LIVER sRNA SYNTHESIS IN ACTINOMYCIN D-TREATED RATS

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Received July 9, 1965

It is well known that actinomycin D inhibits DNA-dependent RNA synthesis (Reich, et al., 1962). In mammalian cells, with moderate doses of the antibiotic synthesis of high molecular weight RNA is nearly completely inhibited, but there is some incorporation of labelled precursors into 4S sRNA. This residual incorporation has been attributed to turnover of the terminal pCpCpA sequence through the action of a pyrophosphorylase (Tamaoki and Mueller, 1962 ; Merits, 1963 ; Franklin, 1963 ; Harel et al., 1964).

Recently Revel and Hiatt (1964) showed that in actinomycin-treated rats label from C^{14}-orotic acid appeared not only in the UMP and CMP residues but also in the ΨUMP of 4S sRNA, indicating that there was synthesis of the polynucleotide chain as well as turnover of the terminal pCpCpA sequence in the presence of actinomycin.

We have studied the nature of the residual incorporation into the sRNA of actinomycin-treated rats. The results show that in addition to turnover of the pCpCpA end, there is incorporation into internal nucleotides amounting to 30 to 50 % of that seen in controls. We have also studied the effect of actinomycin on the incorporation of precursor into the terminal AMP of the pCpCpA sequence.

METHODS

Wistar rats weighing 250 to 300 g were given 15 μC of C^{14}-adenine by intraperitoneal injection. Actinomycin D (Merck, Sharp and Dohme), in a dose of 200 μg unless otherwise indicated, was injected by the same route dissolved in 0.2 ml propylene glycol.

A 10 % homogenate of liver in 0.25 M sucrose was centrifuged for 120 minutes at 145,000 x g (maximum acceleration) in the Spinco model L ultracentrifuge (rotor 40). To the supernatant an equal volume of phenol-tris 0.05 M pH 7.4 containing 0.1 % hydroxyquinoline was added. After 60 minute extraction at 20° the mixture was centrifuged and the aqueous phase extracted twice with ether. sRNA was then precipitated with 2 volumes of alcohol and washed with alcohol and ether.

sRNA was hydrolysed in 0.3 M KOH at 37° for 18 hours. The hydrolysate was adjusted to pH 3.5 and subjected to electrophoresis (1500 volts for 135 minutes). The spots were eluted with 0.1 N HCl and the specific activity of the eluates determined by measurement of radioactivity (Packard liquid scintillation counter) and of UV absorption. The specific activity was expressed in terms of dpm per µg P, after correction for the efficiency of the counter.

RESULTS

In agreement with previous results we found that actinomycin produced a nearly complete inhibition (90 to 95 %) of ribosomal RNA synthesis, while sRNA showed incorporation of radioactive label amounting to 30 to 50 % of that in controls. Analogous results were obtained when other labelled precursors were used. According to Revel and Hiatt (1964), this residual incorporation is not due solely to incorporation into the terminal sequence.

The specific activity of the internal AMP and of the terminal AMP from the pCpCpA end was determined after different periods of labelling with C^{14}-adenine both in actinomycin-treated rats and in control animals. The separation of the two types of AMP was based on the fact that after alkaline hydrolysis the terminal nucleotide appears as the nucleoside (adenosine) whereas all the others yield 2' (3')-nucleotides. C^{14}-adenosine coming from the terminal pCpCpA sequence was separated from the C^{14}-AMP derived from the internal chain by electrophoresis.

In actinomycin-treated animals the appearance of labelling in internal AMP was never completely inhibited, regardless of the duration of the period over which incorporation was measured,

Table 1 Specific activities of internal AMP of liver sRNA in actinomycin-treated rat.

	Incorporation time (hours)						
	0.5	2	6	10	12	14	16
Control	135	250	578	393	851	655	816
Actinomycin	59	63	244	135	200	223	420
% inhibition	56	75	58	66	76	66	48

showing clearly that there is synthesis of sRNA chains in the presence of actinomycin (Table 1). Similar results were obtained with other labelled precursors.

Actinomycin has quite a different effect on the labelling of the terminal AMP (Figure 1). In control animals the curve of incorporation into terminal AMP after a single injection of C^{14}-adenine shows a series of well-defined peaks, and the time of occurence of these peaks is reproducible. A detailed analysis to these findings will appear elsewhere ; here we merely wish to point out that the terminal AMP shows incorporation which is independent of incorporation in the rest of the polynucleotide chain (Scholtissek, 1962 ; Moulé and Landin,

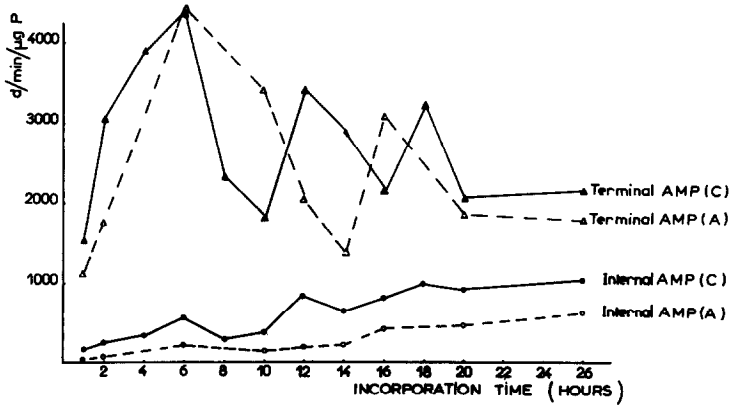

Figure 1 : Time course of C^{14}-adenine incorporation in external and internal AMP of liver sRNA in normal (C) and actinomycin-treated rats (A).

1965). Actinomycin has more of an effect on the time at which the peaks occur in the terminal AMP incorporation curve than on the absolute value of the specific activity of the terminal AMP. Where the peaks coincide in controls and actinomycin-treated animals (for example at 6 hours), there is no difference in incorporation into terminal AMP, while incorporation into the internal AMP is inhibited by 60 % (Table 2). The fact that terminal AMP turnover is due to a pyrophosphorylase and not to RNA-polymerase satisfactorily explains the lack of inhibition of terminal incorporation by actinomycin.

When the dose of actinomycin injected was raised to very toxic levels (1 mg per rat) labelling of internal AMP residues was completely inhibited (Table 2). Labelling of the terminal AMP after treatment with this high dose of actinomycin was still 75 % of that seen in control animals.

Table 2 Specific activities (S.A.) of external and internal AMP of liver sRNA in actinomycin-treated rats (incorporation time : 6 hours)

	External AMP		Internal AMP	
	S.A.	% inhibition	S.A.	% inhibition
Control	5100	-	600	-
Actinomycin 200 µg	4700	8	230	62
1000 µg	3870	24	42	93

DISCUSSION

Inhibition of RNA synthesis by actinomycin is now a well-established fact. This effect is not due to interference with the supply of nucleotide precursors (Harbers and Muller, 1962) but the result of an interaction between the antibiotic and DNA (Reich et al., 1962).

The results reported here distinguish between the action of actinomycin on the terminal AMP and its effects on internal nucleotides. In the presence of actinomycin, synthesis of sRNA chains pro-

ceeds at 30 to 50 % of the rate in controls. Since RNA-polymerase is responsible for the synthesis of both sRNA and ribosomal RNA, it remains to be explained why actinomycin inhibits synthesis of the latter so much more than that of the former. In our opinion, the best explanation is that of Franklin (1963), who suggested that since the cistron for sRNA is much shorter than that for rRNA, the former will be more likely to escape the effect of actinomycin. This explanation is supported by the experiments with high doses of actinomycin reported above, since such doses do inhibit sRNA synthesis completely, perhaps by "saturation" of the receptor sites on DNA.

The effect of actinomycin on the terminal AMP remains to be discussed. It is known that there is no exchange between the terminal nucleotide and the AMP of the amino acyl adenylate, nor turnover coupled to the process of amino acid transfer (Nathans and Lipmann, 1961). By inhibiting RNA-polymerase, and thus the synthesis of messenger RNA, actinomycin presumably alters protein synthesis. If such an alteration is responsible for the changes seen in the incorporation of precursor into terminal AMP, the results reported here may contribute to the understanding of the end turnover of the liver sRNA.

We wish to thank Merck, Sharp and Dohme for a generous gift of actinomycin D.

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