Activity of Distamycin A on the Induction of Adaptive Enzymes in *Escherichia coli*

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SUMMARY

We have studied the effect of distamycin A on the synthesis of inducible enzymes in *Escherichia coli* strain K12. Distamycin A did not inhibit the growth of *E. coli* K12 when the micro-organism already contained constitutive or inducible enzymes for the metabolism of sugars. The growth of *E. coli* was prevented when distamycin A was present during the enzymic induction. Distamycin A inhibited the formation of inducible β -galactosidase; the activity of the antibiotic was directly related to its concentration, but independent of the concentration of the inducer. Thus it does not seem that distamycin A causes a decrease of the concentration of inducer. The enzyme synthesized in the presence of subinhibitory concentrations of distamycin A showed the same kinetic behaviour as β -galactosidase induced under normal conditions.

INTRODUCTION

Distamycin A is an antibiotic produced by *Streptomyces distallicus* isolated in our laboratories, the structure of which was identified and confirmed by total synthesis by Arcamone *et al.* (1964). Partially purified preparations of distamycin A exerted an inhibitory action on the development of experimental tumors (Di Marco *et al.* 1962). The purified crystalline antibiotic shows antiviral activity on vaccinia and herpes simplex viruses *in vitro* or in experimental infections (Casazza & Ghione, 1964; Verini & Ghione, 1964; Werner, Ganter & de Ratuld, 1964). The antibiotic interferes with some phases of the multiplication of the phages T1 and T2 of *Escherichia coli* (Di Marco *et al.* 1963*a, b*). On the basis of the hypothesis that distamycin A blocks some enzymic processes induced by the phages, we investigated the action of the antibiotic on the induction of adaptive enzymes in *E. coli*. For this purpose we studied the activity of distamycin A on bacterial growth which depended on the induced synthesis of carbohydrate-metabolizing enzymes, and the action of the antibiotic on the induction of β -galactosidase.

METHODS

The strain *Escherichia coli* K12 used was grown in a complex medium (peptone broth, nutrient broth, yeast extract broth) or in a chemically defined medium, namely, Davis salt solution (Davis & Mingioli, 1950) supplemented with carbohydrates which were metabolizable by constitutive enzymes (carbohydrates of type I) or by inducible enzymes (carbohydrates of type II) of *E. coli*. Carbohydrates of type I, besides the glucose of the original medium of Davis & Mingioli, are mannose,

fructose; carbohydrates of type II are lactose, trehalose, ribose, arabinose, xylose. The bacteria were previously washed in Davis salt solution and incubated at 37° for 2–3 hr in order to obtain metabolic starvation.

The inoculum was standardized at 10⁶ organisms/ml.; the bacterial growth was measured by a photometric method (Beckman spectrophotometer at wavelength 600 m μ), by the use of a calibration curve. For β -galactosidase induction *Escherichia coli* κ 12 was incubated in peptone broth (peptone, 2 %) containing 0.5 % lactose. Assays for β -galactosidase were made by the colorimetric procedure of Lederberg (1950) with washed organisms resuspended in 0.02 M-Na phosphate buffer (pH 7.5) to a constant extinction value (0.200, at 600 m μ). The organisms were incubated at 25° with the addition of *o*-nitrophenyl- β -galactoside (ONPG) 0.0005 M. The reaction was stopped by adding Na carbonate to 0.2 M final concentration. The concentrations of the released *o*-nitrophenol (ONP) were determined photometrically (Beckman spectrophotometer, at 420 m μ) and represented a measure of the activity of the enzyme.

RESULTS

Activity of distamycin A on growing cultures of Escherichia coli κ 12

Distamycin A even at relatively high concentrations (50 μ g./ml.) did not modify the growth of *E. coli* κ 12 in complete media or in defined media containing as sole carbon source carbohydrates which are metabolized by constitutive enzymes of *E. coli*. On the other hand, distamycin A showed a marked inhibitory action when the bacterial growth depended upon a *de novo* synthesis of induced enzyme, as in the case of the utilization of pentoses or disaccharides by the wild-type strain (Table 1).

Table 1. *Growth of Escherichia coli* κ 12 in presence of distamycin A
20 μ g./ml. after incubation for 36 hr at 37°

Media contained no distamycin A, or 20 μ g./ml. Effect on growth is shown as % of growth without antibiotic. Carbohydrates were added to 0.2 % (w/v).

Media	Growth with antibiotic %
Davis salt solution	
+ glucose	90
+ mannose	87
+ fructose	100
+ xylose	62
+ arabinose	50
+ ribose	40
+ rhamnose	65
+ maltose	70
+ trehalose	25
+ lactose	50
Beef extract broth	100
Yeast extract broth	100
Peptone broth	100

Distamycin A showed no inhibitory effect on the development of a strain of *E. coli* κ 12 which was previously adapted to metabolize the pentoses or disaccharides (Table 2).

At least in the case of arabinose, distamycin A did not act as an antimetabolite

(Woolley, 1952); even high concentrations of arabinose (0.8%, inhibitory *per se*) did not antagonize the action of distamycin A (Table 3).

The inhibitory action of distamycin A was also shown with strains already adapted to utilize substrated different from those actually present in the medium (Table 4).

Table 2. *Growth of induced and non-induced Escherichia coli* κ 12 strains in presence of distamycin A (20 μ g./ml.) and of various carbohydrates (0.2%) in Davis salt solution (DSS)

Maintenance media	Assay media	Growth with distamycin A as % of growth without antibiotic
Peptone broth	DSS + arabinose	50
DSS + arabinose		100
Peptone broth	DSS + xylose	50
DSS + xylose		110
Peptone broth	DSS + rhamnose	65
DSS + rhamnose		105
Peptone broth	DSS + trehalose	26
DSS + trehalose		108
Peptone broth	DSS + maltose	63
DSS + maltose		106

Table 3. *Growth of Escherichia coli* κ 12 (strain routinely maintained in peptone agar) in Davis salt solution with the addition of arabinose and distamycin A

Distamycin A (μ g./ml.)	Arabinose (%)				
	0.5	1	2	4	8
	Turbidity readings*				
80	0	0	0	0	0
40	0	0	0	0	0
20	57	29	43	65	0
10	123	123	110	76	64
0	173	185	154	140	121
ID 50† (μ g./ml.)	15	12	15	10	10

* Turbidity readings at 600 m μ . † Values of median inhibitory dose (ID 50).

Table 4. *Growth of lactose-adapted or unadapted strains of Escherichia coli* κ 12 in presence of distamycin A (20 μ g./ml.) and various carbohydrates (0.2%) in Davis salt solution (DSS)

Maintenance media	Assay media	Growth with distamycin A as % of growth without antibiotic
Peptone broth	DSS + arabinose	55
DSS + lactose		60
Peptone broth	DSS + trehalose	45
DSS + lactose		43
Peptone broth	DSS + lactose	50
DSS + lactose		100

The viability of the bacteria was not affected by distamycin A; the growth of the organisms was restored when the medium containing distamycin A was supplemented with glucose (Table 5).

Table 5. *Growth of Escherichia coli* K 12 (strain routinely maintained in peptone agar) in presence of distamycin A (20 µg./ml.) in Davis salt solution with arabinose (culture A) or with further addition of glucose after incubation for 18 hr (culture B)

Culture	Incubation time (hr)		
	0	18	36
	Relative turbidity readings*		
A	0	45	45
B	0	45	215

* Relative turbidity readings at 600 mµ.

Activity of distamycin A on the β-galactosidase induction

Distamycin A up to 100 µg./ml. did not affect the activity of β-galactosidase, the rate of ONP formation over 18 min. was the same in presence or absence of the antibiotic, but it did interfere with the induction of this enzyme. The action of distamycin A was proportional to its concentration (Fig. 1) and independent of the concentration of inducer within the concentrations tested (Fig. 2). The effect of distamycin A was clearly evident when it was added to the medium together with inducer, or not more than 30 min. later; when added after 30 min. the activity rapidly decreased (Fig. 3).

The β-galactosidase enzymes induced either in the presence of subinhibitory concentrations of distamycin A or in its absence showed the same kinetic characteristics. The value of the Michaelis constant (K_m) calculated according to the procedure of Lineweaver-Burk (1934) was 2.16×10^{-4} M for the enzyme induced in a medium free from antibiotic and 2.26×10^{-4} M for the enzyme induced in the presence of distamycin A.

DISCUSSION

Our results support the hypothesis of an interference of distamycin A with the mechanisms involved in the induction of adaptive enzymes. The reported data show that the growth of *Escherichia coli* K 12 was prevented by distamycin A only when the multiplication of the micro-organisms depended on the formation of an induced enzyme required for the metabolism of a carbohydrate present in the medium as sole carbon source. The growth of *E. coli* was inhibited when the antibiotic was present during induction of the enzyme. These characteristics of the action of distamycin A suggest that it does not inhibit the metabolic processes catalyzed by enzymes (either constitutive or inducible) already present in the organisms, but interferes with the system involved in the enzymic induction. In the light of these findings it seems hazardous to draw any conclusion on the specific site of action of distamycin A in such a complex mechanism as enzymic induction and repression (Monod & Cohn, 1952; Jacob *et al.* 1960; Jacob & Monod, 1961). On the basis of the present results the site of action of distamycin A has to be common to

several pathways since different enzymic adaptive systems are blocked by this antibiotic. The investigation of the distamycin A activity on the induction patterns of some other enzymes (such as penicillinase in *Bacillus subtilis* and serine or threonine deaminase in *Escherichia coli* κ 12) are hindered by the interference displayed by the antibiotic on the assay methods. We cannot deduce from our data

Fig. 1

Fig. 2

Fig. 1. Activity of distamycin A on the induction of β -galactosidase in *Escherichia coli* κ 12. The incubation time in presence of the inducer is plotted against the concentrations of ONP released. Curve 1, control; curve 2, distamycin A 20 μ g./ml.; curve 3, distamycin A 40 μ g./ml.; curve 4, distamycin A 80 μ g./ml.

Fig. 2. Activity of distamycin A (100 μ g./ml.) on the induction of β -galactosidase in *Escherichia coli* κ 12 in presence of various concentrations of inducer. The incubation time in presence of the inducer is plotted against the concentrations of ONP released. Curve 1, lactose 0.1-0.5-2.5%; curve 2, lactose 0.1% + distamycin A; curve 3, lactose 0.5% + distamycin A; curve 4, lactose 2.5% + distamycin A.

Fig. 3. β -Galactosidase activity of cultures of *Escherichia coli* κ 12 to which the distamycin A was added at various intervals during the adaptation period. On the axis of abscissa the times between the addition of the inducer and that of the antibiotic. On the axis of ordinate % of ONP released (activity of untreated culture = 100).

if distamycin A inhibits the induction of permeases and/or kinases. The work is still in progress, and Barr's (1963) considerations on the danger of extreme theorization lead us to continue on the experimental rather than on the merely theoretical basis.

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