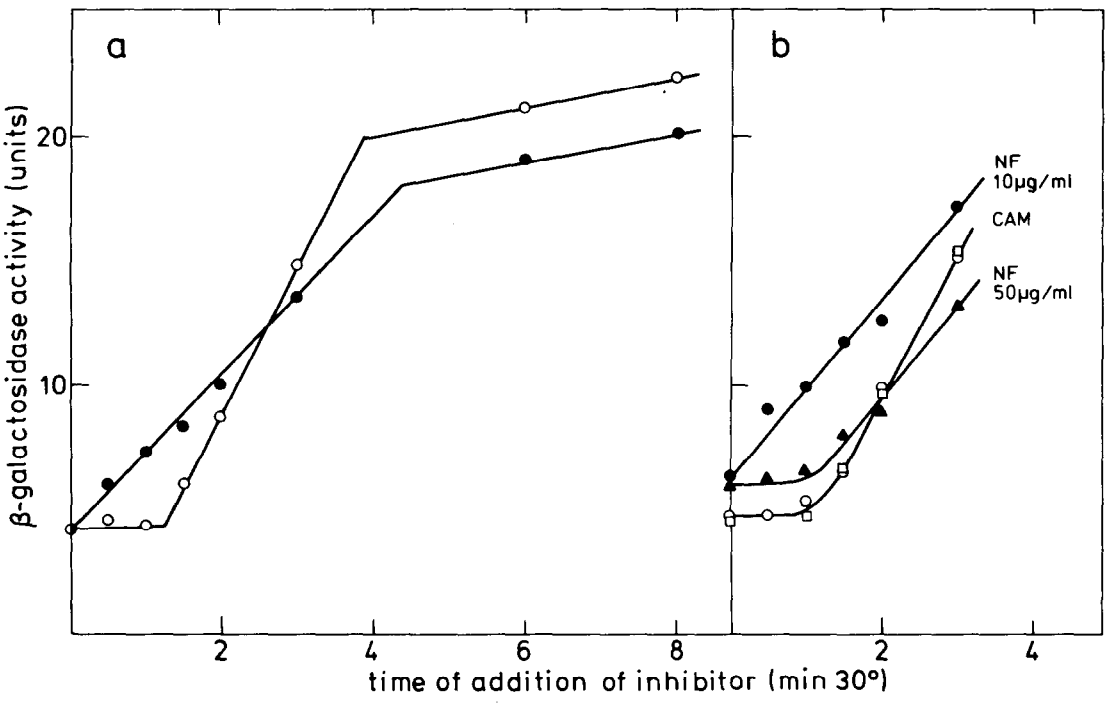

Fig.2. Kinetics of nitrofurantoin action on β-galactosidase translation in vivo. Cells and conditions were the same as in fig.1. 7-Aza-tryptophan and 5-methyltryptophan were added at – 9 min (9 min before time zero of the abscissa), IPTG was added at – 5 min, rifampicin and tryptophan at 0 min. At various times thereafter, aliquots were transferred to tubes containing either nitrofurantoin or chloramphenicol. At 30 min, all samples were harvested and assayed for β-galactosidase activity. (●——●) 10 μg/ml nitrofurantoin; (▲——▲) 50 μg/ml nitrofurantoin; (○——○) 100 μg/ml ((□) 10 μg/ml chloramphenicol. (a) and (b) are two independent experiments. The different kinetics of action at 10 μg/ml and 50 μg/ml do not reflect membrane permeation kinetics since the same results were obtained with permeabilized cells (not shown) and since no such differences were found with other enzyme syntheses such as T7-DNA-ligase (fig.3).

β-galactosidase synthesis (10 μg/ml), yielded curves typical of an inhibitor of initiation (fig.2a,b). At 50 μg/ml, however, nitrofurantoin blocked the elongation of β-galactosidase synthesis (fig.2b).

As demonstrated [1], low doses of nitrofurantoin preferentially inhibit the translation of one class of enzymes. At higher doses such as 50 μg/ml, nitrofurantoin interferes with the synthesis of all *E. coli* proteins and of phage proteins such as T7 DNA-ligase. In a similar kinetic study for T7 DNA ligase, the inhibition by nitrofurantoin at 50 μg/ml turned out to occur during peptide elongation (fig.3). In contrast to the observations with β-galactosidase, there was little inhibition of DNA-ligase synthesis at 10 μg/ml and this slight inhibition was also due to a block in elongation. Thus we conclude that the block to elongation is non-specific and that selectivity is achieved at the initiation stage of translation.

The inhibition of elongation of T7 DNA ligase is caused by premature chain termination since we were measuring complete DNA-ligase peptide (fig.3). The premature termination may occur as a result of an interaction of nitrofurantoin either with an elongation protein or with the RNA itself. A similar explanation could be given for the specific action on initiation of translation. Nitrofurantoin may interact with an initiation protein or with the initiation site on mRNA. Also the inhibition by high doses of nitrofurantoin of replication reported [1] might be mediated either by a direct interaction with nucleic acids or by inhibition of a replication protein. In the latter case, we have shown by experiments with mutants in DNA repair,

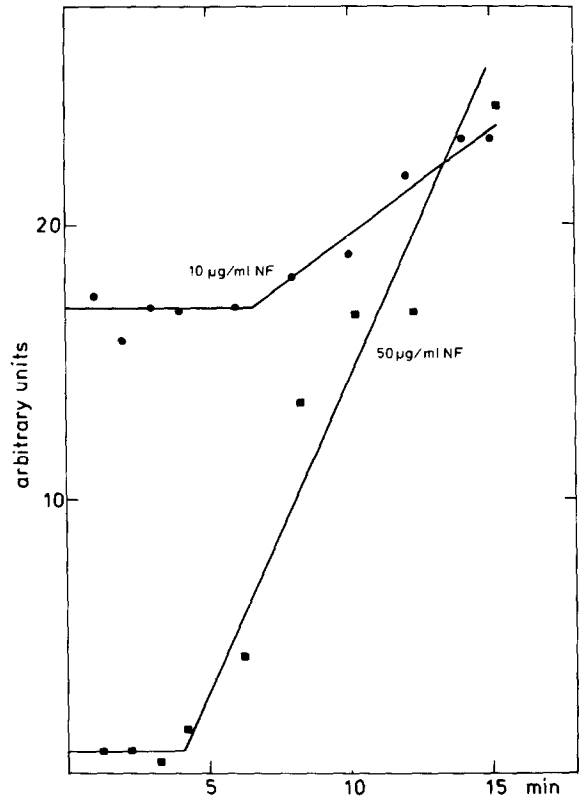

Fig.3. Kinetics of nitrofurantoin action on T7 DNA-ligase synthesis. Part of the culture used for the experiment in fig.2 was irradiated with ultraviolet light and used for infection with T7 H280 (mutant in T7 DNA polymerase and T7 protein kinase). 0.01% casamino acids and 25 µCi/ml amino [^{14}C]acids were added. At various time after infection, 0.2 ml aliquots were transferred to tubes containing nitrofurantoin at either 10 µg/ml (•) or 50 µg/ml (▪) and incubated further until 15 min past infection. Total protein of each sample was resolved by gel electrophoresis and the radioactivity in DNA-ligase evaluated by microdensitometry. Time of inhibitor addition is plotted versus amount of T7 DNA-ligase accumulated at 15 min past infection.

that nitrofurantoin induces lesions on nucleic acid [6]. We, therefore, favour a unifying concept of nitrofurantoin action involving direct interaction with nucleic acids. With respect to the specific action on the initiation of translation, this implies a tremendous sequence preference for the initiation region on mRNA of the nitrofurantoin-sensitive group.

References

[1] Herrlich, P. and Schweiger, M. (1976) Proc. Natl. Acad. Sci. USA 73, 3386–3390.
[2] Ponta, H. Rahmsdorf, H.-J., Pai, S. H., Hirsch-Kauffmann, M., Herrlich, P. and Schweiger, M. (1974) Mol. Gen. Genet. 134, 281–297.
[3] Svenson, S. B. and Karlström, O. H. (1976) J. Virol. 17, 326–334.
[4] Pfennig-Yeh, M., Ponta, H., Hirsch-Kauffmann, M., Rahmsdorf, H.-J., Herrlich, P. and Schweiger, M. (1977) J. Mol. Biol. (submitted).
[5] Pardee, A. B. and Prestidge, L. S. (1958) Biochim. Biophys. Acta 27, 330–344.
[6] Hirsch-Kauffmann, M., Herrlich, P. and Schweiger, M. (1977) Klinische Wochenschrift, in press.
[7] Miller, J. H. (1972) in: Experiments in Molecular Genetics, Cold Spring Harbor, New York.