SOME STRUCTURAL REQUIREMENTS FOR THE ANTIBIOTIC ACTION OF DISTAMYCINS

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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* K_{12} [2] and interferes with the multiplication of some DNA-viruses such as vaccina, 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 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 three residues of 1-methyl-4-aminopyrrole-2-carboxylic acid and two side chains, the first constituted by a formyl group, and the second by a propionamidine chain (fig. 1). Arcamone et al. [9, 10] have recently succeeded in in synthesizing some structural analogues of distamycin A. These structural modifications were obtained by substitution of the formyl group, substitution of the propionamidine side chain and variation of the number of pyrrole residues (fig. 1). The present communication describes the activity of distamycin derivatives with 2, 3, 4 and 5 pyrrole rings 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 Zellstofffabriek Waldhof, Mannheim. Calf thymus DNA was supplied by Serva and Co., Heidelberg. All other chemicals were analytical grade reagents from Merck, Darmstadt.

RNA-polymerase reaction: RNA-polymerase was isolated from *E. coli* K_{12} cells according to the procedure by Burgess [11] 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 5–10 mcg enzyme protein and incubations were carried out for 20 min at 37°. The reaction was stopped by adding 3 ml of 5% trichloro acetic acid (TCA) and serum albumin was used as carrier. The precipitate was collected on a membrane filter (Sar-

torius, Göttingen) 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. [12].

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 [13].

Assay on vaccinia virus: Cultures of HeLa cells (grown in HLS medium) or mouse-embryo cells (grown in HLS medium plus 0.1% yeastolate) infected with vaccinia virus (Strain WR/ATCC) were used. Preliminary assays were made according to Herrmann et al. [14]. 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 containing 2, 3, 4 and 5 pyrrole rings is shown in table 1. The cytotoxicity of the derivatives with 2 and 3 pyrrole residues is the same. However, compounds containing 4 and 5 pyrrole rings (Dist/4 and Dist/5) are less toxic. The cytotoxicities of Dist/4 and Dist/5 are only 50% and 25% of the natural antibiotic (Dist/A) respectively. It seems therefore, that the cytotoxicity decreases as the number of pyrrole rings increases. This is at least true for Dist/A, Dist/4 and Dist/5. Our studies on Dist/6 (distamycin with 6 pyrrole rings) have, however, shown that no such relationship strictly exists. Dist/6 was found to be as toxic as Dist/4.

The antiviral activity of distamycin derivatives is dependent on the pyrrole ring. Taking the antiviral activity of the natural antibiotic (Dist/A) as 100, one observes a 4-fold increase for Dist/4 and a 10-fold increase for Dist/5. On the other hand we found about 85% inhibition of the antiviral activity of Dist/A by removing 1 pyrrole ring (Dist/2).

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 A [6]. This interaction leads to a concentration-dependent inhibition of DNA-dependent RNA polymerase reaction. Table 2 shows the template activity of calf thymus DNA in the presence of the natural antibiotic (Dist/A). In these experiments Dist/A was pipetted into reaction mixture containing DNA, buffer and the triphosphates. The raction was started with DNA-dependent RNA polymerase. One gets about 70% inhibition at 4×10^{-5} M and 84% at 8×10^{-5} M. This is in good agreement with our previous results [6]. The effect of an equimolar concentration (4×10^{-5} M) of various distamycin derivatives on the template activity of calf thymus DNA is shown in table 3. The derivatives were added into the reaction mixture as described above. The inhibition of DNA-dependent RNA synthesis increases as the number of pyrrole residues in the antibiotic molecule increases. The compound with 2 pyrrole rings inhibits ^{3}H-AMP incorporation to 50%, whereas Dist/5 at the same molar concentration to 82%.

Table 1
Cytotoxicity and antiviral activity of the distamycin derivatives.

Compound	Number of pyrrole rings (n)	Inhibition of vaccinia virus multiplication* (% inhibition)	Cytotoxicity*
Dist/2	2	13	100
Dist/A	3	100**	100**
Dist/4	4	400	50
Dist/5	5	1000	25

* Activity calculated with respect to distamycin A considered = 100.
** Absolute values (ID_{50} mcg/ml): Cytotoxicity = 80; WR = 2.

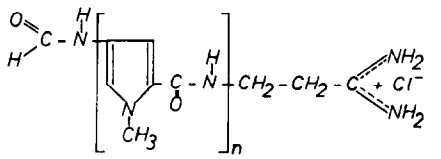
Table 2
Distamycin A inhibition of DNA-dependent RNA polymerase reaction.

System	AMP-^{3}H Incorporation cpm/reaction mixture	% Incorporation
Complete	373	100
Without DNA	11	3
Complete + Distamycin A		
4×10^{-5} M	111	29.6
8×10^{-5} M	60	16.1

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.

Since the antiviral activity of distamycins and their action on the template activity of DNA are dependent on the number of pyrrole rings one may assume that 1-methyl-pyrrole-2-carboxamide group is involved in its binding to DNA. Some preliminary experiments in our laboratory and by others [15] have shown that the stabilizing action of distamycins on DNA increases with increasing number of pyrrole rings of the molecule. This is accompanied by a considerable increase in the hyperchromicity. The melting temperature increase caused by Dist/5 is approximately twice that of Dist/A [15].

None of the compounds mentioned here demonstrated noteworthy activity against RNA viruses. However, equilibrium dialysis studies with calf thymus DNA and polyribonucleotides in the presence of labeled Dist/A have shown a significant binding



n = 2 Dist/2
n = 3 Dist/A
n = 4 Dist/4
n = 5 Dist/5

Fig. 1. Chemical structures of distamycin derivatives.

to polyribonucleotides as well. Among the polyribonucleotides a highest binding was obtained for polyguanylic acid. Some minor changes in the ORD spectrum of RNA have been recently reported by Zimmer and Luck [16].

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

Compound added*	Number of pyrrole rings (*n*)	AMP-^{3}H Incorporation cpm/reaction mixture	% Incorporation
None	–	373	100
Dist/2	2	187	50
Dist/A	3	111	29.6
Dist/4	4	79	21.2
Dist/5	5	67	18.0

* Concentration = 4×10^{-5} M.

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