SOME STRUCTURAL REQUIREMENTS FOR THE ANTIBIOTIC ACTION OF DISTAMYCINS

II. Structural modification of the side chains in distamycin A molecule

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1. Introduction

Distamycin A, an antibiotic substance produced by *Streptomyces distallicus*, is endowed with cytostatic properties evidenced by its activity on some experimental tumors of the mouse and the rat [1]. It suppresses the multiplication of T_1 and T_2 phages in *E. coli* K12 [2] and interferes with the multiplication of some DNA-viruses such as vaccinia, herpes simplex and adenoviruses [3]. Another interesting feature of distamycin A is its ability to prevent the induction of bacterial adaptive enzymes of *E. coli* [4, 5].

In some recent publications [6, 7] we have reported the reaction of this antibiotic on the structure and template activity of DNA. The absorbance of DNA decreases in the presence of distamycin A. This effect is dependent on the antibiotic/DNA-P ratio (r). The melting profile of native DNA shifts towards higher temperatures with increasing antibiotic concentration. The hyperchromicity also increases from 40 to about 60% in the presence of distamycin when r is raised from r=0 to r=1 [6]. These interactions lead to a pronounced inhibition of the incorporation of AMP into RNA in the DNA-directed RNA-polymerase system.

The structure of this antibiotic also obtained by total synthesis [8], is characterized by 3 residues of 1-methyl-4-aminopyrrole-2-carboxylic acid and 2 side chains, the first constituted by a formyl group, and the second by a propionamidine chain (fig. 1, compound I). Arcamone et al. [9, 10] have recently succeeded in synthesizing some structural analogues of distamycin A. These structural modifications were obtained by substitution of the formyl group (fig. 1, R_1), substitution of the propionamidine side chain (fig. 1, R_2) and variation of the number of pyrrole residues. The cytotoxicity, antiviral activity and the inhibition of DNA-dependent RNA synthesis by distamycin derivatives containing 2, 3, 4 and 5 pyrrole rings has been recently studied by Chandra et al. [11]. They found that the antiviral activity of distamycins and their action on the template activity of DNA are dependent on the number of pyrrole rings in the molecule. The present communication describes the activity of distamycin derivatives with a constant number of pyrrole rings, but with various substitutions at the side chains (fig. 1), in various biological systems, and on the DNA-dependent RNA polymerase reaction.

2. Materials and methods

^{14}C- or ^{3}H-ATP was obtained as tetralithium salt from NEN Chemical GmbH, Germany; other triphosphates were obtained from Zellstofffabrik Waldhof,

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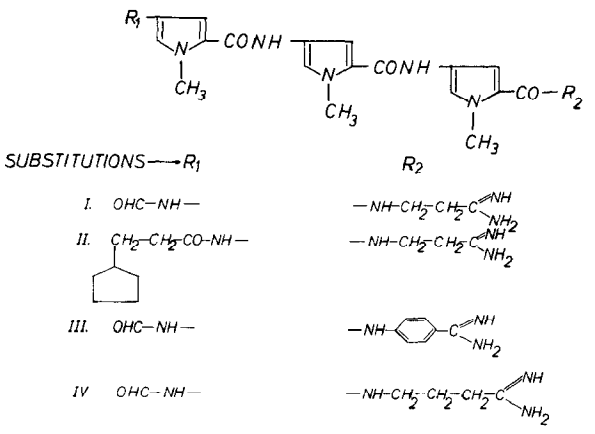

Chemical structures of distamycin derivatives.

Mannheim. Calf thymus DNA was supplied by Serva and Co., Heidelberg. All other chemicals were analytical grade reagents from Merck, Darmstadt.

2.1. *RNA-polymerase reaction*

RNA polymerase was isolated from *E. coli* K12 cells according to the procedure by Burgess [12] and kept in buffer containing 50% glycerol at −20°. The reaction mixture contained, in 0.25 ml, 0.04 M Tris, pH 7.9, 0.01 M $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.15 M KCl, 0.15 mM UTP, CTP and GTP, 0.15 mM ^{3}H-ATP and 0.15 mg per ml of calf thymus DNA. The reaction was started with about 10 μg enzyme protein and incubations were carried out for 20 min at 37°. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid (TCA) and serum albumin was used as carrier. The precipitate was collected on a membrane filter (Sartorius, Göttingen, SM 11306) and washed 4 times with 3 ml of 2% TCA. The filter was dried and counted with toluol scintillation fluid in a Packard liquid scintillation spectrometer. Protein was estimated by the method of Lowry et al. [13].

2.2. *Cytotoxicity assay*

The cytotoxicity of distamycin derivatives was estimated on the basis of the morphological modifications induced in HeLa cell cultures, after incubation for 40 hr in Hanks' saline solution +0.5% lactalbumin hydrolysate +5% calf serum (HLS). The percent inhibition of cellular growth was evaluated according to Morasca [14].

2.3. *Assay on vaccina virus*

Cultures of HeLa cells (grown in HLS medium) or mouse-embryo cells (grown in HLS medium containing 0.1% yeastolate) infected with vaccinia virus (Strain WR/ATCC) were used. Preliminary assays were made according to Herrmann et al. [15]. Subsequent studies were carried out by assessing the inhibition of plaque formation (ECP) as well as the inhibition of infectious virus production in test tube cultures treated with the compounds for 40 hr after the absorption of the virus.

3. Results and discussion

The cytotoxicity and antiviral activity of distamycin derivatives, obtained by substitutions of the formyl group (II) or the propionamidine chain (III and IV), is shown in table 1. Substitution of the formyl group with a cyclopentyl propionyl chain does not influence its cytotoxicity but the compound loses its antiviral activity completely. The substitution of the propionamidine group with a benzamidine moiety doubles the cytotoxicity of the compound. The antiviral activity of this compound is, however, only 44% of that of distamycin A. The analogue containing butyramidine group in place of the propionamidine moiety (IV) also exhibits a higher cytotoxicity than the compound I. This has only 31% of the antiviral activity of compound I.

These results allow the conclusion that the presence of the formyl group in distamycin is necessary for its antiviral activity. Studies with other derivatives, where the formyl group was substituted by a nitro, amino or acetyl group have shown that all these derivatives are completely inactive against viruses. However, substitutions at the formyl group do not influence the cytotoxicity of the compound. Compounds having substitutions at the propionamidine are active against viruses but exhibit a much higher toxicity. An interesting compound of this group is the acetamidine derivative which showed a higher antiviral activity (150%) than distamycin A.

Using the melting behaviour of DNA–antibiotic complexes as a criterion of binding, a drastic increase in the melting temperature of DNA was observed in the presence of distamycin [6]. This interaction leads to a concentration-dependent inhibition of DNA-dependent RNA-polymerase reaction. Table 2 shows

Table 1
Cytotoxicity and antiviral activity of the distamycin derivatives.

Compound tested	Cytotoxicity*	Inhibition of vaccinia virus multiplication. Inhibition (%)*
I	100**	100**
II	100	0
III	200	44
IV	150	31

* Activity calculated with respect to that of compound I (Distamycin A) considered = 100.
** Absolute values (ID_{50} μg/ml): Cytotoxicity = 80; WR=2.

the template activity of calf thymus DNA in the presence of the natural antibiotic (distamycin A). In these experiments compound I (distamycin A) was pipetted into reaction mixtures containing DNA, buffer and the triphosphates. The reaction was started with DNA-dependent RNA-polymerase. Under these conditions we observed in the range 1×10^{-5}–8×10^{-5} M a concentration-dependent inhibition of RNA-polymerase reaction. As follows from table 2, more than 80% of the reaction is inhibited at 8×10^{-5} M concentration of distamycin A.

Chandra et al. [11] have recently shown that the antiviral activity of distamycins and their action on the template activity of DNA are dependent on the number of pyrrole rings in the molecule. The distamycin derivative with 5 pyrrole rings (Dist/5) has 10 times higher antiviral activity than distamycin A (with 3 pyrrole rings), and is a better inhibitor of the RNA-polymerase reaction. It was therefore interesting to study the correlation between the antiviral activity and the inhibition of RNA-polymerase reaction by compounds I, II, III and IV. The effect of an equimolar concentration (8×10^{-5} M) of the distamycin derivatives on the template activity of DNA is shown in table 3. The derivatives were added into the reaction mixture as described above. The highest inhibition was obtained with the natural antibiotic (distamycin A) followed by compounds IV, II and III, respectively. The cyclopentyl–propionyl derivative, having no antiviral activity is still able to inhibit the RNA-polymerase reaction to more than 50%. This indicates that factors, other than its binding to DNA, are responsible for its inactivity against viruses. One of the many possibilities for this result may be the permeability of this compound towards the host cell. The inhibition of RNA-polymerase reaction by compounds III and IV is in good correlation to their antiviral activity, compared to distamycin A. Our preliminary results on the melting behaviour of DNA in the presence of these derivatives are in good agreement with the present data. These studies will be reported later.

Table 2
Distamycin A inhibition of DNA-dependent RNA polymerase reaction.

System	AMP-^{3}H Incorporation (cpm/reaction mixture)	Incorporation (%)
Complete	3843	100
Without DNA	114	2.9
Complete + Compound I (Distamycin-A)		
4×10^{-5} M	1064	28
8×10^{-5} M	718	19

Distamycin A was pipetted into reaction mixture containing calf thymus DNA, buffer and the triphosphates. The reaction was started with DNA-dependent RNA-polymerase. For details see Materials and methods.

Table 3
Inhibition of DNA-dependent RNA polymerase reaction by distamycin derivatives.

Compound added*	AMP-^{3}H Incorporation (cpm/reaction mixture)	Incorporation (%)
None	3801	100
I	650	17
II	1792	47
III	1990	53
IV	980	26

* Concentration = 8×10^{-5} M.

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