IN VITRO EFFECT OF CYCLOHEXIMIDE ON THE NUCLEOLAR AND EXTRANUCLEOLAR NUCLEAR RNA POLYMERASES OF RAT LIVER

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Received January 18, 1972

SUMMARY

Cycloheximide, which has recently been reported to specifically inhibit RNA polymerase I of water mold Blastocladiella emersonii, did not inhibit appreciably RNA polymerase I of rat liver in vitro. This finding indicates that cycloheximide does not serve as a specific inhibitor of nucleolar RNA polymerase molecule of rat liver.

INTRODUCTION

Certain drugs have so far been reported as an inhibitor of DNA-dependent RNA polymerase (E.C.2.7.7.6.) of bacterial and mammalian origin. Rifampicin was reported to be a specific inhibitor of bacterial RNA polymerase (1)(2) while α-amanitin was shown to selectively inhibit the mammalian RNA polymerase II of extranucleolar nuclear origin (3)(4)(5). It may be of practical advantage to have an inhibitor which acts specifically on polymerase I (nucleolar RNA polymerase) molecule just as

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α-amanitin was useful in the determination of the relative activities of polymerase I, II and III in the isolated nuclei (5). Recently Horgen and Griffin reported that cycloheximide, an inhibitor of protein biosynthesis of eukaryotic cells, exerted a specific inhibitory effect on the RNA polymerase I of the water mold Blastocladiella emersonii (6).

The present communication describes our finding that, in disagreement with the results of Horgen and Griffin, cycloheximide is not effective in the inhibition of RNA polymerase I of rat liver.

MATERIALS AND METHODS

Isolation of nuclei and nucleoli Male albino rats were used throughout the experiment. Nuclei and nucleoli were isolated from livers of rats with Mg^{2+}-procedure as described elsewhere (7).

Preparation of RNA polymerase RNA polymerase was extracted from either isolated nuclei (nuclear total RNA polymerase) or isolated nucleoli (fraction IV) according to the method of Roeder and Rutter (8). RNA polymerase I and II were obtained after chromatography on DEAE-Sephadex A-25 of nuclear total RNA polymerase and stored at -70°C in the presence of bovine serum albumin (1 mg/ml).

Assay of RNA polymerase activity RNA synthetic activity of isolated nucleoli or solubilized enzymes was measured by the incorporation of [^{14}C] UMP from [^{14}C] UTP into acid insoluble material. With isolated nucleoli RNA synthetic activity was measured as described previously (9). With purified enzymes, the assay was carried out as follows. The reaction mixture contained in a final volume of 0.15 ml; 0.05 ml of incubation cocktail —— which was composed of 5 μmoles of Tris-HCl (pH 7.9), 0.2 μmole of ATP,

0.05 μmole each of GTP and CTP, 0.16 μmole of $MnCl_2$, 0.025 μmole of dithiothreitol, 20 μg of native calf thymus DNA (Sigma) and 0.1 μc of [^{14}C] UTP (50 mc/mmole, Radiochemical Centre, England) —— 0.05 ml of distilled water (or cycloheximide solution) and 0.05 ml of RNA polymerase solution. After incubation at 37°C for 20 min, the test tubes were chilled in ice and immediately 125 μl of aliquot was charged onto glass fibre disc (Whatman, GF/C). The discs were washed 5 times in cold 5% trichloroacetic acid-1% sodium pyrophosphate, twice in 99.5% ethanol, dried under an infrared lamp and counted in a liquid scintillation spectrometer using toluene scintillator.

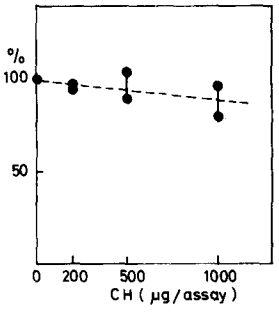

Fig. 1. Effect of cycloheximide on RNA synthetic activity of isolated nucleoli. Assay of the activity was performed as described previously (9). The activity in the presence of cycloheximide was expressed as percentage of the activity without the drug. CH: cycloheximide.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of cycloheximide on the RNA synthetic activity of the isolated nucleoli. Based upon the preliminary finding that at a dose of 200 μg per assay (1.0 ml) the inhibition was, if any, hardly detectable, the amount of the drug was increased up to 1 mg per assay. As seen from Fig. 1, no serious inhibition was noted even at a concentration of 1 mg per assay with inhibition ratio ranging 2 - 20%. In order to

rule out the possibility that cycloheximide might be degraded or metabolized to an inactive form by some enzymatic activities within the nucleolus, the experiments with solubilized (fraction IV of Roeder and Rutter) and chromatographically purified RNA polymerases were carried out. The effect of cycloheximide on the nucleoplasmic RNA polymerase was also examined. That the nucleolar fraction IV contains only polymerase I and nucleoplasmic fraction IV only polymerase II was demonstrated by Roeder and Rutter (8) and confirmed by ourselves (data are not presented).

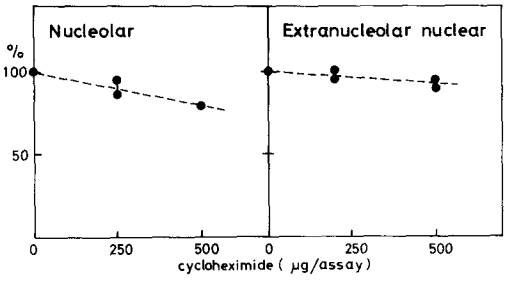

Fig. 2. Effect of cycloheximide on the solubilized (Fraction IV of Roeder and Rutter) nucleolar and extranucleolar nuclear RNA polymerases from corresponding subnuclear fractions. Assay of RNA polymerase activity are presented in MATERIALS AND METHODS.

Fig. 2 and 3 show that both nucleolar and nucleoplasmic RNA polymerases are relatively resistant to cycloheximide. The results with the solubilized nucleolar enzyme were similar to those obtained with isolated nucleoli. In contrast to the finding by Horgen and Griffin that the activity of purified RNA polymerase I of water mold was completely inhibited by 200 µg/ml of cycloheximide (6), rat liver nucleolar enzyme was inhibited only 7% at 300 µg per assay and 28% even at 1 mg per assay (Fig. 3). Taking into account that in Fig. 3 the volume of the assay mixture was 0.15 ml, the concentration of the drug (µg/ml)

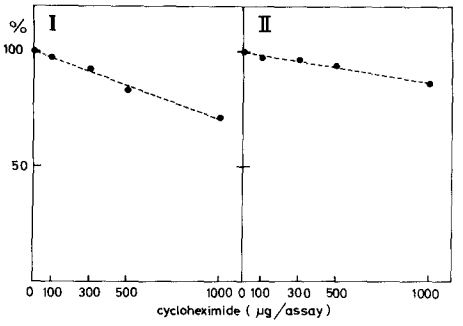

Fig. 3. Effect of cycloheximide on the chromatographically purified nucleolar and extranucleolar nuclear RNA polymerases. Details of the procedure are given in MATERIALS AND METHODS.
I : Nucleolar RNA polymerase
II: Extranucleolar nuclear RNA polymerase

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was much higher. The resistant nature of polymerase II was compatible with the results of Horgen and Griffin (6).

The different results obtained with polymerase I may suggest the structural difference of this enzyme between water mold and rat liver and also indicate that at least in rat liver cycloheximide can not serve as a specific inhibitor of polymerase I molecule.

In addition, these findings do corroborate the previous assumption (9) that the inhibition of nucleolar RNA synthesis of rat liver exerted by cycloheximide in vivo was not the result of direct action of cycloheximide itself, but was mediated by the cessation of protein biosynthesis.

ACKNOWLEDGEMENTS

The authors thank Mr. M. Sakai for excellent technical assistance. This work was supported in part by grants from the Ministry of Education of Japan.

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