EFFECT OF ECHINOMYCIN AND OLIVOMYCIN ON RNA SYNTHESIS IN EHRLICH ASCITES TUMOUR CELLS

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1. Introduction

Partial inhibition of RNA synthesis in animal cells with actinomycin D affects preferentially rRNA synthesis while mRNA synthesis seems to be more resistant [1–3]. This action of actinomycin D has been correlated with a selective binding of the drug to guanine-rich rRNA cistrons [4]. However a similar effect was obtained also with 2,4-dinitrophenol, a presumably non-specific inhibitor of RNA synthesis [3]. It has been pointed out too that any decrease in the overall rate of RNA synthesis in animal cells is accompanied by a relatively higher labelling of mRNA, as compared to rRNA [5]. Therefore, rRNA synthesis appears to be more sensitive to the action of inhibitors than is mRNA. In order to elucidate further the observed phenomenon, we studied the action of two antibiotics, echinomycin and olivomycin, on RNA synthesis in Ehrlich ascites tumor (EAT) cells. As shown by others [6–9] these two antibiotics inhibit RNA synthesis, but they differ in their requirement for the presence of guanine residues in the DNA template. The results obtained in this study show, that both antibiotics inhibit preferentially rRNA synthesis, while the labelling of a heterogeneous, "DNA-like" RNA fraction (presumably mRNA) is more resistant.

2. Experimental

The experiments were carried out on the 7th day after inoculation of mice with EAT cells. Echinomycin (10 μg per animal) or olivomycin (30 μg per animal) dissolved in 50% ethanol were introduced intraperitoneally. Two hours after the antibiotic administration 400 μC per animal of carrier-free $Na_2H^{32}PO_4$ were injected. After two hours labelling *in vivo* the mice were sacrificed and the EAT cells collected by low-speed centrifugation in cold.

Total EAT cells RNA was extracted at 65° by a phenolsodium dodecyl sulfate procedure [3,10]. High molecular weight RNA was freed of soluble RNA by precipitation with 1.7 M NaCl and (^{32}P)-phosphate contaminants were removed by passage through a column of Dowex 1, formate form, as described previously [3]. Characterization of labelled RNA was made by analytical agar gel electrophoresis and subsequent radioautography according to Tsanev et al. [11,12]. The nucleotide composition of RNA preparations was determined by the method of Katz and Comb [13].

All reagents used were analytical grade. Freshly distilled phenol was employed, saturated with 0.14 M NaCl and containing 1% 8-oxychinoline, the pH was adjusted to 5.0. Echinomycin and olivomycin were a gift from Drs. N.Loshkareva and G.Gause, Moscow, USSR. Carrier-free $Na_2H^{32}PO_4$ was purchased from Zentral Institut für Kernforschung, Rossendorf ü. Dresden, DDR.

3. Results

In a first series of experiments the dependence of *in vivo* RNA synthesis in EAT cells on antibiotic concentration was studied. As shown in fig. 1a, echinomycin at a final concentration of 10 μg per animal inhibits about 95% of the (^{32}P)-phosphate incorporation in total high molecular weight RNA. Figures of

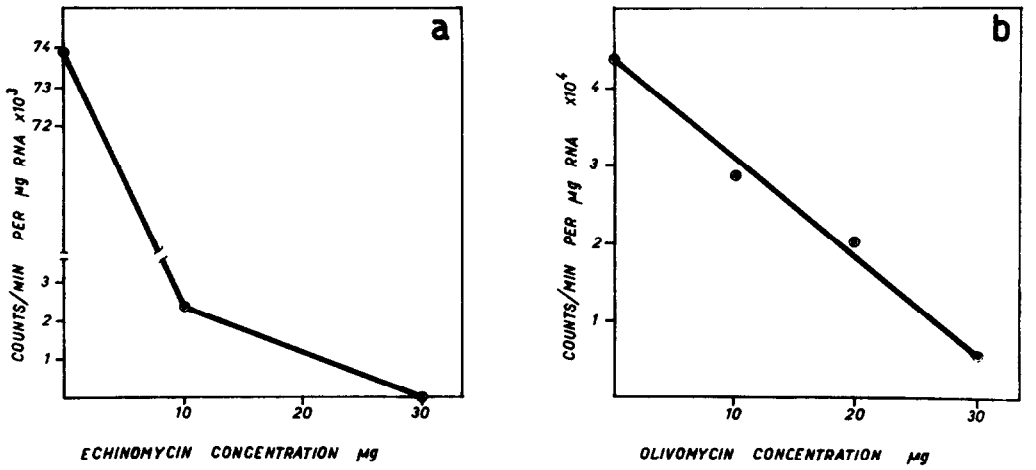

Fig. 1. Inhibition of *in vivo* (^{32}P)-phosphate incorporation in total high molecular weight RNA of EAT cells by echinomycin (a) and olivomycin (b).

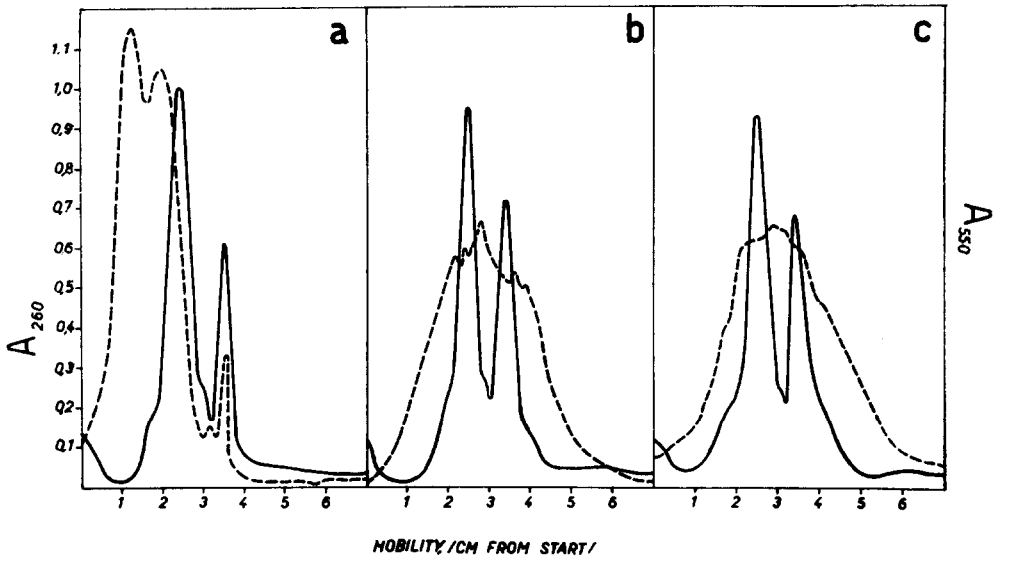

Fig. 2. Agar gel electrophoresis of total high molecular weight RNA from EAT cells after (^{32}P)-phosphate labelling *in vivo* for two hours. The agar gel electrophoretic profiles of control cells (a) and these treated with echinomycin (b) and olivomycin (c). ——A260 mμ, -------A550 mμ.

about 85% inhibition of RNA labelling were attained with 30 μg per animal of olivomycin (fig. 1b).

Agar gel electrophoresis demonstrates that the bulk of total cellular RNA is constituted of the 28S and 18S rRNA peaks (fig. 2a) with an average 28 S/18 S mass ratio of 2.51, calculated from 21 independent assays. Inhibition of EAT cells RNA synthesis by the two antibiotics did not alter the agar gel electrophoretic profiles of total cell RNA (fig. 2b and c).

After two hours labelling *in vivo*, the label in total RNA from control mice is located mainly in the region of the high molecular weight rRNA precursors, namely 45 S and 32 S. The second rapidly labelled fraction forms a shoulder at about 28 S, whereas a well delimited 18 S peak is seen as well (fig. 2a). These results indicate that at two hours labelling *in vivo* part of the label is already transferred to the stable rRNA species of EAT cells.

Table 1
Nucleotide composition of total high molecular weight EAT cells RNA labelled *in vivo* for 2 hours in the presence of inhibitors.

Inhibitor	N	Molar ratio (percent of total)				$\frac{G+C}{A+U}$
		A	U	G	C	
Control						
DIR.	3	17.9	21.4	33.4	27.3	1.54
RAD.	3	18.3	23.2	30.5	28.0	1.41
Echinomycin						
DIR.	3	18.3	20.4	33.4	27.9	1.58
RAD.	3	22.5	28.9	22.9	25.7	0.95
Olivomycin						
DIR.	3	17.9	21.0	33.8	27.3	1.57
RAD.	3	21.0	29.8	24.7	24.5	0.97

DIR. – Direct determination by UV absorption;
RAD. – Determination by (^{32}P)-phosphate distribution;
N – Number of independent experiments.

The distribution of the labelled RNA species is drastically changed in the mice treated with antibiotics where 85 to 90% inhibition of overall RNA synthesis is caused by the antibiotics. Here, the label is distributed heterogeneously in the zone between 40 S and 10 S with somewhat more radioactivity in the area of slower moving RNA fractions. An almost complete disappearance of the label in the region of rRNA precursor components is observed (fig. 2b and c).

The nucleotide composition of total and labelled RNA in control and inhibited cells is shown in table 1. The directly determined nucleotide composition of total EAT cells RNA gives a G + C/A + U molar ratio of 1.57 typical of ribosomal RNA. None of the inhibitors used causes a shift in the directly determined nucleotide composition of total cell RNA. The nucleotide composition of (^{32}P)-phosphate labelled RNA from control EAT cells shows a G + C/A + U molar ratio of 1.41. This figure approaches, but does not attain, the values obtained by direct determination, a finding which is in agreement with previous results from this laboratory on EAT cells RNA labelled *in vivo* [14] and *in vitro* [15].

The nucleotide composition of newly synthesized RNA in the presence of the two antibiotics displays a G + C/A + U molar ratio of 0.95 and 0.97, e.g. there is a clear shift towards "DNA-like" nucleotide composition. In this case the molar ratios of both A and U are significantly higher than in control EAT cells, while the molar ratio of G appears to be much more decreased than that of C.

Assuming a G + C/A + U molar ratio of 1.60 to be typical for ribosomal RNA and of 0.75 for DNA, it may be calculated that the labelled RNA in control experiments contains about 17% "DNA-like" RNA. On the other hand, RNA labelled in the presence of either echinomycin or olivomycin contains about 60–70% "DNA-like" RNA. Consequently, both antibiotics inhibit preferentially ribosomal RNA synthesis, while that of "DNA-like" RNA remains apparently unaltered.

4. Discussion

The results of the present experiments clearly show that 85 to 90% inhibition of overall RNA synthesis in EAT cells by echinomycin or olivomycin leads to a preferential inhibition of rRNA synthesis. This is shown both by the disappearance of the label in the region of rRNA precursors and the shift of the nucleotide composition of rapidly labelled RNA.

The inhibition of RNA synthesis in EAT cells by these two antibiotics is in agreement with previously

reported data by Gause et al. [6–8]. These authors have shown that the action of the two antibiotics is connected with DNA-dependent RNA-polymerase, their effect being most likely due to depression of RNA chain elongation.

The presence of guanine in the DNA template is necessary for the action of olivomycin [6,9] and actinomycin D [4,16] while echinomycin as well as other drugs [9] have no definite requirement for any single base in the template. We have shown previously that 2,4-dinitrophenol, a known uncoupling agent of oxydative phosphorylation, causes also a preferential inhibition of rRNA synthesis [3]. Thus, although these agents have no structural similarities [9] and only some of them have an expressed specific site of action, they all affect preferentially ribosomal RNA synthesis.

These facts may be explained if one assumes that any inhibition of RNA synthesis interferes with some common mechanism of RNA transcription e.g. release of the polynucleotide chain from the template or the transcription rate, the cistrons for rRNA being much more sensitive to different inhibitory agents.

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